CHAPTER – 2

Literature Review

2.1 OBJECTIVE RELATED BACKGROUND

Oral ingestion is the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, cost-effectiveness, least sterility constraints and flexibility in the design of dosage form. As a result, many of the generic drug companies are inclined more to produce bioequivalent oral drug products. The high costs and time involved in new drug development, expiry of patents for a significant number of drug molecules, ease of manufacturing and ready availability of technology for the production of oral drug products are also driving the generic pharmaceutical companies towards the development of bioequivalent oral dosage forms [Wenlock et al., 2003; Vieth et al, 2004]. However, the major challenge with the design of oral dosage forms lies with their poor bioavailability.

The term bioavailability is defined as the rate and extent (amount) of absorption of unchanged drug from its dosage form. The rate of absorption plays crucial role in the treatment of acute conditions like pain while the extent of absorption is critical in the treatment of chronic conditions like asthma. A poorly bioavailable drug is one of the major challenges for formulation scientists, because it can lead to compromised product performance and the drug is unlikely to reach its molecular target. Poorly bioavailable compounds require a high dose but have low systemic exposure, result in adverse effects because of the high dose and may vary in their effect in individual patients. The oral bioavailability depends on several factors including aqueous solubility, drug

permeability, dissolution rate, first-pass metabolism, pre-systemic metabolism and susceptibility to efflux mechanisms [Brahmankar and Jaiswal, 2012].

The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability. The sufficient drug dissolution, solubility in human fluids and permeability in gastrointestinal tract (GIT), ultimately sufficient oral bioavailability of drug, is the key factor to achieve desired drug effect and results in therapy. These three factors affiliate to the rate and the extent of oral drug absorption of drug and are thus, closely related to the oral bioavailability of drugs [Sakaeda et al., 2001].

Based on the pharmacokinetic properties like rate and extent of drug absorption, a classification system, the Biopharmaceutics Classification System (BCS) has been created to sort potentially active pharmaceutical solids. The BCS is a guide for predicting the intestinal drug absorption and is provided by the United States Food and Drug Administration (USFDA). The fundamental basis for the BCS was established by Gordon Amidon, who was presented with a Distinguished Science Award at the International Pharmaceutical Federation Congress in Salvador, Brazil, August 2006, for the same. The BCS is an experimental model that measures permeability and solubility under prescribed conditions. The original purpose of the system was to aid in the regulation of post-approval changes and generics, providing approvals based solely on *in-vitro* data when appropriate. Importantly, the system was designed around oral drug delivery since the majority of drugs is and remains orally dosed. Waivers, permission to skip *in-vivo* bioequivalence studies, are reserved for drug products that meet certain requirements around solubility and permeability and for those that are also rapidly dissolving [Amidon et al., 1995; Folkers et al., 2003].

The BCS formulated by the Food and Drug Administration (FDA) classifies active pharmaceutical ingredients (APIs) into four classes (Figure 2.1) on the basis of solubility and intestinal permeability.

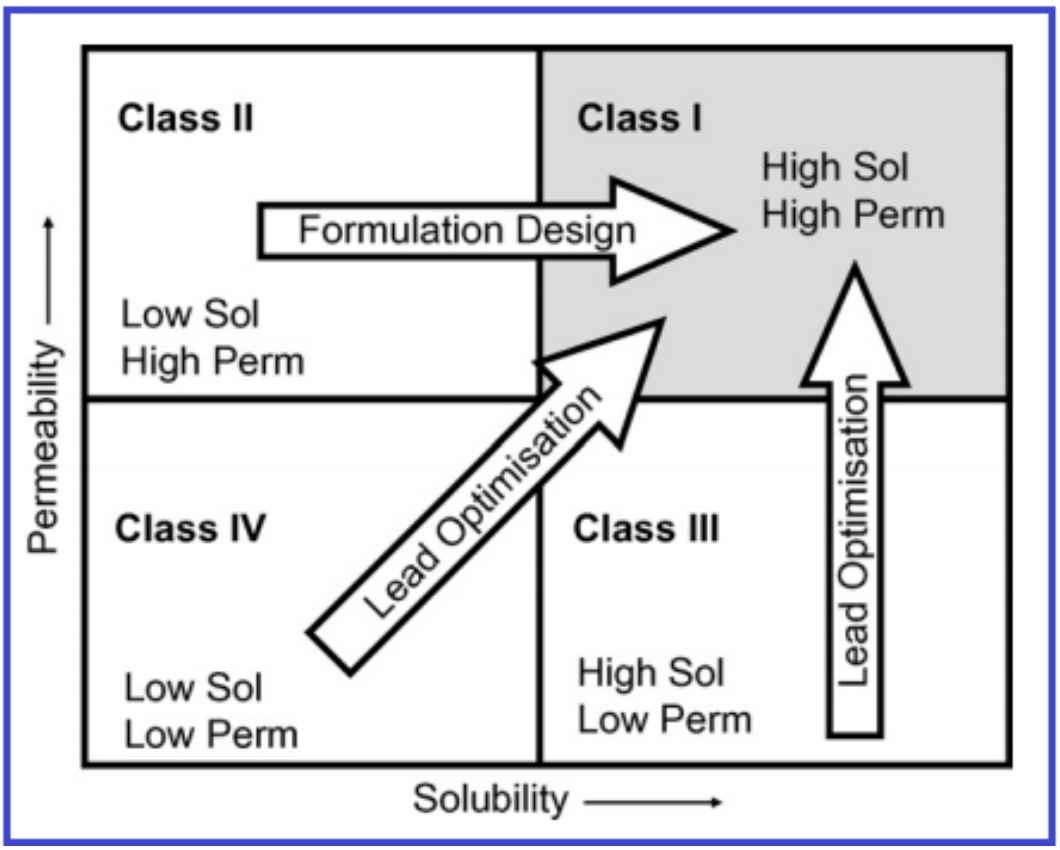


Figure 2.1. BCS Classification system.

According to the BCS, drug substances are classified as follows:

Class I - high permeability, high solubility. Example: metoprolol. These compounds are well absorbed and their absorption rate is usually higher than excretion.

Class II - high permeability, low solubility. Example: glibenclamide. The bioavailability of these products is limited by their solvation rate. A correlation between the *in-vivo* bioavailability and the *in-vitro* solvation can be found.

Class III - low permeability, high solubility. Example: cimetidine. The absorption is limited by the permeation rate but the drug is solvated very fast. If the formulation does not change the permeability or gastro-intestinal duration time, then class I criteria can be applied.

Class IV - low permeability, low solubility. Example: hydrochlorothiazide. These compounds have a poor bioavailability. Usually they are not well absorbed over the intestinal mucosa and a high variability is expected.

The drugs are classified in BCS on the basis of following parameters:

a) Solubility

b) Permeability

c) Dissolution

The class boundaries for these parameters are:

a) Solubility class boundaries

It is based on the highest dose strength of an immediate release product. A drug is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1 to 7.5. The volume estimate of 250 mL is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass of water.

b) Permeability class boundaries

It is based indirectly on the extent of absorption of a drug substance in humans and directly on the measurement of rates of mass transfer across human intestinal membrane. Alternatively, non-human systems capable of predicting drug absorption in humans can be used (such as *in-vitro* culture methods). A drug substance is considered highly permeable when the extent of absorption in humans is determined to be 90% or

more of the administered dose based on a mass-balance determination or in comparison to an intravenous dose.

c) Dissolution class boundaries

An immediate release product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 15 minutes using United States Pharmacopoeia (USP) Dissolution Apparatus 1 at 100 rotations per minute (RPM) or Apparatus 2 at 50 RPM in a volume of 900 mL or less in the following media: 0.1 N HCl or simulated gastric fluid, pH 4.5 buffer and pH 6.8 buffer or simulated intestinal fluid [Amidon et al., 1995; Folkers et al., 2003].

Unfortunately, a lot of promising pharmaceutical substances, currently 40% of the discovered drugs fall under BCS Class II and IV and are inconvenient oral candidates due to their strong hydrophobic character exhibiting low solubility in water thus low bioavailability. So, drug molecules with limited aqueous solubility are currently the major problem for the pharmaceutical industry. Low solubility leads to many disadvantages such as slow dissolution in biological fluids, insufficient and inconsistent systemic exposure and consequent sub-optimal efficacy in patient, particularly when delivered via the oral route of administration [Liu, 2008].

Further discouragingly, most of the entities listed on the World Health Organization's (WHO's) list of essential medicines indicate an evolving trend towards more hydrophobic compounds as shown in Figure 2.2. This means that there is a growing demand for strategies and processing technologies to increase the bioavailability of intrinsically poorly soluble compounds [Müllers, 2015].

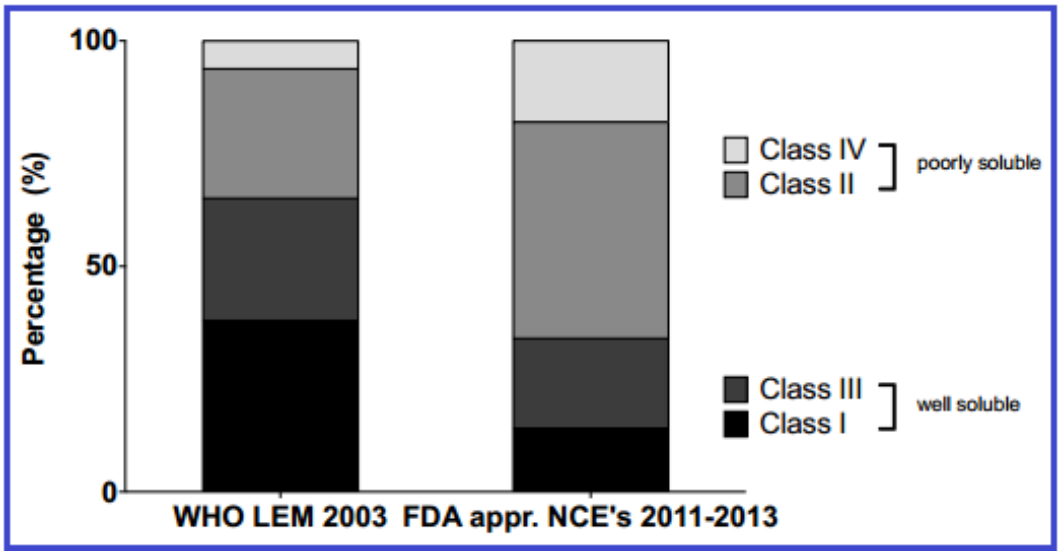


Figure 2.2. Distribution over the BCS classes of API's on the WHO's essential list of medicines (WHO LEM) from 2003 and of new chemical entities approved by the Food and Drug Administration (FDA appr. NCE's) from 2011 – 2013 [Müllers, 2015].

For these reasons, the development of formulations for optimal oral drug delivery is essential and challenging in the current pharmaceutical research. A lot of money and effort is invested to find an appropriate formulation. There are a number of methods how to improve the biopharmaceutical properties of drugs with poor or inconvenient bioavailability or dissolution. For oral drug delivery, a simplified summary of approaches based on properties might look like those presented in the tabulated Figure 2.3 [http://www.particlesciences.com/news/technical-briefs/2011/biopharmaceutical-classification-system.html], shown below. Each approach must then be tailored to meet the other demands of that particular API and desired product profile [http://www.particlesciences.com/news/technical-briefs/2011/biopharmaceutical-classification-system.html].

BCS Class	Solubility	Permeability	Oral Dosage Form Approach	Chances of Non-oral Dosage Form being Required
1	High	High	Simple solid oral dosage form	
2	Low	High	• Techniques to increase surface area like particle size reduction, solid solution, solid dispersion • Solutions using solvents and/ or surfactants	
3	High	Low	Incorporate permeability enhancers, maximize local lumenal concentration	
4	Low	Low	Combine 2 and 3	

Figure 2.3. Increasing order of challenge for oral formulation development.

According to the literature, between 60 and 70 % of the API's in the pipeline show sufficient membrane permeability but a low water solubility and thus belong to BCS class II [Rehder, 2013]. A majority of the recently developed drugs on the market is also classified as poorly soluble BCS II and statistically, 40 % of new molecules are thus excluded from further development at the early stages of pharmaceutical research as their bioavailability may be solubility and dissolution rate-limited after oral administration [Liu, 2008].

All the BCS class II new drug candidates present poor pharmacokinetic properties due to their low solubility and poor dissolution performance. So, aqueous solubility and fast dissolution are key parameters to achieve adequate bioavailability of BCS class II API's [Sakaeda et al., 2001]. The solubility term descriptions as per the USP/National Formulary (NF) and the European Pharmacopoeia (Ph. Eur.) were provided in the Figures, 2.4 and 2.5, respectively.

Descriptive term	Parts of solvent required for 1 part of solute
Very soluble	≤ 1
Freely soluble	1 to 10
soluble	10 to 30
Sparingly soluble	30 to 100
Slightly soluble	100 to 1000
Very slightly soluble	1000 to 10000
Practically insoluble, or insoluble	≥10000

Figure 2.4. Solubility terms given by USP/NF.

Descriptive term	Approximate volume of solvent (mL) per weight of solute (g)	Corresponding solubility (mg/mL)
Very soluble	Less than 1	More than 1000
Freely soluble	From 1 to 10	From 1000 to 100
Soluble	From 10 to 30	From 100 to 33.3
Sparingly soluble	From 30 to 100	From 33.3 to 10
Slightly soluble	From 100 to 1000	From 10 to 1
Very slightly soluble	From 1000 to 10000	From 1to 0.1
Practically insoluble	More than 10000	Less than 0.1

Figure 2.5. Classification of drug solubilities according to Ph. Eur. 7th Edition.

Aqueous solubility and dissolution rate are particularly important in the context of BCS class II drug performance since orally delivered drugs must dissolve from their dosage form within the GIT in order to be absorbed, first by the tissue of the intestines and ultimately into circulation. To increase their water solubility and thus their bioavailability, different formulation approaches may be applied and studied.

2.2 DISEASE RELATED BACKGROUND

Cardiovascular diseases (CVDs) are the world's first leading causes of death. It is a class of disease that involves the heart or blood vessels (arteries, capillaries and veins), which refers to any disease that affects the cardiovascular system, principally cardiac disease,

peripheral arterial disease, and vascular diseases of the brain and kidney. The causes of CVDs are divers but atherosclerosis and / or hypertension are the most common [Lafta, 2014].

Atherosclerosis is a type of *arteriosclerosis*. *Arteriosclerosis* is a general term for the thickening and hardening of arteries. Arteries are the blood vessels that carry oxygen-rich blood to the heart and other parts of the body. Atherosclerosis, or hardening of the arteries, is a condition in which plaque builds up inside the arteries. Plaque is made of cholesterol, fatty substances, cellular waste products, calcium and fibrin (a clotting material in the blood). Over time, plaque hardens and narrows the arteries. This limits the flow of oxygen-rich blood to the organs and other parts of the body. Atherosclerosis can lead to serious problems, including heart attack, stroke, or even death [http://www.nhlbi.nih.gov/health/health-topics/topics/atherosclerosis]. Figure 2.6 describes the relation between high blood cholesterol levels and atherosclerotic plaque formation.

The CVDs and the associated deaths have been increasing at an astonishingly fast rate in low-and middle income countries. Indeed, India is the heart disease capital of the world! There are many things that put Indians at risk — sedentary lifestyles, an unhealthy diet, stress, diabetes and even narrower arteries. Indians reportedly have narrower arteries as compared to their Western counterparts, putting them at greater risk for a heart attack. Dr Tilak Suvarna, interventional cardiologist, Asian Heart Institute, Mumbai, said, "While the diameter of the arteries is between 3-4 mm among Westerners, it is between 2-3 mm among Indians. Clogging and formation of deposits causing obstruction is easier in smaller arteries. Even bypass surgery and grafting become technically challenging". Indians could thus be highly prone to atherosclerosis

like arterial CVDs [http://timesofindia.indiatimes.com/life-style/health-fitness/health-news/Indians-at-risk/articleshow/6629723.cms].

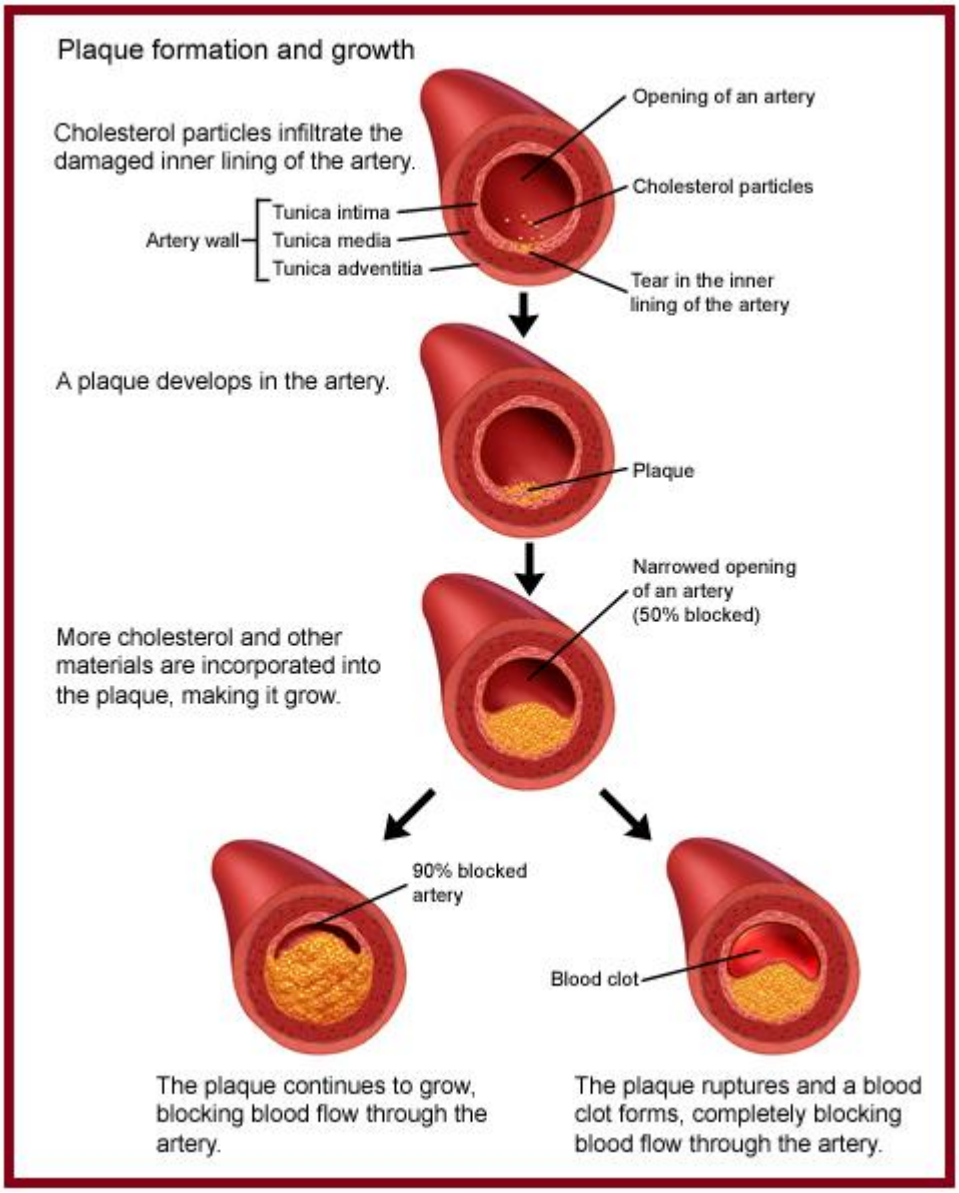


Figure 2.6. Formation of arterial plaque - cholesterol deposition and atherosclerosis.

One of the major risk factor for the development of CVD is hypercholesterolemia. Patients with hypercholesterolemia are at very high risk of coronary artery diseases

(CADs) like atherosclerosis and atherosclerosis-associated conditions. WHO predicts 11.1 million deaths globally and 71% deaths in developing countries due to CAD by 2020 A.D. Hypercholesterolemia is defined as elevated amounts of cholesterol in the blood and usually involves elevated plasma levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL), very low density lipoprotein cholesterol (VLDL) and a low level of high density lipoprotein cholesterol (HDL) [Lafta, 2014].

Cholesterol is a waxy, fat-like substance that is naturally found in the walls of cells. It is a structural component of all cell membranes and is also a precursor to steroid hormones, vitamin D, and bile acids that aid in digestion of fat. If the amounts of cholesterol in the blood are excessive, cholesterol can build up in arteries, which can lead to CAD and many other serious conditions. Nonfamilial (non-inherited) hypercholesterolemia is the most common form of hypercholesterolemia. It occurs in people with a susceptible genotype which is aggravated by excessive intake of saturated fats and cholesterol. Familial hypercholesterolemia (FH) is an inherited genetic disorder. The children of people with familial hypercholesterolemia may inherit either the normal gene or the defective gene, so the prevalence within that family will be approximately 50%. It is the most common potentially fatal genetic disorder. FH is a diagnosis which refers to individuals with very significantly elevated LDL or "bad cholesterol". In heterozygous familial hypercholesterolemia (HeFH), an individual inherits a mutation (alteration) for FH from one (affected) parent. In homozygous familial hypercholesterolemia (HoFH), an individual inherits a causal FH mutation from both parents. HeFH is one of the most common genetic diseases and affects at least 1 in 500 individuals. If DNA testing is performed, most (60-80%) will be found to have a

mutation in one of three relevant genes. Others may express these clinical findings for other reasons or may carry a mutation in a gene or genes that have yet to be discovered. HeFH is characterized by very high levels of LDL, as well as of TC. The condition greatly increases the risk of hardening of the arteries (atherosclerosis), which can lead to heart attacks, strokes and other vascular conditions. Individuals with HeFH have a 20-fold increased risk for CAD. HoFH is very rare (~ 1 in 250,000 to 1 in 1 million). LDL levels are usually, though not always, > 400 mg/dL. Severe vascular disease including CAD and aortic stenosis are often seen by the teenage years. Without very aggressive treatment including LDL apheresis and HoFH specific medications, mortality is common before age 30 [http://rarediseases.org/rare-diseases/familial-hypercholesterolemia/].

Cholesterol is an essential biological molecule that performs many functions within the body. Within membranes, the cholesterol to polar lipid ratios affect stability, permeability, and protein mobility. The hormones produced from cholesterol include androgens, estrogens, and the gluco- and mineralocorticoids. Cholesterol levels in the body are achieved via two sources. Adults with healthy diets will biosynthesize the majority of their cholesterol in the liver and other body tissues and obtain the remainder from the dietary intake of foods high in saturated fatty acids. Free cholesterol is not found in blood; rather it is esterified to fatty acids and packaged in lipoprotein particles. Very low density lipoproteins are produced by the liver and consist of an outer core composed of apolipoproteins; apo-B100, apo-CI, apo-CII, apo-CIII, and apoE surrounding an inner core of phospholipids, TGs, cholesterol, and cholesteryl esters. In the blood, the very low density lipoproteins transfer apolipoprotein-CII to high density lipoprotein and lipoprotein lipase in the capillaries begins to remove the TGs,

transforming the particle into an intermediate density lipoprotein. About 50% of intermediate density lipoprotein particles are removed from the circulation by the liver. The remaining intermediate density lipoproteins are transformed to low density lipoprotein particles by the loss of apolipoprotein E and the further reduction of TG content until it is exceeded by the content of cholesteryl esters. The cholesterol packed in these low density lipoprotein particles, the LDL, is the so-called "bad" cholesterol. Low density lipoproteins particles deliver lipids to the body cells via LDL receptor-mediated endocytosis. When LDL lipids are oxidized by free radicals, they bind more easily to the proteoglycans lining the vascular endothelium, and thus, become incorporated into atherosclerotic plaque. HDL, the so-called "good" cholesterol is composed of apolipoproteins-CII and E surrounding a lipid core. The high density lipoprotein particles circulate through the capillaries collecting lipids including cholesterol and cholesteryl esters and returning them to the liver for further metabolism. Cholesterol returned to the liver in form of HDL by high density lipoproteins is synthesized into bile acids. Bile acids facilitate the digestion of lipids by acting as emulsifying agents and also aid in the absorption of fat-soluble vitamins. Cholesterol is ultimately excreted from the body as bile acids.

Excessive levels of oxidized LDL in the blood can lead to potential health risks. Normally, cholesterol levels are tightly controlled by complex mechanisms. When dietary intake of cholesterol is high, biosynthesis is reduced. However, the body's homeostatic mechanisms can be inadequate when baseline endogenous cholesterol biosynthesis becomes excessive or when dietary cholesterol intake is overwhelming. For these instances, drugs have been discovered that can reduce cholesterol biosynthesis (statins), reduce the intestinal absorption of dietary cholesterol and other lipids

(ezetimibe), or enhance the metabolic utilization of lipids in the liver (fibrates). These drugs serve to keep the blood levels of LDL in check to avoid the deleterious effects that can arise from the accumulation of vascular plaque, and thus help in avoiding the associated serious medical conditions such as atherosclerosis, CAD, and stroke [https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/General_Information/2/biofiles_issue12.pdf].

2.3 FORMULATION RELATED BACKGROUND

As already mentioned under the objective related background, there are a few limitations wherewith the scientists and pharmaceutical industry have met in oral drug administration. The first and major ones are the unsatisfactory pharmacokinetic properties resulting in poor or inconvenient oral bioavailability. According to the literature, between 60 and 70 % of the APIs in the pipeline show sufficient membrane permeability but a low water solubility and thus belong to BCS class II [Rehder, 2013]. Their bioavailability may be solubility or dissolution rate-limited after oral administration. To increase their water solubility and thus their bioavailability, different formulation approaches may be applied and studied. The introduction of large numbers of poorly bioavailable drugs creates the demand for updated pharmaceutical technologies to meet these challenges. The market for formulation technologies is likely to increase because improvements in the formulation technologies have provided new directions for product development. However, there is no universal technology that is appropriate for all poorly soluble drugs.

Solubility and dissolution rate are very important properties to the pharmaceutical industry since the bioavailability of an API is often related to them. For BCS class II

drugs with low solubility and reasonable permeability, drug solvation and dissolution steps are the rate-liming processes of drug absorption. To be efficacious, the active drug substance must be released from the drug product and absorbed into the systemic circulation so that it can be transported to its site of activity. Upon oral administration, if drugs are not completely released in the gastrointestinal area, they will have a low bioavailability. Therefore, drug solvation and release are crucial and limiting steps for drug bioavailability particularly for BCS class II drugs. By improving the drug solubility and release profile of these drugs, it is possible to enhance their bioavailability and reduce side effects [Giliyar et al., 2006].

When administered as oral dosage forms, the pharmaceutical formulation plays a critical role in the absorption of BCS class II drugs from GIT. A variety of pharmaceutical formulation technologies are used to enhance the oral bioavailability of BCS class II drugs. They use already approved excipients and Generally Regarded as Safe (GRAS) materials. This in turn reduces the cost and development time. The main technologies to achieve the enhanced oral bioavailability of drugs with poor aqueous solubility include the use of micronization, nanosizing, crystal engineering, solid dispersions, cyclodextrins, polymeric nanoparticles, solid lipid nanoparticles and other colloidal drug delivery systems such as microemulsions, self-emulsifying drug delivery systems, self microemulsifying drug delivery systems, liposomes and drug nanocrystals. All these approaches have benefits and drawbacks and no approach can be used universally. In other words, there is no universal technology that is appropriate for all poorly soluble drugs [Gomez-Orellana, 2005].

The tremendous pharmaceutical research in understanding the causes of low oral bioavailability has led to the development of the above listed novel technologies to

address the challenge. The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability. In addition, subject to the drug properties, its oral bioavailability may be further enhanced by using one or more of the following approaches individually or in combination: improving its solubility and dissolution, inhibiting efflux transport, bypassing first-pass metabolism, increasing its retention time in the intestine, increasing the intestinal permeability of the drug, preventing its pH-dependent precipitation, or increasing its stability in the intestine by changing its site of absorption, etc [Alam et al., 2013].

The drug chosen for the present study, ezetimibe (Eze), is a BCS class II drug. Three different formulation approaches, pharmaceutical cocrystals (CoCs), ternary cyclodextrin (CD) complexes and drug nanocrystals (NCs), were exploited in this research work and the same were discussed in this section to provide a formulation related background review.

2.3.1 Cocrystals (CoCs)

In the pharmaceutical industry, it is the poor biopharmaceutical properties rather than toxicity or lack of efficacy that are the main reasons why less than 1% of active pharmaceutical compounds reach the marketplace. Among these biopharmaceutical properties, solubility remains a key property, with drugs often damaged during commercial production due to their low solubility. The improvement of solubility and dissolution profiles of these drug molecules without changing the molecular structure is a special challenge for the successful development of pharmaceutical products. Cocrystallization is one such useful method. Since a long time ago, there is an interest in the design of pharmaceutical CoCs, which becomes as a potential method for

improving the bioavailability of drugs with low aqueous solubility without changing the molecular structure of the drug [Thakuria et al., 2013]. The structural composition of a simple CoC includes two components, a drug and a coformer. CoCs with the same API can have remarkably different pharmaceutical properties depending on the nature of the second component. The CoCs are a homogeneous phase of stoichiometric composition and not a mixture of pure component crystalline phases. The CoCs are stabilized mainly through intermolecular hydrogen bonding interactions between the API and the guest molecule. The formulation of CoCs is very attractive in pharmaceutical chemistry due to the possibility of influencing the solubility and dissolution of drugs without changing the molecular structure of the API [Blagden et al., 2007].

CoCs are the latest material being explored in order to enhance drug properties. When using CoCs, the bulk material and the physicochemical properties of the API can be modified while still maintaining the intrinsic activity of the drug molecule. Cocrystallization is one of the emerging crystal engineering techniques for modulating the pharmaceutical performance through controlling solid-state properties of APIs. This is possible because cocrystallization significantly expands the access to new solid forms. Recognizing the growing interest in using CoCs for drug product development, the USFDA released draft guidance on the subject of regulatory classification of pharmaceutical CoCs. The FDA guidance released on pharmaceutical CoCs specifies that the CoC components exist in their neutral states and interact via non-ionic interactions, as opposed to ionic interactions, which would classify this crystalline solid as a salt form. [http://www.fda.gov/downloads/Drugs/.../Guidances/UCM281764.pdf].

The API and coformer can interact through hydrogen bonding, or van der Waals forces. In theory, all types of drug molecules have the capability to form CoCs; therefore, CoCs

have advantages over traditional solid-state modification techniques (e.g., salts, solvates, hydrates, and polymorphs). For example, CoCs provide an alternative for APIs that are unable to form salts due to lack of ionization moieties. Since the formation of CoCs involves supramolecular bonding like the hydrogen bonding, or van der Waals forces, a brief note on the supramolecular chemistry has been provided below.

The term of supramolecular chemistry has been defined by Jean-Marie Lehn in 1978. The Nobel laureate of 1987 has defined it as chemistry of non-covalent interactions between host and guest molecules. Supramolecular chemistry refers to the domain of chemistry beyond that of molecules and focuses on the chemical systems made up of a discrete number of assembled molecular subunits or components. There are two main subsets of supramolecular chemistry, namely (i) host-guest chemistry and (ii) self-assembly. In host-guest systems, the smaller molecule 'guest' becomes enveloped by the larger molecule called 'host' in a binding region, while in a self-assembled system, two or more species with no significant difference in size are joined together by non-covalent bonds [Shattock, 2007]. The selectivity phenomenon observed in supramolecular chemistry is shown in Figure 2.7.

The intermolecular interactions are typically hydrogen bonds and van der Waals interactions. Speaking in relevance to the pharmaceutical industrial applications, one of the frequently utilized host compounds in supramolecular chemistry are cyclodextrins (CDs) that are the focus of the next subsection. The most pharmaceutically relevant supramolecular systems, next only to CD complexes are CoCs.

Considering the increasing interest on CoCs in the pharmaceutical industry and academia, this formulation approach was exploited in the present research work.

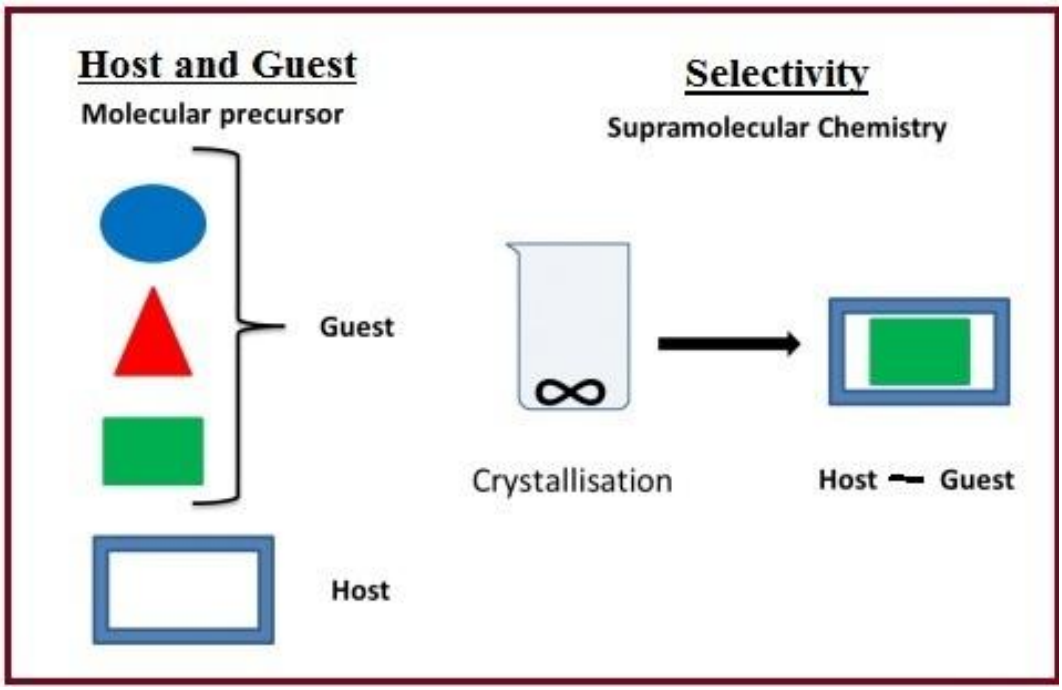


Figure 2.7. Schematic representation of supramolecular chemistry showing the phenomenon of selectivity.

Supramolecular chemistry can be viewed as chemistry beyond the molecule: while traditional molecular chemistry focuses on the intramolecular covalent bond, supramolecular chemistry examines intermolecular bonding, the weaker and reversible noncovalent interactions between molecules, mainly hydrogen bonding, hydrophobic forces, and van der Waals forces. The study of non-covalent interactions is crucial to understanding many biological processes from cell structure to vision that rely on these forces for structure and function. Biological systems are often the inspiration for supramolecular research. Hydrogen bonds play a key role in biological systems. The three-dimensional structures in proteins and nucleic acids are formed through hydrogen bonds. In these macromolecules, bonding between parts of the same macromolecule causes it to fold into a specific shape, which helps to determine the molecule's

physiological or biochemical role. The ultimate example of a hydrogen bonded array is provided by nature in the form of the double helix DNA, which is formed by complementary base pairing between cytosine and guanine; and adenine and thymine; and enables replication [Martin, 2011; Schmidt, 2013].

Solid state supramolecular forms are manifestations of self-assembly. Hydrogen bonding is one of the most important and most often occurring intermolecular interactions in organic crystalline materials. In supramolecular chemistry, hydrogen bonding has been described as the 'master key interaction' because of its strength and highly directional nature. The definition for hydrogen bond is "a bond that forms when a hydrogen atom attached to an electronegative atom (or electron withdrawing group) is attracted to a neighbouring dipole on an adjacent molecule or functional group". According to the International Union of Pure and Applied Chemistry definition, "the hydrogen bond is an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X-H in which X is more electronegative than H, and an atom or a group of atoms in the same or different molecule, in which there is evidence of bond formation". The latter definition is more precise from that aspect that includes the intramolecular hydrogen bonds also. They can be represented as D-H···A where D is a donor and A is the acceptor. Hydrogen bonding occurs most commonly between donor groups such as C-H, N-H, O-H, F-H, P-H, S-H, Cl-H, Br-H, I-H and acceptors groups of N, O, P, S, Cl, Br, I, alkenes, alkynes, and aromatic π-systems [Desiraju, 1989; Lu and Rohani, 2009; Gao et al., 2012].

Crystal engineering, a sub-discipline of supramolecular chemistry, is the study of the recognition of molecules by one another during crystallization. Crystalline solids are actual manifestations of self-assembly. Crystal engineering involves modification of the

crystal packing of a solid material by changing the intermolecular interactions that regulate the breaking and formation of non-covalent bonds, particularly hydrogen bonding. 'Synthone' (connector between molecules) is a term widely used to describe the bonding between the components of a CoC. In 1995, a review article by Desiraju introduced the formal literature definition of the supramolecular synthon, based on analogy with the definition of synthons [Desiraju, 1995]. Supramolecular synthons are helpful for understanding and designing CoCs. Two main types of supramolecular synthons can be identified in crystals: supramolecular homosynthons and supramolecular heterosynthons. Supramolecular homosynthons occur as a consequence of the interaction between identical, complementary functional groups as in the case of the carboxylic acid dimer or an amide dimer. Supramolecular heterosynthons result from the interaction between different but complementary functional groups. Examples of supramolecular heterosynthons include: carboxylic acid···pyridine, alcohol···pyridine and carboxylic acid···amide [Kilinkissa, 2014].

The classification of solid state forms and crystalline forms were shown in Figures, 2.8 and 2.9, respectively. The classification describes the long known differentiation of a solid state form substance as crystalline form or amorphous form. Crystalline forms are the most preferred state on account of their long term physicochemical stability (the mobility of drug particles in crystalline phase is minimal when compared to amorphous phase). The crystalline form solid substance could further exist as single component polymorphs or as multicomponent structures like salts or hydrates or solvates or CoCs. On account of the crystalline state advantage, involvement of non-ionic or non-covalent bonding and lack of a liquid component in the structural composition (an API hydrate or

solvate may still form a CoC with a suitable coformer), CoCs have gained increased interest in the recent years.

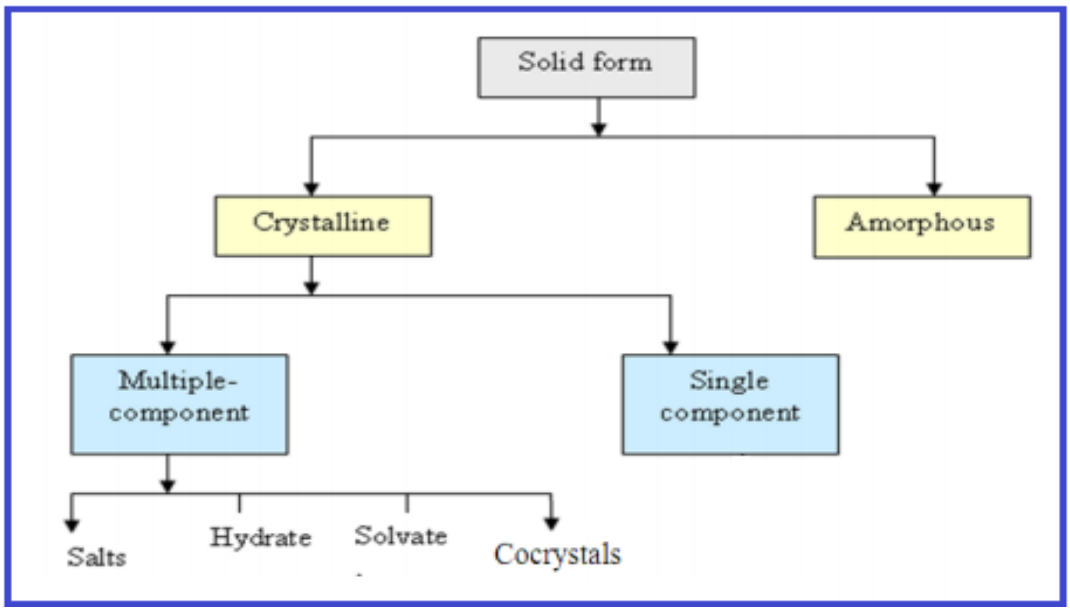


Figure 2.8. Classification of solid state forms.

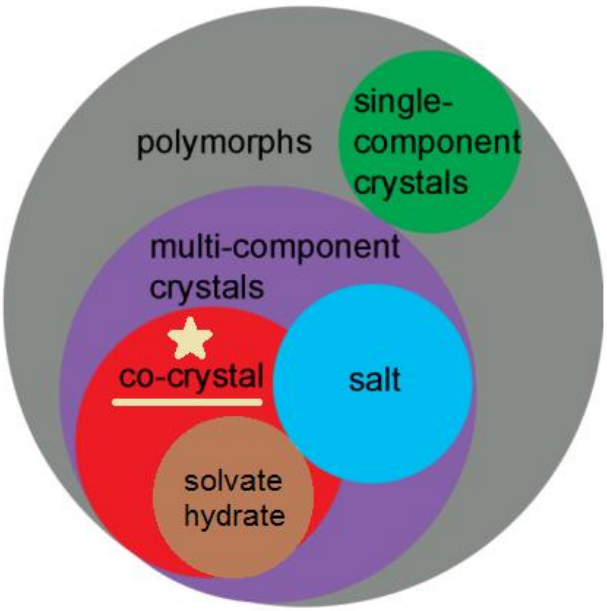


Figure 2.9. Classification of crystalline forms.

Pharmaceutical CoCs, long-known but underutilized, are multiple component crystals made from an API and a coformer that are solids at ambient conditions. The CoC former should be non-toxic, and the GRAS list provides one source of potential compounds for this purpose. Typical examples of GRAS and FDA approved coformers are nicotinic acid and nicotinamide [Schultheiss and Newman, 2009].

Within the last decade, CoCs found their place in pharmaceuticals due to their ability to control and alter physico-chemical properties without compromising the structural integrity and altering the biological activity of the API [Vishweshwar et al., 2006]. CoCs may also play a role in drug development. The role of cocrystallisation in the pharmaceutical industry may be defined as to identify and develop new solid forms of already existing APIs. CoCs have generated tremendous interest in pharmaceutical research and development because of the potentital to customize physicochemical properties of the solid while maintaining the chemical integrity of the drug. CoCs are part of a broader class of multicomponent crystals, where two or more molecules (commonly referred to as drug and coformer) populate a homogeneous crystalline lattice in a well defined stoichiometry. What distinguishes CoCs from other types of multicomponent crystals such as salts and solvates is that drug and coformer are solids at ambient temperature and that the intermolecular interactions are nonionic in nature [Gagniere et al., 2009b]. The distinguished constitution of a CoC was shown in Figure 2.10 and the schematic classification of multicomponent crystals was shown in Figure 2.11.

The diversity of solid forms that can be generated from a drug greatly increases through cocrystallization; the physicochemical properties of the CoCs can vary depending on the characteristics of its constituent molecules. Pharmaceutically relevant properties that

can change via cocrystallization include but are not limited to solubility, dissolution, bioavailability; physical and chemical stability, hygroscopicity, flowability and compressability [Gagniere et al., 2009a]. Of these properties, solubility is the most widely appreciated in the literature. CoCs have the potential to address the solubility limitations of poorly soluble pharmaceutical compounds, a problem which can pose a serious challenge to successful formulation. Poor aqueous solubility can result in poor dissolution, which can then affect bioavailability and pharmacokinetics [Mulye et al., 2012; Childs et al., 2013].

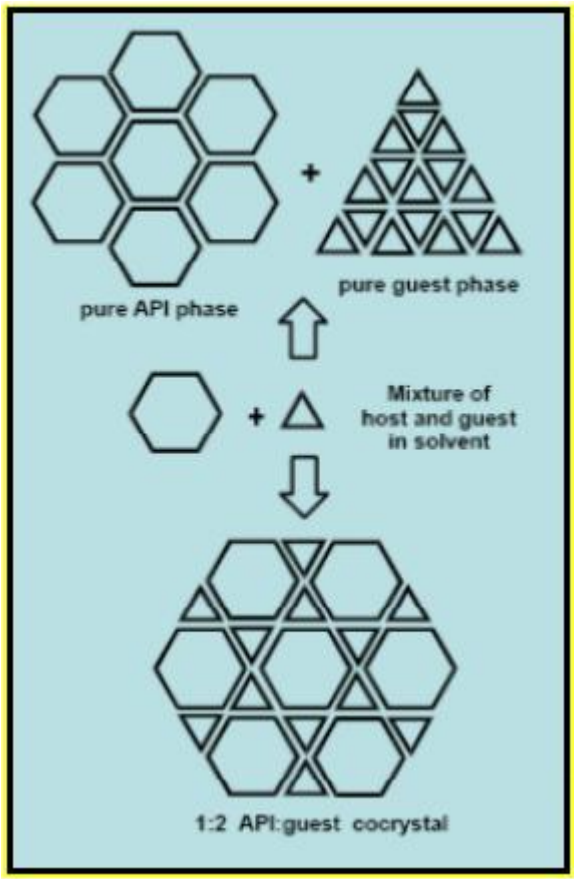


Figure 2.10. Formation of a CoC.

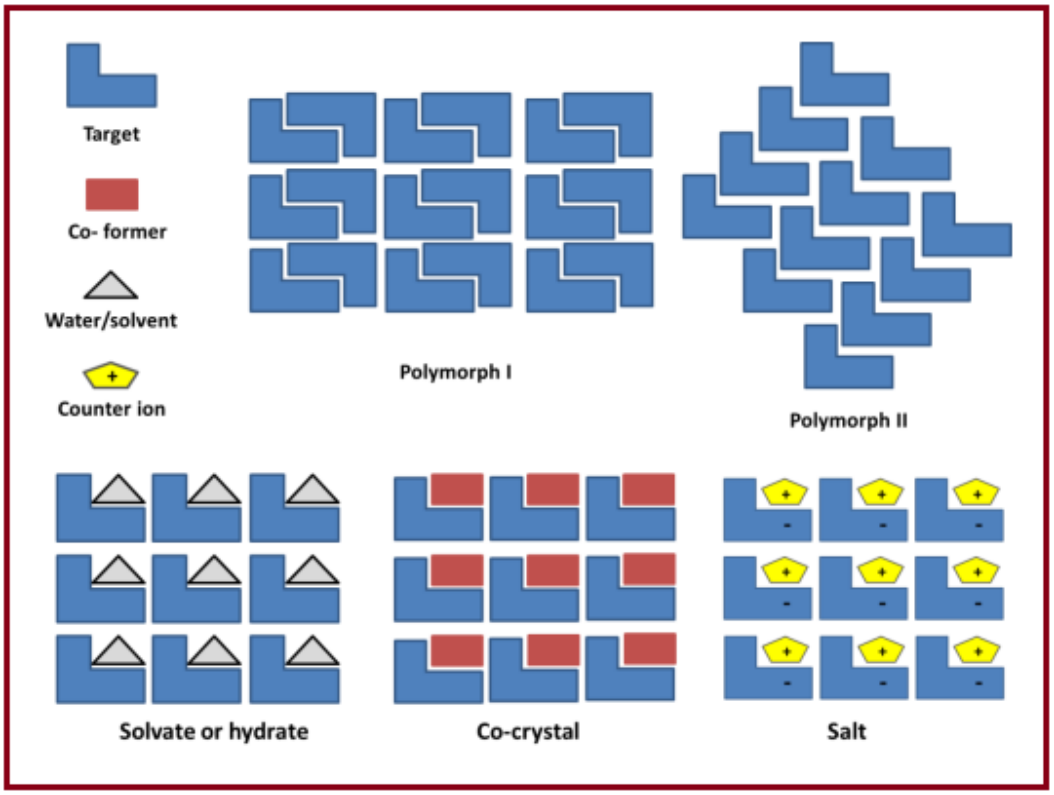


Figure 2.11. Schematic representation of polymorph, solvate or hydrate, CoC and salt [Kilinkissa, 2014].

CoCs are structurally homogeneous crystalline materials containing two or more components present in definite stoichiometric amounts. The CoC components are discrete neutral molecular reactants which are solids at ambient temperature. Based on this definition of CoCs, a pharmaceutical CoC means a CoC with one of the CoC components as an API and the other components are called coformers. From the definition, it is clearly shown that an API hydrate is not a CoC, however a solid-state API hydrate can cocrystallise with a solid coformer to form a CoC. Currently, the CoC approach is a method of great interest for the pharmaceutical industry.

Compared to other solid-state modification techniques employed by pharmaceutical industry, CoC formation appears to be an advantageous alternative for drug discovery

(e.g. new molecule synthesis, nutraceutical CoCs) and drug delivery (solubility, bioavailability). Pharmaceutical CoCs are an example on how crystal-engineering concept can be utilised to address physical and intellectual property issues in the context of drug development and delivery. Experts are of the opinion that pharmaceutical intellectual property landscape may benefit through cocrystallization [Trask, 2007; Almarsson et al., 2011].

To date, many ways of producing CoCs have been reported. The most common formation methods are based on solution and grinding. Solution methods include evaporation of a heterometric solution method, reaction crystallisation method, and cooling crystallisation. Grinding methods include neat grinding and solvent drop grinding. Apart from solution and grinding methods, there are also many newly emerging methods, such as cocrystallisation using supercritical fluid technology (SCF), hot-stage microscopy, and ultrasound assisted cocrystallisation [Qiao et al., 2011]. CoCs are characterized for solid state properties and structural morphology by different techniques as mentioned in Table 2.1.

Table 2.1. Most commonly employed characterization techniques for CoCs.

Characterization parameter	**Examples of analytical methods**
Structural characterization	FTIR; Raman spectroscopy; solid or liquid state NMR
Solid state analysis	Powder XRD, DSC; single crystal XRD
Structure and morphology	Light microscopy; SEM; transmission electron microscopy

CoCs, a well known but understudied class of crystalline solids, have attracted interest from crystal engineers and pharmaceutical scientists in the past decade and are now an

integral part of the preformulation stage of drug development. This is largely because CoCs that contain a drug molecule can modify the physicochemical properties without the need for covalent modification of the drug molecule. Regardless of the enhancement of physical properties, there remains no marketed drug product that manipulates the advantages of cocrystallisation (although there are products close to release). Thus far there are a limited number of pharmaceutical CoCs approved by the FDA as drug products. A CoC of tramadol (hydrochloride) and celecoxib received EMEA approval as EMEA-001279-PIP01-12, back in 2012 [Yan, 2014]. There is a pharmaceutical CoC in late-stage clinical development: ertugliflozin pyroglutamic acid. The diabetes drug candidate ertugliflozin belongs to a class called SGLT-2 inhibitors, which promote excretion of glucose into urine and thus aid in the treatment of diabetes. Ertugliflozin reportedly did not exist in a suitable crystal form for development until the CoC approach was employed to improve physicochemical properties. L-Pyroglutamic acid (also known as 5-oxo-proline) qualifies as a pharmaceutically acceptable choice given its occurrence in proteins. This CoC material is the basis of a drug product that is in late-stage (phase 3) trials. The drug is subject to collaboration between Pfizer, the company that originally developed ertugliflozin, and Merck, which presumably reflects the value of the drug candidate [Duggirala et al., 2016].

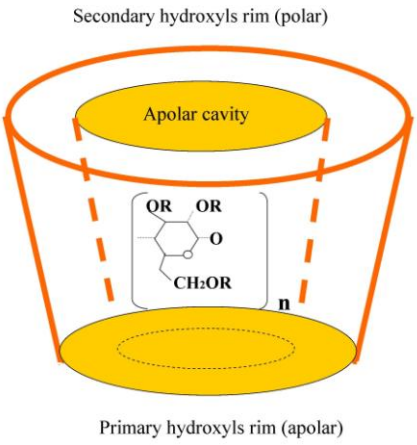
2.3.2 Ternary cyclodextrin (CD) complexes

CDs have emerged as an important tool in the formulator's armamentarium to improve apparent solubility and dissolution rate of poorly water-soluble drug candidates. The continued interest and productivity of these materials bode well for future application and their currency as excipients in research, development and drug product marketing

[Augustijns et al., 2009]. CDs are cyclic oligosaccharides composed of D-glucopyranoside units (glucose) linked by α – 1, 4 – glycosidic bonds. They are obtained from biotechnological processes involving the enzymatic degradation of corn starch and offer greater yield with 6, 7 and 8 units of glucose, known as α-CD, β-CD and γ-CD, respectively. Figure 2.12 shows the CD structure and composition. CDs with less than 6 units of glucose do not exist for stoichiometric reasons and those with more than 8 units offer low yields and weak complexing properties, thus making them unsuitable for the pharmaceutical industry. The safety profile of natural CDs and their derivatives has been widely studied, and they have generally proven to be atoxic, because they only manage to cross biological membranes with some degree of difficulty. Thus, oral administration of CDs should not be regarded as a problem. CDs are listed in a number of pharmacopoeias and are accepted as pharmaceutical excipients and food additives by various regulatory agencies [Szejtli, 2004]. CDs can be found in the USP/NF and are included in the USFDA GRAS list. For example, 2-Hydroxypropyl-β-cyclodextrin is compendial in the USP and Ph. Eur. and is cited in the FDA's list of pharmaceutical ingredients, also included in compendial sources, e.g. the Handbook of Pharmaceutical Excipients [Zoeller et al., 2012]. CDs can be found in over 35 commercially available drug products, including tablets, parenteral solutions, eye drops, ointments, and suppositories [Loftsson and Brewster, 2012].

The oral bioavailability of CDs is very low meaning that they act as true drug carriers. Toxicological studies have shown that orally administered CDs are practically nontoxic because of their low absorption into the systemic blood circulation. The most important property of CDs is their ability to modify the physicochemical and biological characteristics of drugs. Their cavity can establish interactions through intermolecular

forces with molecules, ions or radicals, acting as a host substance [Stella and Quanrenhe, 2008]. The resulting molecular complex is called an inclusion compound or a supramolecular compound which usually presents solubility of several orders of magnitude higher than that of pure host or drug molecule. The only major obstacle to pharmaceutical exploitation of CDs is their formulation bulk [Loftsson and Brewster, 2012].



Cyclodextrin	R	n
α-cyclodextrin	H	6
β-cyclodextrin	H	7
γ-cyclodextrin	H	8
Hydroxyl propyl β-cyclodextrin	$-CH_2CHOHCH_3$	7
Hydroxyl propyl γ-cyclodextrin	$-CH_2CHOHCH_3$	8
Sulphabutyl ether β-cyclodextrin sodium salt	$-(CH_2)_4SO_3^- Na^+$	7
Randomly methylated β-cyclodextrin	$-CH_3$	7

Figure 2.12. CD composition.

The truncated cone structure of CDs, which are open at both ends, enables the inclusion of a wide variety of organic molecules (apolar drugs) in their central cavities. Host-guest complexes, or drug-CD complexes also known as inclusion complexes, result from the association between host molecules (CDs) and encapsulated guest molecules (drugs). The formation of a complex in an aqueous solution takes place when water molecules are removed from the apolar cavity of CDs (which are in an energetically unfavorable environment due to the nature of the polar-polar interaction) and substituted

for a guest molecule or lipophilic group with polarity, size and shape compatible with that of the CD structure. CDs can form inclusion complexes with many drugs by taking up lipophilic substructures of drug molecules into their central cavity. No covalent bonds are formed or broken during the complex formation and in aqueous solutions, the drug molecules bound within the CD cavity are in dynamic equilibrium with free drug molecules in the solution. Complexes are continuously being formed and dissociated at rates close to the diffusion-controlled limit [Szejtli, 2004].

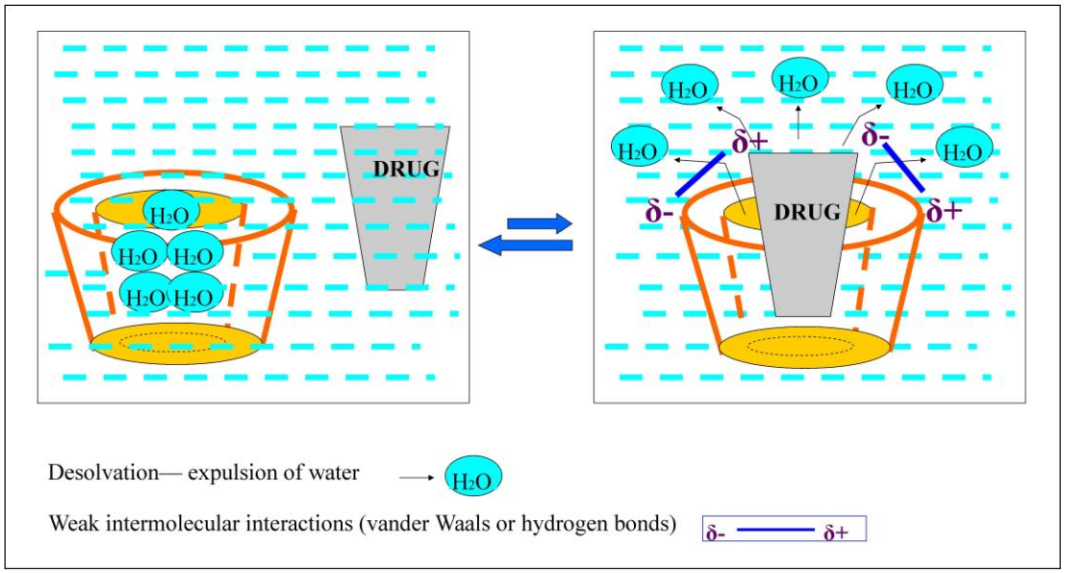


Figure 2.13. Formation of binary CD inclusion complexes.

This process is energetically favorable and contributes to an increase in complex stability, because it causes changes in enthalpy and a reduction in the total energy of the system. Furthermore, other forces are involved in the formation and stabilization of inclusion complexes, such as van der Waals interactions, hydrogen bonds (between guest molecule and CD hydroxyl groups), hydrophobic interactions, release of deformation energy from the macromolecular ring of CDs, and steric effects. The

complexes formed are usually more water soluble than the active ingredients they contain as well as more stable in solution form. They also dissociate easily in order to release the drug molecule [Valle, 2004]. Figure 2.13 represents the formation of a binary drug and CD complex.

2.3.2.1 Need for ternary complexes

Use of CDs is one of several technologies available to improve the solubility of poorly water-soluble drugs. The most remarkable property of CDs is their ability to modify the physicochemical characteristics of molecules that are accommodated within their internal cavity to form the so-called inclusion complexes. Typical characteristics of formulations containing inclusion complexes include a faster dissolution rate and shorter drug release time, as well as more efficient absorption. This translates into greater oral bioavailability of the drugs involved and an increase in biological activity, which may result in a reduction in drug dosage. However, the use of CDs is limited in some cases, because guest molecules need to fit completely or partially within the CD cavity. This adjustment is directly related to the physicochemical properties of the guest and host molecules, easy accommodation of guest molecules within the CD cavity, stoichiometry, and therapeutic dose. An increase in formulation volume represents a critical stage in the applicability of CD inclusion complexes. We can consider that 1 g of a solid complex corresponds to 100-250 mg of a drug (when the molecular weights of the drug and the CD are 200-400 g/mol and 1200-1500 g/mol, respectively). Therefore, the use of CDs in oral solid dosage forms is limited to drug doses less than 200 mg that have good complexation properties.

A strategy often used to improve complexation between drugs and CDs is the addition of small amounts of water-soluble substances (e.g. a hydrophilic polymer) to the

system, which causes an increase in solubilization efficiency, while requiring smaller amounts of CD. These results can be attributed to the synergistic effect of polymer and CD solubilization on the formation of drug:CD:water soluble polymer ternary complexes. Water-soluble substances are able to interact with drugs, CD molecules, and even with drug:CD complexes. The mechanism involved in increasing CD complexation efficiency in the presence of water-soluble substances is not yet fully understood; however, it is believed that water-soluble substances can reduce CD mobility and increase the complex solubility. The addition of water-soluble substances has been shown to increase drug bioavailability and cause an up to 80% reduction in the amount of CD required [Miranda et al., 2011].

Both, the water-soluble ternary components and CDs can form water-soluble complexes with poorly soluble lipophilic drugs but when used in combination, a synergistic solubilization effect is observed, that is, the apparent drug solubility is greater than the sum of water-soluble ternary component and CD solubilization when assessed individually. It is known that water-soluble substances such as polymers and surfactants are able to stabilize self-assembled nanostructures [Loftsson et al., 2005].

Water-soluble substances enhance aqueous solubility of CDs and CD complexes. Furthermore, CDs have been reported to solubilize poorly soluble compounds through formation of aggregates or micellar-like structures and the solubilizing effect of some CD complexes exceeds that of the corresponding pure CD. Water-soluble substances enhance the stability of the CD complex aggregates and perhaps the ability of the aggregates to solubilize poorly soluble drugs through micellar type solubilization [Loftsson and Brewster, 2012].

Obtaining binary or ternary complexes with CDs may occur in the liquid, semi-solid or solid phases. In the liquid phase, the following methods have been suggested: coprecipitation, coevaporation, neutralization, freeze-drying, and drying by pulverization. In the solid phase, the most common methods are grinding or SCF, while malaxation is employed in the semi-solid phase [Carrier et al., 2007]. The characterization techniques widely used were given in Table 2.2.

Table 2.2. Most commonly employed characterization techniques for CD complexes and ternary CD complexes.

Characterization parameter	**Examples of analytical methods**
Structural characterization	FTIR; Raman spectroscopy; solid or liquid state NMR
Solid state analysis	Powder XRD, DSC
Structure and morphology	Light microscopy, SEM, transmission electron microscopy

2.3.2.2 Phase solubility profiles

Phase solubility profiles describe how the increasing CD concentration influences the drug solubility and the different possible types of profiles were shown in Figure 2.14. Higuchi and Connors have classified drug-CD complexes based on the effect of CD on their substrate (drug, the guest) solubility as indicated by phase solubility profiles. A-type phase solubility profiles are obtained when the solubility of the substrate (i.e. drug) increases with increasing ligand (i.e. CD) concentration. When the complex is first order with respect to ligand and first or higher order with respect to substrate then A_L type phase solubility profiles is obtained. If the complex is first order with respect to the substrate but second or higher order with respect to the ligand then A_P-type phase

solubility profile is obtained. A_N-type phase solubility profiles can be difficult to interpret. B-type phase solubility profiles indicate formation of complexes with limited solubility in the aqueous complexation medium. In general, the water-soluble CD derivatives form A-type phase solubility profiles while the less soluble natural CDs frequently form B-type profiles [Loftsson et al., 2005].

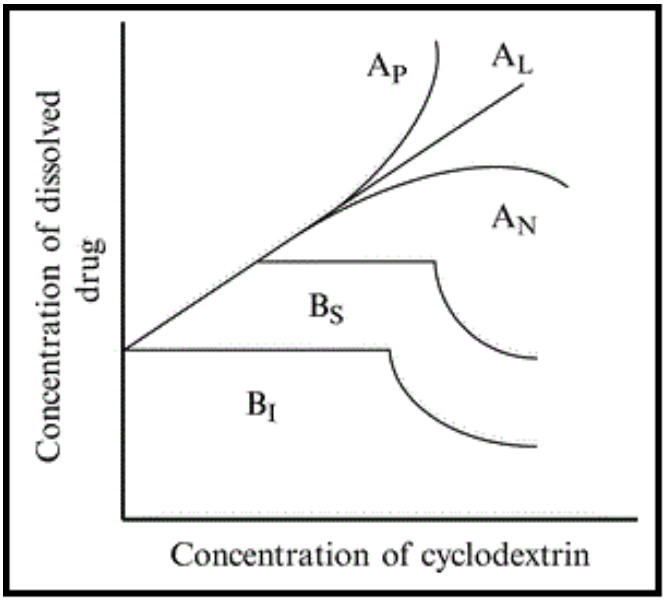


Figure 2.14. Phase solubility behavior and profiles.

Most drug:CD complexes are thought to be inclusion complexes but CDs are also known to form non-inclusion complexes and complex aggregates capable to dissolve drugs through micelle-like structures. The phase solubility profiles do not verify formation of inclusion complexes. They only describe how the increasing CD concentration influences drug solubility. Additionally, these profiles also help in determining whether or not the addition of water-soluble ternary components to the system improves the phase solubility behavior and actually results in greater interaction between the CD and drug components [Loftsson et al., 2005].

2.3.2.3 The stability constant

The stability constant (K_C or simply, K), calculated from the phase solubility diagram (drug concentration v/s CD concentration), can be considered an apparent stability constant for several complexes, describing the combined effect of various structures on the solubility of a drug. The stability constant of a complex is determined from the slope of the phase solubility diagram and the intrinsic solubility of a drug (S_0) as shown in Equation (2.1). Some researchers consider that complexes with K_C values ranging between 200 and 5000 M^{-1} are applicable to dosage formulations, while K_C values between 7 and 100 M^{-1} were deemed sufficient by others, because they were able to improve the physical and chemical properties of drugs compared to non-complexed forms. K_C values are widely used to compare the affinity of drugs for CDs, thus determining whether the addition of water-soluble ternary components to the system actually results in greater interaction between the components [Loftsson and Brewster, 2010].

$K_C = slope/S_0(1\text{-}slope)$ …… **Equation 2.1.**

(S_0 is the intrinsic solubility of the drug or guest).

2.3.2.4 Formation of ternary complexes

When a water-soluble ternary component (WTC) such as a hydrophilic polymer or surfactant, a CD and a drug are mixed together in a solution to obtain the so-called ternary complexes, it is possible to increase drug solubilization, when compared to the WTC and CD separately, which is a result of the synergistic effect between these components. Formulations containing drug:CD complexes with the addition of a WTC have proven to be capable of increasing the bioavailability of formulations while reducing the amount of CD by up to 80%. In the presence of water, the hydrophilic

polymer or surfactant aids in the wettability of particles, resulting in accelerated dissolution and increased amount of drug delivered *in-vitro*. The interaction of WTCs with drug molecules may occur by means of van der Waals interactions, hydrogen bonds (between guest molecule and CD hydroxyl groups), and hydrophobic interactions. Similarly, the interaction between WTCs and CDs and drug:CD complexes begins to occur on the external surface of the CD molecule. CDs, WTCs and drug:CD complexes form aggregates capable of solubilizing drugs and other hydrophobic molecules [Miranda et al., 2011]. Table 2.3 gives examples of marketed products using HPBCD drug complexes.

Table 2.3. Examples of commercialized HPBCD products.

Product	**Active**	**Therapeutic class**	**Route of administration; Dosage form**
Prepulsid	Cisapride	Gastroprokinetic agent	Rectal; Suppository
Dyolect	Diclofenac	Anti-inflammtory and analegesic	Intravenous and Intramuscular; Solution
Dexacort	Hydrocortisone	Anti-inflammtory	Mouth wash; Oral solution
Sporanox	Itraconazole	Anti fungal agent	Intravenous; Solution
Vorzu	Voriconazole	Anti fungal agent	Oral; Tablet
MitoExtra	Mitomycin	Antineoplastic	Intravenous; Infusion
Indocid/ Indocyllir	Indomethacin	Non-steroidal anti-inflammatory drug	Eye drop
Adapted [Brewster, 2012; www.cyclolab.hu]			

Several types of interactions between WTCs and drugs may be established as a result of the structural difference and polarity of CD molecules, which may give rise to various

complexation efficiencies. The resulting chemical structure of the drug is still unknown, as is the nature of the interaction between CDs and the WTCs, but it is recognized that, in aqueous solutions, the ternary components stabilize micelles and other types of aggregates, reduce CD mobility and increase the solubility of complexes by changing the hydration properties of CD molecules. The effect of hydrophilic ternary components' association with CDs on the increased solubility of insoluble drugs is well established in the literature. However, the involved mechanism is not yet totally clear. It was reported that the hydrophilic ternary components could improve the complexation efficiency and the stability constants of CD inclusions of drugs [Loftsson and Brewster, 2012].

WTCs like hydrophilic polymers are most commonly employed to prepare solid dispersion of water insoluble drugs. Generally, hydrophilic polymers may increase dissolution rate of poor soluble drug from solid dispersions by three different mechanisms: (a) drug may be dispersed into polymeric matrix in an amorphous or disorganized crystalline phase; (b) polymer contributes to an increase in the interaction of water with drug molecules or (c) the dissolution of polymer leads to an increase in drug solubility in aqueous environment by the occurrence of soluble complex formation or by cosolvency effect. Thus, to explain the mechanism involved is very important to predict the contribution of the hydrophilic polymer in the increment of drug solubility in solid dispersions or from a multicomponent complex. Polymers are known to interact with the outer surface of CDs and with drug-CD complexes, forming co-complexes or aggregates that show higher stability constant (K_C) values than those for the binary drug CD systems. They increase the complexation efficiency, and therefore a smaller amount of CD can be used in the preparation of the complex [Loh et al., 2014].

WTCs are known to enhance the complexation efficiency of CDs. Both the WTCs and CDs can form water-soluble complexes with poorly soluble, lipophilic drugs but when used in combination, a synergistic solubilization effect is observed, that is, the apparent drug solubility is greater than the sum of WTC and CD solubilization when assessed individually. The maximum complexation efficiency is typically obtained at relatively low WTC concentrations or between 0.1% and 1% (w/v). The enhancement of complexation efficiency is due to an increase in the apparent stability constant of the complex. WTCs are known to form water-soluble complexes with poorly soluble drugs. However, only free drug molecules, that is, molecules not bound to WTCs, are able to form complex with CDs. In aqueous WTC solutions saturated with a given drug, the concentration of free drug is equal to the solubility of the drug in the pure aqueous media. Thus, the concentration of available drug molecules should not be affected by the WTCs and S_0 in Eq. 2.1 would be expected to be constant. The observed increase in complexation efficiency is due to an increase in the K_C value. It is known that WTCs such as polymers, as well as surfactants, are able to stabilize self-assembled nanostructures. Polymers stabilize and enhance the solubilizing effects of micelles, and polymers are used to stabilize particulated pharmaceutical systems of various types. Water-soluble ternary components are thus known to enhance aqueous solubility of CDs and CD complexes. These observations together with the fact that the enhancement in complexation efficiency is due to an increase in the apparent stability constant of the complex suggest that the WTCs enhance the stability of the CD complex aggregates and perhaps the ability of the aggregates to solubilize poorly soluble drugs through micellar type solubilization [Loftsson et al., 1994].

Improving the solubility of poorly soluble drugs is one of the main applications of CDs and their derivatives, which have the ability to encapsulate organic molecules in their cavities, thus forming inclusion complexes, which in turn modify the physicochemical characteristics of such drugs. The drug:CD:water-soluble substance complex represents an attractive alternative, especially in cases where a high amount of CD is required for complexation, which significantly increases the volume of dosage forms. Thus, it is possible to obtain solid-form medications with an optimized dissolution profile, which may result in improved bioavailability. Most drugs with low aqueous solubility have organic solvents, emulsifiers and extreme pH conditions in their formulations, which can cause irritation and other adverse reactions. The drug:CD:water-soluble substance complexes can be administered in any dosage form for the treatment of a variety of ailments, depending on the biological activity of the complexed drug. Research on ternary complexes has gained prominence in recent decades, and it is therefore possible to find a considerable number of studies in which drug:CD:water-soluble ternary components were studied [Srivalli and Mishra 2016].

2.3.3 Drug nanocrystals (NCs)

NCs offer 100% drug loading and they are different from polymer- or lipid-nanoparticles that involve encapsulation of API and present a lower drug load. In NCs, the drug is not encapsulated or entrapped in any carrier and this is what makes their manufacturing and optimization simpler since there is no need to study the entrapment efficiency. An NC formulation is a drug dispersed in either aqueous or non-aqueous media containing one or more stabilizers. Stabilizers could be one or more generally regarded as safe excipients (surfactants or buffers, phospholipids, salts or sugars). NCs

can be formulated as solid drug nanoparticles or liquid drug nanosuspensions. NCs offer drug loading ranges from 1 to 400 mg/mL and are basically formulated as liquid dispersions. The liquid forms thus obtained can be administered as oral suspensions or post processed into solid or injectable dosage forms. For injectable purposes, the formulations can be terminally sterilized by heat, gamma radiation, filtration or by asceptic approach following which the formulation can be stored as a liquid or as a lyophilized powder to be redispersed at the time of injection. NCs, which can be administered by different routes, offer enhanced solubility and dissolution rate, maximum absorption, enhanced bioavailability with the elimination of food effects, safe dose escalation, enhanced safety, better efficacy and tolerability profiles and improved patient compliance. Such enhanced performance is due to their small particle size (PS) and large surface area. The ease of their manufacturing makes them the choicest nanoparticles if one needs to check the existence of any correlation between the PS and drug candidate bioavailability even at the initial screening and basic formulation development stages. NCs also offer flexibility in scaling up or down which would be of great value whenever alterations with respect to unit operation functions or formulation are desired during scale up process. Above all, the greatest advantage associated with NC formulations that also contributes to the commercial success of NCs is that an NC is considered a new drug product and not a "generic" to any other approved product by the FDA because its pharmacokinetic profile is not bioequivalent to any other solubilized form of the same drug, not even to the drug's own micronized form, administered at the same dose. Therefore, an NC can be patented as a "new drug" which offers a product line extension for the already existing drug formulations and can serve as a new, rational and beneficial dosage form [Srivalli and Mishra, 2015a].

Commonly used polymeric stabilizers (non-ionic) for nanosuspensions include cellulose ethers, such as hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), povidone, Tweens, and poloxamers (types 188, 407 and 338). Commonly used surfactant stabilizers are either non-ionic, such as the polysorbate types, or anionic, such as sodium dodecyl sulfate or sodium lauryl sulfate (SLS), sodium cholate and docusate sodium. For effective nanosuspension stabilization, the drug substance:stabilizer ratio may vary between 20:1 to 2:1, (w/w). While insufficient amounts of stabilizers remain ineffective for preventing particle agglomeration, excessive quantities may promote crystal growth by Ostwald ripening [Merisko-Liversidge et al., 2003]. Naturally, only excipients with established safety profiles should be used for the stabilization of nanosuspensions [Kesisoglou et al., 2007].

Briefly, NCs are defined as particles consisting of a pure drug stabilized with ionic or non-ionic surfactants. The higher drug loads of the NCs make them pharmacologically effective drug delivery systems. The size of the NCs is below 1000 nm, typically 100-300 nm or lower. The term "nanosuspension" refers to the NCs being dispersed in a liquid. Depending on the drug properties and the production parameters, the NCs can be obtained in the crystalline or amorphous state. The solid state behavior of the drug particles has a great influence on their solubility. The unique physicochemical properties of NCs may be explained by three equations - Noyes Whitney, Ostwald Freundlich and Prandtl [Salazar, 2013]. Figure 2.15 explains the distinctive physicochemical properties of the NCs described by each of the above mentioned three equations.

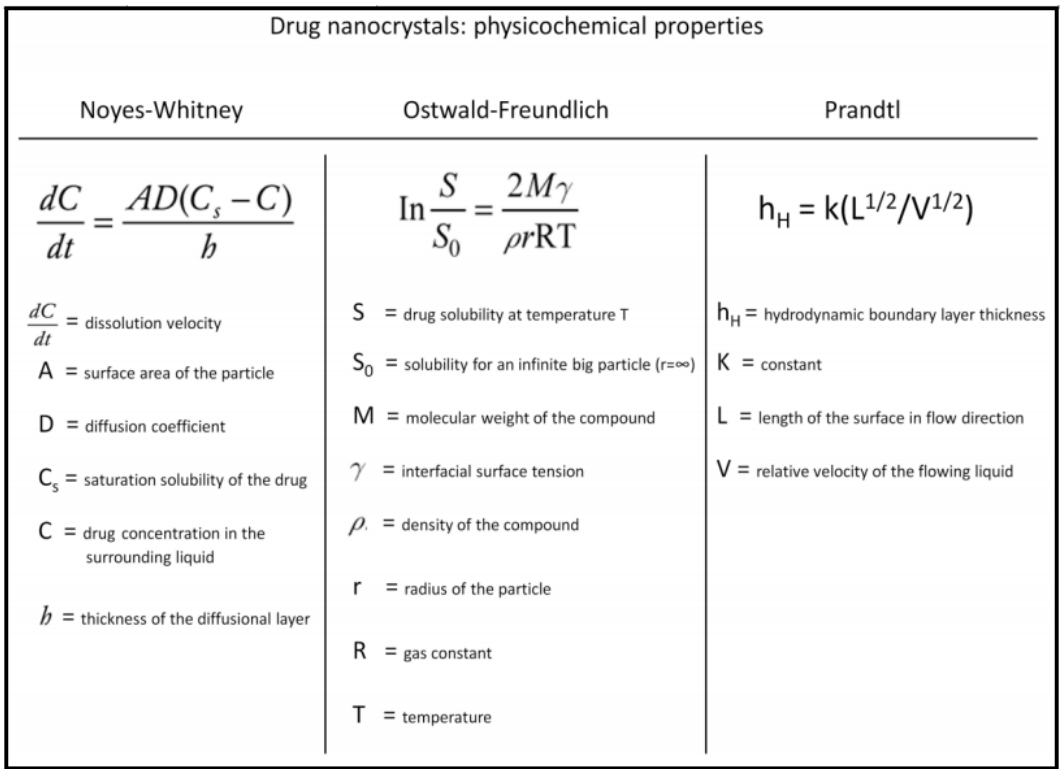


Figure 2.15. Distinctive physicochemical properties of NCs [Salazar, 2013].

The dissolution velocity of a compound is directly proportional to its diffusion coefficient (D), the difference between saturation solubility (Cs) and drug concentration in the bulk medium (C) and the surface area (A) presented by the particles (the Noyes-Whitney equation). Due to the extremely small PS of the NCs, their surface area is increased compared to the micronized material. This increase in surface area leads to an enhanced dissolution rate according to the Noyes Whitney equation. The enhanced dissolution rate can lead to a higher oral bioavailability of poorly soluble compounds after oral administration.

The small particle radius of the NCs increases the saturation solubility of the nanosized compounds according to the Ostwald Freundlich equation (Figure 2.15), which

describes the vapor pressure as a function of the curvature of solid particles in a liquid phase. S is the drug solubility at the given temperature, T; S_0 is the solubility for an infinite big particle (radius $r=\infty$), M is the molecular weight of the compound, γ is the interfacial surface tension, ρ is the density of the compound and R is the gas constant. The dissolution pressure of a particle in a liquid improves by increasing the curvature (i.e., by reducing the PS) of the particle. However, the influence of the PS on the saturation solubility begins with particle sizes below 1 μm. Another factor influencing the solubility is the crystallinity. Drugs having a high solid density (with a high melting point) are less soluble than amorphous compounds with a lower density.

Finally, the Prandtl equation demonstrates how the increased curvature of the NCs reduces the diffussional distance, h, which improves the dissolution velocity (Figure 2.15). Besides the enhanced dissolution rate and saturation solubility, there are some other benefits of employing NCs as formulation tools. These benefits include reduced fed/fasted variability, reduced absorption variability between patients, faster action onset, an enhanced absorption rate and better compliance due to reduced oral unit intake. Compounds belonging to BCS class II are poorly soluble (aqueous solubility < 0.1 mg/mL) and highly permeable. Class II drugs, in general, show a bioavailability that depends on the dissolution rate as a limiting factor. Consequently, their formulation as NCs for dissolution rate enhancement could eventually solve their oral bioavailability issues [Salazar, 2013].

The preparation techniques for NCs could be mainly classified into three categories namely top down, bottom up, and combination methods. In short, the top down methods are physicomechanical processes mainly involving crushing or attrition principles (fragmentation) while bottom up methods are physicochemical processes involving the

principles of atomic or molecular level self organization (amalgamation) as demonstrated in Figure 2.16.

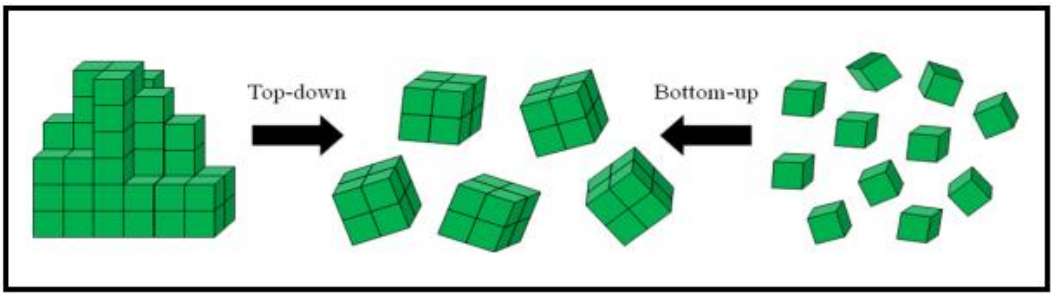


Figure 2.16. Production of NCs.

The top down methods mainly involve milling or homogenization while the bottom up methods are primarily based on the principle of precipitation. The combination approaches involve bottom up plus top down method combinations [Srivalli and Mishra, 2014]. A brief summary of the most commonly employed characterization methods for NC products is presented in Table 2.4.

Media Milling (MM), the wet ball milling comminutes material loaded into milling chamber with an agitator. The milling material, the drug to be nanosized is normally provided as a treated (micronised) or untreated solid dispersed in a liquid medium (usually water) with the aid of surfactants as stabilizers. The comminution principle involved is the mechanical attrition and shear that arises due to collision between milling media and drug particles or between two drug particles or also between a drug particle and the walls of the milling chamber. The milling media are small beads or pearls made of ceramic (e.g., yttrium stabilized zirconium dioxide) or highly cross-linked polystyrene resin or stainless steel or glass having different sizes (0.3 mm or higher). However, the first two ensure minimal contamination to the product. The size

reduction effectiveness could be further determined by the concentration of drug and surfactant, viscosity of the dispersion medium, temperature conditions and by the initial PS and hardness of the drug.

Table 2.4. Most commonly employed characterization techniques for NCs.

Characterization parameter	**Examples of analytical methods**
Particle size and particle size distribution	Photon correlation spectroscopy (based on dynamic laser light scattering), laser diffraction (static laser light scattering), microscopic methods
Surface charge	Zeta potential
Structural characterization	FTIR; Raman spectroscopy; solid or liquid state NMR
Solid state analysis (crystallinity)	Powder XRD, DSC
Structure and morphology	Light microscopy, SEM, transmission electron microscopy, atomic force microscopy
Rheological properties (for liquid nanosuspensions)	Viscometer, rheometer

High pressure homogenization (HPH) is another top down process where in the PS reduction is brought about by shear forces, cavitation forces and particle collision aided by high pressure conditions. It is of two types namely the microfluidization and piston gap homogenization. Microfluidization is also called as air-jet milling or jet stream homogenization wherein the particles are fragmented in a high pressure air jet induced by collision of two fluid streams. Piston-gap homogenization employs high pressure to force a liquid suspension through a gap or narrow channel inside a pipe. If the media is aqueous, bubbles are formed inside the gap due to reduced static pressure in the gap

region which later collapse upon exiting the narrow gap. The break-up of particles is achieved by the consequently generated cavitation energy. On the other hand if the media is oil or a non aqueous solvent, the particle comminution is facilitated by the high shear and collision through the gap.

All bottom up approaches employ two basic principles namely precipitation and evaporation. Accordingly there are numerous variations available which incorporate either of the two principles or a combination of both. 'Cryogenic solvent evaporation' is a bottom up method which involves spraying of drug solution into cryogenic liquids using 'spray freezing into liquid' technology. Here the drug solution droplets are frozen upon contact with cryogenic liquid (liquid nitrogen) and the organic solvent is removed by lyophilization. Precipitation when performed in conjugation with centrifugation is termed 'high gravity controlled precipitation' technique. Performing precipitation by evaporation of organic solvent is called 'evaporation precipitation into aqueous solution'. A technique such as 'controlled crystallization during freeze drying' is also available. There are several methods involving precipitation based on SCF technology. If the drug is soluble in SCF, the method employed is called 'rapid expansion of supercritical solution'. If SCF is used as antisolvent, there are other variations possible such as 'gas antisolvent process', 'supercritical antisolvent process' and 'solution enhanced dispersion of solids'. Literature presents the reports of some positive results with the specialized bottom up approaches too but their application is mainly limited due to the requirement of special processing expertise and custom designed equipment as well as the high costs associated with such production equipment.

Solvent-antisolvent precipitation is the simplest and single step precipitation process involving low energy, less expense and simpler instruments. The process may be

designed more efficiently with the incorporation of high speed homogenization or sonication and subsequent solidification. The use of evaporation processes like spray or freeze drying operable at low temperature (suitable for thermolabile drugs) or fluid bed drying for solidification purpose would still constitute cost-efficient processes when compared to other high energy and sophisticated precipitation processes.

All the combination approaches are uniquely referred to as smartCrystals®. Examples of smartCrystals® technologies so far explored include Nanoedge™ technology which involves microprecipitation plus HPH; H42 technology involving non-aqueous spray drying followed by HPH; H96 technology which involves freeze-drying followed by HPH. There is also one H69 technology which employs the same combination approach as Nanoedge™ but inorder to save time between the precipitation and homogenization processes and to yield smaller drug NCs, the precipitation process is carried out directly within the zone of dissipation of homogenizer. Combination Technology is another smartCrystal technology which involves media milling followed by HPH [Srivalli and Mishra, 2014 and 2015a].

A nanoformulation should be robust as well as cost effective. Pharmaceutical formulators, not surprisingly tend to initiate the formulation development with a trial-and-error method since serendipity has a huge role to play in the pursuit of a successful scale up. In order to understand the statistics of the literature or the compiled experimental data and to understand the physics behind the problem, the application of trial and error experimental methods becomes a necessity. Experimental designs are in great demand in the current scenario to handle the formulation development process and to arrive at a rationale formulation. The trial and error experimental approach before the start up and the design based experimentation in the subsequent phase equips the

formulator with certain practical experience which is a prerequisite to the further creative proceedings. An ideal formulation is the one that presents acceptable quality, efficacy (and bioavailability) and safety profiles. "Quality by design" applied to pharmaceutical product development involves identification of critical quality attributes, design of experiments, possible risk assessment and process analytical technology to control the risk precipitating factors. Through "quality by design" approach, experimental designs are applied and the correlation between and among all the input and output variants could be disclosed. The effect of input or independent or predetermined variables constituting the choice of production method and process parameters (homogenization pressure, stirring rate, cooling speed, etc); choice of excipients and material properties (chemical nature and properties) and the effect of interaction among them on the output or dependent variables or critical quality attributes such as PS, zeta potential, polydispersity index, and release characteristics could be studied [Srivalli and Mishra, 2014 and 2015a].

Nanomaterials exhibit special physical properties being different to the bulk material. The special properties of NCs are increased saturation solubility and increased surface area, both leading to an increase in the dissolution velocity. Nanonisation is therefore the ultimate solution to increase the oral bioavailability in cases where the dissolution velocity is the rate limiting step. In addition, the concentration gradient between lumen of the gut and the blood is increased. In case the drug being dealt with is a P-glycoprotein (P-gp) substrate, one more ideal conception would be to combine the NCs with the P-gp inhibition strategy, i.e. making "smart nanocrystals". The challenge is to create "smarter" nanocrystals that not only dissolve fast but are combined with a formulation technology to inhibit P-gp [Srivalli and Mishra, 2015b].

The market profile of NCs accentuates how readily these products were accepted. Having had seen the light of development in the 1990s, the first NC product entered the market as early as 2000. So far, there are six NC products launched in the market. Products like Semapimod® (guanylhydrazone), Paxceed® (paclitaxel), Theralux® (thymectacin) and Nucryst® (silver) are currently in clinical trials and there are many more products in preclinical studies. The scientific rationale of developing an NC formulation based dosage form is reduced dose or dosing frequency, enhancement of bioavailability, reduction of variations in bioavailability and improved patient compliance [Srivalli and Mishra, 2014 and 2015a]. Table 2.5 provides details of the commercialized NC products.

Table 2.5. Examples of commercialized NC products.

Product	**Active**	**Therapeutic class**	**Route of administration; Dosage form**
Rapamune®	Sirolimus (rapamycin)	Immunosuppressant	Oral; Tablet
Emend®	Aprepitant	Antiemetic	Oral; Capsule
TriCor®	Fenofibrate	Antihypercholesterolemic	Oral; Tablet
Triglide®	Fenofibrate	Antihypercholesterolemic	Oral; Tablet
Megace ES®	Megestrol acetate	Antianorexic	Oral; Suspension
Invega Sustenna®	Paliperidone palmitate	Antipsychotic	Intramuscular (deltoid/gluteal); Suspension
Adapted [Srivalli and Mishra, 2014 and 2015a]			

2.4 FORMULATION INGREDIENTS SPECIFIC REVIEW

2.4.1 Drug profile

Drug: Ezetimibe (Eze).

Chemical Name: 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone. Chemically, Eze is an aromatic heteromonocyclic compound and belongs to the class of organic compounds known as monobactams. These are compounds comprising beta-lactam alone and not fused to another ring. Figure 2.17 shows the structural formula of Eze.

Empirical Formula: $C_{24}H_{21}F_2NO_3$

Molecular Weight: Average = 409.43 g/mol.

Biopharmaceutics classification system class: Class-II (High Permeability & Low Solubility).

Description: Eze is a white, crystalline powder.

Melting point: 163°C.

Solubility: Eze is freely to very soluble in ethanol, methanol, and acetone and practically insoluble in water.

Stability and Storage: Stable under ordinary conditions (room temperature) [http://www.drugbank.ca/drugs/DB00973].

Clinical Background:

Eze is an anti-hyperlipidemic medication which is used to lower cholesterol levels. Specifically, it appears to bind to a critical mediator of cholesterol absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein on the GIT epithelial cells as well as in

hepatocytes. It belongs to a class of lipid-lowering compounds that selectively inhibits the intestinal absorption of cholesterol and related phytosterols [Catapano, 2001].

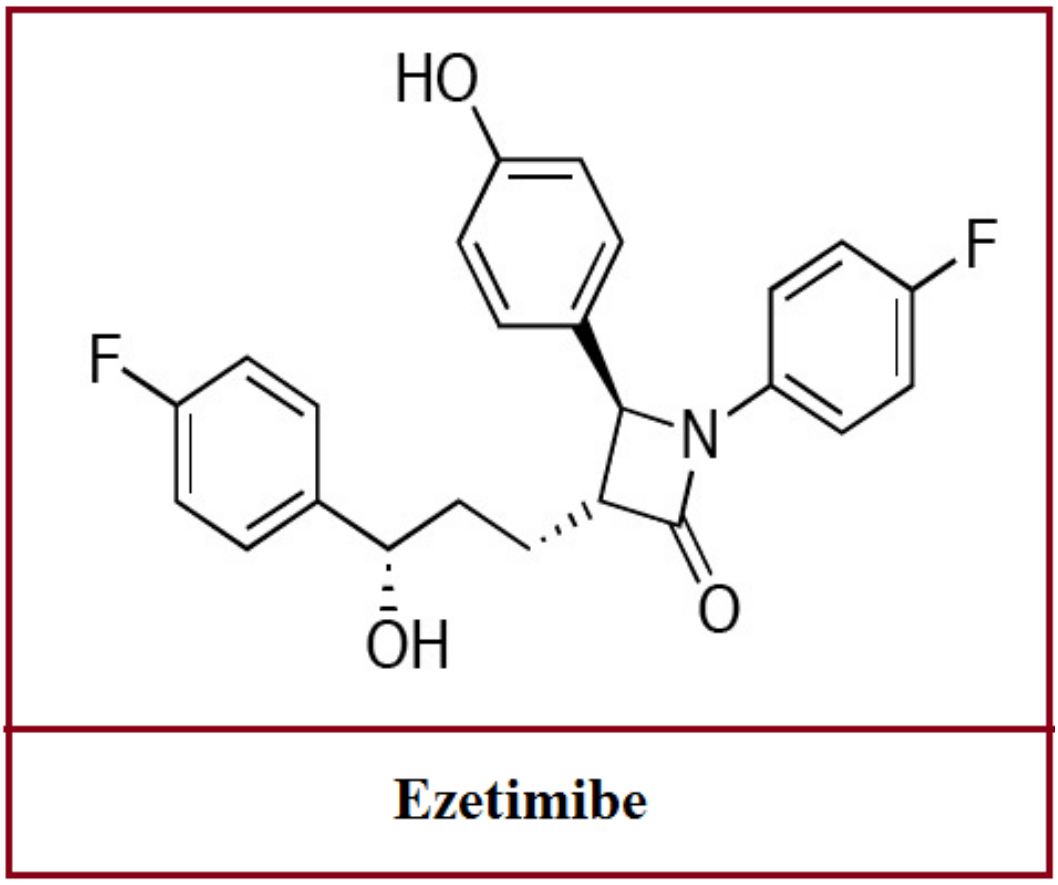


Figure 2.17. Eze structural formula.

Mechanism of action:

Eze localizes and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol. This leads to a decrease in the delivery of intestinal cholesterol to the liver. Eze's mechanism of action involves reducing blood cholesterol by inhibiting the absorption of cholesterol in the small intestine. Unlike other cholesterol-reducing agents, Eze localizes and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol, leading to a decrease in the

delivery of intestinal cholesterol to the liver. This leads to a reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood. Eze has been demonstrated to have no significant effect on the plasma concentrations of the fat-soluble vitamins [Catapano, 2001].

Indication:

Eze is indicated for use as adjunctive therapy to diet for the reduction of elevated TC, LDL, and Apo B in patients with primary (heterozygous familial and non-familial) hypercholesterolemia [Zetia - prescribing information].

Dosing and administration:

Eze has a long half-life that allows for once-daily dosing. It is available as a 10-mg tablet. Eze 10 mg daily may be given with or without food along with a standard cholesterol lowering diet. To obtain a greater effect, Eze may be given with a statin. Both agents may be given simultaneously for convenience. No dosage adjustment is needed in patients with mild hepatic insufficiency. Use is not recommended in patients with moderate to severe hepatic insufficiency because no studies have been conducted in this patient population. No dosage adjustment is needed in patients with mild renal insufficiency. In patients with severe renal insufficiency, the mean area under the curve for total Eze after a single dose was increased by 1.5-fold. Eze is a pregnancy category C agent. There are no well controlled studies of Eze in pregnant women. Eze has been demonstrated to cross the placenta when given to pregnant rats and rabbits in multiple oral doses. So, Eze is indicated in pregnant women only if the potential benefit outweighs the risk to the fetus. It is not known whether Eze is excreted into human breast milk, so it should not be used in nursing mothers unless the potential benefit justifies the potential risk to the infant [Zetia - prescribing information].

Drug interactions:

Eze is neither an inhibitor nor an inducer of cytochrome P450 isoenzymes, so metabolism of other agents is not affected. The mean area under the curve of total Eze was reduced by approximately 55% when given concurrently with cholestyramine. The Eze concentration was increased by about 1.5-fold when given with fenofibrate. Concurrent administration with gemfibrozil increases Eze's concentration by approximately 1.7-fold. The level of Eze was increased 12-fold in one renal transplant patient who was also taking cyclosporine [https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=582e1593-1e6c-4ce8-8bae-134fbc3a66f0].

Pharmacokinetics

The extent of Eze absorption is not affected by taking it with high-fat or nonfat meals. However, the C_{max} value of Eze is increased by 38% when taken with a high-fat meal. Overall, Eze may be taken with or without food. Eze is metabolized mainly in the small intestine and liver via glucuronide conjugation, with subsequent biliary and renal excretion. Eze is rapidly metabolized to Eze-glucuronide in humans. Both Eze and its conjugate are the major drug-derived compounds detected in plasma, making up 10% to 20% and 80% to 90% of the total drug in plasma, respectively. Both forms have a long half-life of about 22 hours, accounting for their slow elimination from plasma. Also, multiple peaks are seen on plasma concentration-time profiles, implying enterohepatic recycling. Eze was the major component in feces and accounted for 69% of the administered dose, while its conjugate was the major component in urine and accounted for 9% of the administered dose.

Absorption:

Eze, which is water insoluble, is absorbed and extensively conjugated to an active phenolic glucuronide (Eze-glucuronide) after oral intake. After a single dose of 10 mg, mean Eze peak plasma concentrations (C_{max}) of 3.4 to 5.5 ng/mL were obtained within 4 to 12 hours (T_{max}). The pharmacokinetic parameters for Eze-glucuronide are as follows: C_{max} = 45 - 71 ng/mL; T_{max} = 1-2 hours. The extent of Eze absorption is not affected by taking it with high-fat or nonfat meals. However, the C_{max} value of Eze is increased by 38% when taken with a high-fat meal. Overall, Eze may be taken with or without food. There was no substantial deviation from dose proportionality between 5 and 20 mg. The absolute bioavailability of Eze cannot be determined, as the compound is virtually insoluble in aqueous media suitable for injection.

Distribution:

Both Eze and Eze glucuronide are highly bound (> 90%) to human plasma proteins. Eze and Eze-glucuronide are bound 99.7% and 88 to 92% to human plasma proteins, respectively.

Metabolism:

Eze is metabolized primarily in the small intestine and liver via glucuronide conjugation (a phase II reaction) with subsequent biliary and renal excretion. Minimal oxidative metabolism (a phase I reaction) has been observed in all species evaluated. Eze and Eze glucuronide are the major compounds detected in plasma. The conjugated Eze-glucuronide constitutes 80–90% of plasma drug levels with Eze the remaining 10–20%. Both Eze and Eze-glucuronide are slowly eliminated from plasma with evidence of significant enterohepatic recycling. The half-life for Eze and Eze-glucuronide is approximately 22 hours.

Excretion:

Following oral administration of ^{14}C-Eze (20 mg) to human subjects, total Eze (Eze + Eze-glucuronide) accounted for approximately 93% of the total radioactivity in plasma. Approximately 78% and 11% of the administered radioactivity were recovered in the faeces and urine, respectively, over a 10-day collection period. After 48 hours, there were no detectable levels of radioactivity in the plasma. Eze was the major component in faeces (69% of the administered dose) while Eze-glucuronide was the major component in urine and accounted for 9% of the administered dose.

Pharmacodynamics:

Eze is in a class of lipid-lowering compounds that selectively inhibits the intestinal absorption of cholesterol and related phytosterols. Eze, administered alone is indicated as adjunctive therapy to diet for the reduction of elevated TC, LDL, and Apo B in patients with primary (heterozygous familial and non-familial) hypercholesterolemia. It is also used in combination therapy with HMG-CoA reductase inhibitors. Eze has a mechanism of action that differs from those of other classes of cholesterol-reducing compounds (HMG-CoA reductase inhibitors, bile acid sequestrants, fibric acid derivatives, and plant stanols). The cholesterol content of the liver is derived predominantly from three sources. The liver can synthesize cholesterol, take up cholesterol from the blood from circulating lipoproteins, or take up cholesterol absorbed by the small intestine. Intestinal cholesterol is derived primarily from cholesterol secreted in the bile and from dietary cholesterol. The molecular target of Eze has been shown to be the sterol transporter, NPC1L1, which is involved in the intestinal uptake of cholesterol and phytosterols. Eze does not inhibit cholesterol synthesis in the liver, or increase bile acid excretion. Instead, Eze localizes at the brush border of the small

intestine and inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. This causes a reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood; this distinct mechanism is complementary to that of statins and of fenofibrate.

Clinical studies have demonstrated that elevated levels of TC, LDL and Apo B, the major protein constituent of low density lipoprotein, promote human atherosclerosis. In addition, decreased levels of HDL are associated with the development of atherosclerosis. Epidemiologic studies have established that cardiovascular morbidity and mortality vary directly with the level of TC and LDL and inversely with the level of HDL. Like low density lipoprotein, cholesterol-enriched TG-rich lipoproteins, including very-low-density lipoproteins, intermediate-density lipoproteins, and remnants, can also promote atherosclerosis. The independent effect of raising HDL or lowering TG on the risk of coronary and cardiovascular morbidity and mortality has not been determined. Eze reduces TC, LDL, Apo B, non-HDL cholesterol, and TG, and increases HDL in patients with hyperlipidemia

[http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021445s020s021lbl.pdf].

Toxicity:

In animals, no toxicity was observed after single oral doses of 5000 mg/kg of Eze in rats and mice and 3000 mg/kg in dogs. Eze was well tolerated by mice, rats and dogs. No target organs of toxicity were identified in chronic studies at daily doses up to 1500 and 500 mg/kg in male and female rats, respectively, up to 500 mg/kg in mice, or up to 300 mg/kg in dogs.

Carcinogenicity:

In two-year studies conducted in mice and rats, Eze was not carcinogenic. A 104-week oral carcinogenicity study with Eze was conducted in mice at doses up to 500 mg/kg (> 150 times the human exposure at 10 mg daily based on $AUC_{0\text{-}24hr}$ for total Eze). A 104-week oral carcinogenicity study with Eze was conducted in rats at doses up to 1500 mg/kg (males) and 500 mg/kg (females) (~14 and ~17 times the human exposure at 10 mg daily based on $AUC_{0\text{-}24hr}$ for total Eze). There were no statistically significant increases in tumor incidences in drug-treated rats or mice.

Mutagenicity:

No evidence of mutagenicity was observed *in-vitro* in a microbial mutagenicity (Ames) test with Salmonella typhimurium and Escherichia coli with or without metabolic activation. No evidence of clastogenicity was observed *in-vitro* in a chromosomal aberration assay in human peripheral blood lymphocytes with or without metabolic activation. In addition, there was no evidence of genotoxicity in the *in-vivo* mouse micronucleus test.

Reproductive and Teratogenicity Studies:

In oral (gavage) fertility studies of Eze conducted in rats, there was no evidence of reproductive toxicity at doses up to 1000 mg/kg/day in male or female rats (~1181 [males] times the human dose at 10 mg daily based on surface area and ~7 [females] times the human exposure at 10 mg daily based on $AUC_{0\text{-}24hr}$ for total Eze). Eze, at doses up to 1000 mg/kg (the highest feasible dose), was not maternotoxic in embryo-fetal development studies in rats and rabbits. Eze was not teratogenic in rats or rabbits and had no effect on prenatal or postnatal development

[http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21445_Zetia_biopharmr_P1.pdf].

Research works carried so far in the literature:

Eze is a model low soluble and highly permeable drug. Pharmacologically, Eze is a hypocholesterolemic agent. The oral absorption of Eze shows inter-subject variability and its bioavailability could be as low as 35% due to its poor solubility and P-gp efflux. Eze acts by inhibiting the small intestinal absorption of cholesterol. The P-gp molecules at the intestinal brush border cause P-gp efflux of Eze and thus interfere with the absorption of Eze. So far, Eze has been formulated as CD complexes [Patel et al., 2008], CoC formulations [Snehal et al., 2012] and CDDS [Dixit and Nagarsenker, 2008; Bali et al., 2010 and 2011; Bandyopadhyay et al., 2012]. While the studies on CD complexes and CoC formulations were limited only to *in-vitro* characterization, the CDDS formulations were studied extensively at *in-vivo* level too. Therefore, the *in-vivo* behavior of Eze CD complexes and CoC systems lack an in-depth study report.

The CDDS formulations reported improvement in *in-vitro* dissolution as well as *in-vivo* bioavailability of Eze which signified the effect of nanosize on the improved performance of Eze. Among the different CDDS, Eze was formulated as self nanoemulsifying systems reported in liquid and solid forms and as nanoemulsion formulations. All these formulations contained several components which made the optimization of their preparation laborious and time taking. Furthermore, their preparation involved use of large amounts of surfactants and cosurfactants, which, from the toxicological stand point, is a legitimate concern. So, though nanosize proved advantageous in improving the performance of Eze, there is still a need to develop a

simpler, less toxic, less laborious and economic nanoformulation. Table 2.6 lists the research works on Eze and their unmet needs.

Table 2.6. Formulation research works on Eze and their drawbacks.

Formulation	**The unmet need [Reference]**
Binary cyclodextrin complexes	*In-vivo* studies have not been reported [Patel et al., 2008]
Cocrystals – salicilic and benzoic acids	Conflicting findings compared to previously reported works; *In-vivo* studies have not been reported [Mulye et al., 2012]
Cocrystal – methyl paraben	Conflicting findings compared to previously reported works; *In-vivo* studies have not been reported [Sugandha et al., 2014]
Cocrystals – l-proline and imidazole	*In-vivo* studies have not been reported [Shimpi et al., 2014]
Liquid self nanoemulsifying systems	Incorporation of several excipients raises issues [Bandyopadhyay et al., 2012]
Solid self nanoemulsifying systems	Incorporation of several excipients raises issues [Dixit and Nagarsenker, 2008]
Nanoemulsion	Incorporation of several excipients raises issues [Bali et al., 2010 and 2011]

2.4.2 Excipients' profile

2.4.2.1 Nicotinic acid (NA)

Other names: Niacin; Bionic; Vitamin B_3**,** Pyridine-3-carboxylic acid (chemically).

Functional Category: NA is a water soluble vitamin of the B complex and is required by the body for the formation of coenzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).

Solubility: Solubility in water is 15 g/L at 20 ˚C.

Description: NA is an odorless white crystalline powder. Figure 2.18 shows the structure of NA.

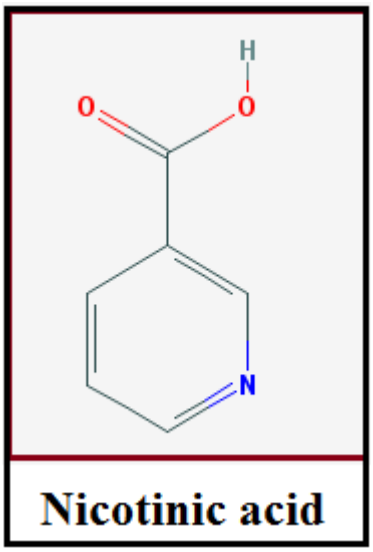


Figure 2.18. Structure of NA.

Applications in pharmaceutical formulation or technology:

NA occurs naturally in plants and animals, and is also added to many foods as a vitamin supplement. It is also present in many multiple vitamins and nutritional supplements. It

has pellagra-curative, vasodilating and antilipemic properties. NA is used to treat and prevent a lack of natural NA in the body, and to lower cholesterol and TG in the blood. It is also used to lower the risk of heart attack in people with high cholesterol who have already had a heart attack. It is sometimes used to treat CAD like atherosclerosis [http://www.drugs.com/niacin.html].

Research works where NA was employed in drug formulations (in CoC systems):

NA is a lipid lowering agent that acts by inhibiting lipolysis in adipose tissue [Athimoolam and Rajaram, 2005]. NA has been in use in the preparation of CoCs [Kavuru et al., 2010; Sanphui and Rajput, 2014] as described below. Hydrochlorothiazide:NA CoC in the ratio 1:1 formed upon cogrinding and subsequent crystallization from methanol [Sanphui and Rajput, 2014]. Similarly, Hesperetin:NA and L-Ascorbic acid:NA 1:1 CoCs were generated by slow evaporation and cooling crystallization from methanol [Kavuru et al., 2010].

2.4.2.2 Nicotinamide (ND)

Other names: Niacinamide, Nicotinic acid amide; Vitamin PP; Pyridine-3-carboxamide (chemically). Figure 2.19 shows the structure of ND.

Functional Category: ND has anti-inflammatory actions. ND is a water-soluble component of the vitamin B complex group. These may be of benefit to patients with inflammatory skin conditions. These conditions include acne vulgaris, and the compound can suppress antigen-induced lymphocytic transformation and inhibit 3', 5'-cyclic-AMP phosphodiesterase. ND has demonstrated the ability to block the inflammatory actions of iodides known to precipitate or exacerbate inflammatory acne.

Solubility: Solubility in water is 1000g/L at 20 ˚C.

Description: ND is an odorless white crystalline powder.

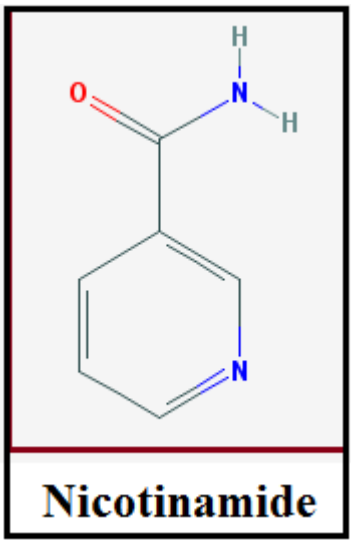


Figure 2.19. Structure of ND.

Applications in pharmaceutical formulation or technology:

NA is converted to ND *in-vivo*, and, though the two are identical in their vitamin functions, ND does not have the same pharmacological and toxic effects of niacin, which occur incidental to niacin's conversion. Thus, ND does not reduce cholesterol or cause flushing. *In-vivo*, ND is also incorporated into NAD and NADP. NAD and NADP function as coenzymes in a wide variety of enzymatic oxidation-reduction reactions essential for tissue respiration, lipid metabolism, and glycogenolysis. The pathways for ND and NA are very similar but ND lacks the vasodilator, gastrointestinal, hepatic, and hypolipemic actions of NA [https://www.drugs.com/drp/nicotinamide.html]. Table 2.7 presents examples of research works that used ND in CoC systems.

Table 2.7. Examples of research works employing ND in CoC systems.

CoC	Preparation method	Drug:coformer ratio [Reference]
Theophylline:Nicotinamide	Neat grinding and Slow evaporation in solvent, ethanol	1:1 [Lu and Rohani, 2009]
Carbamazepine:Nicotinamide	Slow evaporation in solvent, ethanol	1:1 [Gagniere et al., 2009b]
Adefovir dipivoxil:Nicotinamide	Solution cooling crystallization in solvent, ethanol	1:1 [Gao et al., 2012]
Salicylic acid:Nicotinamide	Slow evaporation in solvent, ethanol	1:1 [Berry et al., 2008]
Flurbiprofen:Nicotinamide	Solution cooling crystallization in solvent, ethanol	
Fenbufen:Nicotinamide	Solvent evaporation in solvent, ethanol	
Ibuprofen:Nicotinamide	Solution cooling crystallization in solvent, methanol	

2.4.2.3 2-Hydroxypropyl-β-Cyclodextrin (HPBCD)

Other names: Hydroxypropyl Betadex

Functional Category: Complexing agent; dissolution enhancer; release-modifying agent; sequestering agent; solubilizing agent; stabilizing agent; tonicity agent.

Solubility: Freely soluble in water.

Description: White or almost white, amorphous or crystalline powder. Figure 2.20 shows the structure of HPBCD.

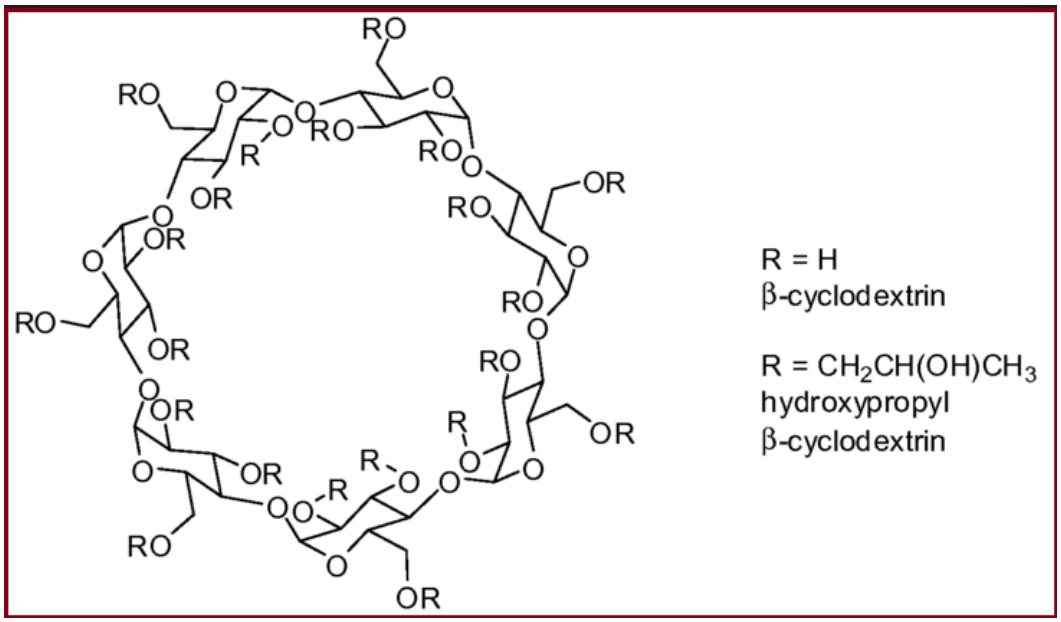


Figure 2.20. Structure of HPBCD.

Table 2.8. Examples of research works employing HPBCD ternary systems.

Ternary component	Drug	Effect [Reference]
Polyvinyl pyrrolidone, HPMC, Polyethylene glycol	Celocoxib	Positive [Chowdary and Srinivas, 2006]
L-arginine	Cefixime	Positive [Jadhav et al., 2013]
Tartaric acid	Carvedilol	Positive [Yuvaraja and Khanam, 2014]
Sucrose	Midazolam	Positive [Kaartama et al., 2012]
Kollicoat IR	Glyburide	Positive [Zoeller et al., 2012]
Soy bean lecithin	Dihydroartemisinin	Positive [Wang et al., 2013]
Soluplus	Pioglitazone/glimepiride (fixed dose combination)	Positive [Taupitz et al., 2013]

Applications in pharmaceutical formulation or technology:

HPBCD has been widely investigated in pharmaceutics and has principally been used as a solubilizer for hydrophobic molecules in oral liquids, oral solids, parenterals, pressurized metered dose inhalers, dry powder inhalers, and topical formulations. It has also been shown to act as a stabilizer during processing and storage of formulations. HPBCD inclusion complexes have been reported to show mechanical properties distinct from the pure materials. The reported advantage of HPBCD over unsubstituted β-cyclodextrin is its greater water solubility [Rowe et al., 2009]. Table 2.8 presents examples of research works that used HPBCD in drug-CD ternary systems.

2.4.2.4 Ascorbic Acid 2-Glucoside (AA2G)

Other Names: L-ascorbic acid 2-glucoside; Ascorbyl Glucoside; 2-O-α-D-glucopyranosyl-L-ascorbic acid (chemically).

Functional Category: Fortifying agent.

Solubility: Solubility in water is 125 g/100 g water (25℃).

Description: White to yellowish white powder, or crystalline powder. Figure 2.21 shows the structure of AA2G.

Applications in pharmaceutical formulation or technology:

AA2G was originally developed as a quasi-drug cosmetic product in Japan to lighten the overall tone of the skin and reduce the pigmentation in age spots and freckles. Further research has shown other dramatic benefits and today AA2G is used all over the world – not only for whitening but also for brightening dull looking skin, reversing the effects of aging, and in sunscreen products for protection. When products containing AA2G are used on the skin, the action of glucosidase gradually releases vitamin C,

providing the benefits of vitamin C effectively over a prolonged period of time. AA2G has glucose bound to the hydroxyl group of the second carbon (C2) of the ascorbic acid. The C2 hydroxyl group is the primary site of natural vitamin C's beneficial activity; however, this is the site where vitamin C is degraded. The glucose protects vitamin C from high temperatures, pH, metal ions and other mechanisms of degradation. AA2G is natural vitamin C (ascorbic acid) stabilized with glucose. This combination allows the benefits of vitamin C to be conveniently and effectively used in cosmetic products. When creams and lotions containing AA2G are applied to the skin, an enzyme present in the skin, glucosidase, acts on the AA2G to slowly release the healthful benefits of vitamin C [Moribe et al., 2011].

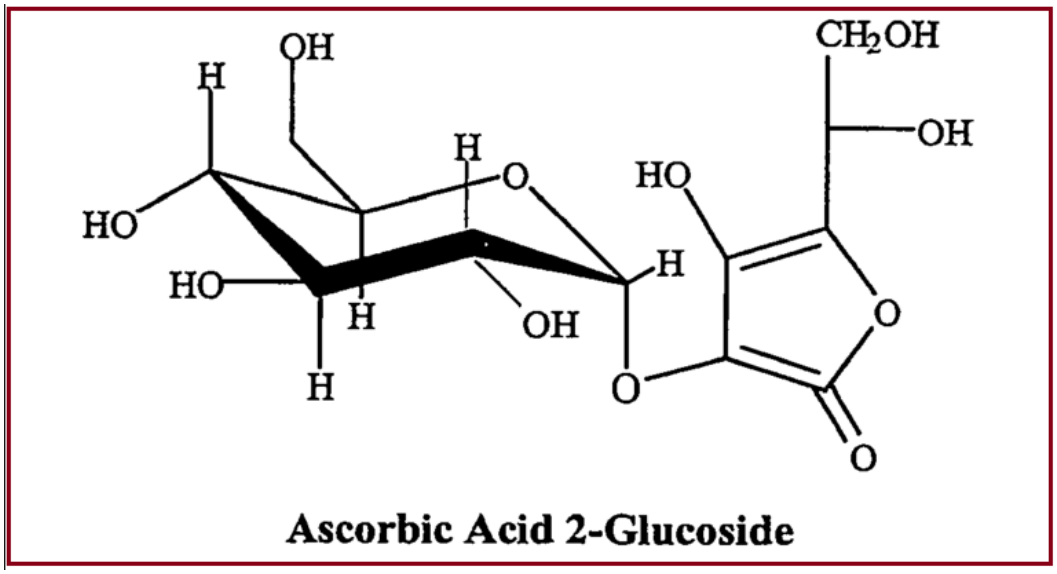


Figure 2.21. Structure of AA2G.

Research works where AA2G was employed in drug formulation (in NC systems):

AA2G is a novel hydrophilic (non-surfactant) excipient that has been approved as a food additive and is expected to be used as a principle solubilizer in fat-soluble vitamin

formulations and in other cosmetic products [Inoue et al., 2007]. Inoue et al. [2007] reported formation of nanoparticles and improvement in aqueous solubility and dissolution properties of clarithromycin on co-grinding with AA2G.

2.4.2.5 Tocopheryl Polyethylene Glycol Succinate (TPGS)

Other Names: Tocophersolan; D-α-tocopheryl polyethylene glycol succinate; Vitamin E TPGS or simply TPGS.

Functional Category: Pharmaceutical solubilizer and absorption enhancer; water-soluble antioxidant; useful in the treatment of vitamin E deficiency and chronic cholestasis.

Solubility: ≈ 20% w/w solubility in water at 25 °C.

Description: White to slightly yellow waxy solid. Figure 2.22 shows the structure of TPGS.

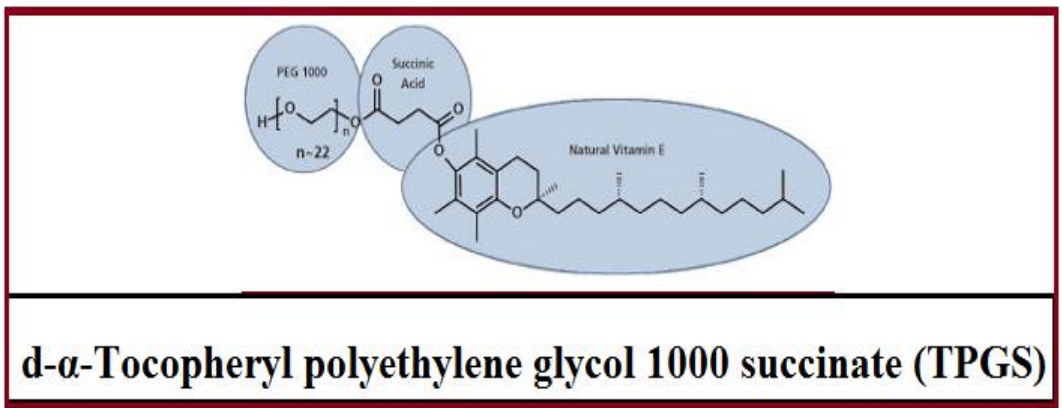


Figure 2.22. Structure of TPGS.

Applications in pharmaceutical formulation or technology:

The solubility of TPGS is high compared to the low water solubility of vitamin E, which is classified as an oil-soluble vitamin. This physical characteristic of TPGS is both the starting point and the core advantage of TPGS based innovations, especially in the pharmaceutical field. The TPGS structure shows a hydrophilic head (polyethylene glycol chain) and a lipophilic tail (tocopheryl group) (Figure 2.22). This amphiphilic skeleton gives TPGS its non-ionic surfactant properties as reflected by the hydrophilic lipohilic balance (HLB) number. The HLB of TPGS is 13, which is within the 0–20 range for non-ionic surfactants. Consequently, it can both act to increase solubility parameters and to allow making emulsions of lipophilic compounds in aqueous systems. As novel nonionic surfactant, it exhibits amphipathic properties and can form stable micelles in aqueous vehicles at concentration as low as 0.02 mM. It has been widely investigated for its emulsifying, dispersing, gelling, and solubilizing effects on poorly water-soluble drugs. It can also act as a P-gp inhibitor and has been used as an excipient for overcoming multidrug resistance and for increasing the oral bioavailability of many drugs. Since TPGS has been approved by FDA as a safe pharmaceutical adjuvant, many TPGS based drug delivery systems have been developed. In fact, α-tocopherol (also called vitamin E) is a physiological lipid-soluble antioxidant present in the human body, and its antioxidant activity has been also observed when incorporated into phospholipid membranes. Another interesting property of this substance is that the hydrophilic portion of the molecule is polyethylene glycol, a polymer well known for its stealth feature when associated to liposomes and nanoparticles in the blood stream [Guo et al., 2013]. Table 2.9 presents examples of research works that used TPGS as stabilizer in NC systems.

Table 2.9. Examples of research works employing TPGS in NC systems.

Stabilizer	Drug	Preparation method [Reference]
TPGS alone	Paclitaxel	Precipitation [Liu et al., 2010]
TPGS alone	Paclitaxel	Precipitation [Gao et al., 2013]
TPGS alone	9 model drug compounds, cinnarizine, griseofulvin, indomethacin, itraconazole, loviride, mebendazole, naproxen, phenylbutazone and phenytoin	MM [Eerdenburgh et al., 2008a and 2009]
TPGS alone	Rilpivirine	MM [Baert et al., 2009]
TPGS alone	Baicalin	HPH [Yue et al., 2013]
TPGS with HPMC as secondary stabilizer	NVS-102	MM [Ghosh et al., 2012]
TPGS alone	Naproxen	MM [George and Ghosh, 2013]

2.4.2.6 Sodium Lauryl Sulfate (SLS)

Other Names: Sodium dodecyl sulfate (SDS)

Functional Category: Anionic surfactant; detergent; emulsifying agent; skin penetrant; tablet and capsule lubricant; wetting agent.

Solubility: Freely soluble in water, giving an opalescent solution; practically insoluble in chloroform and ether.

Description: White or cream to pale yellow colored crystals, flakes, or powder having a smooth feel, a soapy, bitter taste, and a faint odor of fatty substances. Figure 2.23 shows the structure of SLS.

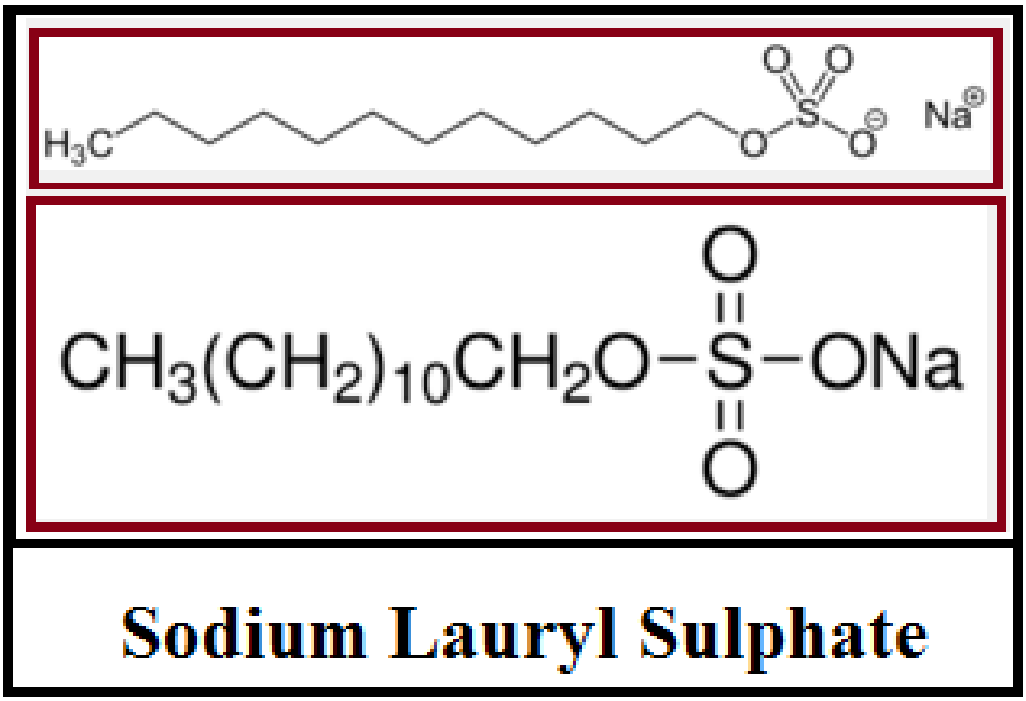


Figure 2.23. Structure of SLS.

Table 2.10. Examples of research works employing SLS in NC systems.

Use of SLS as primary or secondary stabilizer	**Drug**	**Preparation method [Reference]**
SLS alone	Albendazole	HPH [Kumar et al., 2008]
SLS alone	Spironolactone	HPH [Langguth et al., 2005]
Poloxamer and SLS	Albendazole	HPH [Kumar et al., 2008]
Tween 80 and SLS		
HPC and SLS	MK0869	MM [Wu et al., 2004]
Povidone K15 and SLS	AZ68	MM [Sigfridsson et al., 2007]
Polyvinylpyrrolidone/vinyl acetate and SLS	Undisclosed	MM [Deng et al., 2008]

Applications in pharmaceutical formulation or technology:

SLS is an anionic surfactant employed in a wide range of nonparenteral pharmaceutical formulations, toothpastes and cosmetics. It is a detergent and wetting agent effective in both alkaline and acidic conditions [Rowe et al., 2009]. Table 2.10 presents examples of research works that used SLS as one of the stabilizers in NC systems.