2.1 What is nanotechnology?

'Nanotechnology is the design, characterization, production and application of structures, devices and systems by controlling their shape and size at the nanometre scale'. In other words we can say that "'Nanotechnology' is the production technology to get the extra high accuracy and ultra fine dimensions, i.e. the preciseness and fineness on the order of 1 nm (nanometer), which is 10^{-9} meter in length" (Royal Society-Great Britain., 2004).

The nanometer scale is conventionally defined as 1 to 1000 nm. One nanometer is one billionth of a meter (10^{-9} m). The size range is normally set to a minimum of 1 nm to avoid single atoms or small groups of atoms being designated as nano-objects (Royal Society-Great Britain., 2004).

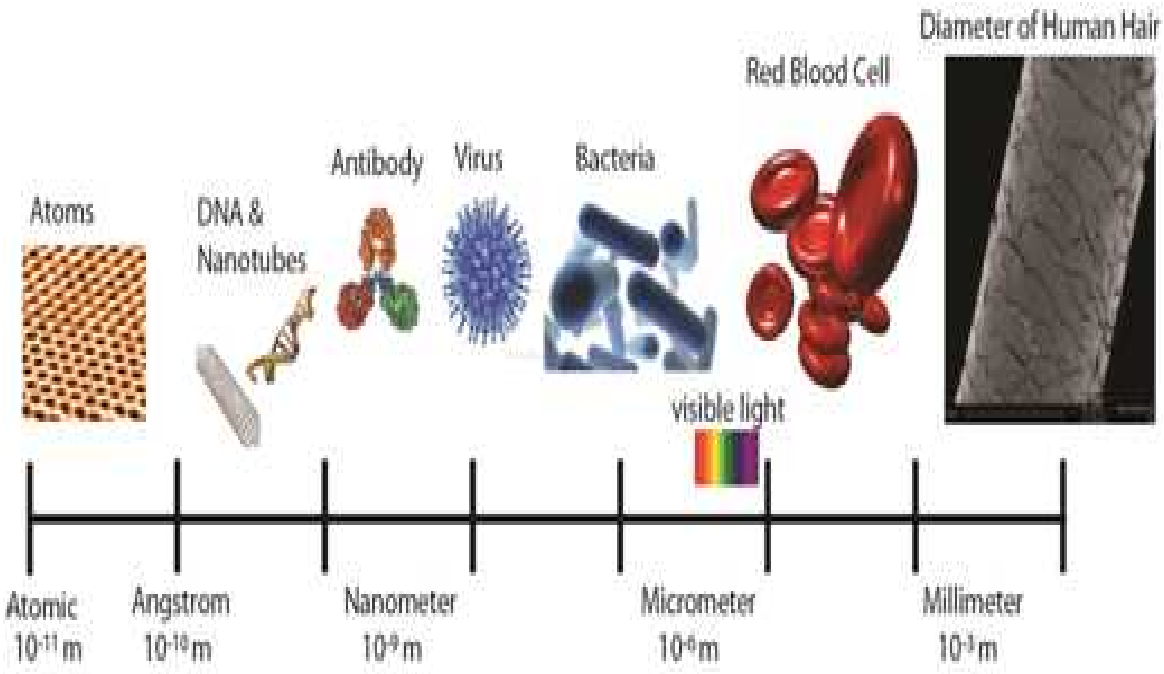


Figure.1: The nanometer length scale is a thousand times smaller than bacteria and ten million times smaller than the width of a single human hair.
(https://www.emersonprocessxperts.com/2014/01/process-engineering-of nanotechnology/)

Nanotechnology employs engineered materials or devices with the smallest functional organization on the nanometer scale (1–1000 nm) which are able to interact with biological systems at the molecular level. Thus, they may stimulate, respond or interact with target cells as well as tissues in order to induce desired physiological responses together minimizing undesirable side effects. Furthermore, nanotechnology offers ways to manipulate complex biological systems with greater selectivity and timing as compared to conventional pharmacological approaches (Modi et al., 2009).

Today, nanotechnology is found in a wide range of applications in the pharmaceutical industry. Due to new advances in nanotechnology, it is now possible to produce drug loaded nanoparticles that can be utilized in a variety of innovative ways (Gupta et al., 2006; Jain et al., 2008).

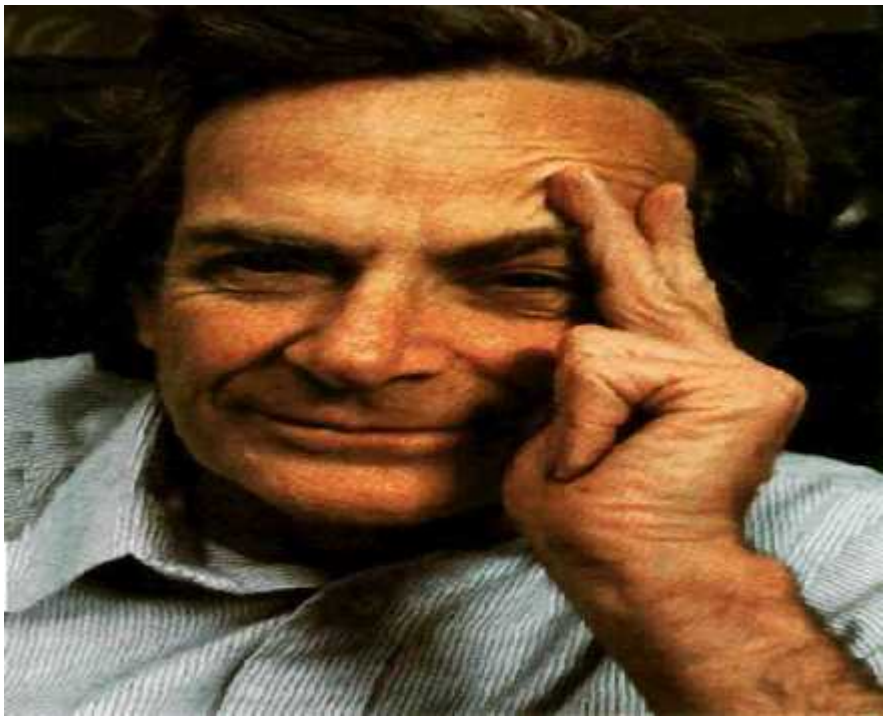
Nanotechnology mediated drug delivery has been reported to enhance the drug efficacy, bioavailability, reduce toxicity, higher therapeutic index and improve patient compliance by targeting the cells and tissues to produce desired pharmacological action (Preet Kaur et al., 2011).

2.2 History of nanotechnology

“But I am not afraid to consider the final question as to whether, ultimately in the great future we can arrange the atoms the way we want; the very atoms, all the way down!” – By Richard Feynman, There’s plenty of Rooms at the Bottom.

The first time the idea of nanotechnology was introduced in the year 1959, when Richard Feynman, a physicist at Caltech, gave a talk called “**There’s Plenty of Rooms at the Bottom**”. Though he never explicitly mentioned “nanotechnology,” Feynman suggested that it will eventually be possible to precisely manipulate atoms and molecules. Moreover, in an even more radical proposition, he thought that, in principle, it was possible to create “nano-scale” machines, with the help of a cascade of billions of factories. According to the physicist, these factories would be progressively smaller scaled versions of machine hands and tools. He proposed that these tiny “machine shops” would be then able to create billions of tinier factories. In these speculations, he also suggested that there are various factors, which uniquely affect the nano-

scale level. Specifically, he suggested that as the scale got smaller and smaller, gravity would become more negligible, while both Van Der Waals attraction and surface tension would become very important. In the end, Feynman's talk has been viewed as the first academic talk that dealt with a main tenet of nanotechnology, the direct manipulation of individual atoms (Fanfair et al., 2007; Feynman et al., 1959; Gispert., 2011).



In his Nobel Prize Lecture (11 Dec 1965), 'The Development of the Space-Time View of Quantum Electrodynamics'. Collected in Stig Lundqvist, Nobel *Lectures: Physics, 1963-1970* (1998), 177.

The term "nanotechnology" was first devised by Norio Taniguchi in the year 1974 (Webster., 2007) whereas the term "nanomedicine" was coined by Drexler and colleagues in 1980's (Tibbals., 2011). The first development in the field was reported in early 70s at the ETH Zurich, when the first controlled released system was developed along with other several drugs that showed the capability to cross the blood brain barrier with much improved pharmacokinetics (Najlah et al., 2007). However, it wasn't until 2006 when the first international journal in nano-medicine was introduced called 'International Journal of Nano-medicine' to cater to the emerging needs of nanotechnology in medicine (Cevc et al., 2004).

2.3 Nanotechnology in drug delivery

Delivering therapeutic compound to the target site is a major problem in treatment of many diseases. A conventional application of drugs is characterized by limited effectiveness, solubility, poor biodistribution, *in-vivo* stability, intestinal absorption, plasma fluctuations of drug, targeted delivery to the site of action and lack of selectivity (Nevozhay et al., 2007).

Of recent, several researches in nano drug delivery have been designed to overcome these above mentioned challenges through controlling drug delivery. In controlled drug delivery systems or nanostructures the drug is transported to the place of action, thus, its influence on vital tissues and undesirable side effects can be lowered. In addition, nanostructures protects the drug from rapid degradation or clearance and enhances drug concentration in target tissues, therefore, lower doses of drugs are required (Maeda et al., 2000). This modern form of therapy is especially important when there is a discrepancy between a dose and concentration of a drug and its therapeutic results or toxic effects. The technology enables the delivery of drugs that are poorly water soluble or lipophilic in nature and can provide means of bypassing the liver, thereby preventing the first pass metabolism. Nanotechnology increases oral bioavailability of drugs due to their specialized uptake mechanisms such as absorptive endocytosis and are also able to remain in the blood circulation for a longer period of time, releasing the incorporated drug in a controlled manner, leading to less plasma fluctuations, in-toxicity and minimized side-effects (Palmer et al., 1984; Torchilin., 2004; Vasir et al., 2005) .

Nanotechnology is an ideal targeting system should have long circulating time, it should be present at appropriate concentrations at the target site, and it should not lose its activity or therapeutic efficacy while in circulation. Various nanosystems, as a result of their larger size, are accumulated at higher concentrations than normal drugs (Matsumura et al., 1986).

In addition, the increased vascular permeability coupled with an impaired lymphatic drainage in tumor allows an enhanced permeability and retention effect of the nanosystems in the tumor or inflamed tissue. Thus, this patho-physiological opportunity allows extravasations of the nanosystems and their selective localization in the inflamed tissues. The tendency of nanosystems to specifically localize in the reticulo-endothelial system also presents an excellent

opportunity for passive targeting of drugs to the macrophages present in the liver and spleen. Thus, this natural system can be used for targeting drugs for intracellular infections. The therapeutic value of many promising drugs for the treatment of various neurological disorders is diminished by the presence of the blood-brain barrier. The blood-brain barrier is a unique membrane that tightly segregates the brain from the circulating blood. Thus, drug delivery to this organ is a challenge, because the brain benefits from very efficient protection.

Nanotechnology offers a solution for using the numerous chemical entities for treating brain disorders that are not clinically useful because of the presence of the blood-brain barrier. Nanoparticles can be effectively used to deliver relevant drugs to the brain (Pardridge., 1999; Duggal., 2011).

Nanoparticles can be used in targeted drug delivery at the site of disease to improve:

- The uptake of poorly soluble drugs
- The targeting of drugs to a specific site
- Drug bioavailability

Advantages of Nanoparticles

- Increased bioavailability
- Dose proportionality
- Decreased toxicity
- Smaller dosage form (i.e., smaller tablet)
- Stable dosage forms of drugs which are either unstable or have unacceptably low bioavailability in non-nanoparticulate dosage forms.
- Increased active agent surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body. Faster dissolution generally equates with greater bioavailability, smaller drug doses, less toxicity.
- Reduction in fed/fasted variability (Kelsall., 2005; Moghimi et al., 2005; Shi., 2010; Wilczewska et al., 2012).
- Nanoforms with optimized physicochemical and biological properties are taken up by the cells more easily as compared to larger molecules, so they can be successfully used as delivery vehicles for currently available therapeutics and drugs. Liposomes, solid lipids

nanoparticles, dendrimers, polymeric nanoparticles, silicon or carbon materials and magnetic nanoparticles are the examples of nanoforms that have been tested as drug delivery systems (Maroof K et al., 2016).

2.4 Nanotechnology for the poorly soluble drugs

Over the past ten years, the number of drug candidates with solubility problems has steadily increased as a result of using combinatory chemistry and high throughput screening in drug discovery. At present it is estimated that approximately 70 % of the new chemical entities are poorly soluble in aqueous as well as in organic media, and approximately 40 % of currently marketed immediate release oral drugs are considered practically insoluble (solubility less than 100 g/ml) in water observed by Lipinski in 2000, and by MeriskoLiversidge and Liversidge in 2008. Poor solubility leads to a variety of issues. Low solubility limits the drug dissolution rate, which frequently results in low bioavailability of the orally administered drug demonstrated by Horter and Dressman in 2001. In such a case the therapeutic drug concentration in the blood can be achieved by dose escalation. However, dose escalation is often undesirable because of the following reasons: (1) possibility of increased toxicity and therefore decreased patient compliance; (2) difficulty in designing formulations for drug product with high drug load; and (3) increase in manufacturing costs associated with higher consumption of active pharmaceutical ingredients (API). Low solubility also may result in erratic absorption patterns, which detract from the clinical efficacy of the drugs. Consequently one of the major challenges of the pharmaceutical industry is developing strategies to enhance the aqueous solubility of drugs. The current advancement in the delivery of water-insoluble drugs and biopharmaceuticals are expected to notably influence the pharmaceutical and biotechnological industries. Furthermore, the relentless advancement in the current drug delivery nanotechnology which scientists could use to develop formulations has had a remarkable impact on reducing cost and improving the efficiency and productivity (Lipinski., 2000; Merisko-Liversidge et al., 2008; Hörter et al., 2001; Lu et al., 2013; Bamrungsap et al., 2012)

Various nanoparticles have been investigated as drug delivery systems, from biological (albumin, gelatin and phospholipids), to chemical (polymers and solid metal-containing Nanoparticles (NPs) (Suri et al., 2007). Nanoparticles are taken up by the cells more efficiently

as compared to the larger macromolecules; therefore, they could be used as an effective transport as well as delivery systems. Nanoparticles can be used in targeted drug delivery to improve the uptake of poorly soluble drugs and also improve oral drug bioavailability (Takagi et al., 2006).

Currently, 40 % of the marketed immediate release drugs are categorized under poorly soluble category (Hörter et al., 2001). There are many problems arising from the poor solubility of drug candidates in drug research as well as development. The aqueous solubility of a drug is a critical determinant of its dissolution rate. The limited dissolution rate arising from low solubility frequently results in the low bioavailability of orally administered drugs, and compounds with aqueous solubility lower than 100 g/mL generally present dissolution-limited absorption (Stella et al., 2007). In such cases, dose escalation would be required until the blood drug concentration reaches the therapeutic drug concentration range. This dose escalation sometimes causes topical toxicity in the gastrointestinal tract upon oral administration, and such toxicity could lead to a reduction in patient compliance. In drug product development, the formulation design of a drug product with high drug load is generally difficult. Increasing drug load might result in poor powder properties, such as poor powder flow ability and sticking tendency during granulation and tablet formation. In addition, the manufacturing cost would increase since a large amount of active pharmaceutical ingredient (API) might be consumed to develop and manufacture the drug product. The poor solubility of new drug candidates might also affect in vitro assay performance in drug discovery stage. In drug discovery, a number of in vitro cell culture assays are conducted to evaluate several biological properties of drug candidates, such as efficacy, membrane permeation genotoxic properties. The solubility limitation or precipitation of a drug in the test medium may yield invalid information on the drug properties in *in-vitro* condition. In preclinical development, the solubility limitation could also impair data quality on in vivo toxicity assessments since toxicological studies usually require higher exposure than that in pharmacological or pharmacokinetic studies to assure its safety. In clinical use, the poor bioavailability of a drug substance might result in limited therapeutic potential, thereby leading to insufficient clinical outcomes.

Various approaches to overcome the poor aqueous solubility of drug candidates have been investigated in drug research and development. Changing the chemical structure in the lead

optimization phase is considered to be an option to increase the solubility of drug candidates. Prodrug approaches might also enhance the aqueous solubility of drug candidates by introducing a polar functional group into the structure of a molecule (Müller et al., 1998). In addition to these attempts, a number of approaches have been investigated to increase the dissolution of poorly water-soluble drugs. Basic approaches for poorly water-soluble drugs are also reviewed with an emphasis on enhancing solubility, dissolution rate, and oral bioavailability. Literature-based examples of the formulation options for poorly water-soluble compounds and their practical application to marketed products are also provided in this thesis.

Particle size reduction to nanometer range (<1m) is an attractive approach for poorly water-soluble drugs. Particle size reduction could lead to an increase of the surface area and a decrease of the diffusion layer thickness, which could provide an enhanced dissolution rate for drugs. In addition to these factors, an increase in the saturation solubility is also expected by reducing the particle size to less than 1m, as described by Ostwald–Freundlich's equation (Reddy et al., 2013).

Importance of solubility enhancement of poorly soluble drugs:

- Solubility is one of the important parameters to achieve preferred concentration of drug in systemic circulation for achieving required pharmacological response.
- Poorly water soluble drugs frequently require high doses and need high dosage regimens in order to influence therapeutic plasma concentrations after oral administration.
- Low aqueous solubility is the main problem encountered with preparation and development of new chemical entities as well as for generic drugs.
- For orally administered drugs solubility is the one of the important rate limiting parameter to reach their desired concentration in complete circulation for pharmacological response.
- Water is an excellent solvent for liquid pharmaceutical formulations.
- Most of the drugs like weakly acidic or weakly basic have poor aqueous solubility.
- Poorly water soluble drugs having slow drug absorption leads to gastrointestinal mucosal toxicity and variable bioavailability (Vemula et al., 2010; Dhillon et al., 2014; Vijaykumar Nekkanti et al., 2016).

2.5 Saturation Solubility for poorly soluble drugs

The principle of nanonization is based on the increase in surface area of drug particles. According to Noyes-Whitney equation (Dizaj et al., 2015), the dissolution rate of poorly water soluble drug could be increased by reducing the drug particle size to nano scale and increasing its surface area (Lu et al., 2015). Increasing the surface area by reducing the particle size generally correlates with improved dissolution and drug absorption. Development of nanoparticle formulation for poorly water soluble drugs results in enhanced dissolution rate which is the driving force for its improved pharmacokinetic properties. Particle size and intrinsic solubility are the important parameters influencing the dissolution rate of a drug. As described by the Nernst-Brunner and Levich modification of Noyes-Whitney model the rate of drug dissolution is directly proportional to surface area;

$$dx/dt = (A \times D/d) \times (C-X/V)$$

Where X is the amount of drug in solution, t is time, A is the effective surface area, D is the diffusion coefficient of the drug, d is the effective diffusion boundary layer, C is the saturation solubility of the drug, and V is the volume of dissolution medium. Saturation solubility of a drug depends on the dissolution pressure and temperature. The dissolution pressure is a function of the curvature of the nanoparticle surface. Greater the curved surface of the particles, stronger will be the dissolution pressure. For particles below a size of 1 µm, the dissolution pressure increases significantly leading to an increase in the saturation solubility. In addition the concentration gradient is increased due to decreased diffusional distance on the surface of the drug nanoparticle. This increase in surface area and increase in concentration gradient results in enhanced dissolution velocity and saturation solubility compared to the products containing micronized particles (Vijaykumar Nekkanti et al., 2016). Saturation solubility and dissolution velocity are important parameters that affect the bioavailability of poorly soluble drugs administered orally (Müller et al., 2001; Çağdaş et al., 2014).

At present, there are limited formulation approaches available to address the problems associated with drug's poor aqueous solubility and bioavailability. The most commonly used approaches are incorporation of drug into complexing agents (cyclodextrins), using lipid carriers (liposomes, self-emulsifying systems), micronization, and solid dispersions of the drug in water-soluble

carriers, etc. However the success of these techniques is mostly dependent on specific properties of drug molecule therefore, have limited scope for general application.

For example, ability to ionization, solubility in oils, lipids, molecular size, structure and shape to fit into the hydrophobic cavities, etc. Liposomes have demonstrated reasonable success in formulating poorly soluble drugs however because of the poor stability issues and expensive product costs, these approaches are not suitable for all the drugs, particularly of those which are not soluble in either aqueous or organic solvents (Jatwani et al., 2012). For many years, micronization was successfully applied in the formulation of poorly soluble drugs. Micronization often results in colloidal drug particles with a particle size > 1 µm with less fraction in the sub-micron range. Micronization of drug shall result in a moderate increase in surface area that may not be significant in terms of improving the dissolution rate or saturation solubility to impact the bioavailability (Huang et al., 2014).

Solid dispersions comprise of dispersion of the drug in a solid matrix, where the matrix can be a polymer or lipid based surface active carrier that can rapidly emulsify, upon contact with the dissolution media. Formation of molecular dispersions (solid solution) provides a means of reducing the particle size of the drug to nearly molecular level. As the carrier dissolves, the drug is exposed to the dissolution media as fine colloidal particles in amorphous form.

The reduced particle size and increased surface area, results in improved dissolution rate and oral absorption. There are several formulations available in the market, e.g., Sandimmune/Neoral (cyclosporine microemulsion), Norvir (Ritnovir) and Fortovase (Saquinavir). This approach is suitable for highly potent compounds with low dose requirement and thus not applicable for drugs with low potency where the dose requirements are relatively high (Shegokar et al., 2010). Thus there is a need for a versatile technology that can address formulation issues associated with poorly soluble drugs.

A list of approved products developed using nanoparticle technology is summarized in Table 1 (Vojvodic et al., 2015; Ogden et al., 2005). Some of the key nanotechnology based approaches

for the enhancement of drug solubility and oral bioavailability according to Saffie Siebert and co-workers (Lvov et al., 2008) are highlighted in Table 1.

Table.1: An overview of nanoparticle technology based marketed products (Lvov et al., 2008).

Trade Name	**Drug**	**Indication**	**Drug Delivery Company**	**Innovator Company**	**Status**
Rapamune	Rapamycin, Sirolimus	Immunosuppressant	Elan Nanosystems	Wyeth	Marketed
Emend	Aprepitant	Anti-emetic	Elan Nanosystems	Merck & Co.	Marketed
Tricor	Fenofibrate	Hypercholesterolemia	Abbott Laboratories	Abbott Laboratories	Marketed
Megace ES	Megestrol	Anti anorexic	Elan Nanosystems	Par Pharmaceuticals	Marketed
Triglide	Fenofibrate	Hypercholesterolemia	IDD-P Skyepharma	Schiele Pharma Inc.	Marketed
Avinza	Morphine sulphate	Phychostimulant drug	Elan Nanosystems	King Pharmaceuticals	Marketed
Focalin	Dexmethyl-Phenidate HCl	Attention Deficit Hyperactivity Disorder (ADHD).	Elan Nanosystems	Novartis	Marketed
Ritalin	Methyl Phenidate HCl	CNS Stimulant	Elan Nanosystems	Novartis	Marketed
Zanaflex Capusules	Tizanidine HCl	Muscle relaxant	Elan Nanosystems	Acorda	Marketed

Table.2: Key nanotechnology-based approaches for the enhancement of drug solubility and oral bioavailability

Company	Nanotechnology-based formulation Approach	Description
American Biosciences (Blauvelt, NY, USA)	Nanoparticle albumin-bound technology. e.g. paclitaxel-albumin nanoparticles	Paclitaxel albumin nanoparticle (Birrenbach et al,1976)
Baxter Pharmaceuticals (Deerfield,Illinois)	Nanoedge technology: Particle size reduction was achieved by homogenization, micro precipitation, lipid emulsion.	Nano lipid Emulsion (He et al, 2002)
BioSante Pharmaceuticals (Lincolnshire,Illinois)	Calcium phosphate based nanoparticles were produced for improved oral bio- availability of hormones/proteins	Calcium phosphate nanoparticles (Santos et al, 2012)
ElanPharma International (Dublin, Ireland)	Nanoparticles (< 1µ) were produced by wet milling technique using surfactants and stabilizers. The technology was applied successfully in developing of apprepitant and reformulation of siroliums.	Nanocrystal drug particle (Santos et al, 2012)
Eurand Pharmaceuticals (Vandalia, Ohio USA)	Nanocrystal or amorphous drug is produced by breakdown of crystal lattice and stabilized by using biocompatible carriers (swellable microparticles or cyclodextrins)	Cyclodextrin nanoparticle (Lvov et al, 2008)
iMeddInc (Burlingame,CA,USA)	Implantable drug delivery system using silicon membrane with nano pores(10–100 nm)	Stretchable silicon nanomembrane (Costas K et al,2006)
pSivida Ltd (Watertown, MA, USA)	The solubility and bioavailability of hydrophobic drugs was achieved by incor- porating drug particles within the nano-width pores of biocompatible silicon membranes or fibers.	Silicon nanoparticles (Costas K et al,2006)
SkyePharma Plc, (Piccadilly, London, UK)	Nanoparticulate systems of water insoluble drugs were produced by applying high shear or impaction and stabilization was achieved by using phospholipids.	A polymer stabilizing nano reactor with the encapsulated drug core (Gao et al,2008; Bennet et al, 2014)

2.6 Polymeric Nanoparticle

The ideal requirements for designing of nanoparticle delivery system is how they effectively control particle size, surface character, enhanced permeation, flexibility, solubility and release of therapeutically active agents in order to attain the target and specific activity at a predetermined rate and time (Allemann et al., 1993; Kawashima., 2001; Soppimath et al., 2001; Birrenbach G et al., 1976).

Polymeric nanoparticles have been investigated extensively over the last two decades after the first report was published in 1976. These particles as drug carrier or the carrier of tracer molecules have been presented as solid nanoparticles/nanospheres/nanocapsules where the active substance have been incorporated either inside the spheres or have been adsorbed on their surfaces with the help of chemical bonding or both (Allemann et al., 1993; Illum et al., 1987).

Delivering drugs by means of polymeric nanocarriers is considered to assist the shortcomings of the conventional drugs, such as unfavorable pharmacokinetics, poor solubility, instability, high toxicity, drug resistance, low cellular uptake and other side effects. The polymeric carriers have to be chosen so as to have several characteristics that could be identified and summarized by some investigators as under (Davis S S et al., 1985; Miyazaki et al., 1986; Verdun C et al., 1986; Jalil et al., 1990; Marengo et al., 2000):

1. Polymers should be compatible with the body in terms of adaptability and should be biodegradable and biocompatible.
2. Particle should be able to preserve and protect the drug and should not release them till they reach the site of action.
3. Nanoparticles also should not interact or should not have any harmful effects on the body cells or tissues.
4. Drug should be released at rate so as to achieve the desirable therapeutic index.
5. After the release of drugs, nanoparticles/polymers should be degraded or eliminated from the body.

Polymeric nanocarriers are prepared using different polymers such as synthetic polymers; polyalkylcyanoacrylate and polylactides (Farrugia et al., 1999) and natural polymers; chitosan, gelatin, sodium alginate and albumin (Fernández-Urrusuno et al., 1999; Aynie et al., 1998; Luppi et al., 2011; Peer et al., 2007). Polymeric nanoparticles are promising vehicles used for drug delivery to distribute effectively the drugs to the specific target sites (Dong et al., 2004). It has been suggested that by regulating the particle size, different organs in the body system can also be targeted (Muhamad II et al., 2014).

This targeted approach leads to the drug delivery in lower dosages and in a controlled manner, and thus reduces unwanted side effects of drugs (Ghosh PK et al., 2000). The effectiveness of the polymeric nanoparticles is mainly due to their nanometer size that promotes efficient/effective permeation through the cell membranes and stability in the bloodstream. There are three general physico-chemical mechanisms for polymeric drug carriers to deliver the drug to the targeted tissue/cell.

Polymers are used only because they are very convenient materials for countless and varied molecular designs that can be incorporated into unique nanoparticles preparation with many medical applications (Dong et al., 2004). There are some aspects that need to be considered before using the polymers, which are their adaptability (non-toxicity and non-antigenicity), biodegradability, bioadhesivity and biocompatibility (Soppimath et al., 2001).

2.7 Types of polymers in nanodelivery

Two types of polymers can be used in nanodelivery which is natural and synthetic. Natural polymers or biopolymers may be naturally occurring materials which is formed in nature during the life cycles of green plants, animals, bacteria and fungi are polymers or polymer matrix composites. Collagen while synthetic polymers from the ester family.

Table.3 shows examples of natural and synthetic polymers and Table.4 is showing advantages and disadvantages of natural and synthetic polymers (Zaidi et al., 2017).

Table.3: Example of Natural and Synthetic Polymer

Natural	Synthetic
cellulose, starch, chitosan, carrageenan, alginates, xantham gum, gellan gum, pectin	poly(lactic acid) (PLA), poly(cyanoacrylates) (PACA), poly(acrylic acid), poly(anhydrides), poly(amides), poly (ortho esters), poly(ethylene glycol), and poly(vinyl alcohol) (PVA) and other like poly(isobutylcynoacrylate) (PIBCA), poly(ethylene oxide) (PEO), poly(å- caprolac- tone) (PCL).

Table.4: Advantages and Disadvantages of Natural and Synthetic Polymers (Katas et al., 2012)

Natural polymers		Synthetic polymers
• Less toxic • Biocompatibility • Biodegradable • Easily available	**ADVANTAGES**	• Biocompatibility
• High degree of variability in natural materials derived from animal sources • Structurally more complex • Extraction process very complicated and high cost	**DISADVANTAGES**	• Toxicity • Non degradable • Synthetic process is very complicated and high cost

Biodegradable polymeric nanoparticles have attracted prominent interest in the past few decades as a novel drug carrier due to their long half-life and excellent drug entrapment efficiency (Katas et al., 2012). Furthermore, the polymeric nanoparticles because of their nanoscale particle size have embraced the site-specific targeting and tend to permeate deeply into the skin substructures. Moreover, the biodegradable NPs could also protect the drugs used in oral administration (e.g., hydrocortisone) from the harsh environmental conditions and genes from degradation in the biological media. These nanoparticles can also be responsible for greater mucoadhesivity at the site of target tissues (Katas et al., 2012).

Many of the drugs have problems of poor stability, water insolubility, low selectivity, high toxicity, side effects and so on. Clinically useful drug delivery systems need also to deliver a certain amount of a drug that can be therapeutically effective over an extended period of time. Such requirements can be met by the nano-scale drug delivery systems. Chitosan nanoparticles are good drug carriers because of their good biocompatibility, biodegradability, mucoadhesivity and non toxicity and can be readily modified. As a new drug delivery system, they have attracted increasing attention for their wide applications. Chitosan nanoparticles are now being modified for sustained/controlled release and targeting. Although chitosan nanoparticles can be easily modified to carrier, coat and encapsulate hydrophobic drugs, further investigation is required on the biocompatibility of chitosan nanoparticles and its derivatives (Sharare Najafi et al., 2014).

2.8 Chitin

Chitin and chitosan (CS) polymers are natural amino polysaccharides having unique structures, multidimensional properties, highly sophisticated functions and wide ranging applications in biomedical and other industrial areas (Chandy et al., 1990; Paul et al., 2000; Muzzarelli et al., 2005).

Chitin is similar to cellulose both in chemical structure and in biological function as a structural polymer. The crystalline structure of chitin has been shown to be similar to cellulose in the arrangements of inter- and intra chain hydrogen bonding. Chitosan is made by alkaline N-deacetylation of chitin. The term chitosan does not refer to a uniquely defined compound; it merely refers to a family of copolymers with various fractions of acetylated units. It consists of two types of monomers; chitin-monomers and chitosan-monomers. Chitin is a linear

polysaccharide consisting of (1-4)-linked 2-acetamido-2-deoxy-b-D glucopyranose. Chitosan is a linear polysaccharide consisting of (1-4)-linked2-amino-2-deoxy-b-D glucopyranose. Commercial chitin and chitosan consists of both types of monomers. Chitosan is found in nature, to a lesser extent than chitin, in the cell walls of fungi. Chitin is believed to be the second most abundant biomaterial after cellulose. The annual biosynthesis of chitin has been estimated to 109 to 1011 tons. Chitin is widely distributed in nature. Among several sources, the exoskeleton of crustaceans consists of 15 % to 20 % chitin of dry weight. Chitin found in nature is a renewable bioresource (Miyazaki S et al., 1981, Phaechamud et al., 2008).

2.9 Chitosan

Chitosan is a polysaccharide which is derived from naturally occurring chitin (Khor et al., 2003) (Fig 2). Its unique properties make it attractive for industrial as well as biomedical applications (including controlled drug release, wound healing, nutrition supplements, water purification, removal of toxins, scaffolds for tissue engineering, and semi permeable membranes). Due to its pH dependent solubility, it forms stable films on various surfaces under neutral and basic pH ranges. Its amine groups serve as a covalent attachment for biomolecules, and it can be co-deposited with other polymers or nanoparticles (Koev et al., 2010). Composed of 2-amino-2-deoxy-ß-D-glucan combined with glycosidic linkages, chitosan is considered as perfect material for developing micro/nanoparticles (Agnihotri et al., 2004).

Chitosan is a more versatile form of chitin, which is the second most abundant natural polymer on earth after cellulose. An important application of chitosan is the development of drug delivery systems, with a controlled drug release rate and a reduced frequency of administration of the drug (Knapczyk et al., 1984) due to its gel-forming ability in low pH range together with intoxicity (Knapczyk et al., 1993). The hydrogen bonding and ionic interactions are responsible for the bioadhesive nature of chitosan and different substrates (He et al., 1998; Stoica et al., 2013).

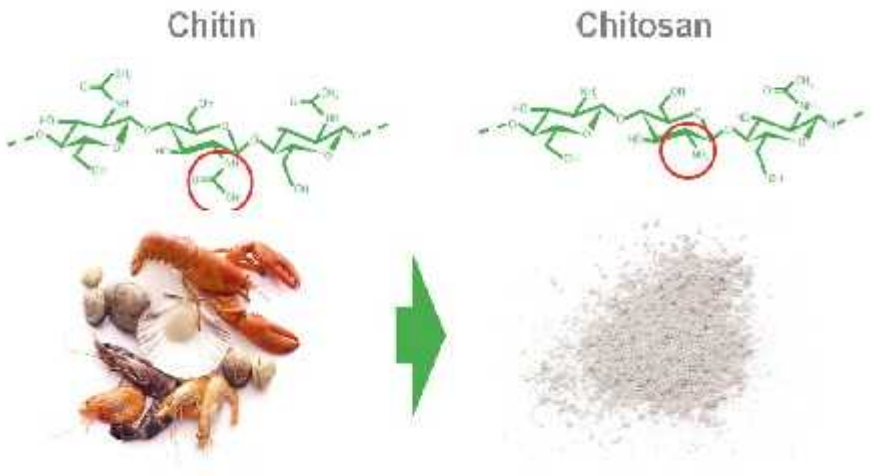


Figure.2: Synthesis of chitosan from chitin through deacetylation (boiling in presence of 40% NaOH) (Zuber et al., 2013).

Chitosan is also found in some microorganisms, yeast and fungus (Illum., 1998). The primary unit present in the chitin polymer is 2-deoxy-2 (acetylamino) glucose. These units combined by ↓-(1, 4) glycosidic linkages, forming a long linear chain polymer. Although chitin is insoluble in most solvents but chitosan is soluble in almost all organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric it is insoluble in phosphoric and sulfuric acid (Roberts et al., 1992; Muzzarelli et al., 1988).

2.10 Solubility of chitosan

Solubility of chitosan in organic acids was limited, but it is greatly soluble in acids like acetic acid, formic acid, and lactic acid. The effect of pH was found to be more predominant in dissolving chitosan. While preparing the solution the pH must be adjusted to below 6, at this pH the protonation of amino groups leads to release of NH ion which is an important prerequisite for dissolving chitosan. 1% acetic acid concentration is the most commonly used for dissolving; also it is soluble in 1% hydrochloric acid. But it is insoluble in inorganic acids like sulphuric acid, and phosphoric acid. As previously mentioned solubility of chitosan solution is mainly affected by pH, and its solution stability also affected by pH, above pH 7 the stability of chitosan solution was found poor. At pH below 4 it forms a stable solution. It forms a precipitation or gelation and poly ion complex which results in formation of gel at higher pH (>7) (Van Toan et al., 2013; Rajalakshmi et al., 2014).

2.11 Chitosan Nanoparticles

2.11.1 Chitosan nanoparticles in drug delivery

Chitosan is a cationic linear copolymer polysaccharide (Fig 3). It is typically obtained by extensive deacetylation of chitin, an abundant polysaccharide found in crustacean's shell.

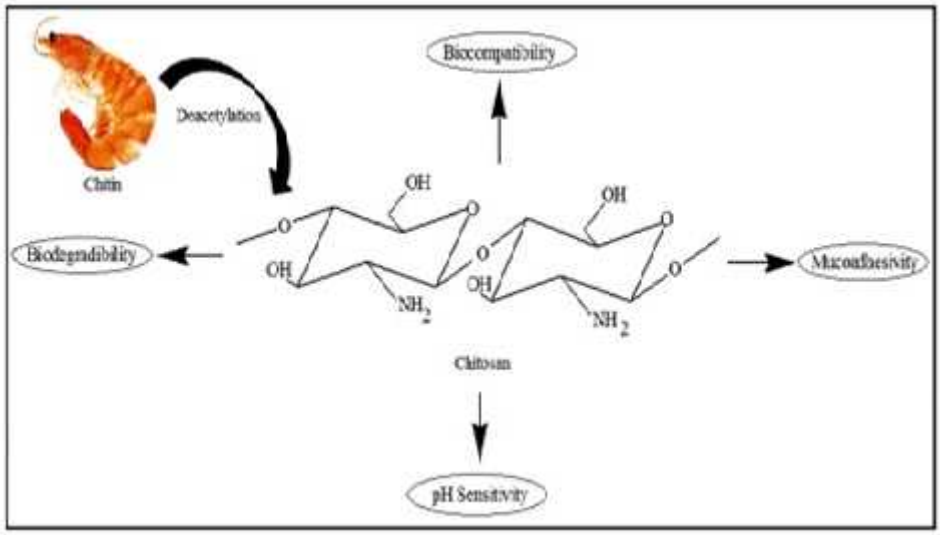


Figure.3: Structure of chitosan along with characteristic properties (Saikia et al., 2015).

Chitosan and chitosan derivatives based systems considered as promising material for the development of safe and effective drug delivery systems owing to their unique physiochemical characteristics. Chitosan also enhances the residence time of the system and consequently the bioavailability of drug because of its mucoadhesivity (Felt et al., 1998).

Chitosan has a cationic character because of its primary amino groups which imparts its lots of properties such as controlled drug release, mucoadhesion, *in-situ* gelation, permeation enhancement and transfection (Park et al., 2010). Modified forms of chitosan are also available such as thiolated chitosan and this exhibits higher mucoadhesivity as compared to unmodified chitosan because thiolated chitosan interacts with the mucus layer of gastrointestinal tract through covalent binding with the cysteine rich glycoprotein and leads to strong attachment with the mucin or mucus and therefore results in sustained delivery.

Other important feature of chitosan as a drug delivery carrier is its metabolic degradation in the body easily. It provides easy elimination process after drug administration, generally by renal clearance; however this applies for chitosan with suitable molecular weight. For large molecular weight chitosan, enzyme degradation is required. The possible site of degradation may be liver and kidney (Nanaki et al., 2012) (Fig 4).

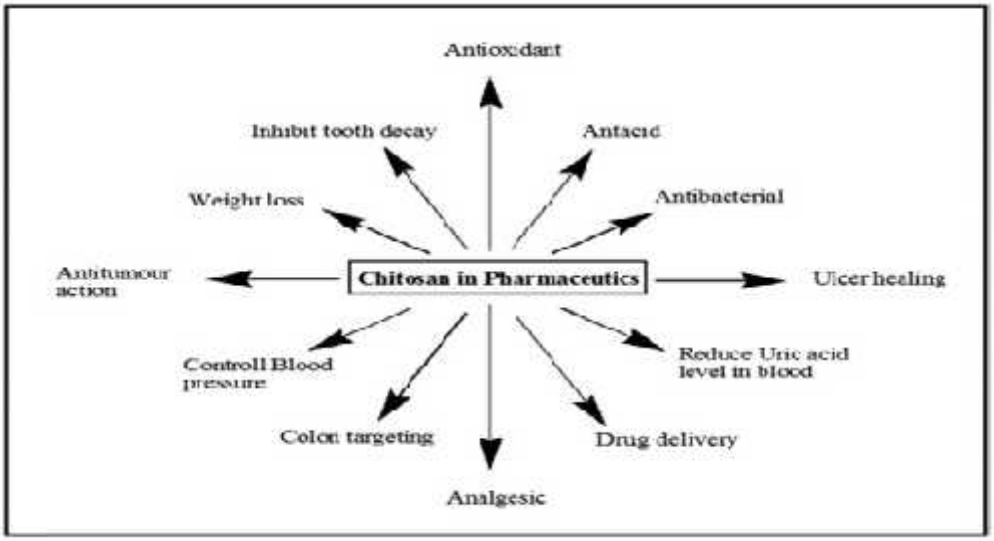


Figure.4: Pharmaceutical properties of chitosan

Other than biomedical applications, chitosan has much other commercial application as shown in figure 5.

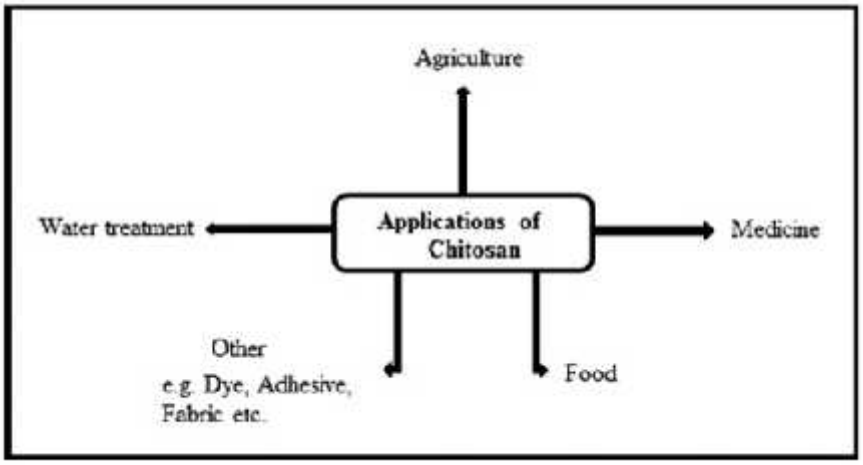


Figure.5: Commercial applications of chitosan

It was found that chitosan can increase photosynthesis process in plants, promoted plant growth, nutrient uptake, germinations and sprouting.

2.12 Controlled Drug Release

Delivery of a pharmaceutically active agent at a right place, at the right concentration for the right period of time is still a challenge. To achieve this objective "drug delivery system" has introduced which are based on association of the active agent with a suitable carrier molecule.

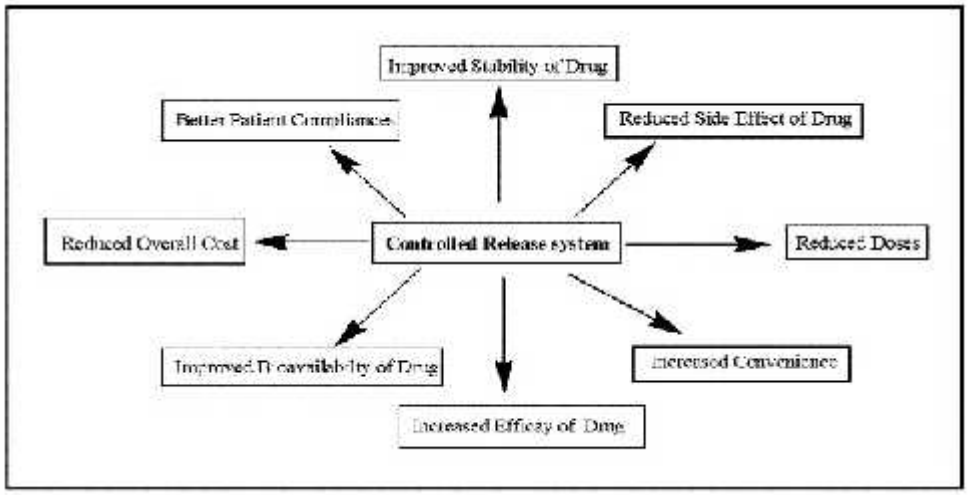


Figure.6: Advantages of Controlled drug delivery systems

Delivery system with "controlled drug release" characteristics has emerged as a promising tool in treatment of various diseases (Smolen et al., 1978) (Fig 6).

Chitosan nanoparticles have been shown to provide sustained release of both hydrophilic and hydrophobic drugs and are prepared by three distinct methods including ionic gelation, precipitation using tripolyphosphate and cross-linking methods using gluteraldehyde. The method used for preparation determines the entrapment efficiency, loading efficiency, and particle size. Particle size of the Chitosan nanoparticles generally depends on molecular weight of chitosan used, concentration of chitosan solution and amount of cross linker. Increasing the concentration of chitosan increases the viscosity of chitosan solution thus making smaller sized particle formation difficult. An additional advantage of this type of system is that they can be produced under aqueous and fairly mild conditions, thus effectively, being especially suitable to preserve the bioactive conformation of delicate macromolecules (e.g. hormones, antigens, DNA,

RNA, growth factors), that otherwise would be prone to enzymatic degradation and hydrolysis. Most frequently chitosan nanoparticles are formed according to a bottom–up approach as a result of a self–assembling or cross-linking processes in which the molecules arrange themselves into ordered nanoscale structures either by physical or covalent inter or intramolecular interactions. In these nanostructures, the drug can be entrapped or attached to the nanoparticles matrix. Chitosan nanoparticles have been prepared by several methodologies, including physical cross-linking by ionic gelation by specific ions such as pentasodium tripolyphosphate (TPP) or EDTA. In particular, chitosan –TPP nanoparticles have been utilized as a drug delivery platform for a wide range of active molecules (Kosta et al., 2012).

The unique character of nanoparticles such as their small size and quantum size effect could make chitosan nanoparticles exhibit superior activities. They are simple and inexpensive to manufacture and scale-up and have unique size and large surface-to-volume ratio. They are mucoadhesive and hydrophilic in nature due to which they provide good protection to the encapsulated drug and increase its clearance time and stability in the body. Thus they are applicable to a broad range of drugs, small molecules, proteins and polynucleotides. The benefits of encapsulating active agents in a polymer matrix are their protection from the surrounding medium or processing conditions and their controlled/sustained release (Agarwal et al., 2015).

2.13 Physical Properties of chitosan nanoparticles

2.13.1. The properties of chitosan are completely dependent on the molecular weight, degree of deacetylation (DDA) and viscosity (Sabarikumar K et al., 2002).

2.13.2. The degree of deacetylation affects the solubility, hydrophobicity and its ability to interact electrostatically with polyanions by affecting the number of protonatable amine groups present in chitosan (Hoppe-Seyler et al., 1894).

2.13.3. It has also been reported that chitosan having a low degree of deacetylation (DDA), which are active as absorption enhancer at both low and high molecular weights, shows a clear dose dependent toxicity (Li et al., 2001). However, chitosan having a higher DDA is active enhancer at high molecular weight, but are less toxic at low molecular weight. As far as toxicity is concerned, it depends on the structural features of the chitosan polymer and not always related

to its absorption enhancing effect. The molecular weight of chitosan also displays fundamental importance. Generally chitosan with low molecular weight and low DDA, exhibits greater solubility with faster degradation rate as compared to its high molecular-weight counterparts (Kwon et al., 2001).

2.13.4. Chitosan has a p*K*a of approximately 6.5 on the amine groups. At pH less than 6, chitosan amines reflect polycationic behavior of chitosan. Therefore, at pH less than 6.5, chitosan is soluble in most organic acidic solutions including formic, acetic, tartaric, and citric acid (Zhang et al, 2001). It is insoluble in phosphoric and sulfuric acid (Ren et al., 2005). The solubility of chitosan in neutral and basic pH can be improved by the process of quaternization to form trimethyl chitosan derivatives (Tiyaboonchai., 2003).

2.13.5. Trimethyl chitosan derivatives are highly soluble at higher pH than unmodified chitosan (Singla et al., 2001). At pH above about 6.5, chitosan's amines are deprontonated and are also reactive and thus can undergo inter polymer linkage leading to fiber and network (*i.e.*, film and gel) formation (Van der Merwe et al., 2004). Despite of tremendous efforts in investigating the suitability of chitosan in drug delivery and the large number of chitosan manufacturers, it is still very difficult to obtain chitosan which is fully standardized with respect to molecular weight and degree of deacetylation for pharmaceutical research purpose (Kommareddy et al., 2005).

2.14 Biological properties of chitosan nanoparticles

Chitosan has properties such as hydrophilicity, biocompatibility, biodegradability, antimicrobial, bioadhesivity, adsorption applications, and non- toxicity. The biocompatibility of chitosan is generally regarded as the ability of the newly developed material to interact with living cells, tissues, or organs by not being toxic or injurious and not triggering immunological reactions or rejections while functioning appropriately in vitro and in vivo conditions. According to the features mentioned above, besides the chitosan being used for drug delivery, it is also used in tissue engineering, gene delivery, nasal drug and vaccine delivery. The formulation of chitosan with a drug may alter the pharmacokinetic and biodistribution profiles, and for pharmaceutical applications it is necessary to take in account the route of administration, its concentration, contact time and cell types that are in contact with chitosan or chitosan complexes (Nishanth Kumar et al., 2015).

2.15. Preparation methods of chitosan nanoparticles

2.15.1 Emulsion solvent diffusion method

This technique is based on partial miscibility of an organic solvent into water. The chitosan nanoparticle was prepared by o/w emulsion by injecting an organic phase into chitosan solution containing a stabilizing agent (*i.e.* poloxamer). Then, under magnetic stirring and high pressure homogenization, the emulsion was diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation then leads to the formation of nanoparticles (Lee et al., 2005).

2.15.2 Microemulsion method

Chitosan nanoparticle can also be prepared by using surfactant in *n*-hexane; chitosan solution (dissolved in acetic acid) and glutraldehyde added under continuous stirring at room temperature. The resulting nanoparticles were stirred overnight. Then the organic solvent removed by evaporation under low pressure and the excess of surfactant removed through precipitation by $CaCl_2$ followed by centrifugation, dialysis and lyophillization (Ren et al., 2005; Nishanth Kumar et al., 2015).

2.15.3 Ionotropic Gelation Method

Chitosan nanoparticles prepared by ionotropic gelation technique were first reported by Calvo and have been widely examined and developed by Janes [Figure 10]. This method is completely based on electrostatic interaction between amine groups of chitosan and negatively charged polyanion such as tripolyphosphate (TPP). The chitosan was dissolved in acetic acid (presence / absence of stabiliser) followed by the addition of polyanion or anionic polymer under magnetic stirring at room temperature (Yi et al., 2005). Despite of its superiority as a biomaterial, chitosan is not fully soluble in water but in acidic solution. Aqueous solubility of chitosan only in acidic solution is mainly because of its rigid crystalline structure and the degree of deacetylation which limits its application to bioactive agents such as gene delivery carriers, peptide carriers, and drug carriers. Water-soluble chitosan is easily soluble in neutral aqueous solution. Its advantages are ease of modification, useful as gene, peptide and lipophilic drug carrier. Chitosan nanoparticles are good drug carriers because of their good biocompatibility and biodegradability, and can be readily modified. As a new drug delivery system, they have

attracted increasing attention for their wide applications, for example, loading of lipophilic drugs, protein drugs, gene drugs, anticancer drugs via various routes of administration including oral, nasal, intravenous, and ocular.

2.15.4 Polyelectrolyte Complex (PEC)

This is the simple and mild method for the preparation of nanoparticles since no harsh conditions are applied. Polyelectrolyte complexes are formed by self-assembly of the cationic charged polymer as well as alginate. This method involved in the formation of complex involves the charge neutralization between cationic polymer as well as alginate due to the polyelectrolyte component self assembly leading to a fall in hydrophobicity. Several cationic polymers (i.e. gelatin, polyethyleneimine) also possess this property. The nanoparticles were spontaneously formed after addition of alginate solution into chitosan which was priorly dissolved in acetic acid solution, under mechanical stirring at room temperature. The complexes size range from 50 nm to 700 nm (Erbacher et al., 1998).

2.15.5 Reverse Micellar Technique

Preparation of ultrafine polymeric nanoparticles with narrow size distribution can be obtained using reverse micellar medium (Leong et al., 1982). Aqueous core of the reverse micellar droplets can be used as a nanoreactor to prepare such particles, since the size of the reverse micellar droplets usually lies between 1 and 10 nm and are highly monodispersed (Maitra., 1984). In this method, the surfactant is dissolved in an organic solvent in order to prepare reverse micelles. To this, aqueous solutions of chitosan and drug are added with constant vortexing to avoid any turbidity. Additional amount of water may be added to obtain nanoparticles of larger size. To this transparent solution, a cross-linking agent is added and cross-linking is achieved after stirring overnight. The material is dispersed in water and then adding a suitable salt, precipitates the surfactant out and the mixture is subjected to centrifugation. The supernatant solution is decanted which contains the drug loaded nanoparticles. The aqueous dispersion is immediately dialyzed using dialysis membrane for about 1 h and the obtained liquid is lyophilized to get dry powder [Figure 11].

2.15.6 Emulsion Droplet Coalescence Method

A nanoparticle formed under this technique involves/utilizes the mechanism of both emulsion cross-linking and precipitation methods. However, in this method, instead of cross linking the stable droplets, precipitation is induced by allowing coalescence of chitosan droplets with sodium hydroxide droplets. First, a stable emulsion containing chitosan solution along with drug is produced n liquid paraffin oil and then, another stable emulsion containing chitosan aqueous solution made of sodium hydroxide is produced in the same manner. When both emulsions are mixed under high speed stirring, droplets of each emulsion would collide at random and coalesce, thereby precipitating chitosan droplets to give small size particles of chitosan nanoparticles [Figure 9] (Tokumitsu et al., 1999).

2.15.7. Spray drying

Spray-drying method can be used as a one-step preparation method of nanoparticle powder. Mannitol microspheres containing chisosan nanoparticles-loaded protein was prepared by this technique (Grenha et al., 2007). Chitosan iron oxide nanoparticles with various chitosan: Iron oxide ratios by spray-drying (Huang et al., 2010). Atomic absorption spectrometry results implied that chitosan had a strong chelation with iron metal. Meanwhile, Fe_3O_4 was crystallized and distributed in the chitosan matrix. These chitosan iron oxide nanoparticles were stable in water with the property of strong super paramagnetism [Figure8].

2.15.8. Emulsification and cross-linking

In this method, chitosan nanoparticles were prepared by addition of cross linking agents to the w/o emulsion to hardened the particles. The reactive amino groups of chitosan undergo a covalent cross-linking with the aldehyde groups present in glutaraldehyde, which is added after the emulsion formation and, consequently, after nanoparticle production. The final particle size was demonstrated to be highly dependent on stirring speed, as well as on the extent of cross-linking. It has many disadvantages like tedious procedures and also the application of harsh cross-linking agents (Ohya et al., 1994; Janes et al., 2001).

2.15.9. Sieving method

In this method nanoparticles are prepared by cross-linking chitosan to obtain a non-sticky glassy hydrogel followed by passing through a sieve [Figure12]. A suitable quantity of CS is dissolved in 4% acetic acid solution to form a thick jelly mass that is cross-linked by adding glutaraldehyde. The non-sticky cross-linked mass is passed through a sieve with a suitable mesh size to get nanoparticles. Nanoparticles are washed with 0.1 N NaOH solutions to remove the nonreacted excess glutaraldehyde and dried overnight in an oven at 40°C (Agnihotri et al., 2004).

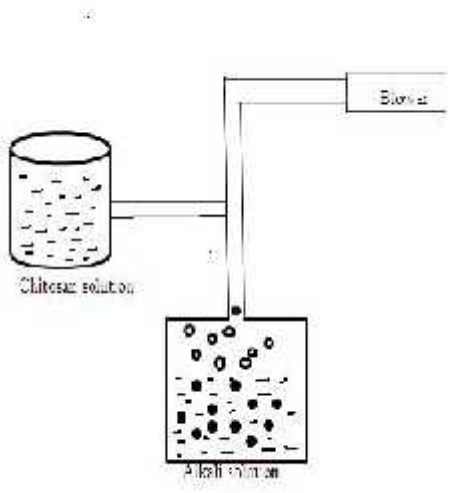


Figure.7: Coacervation phase separation method

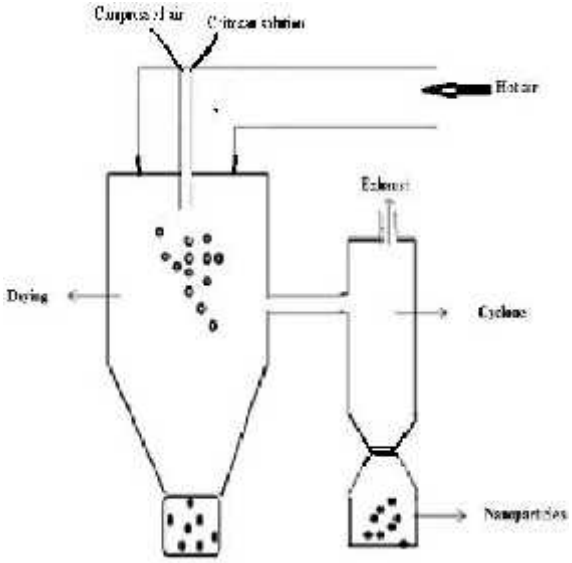


Figure.8: Spray drying method

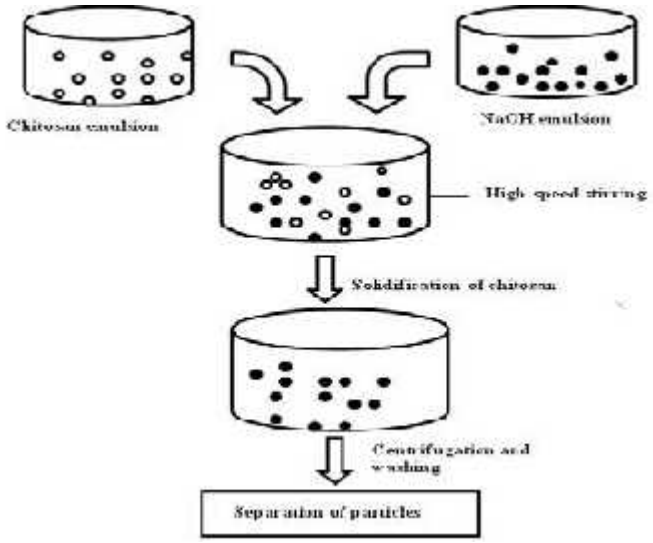


Figure.9: Emulsion-droplet coalescence method

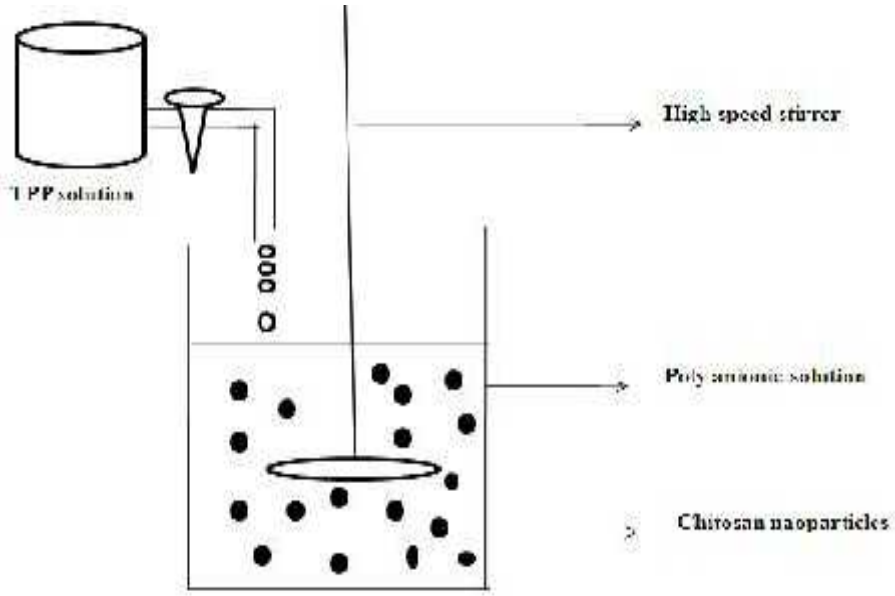


Figure.10: Iontropic gelation method

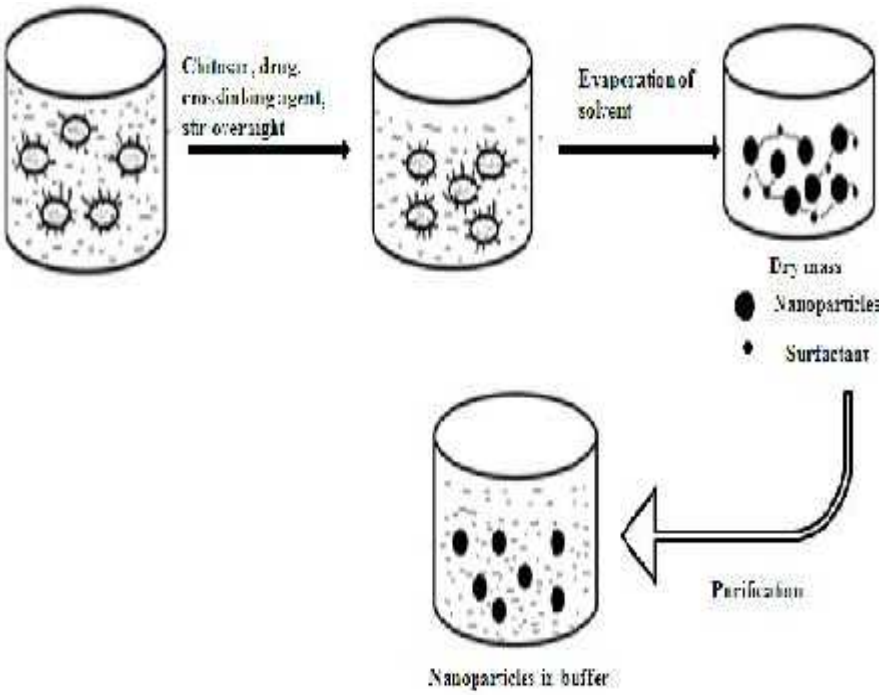


Figure.11: Reverse Micellar method

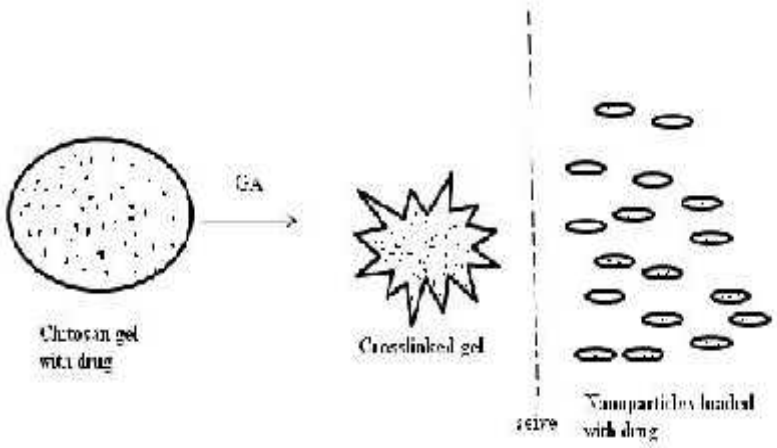


Figure.12: Sieving method

2.16. Poorly soluble oral drugs

2.16.1. Amphotericin B

Amphotericin B (AmB) is a macrocyclic polyene antibiotic. A deoxycholate-soluble salt of AmB (Fungizone) is marketed for use as an intravenous infusion (IV) formulation and has been the gold standard drug treatment for systemic fungal infections since 1953 (J.D. Dutcher, 1986). This parenteral formulation is usually associated with several adverse effects (AE) including fever, chilling, vomiting, headache and nausea during administration (in 80% of the patients) and nephrotoxicity (in 30% of the patients) (Torrado et al., 2008; Van de Ven et al., 2012).

AmB possesses both hydrophobic (polyene hydrocarbon chain) and hydrophilic (polyhydroxyl chain) domains (Lemke et al., 2005). This amphoteric nature is responsible for its poor solubility in both aqueous and organic solvents. It is classified (Biopharmaceutical Classification System - BCS) as a class IV compound (Ménez et al., 2007) with limited solubility and permeability properties due to its high molecular weight of 924 Daltons, leading to a low bioavailability if given orally 0.3% (Ouellette et al., 2004).

2.16.2. Ketoconazole

Ketoconazole is a broad spectrum imidazole antifungal agent marketed as creams and tablets. It interacts with 14-demethylase, a cytochrome P-450 enzyme and inhibits ergosterol synthesis and increased fungal cellular permeability and is used against a wide variety of fungi and yeasts. It is readily but incompletely absorbed after oral dosing and is highly variable. Ketoconazole belongs to BCS class II i.e. poorly soluble and highly permeable drug. Due to poor solubility, it is incompletely absorbed after oral dosing and bioavailability varies among individuals. To overcome these shortcomings novel drug delivery system (NDDS) plays a crucial role (Ravi Shankar et al., 2015; Lyman et al., 1992; Como et al., 1994).

2.16.3. Ciprofloxacin

Ciprofloxacin was developed by Bayer in 1981, is the first oral antimicrobial drug with broad spectrum activity for treating severe infections caused by both Gram-negative and Gram-positive bacteria, including Pseudomonas spp. and Staphylococcus spp. (Sherwood et al., 1997). It is a synthetic chemotherapeutic antibacterial of the floroquinolone drug class. It selectively inhibits bacterial DNA synthesis by acting on enzyme DNA gyrase, one of the groups of topoisomerases, which inserts negative supercoils in DNA. Ciprofloxacin is practically insoluble in water, very slightly soluble in ethanol and in methylene chloride (British Pharmacopia., 2009). The bioavailability of ciprofloxacin may be enhanced by formulating them in suitable delivery vehicle.

2.16.4. Vancomycin (VCM)

Vancomycin (VCM) is a glycopeptides antibiotic that is used for the treatment of infections caused by methicillin-resistant staphylococci (S. Arjun et al., 2002; Levine., 2006). It acts by inhibiting bacterial cell wall synthesis at an earlier stage when compared to other beta-lactam antibiotic. It is usually given IV due to its minimal oral absorption (Hachicha et al., 2006). It is a water soluble high molecular weight compound and hence poorly absorbable from the gastrointestinal tract (Okochi et al., 2000). The physicochemical properties that have been associated with poor membrane permeability of highly polar and macromolecular drugs are low octanol/aqueous partitioning, the presence of strongly charged functional groups, high molecular weight, a substantial number of hydrogen-bonding functional groups and high polar surface area

(Gavini et al., 2004; Mahboubian et al., 2010) . There are few reports showing the encapsulation of VCM in liposomes and microspheres / nanoparticles may show a better bioavailability than the free drug but they are found to be less stable formulations (Lankalapalli et al., 2014).

2.16.5. Chloramphenicol

Chloramphenicol is a well-known broad-spectrum bacteriostatic antibiotic that has been used since 1949, but due to its hydrophobicity, poor penetration in skin, fast degradation, and toxicity, its application has been hindered. Furthermore, it has been demonstrated that old antibiotics such as chloramphenicol remained active against a large number of currently prevalent resistant bacteria isolates due to their low-level use in the past. Recently, the novel nanoparticulate drug-delivery system has been used and reported to be exceptionally useful for topical therapeutics, due to its distinctive physical characteristics such as a high surface-to-volume ratio and minuscule size. It helps to achieve better hydrophilicity, bioavailability, and controlled delivery with enhanced therapeutic index, which has resulted in decreased toxicity levels compared to the crude drug (Kalita et al., 2015).

Solubility is a significant physicochemical factor that affects absorption of drug and its therapeutic effectiveness. Formulation development would lead to be failure if drug having poor aqueous solubility. The low dissolution rate and low solubility of drug substances in aqueous G.I.T fluid frequently leads to inadequate bioavailability. The venture to improve the solubility and dissolution of hydrophobic drugs remain one of the trickiest tasks in drug development. Therefore formulation of mentioned poorly soluble drugs loaded with chitosan nanoparticles could result in enhanced bioavailability (Leuner et al., 2000; Ford et al., 1986).

2.17. Drug release kinetic modeling

There are number of kinetic models, which described the overall release of drug from the dosage forms. Because qualitative and quantitative changes in a formulation may alter drug release and in vivo performance, developing tools that facilitate product development by reducing the necessity of bio-studies is always desirable. In this regard, the use of in vitro drug dissolution data to predict in vivo bio-performance can be considered as the rational development of controlled release formulations (Ozturk S.S et al., 1988; Dressman J.B et al., 1986; Dressman J.B et al., 1984).

2.17.1. Model dependent methods

Model dependent methods are based on different mathematical functions, which describe the dissolution profile. Once a suitable function has been selected, the dissolution profiles are evaluated depending on the derived model parameters. In order to determine the suitable drug release kinetic model describing the dissolution profile, the nonlinear regression module of Statistica 5.0 was used. In non-linear regression analysis the Quasi-Newton and Simplex methods minimized the least squares. The model dependent approaches included zero order, first order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas (Costa P et al., 2001; Shah et al., 1997; Crank et al., 1975; Arhewoh M.I et al., 2004).

2.17.2 Zero order kinetic model

Zero order describes the system where the release rate of drug is independent of its concentration. The equation is….

$$C = C_0 - K_0 t$$

Where, C = Amount of drug release or dissolved (assuming that release occur rapidly after the drug dissolved.) C_0 = Initial amount of drug in solution (it is usually zero) K_0= Zero order rate constant t = time for study of release kinetics, the graph plotted between cumulative amount of drug released verses time.

This relationship can be applied to describe the drug dissolution of drug from several types of modified release Pharmaceutical dosage form as in the case of some transdermal system as well as matrix tablet with low soluble drugs in coated forms. This model is important in certain classes of medicines intended, example for antibiotic delivery, heart and blood pressure

maintenance, pain control and antidepressant (Gautam Singhavi., 2011; Lokhandwala et al., 2013).

2.17.3. First order kinetic model

This model is used to describe the absorption and elimination of some drugs, although it is difficult to understand the mechanism on the theoretical basis. The drug release which follows the first order kinetic can be expressed by the equation:

$$\text{Log } C = \text{Log } C_0 - Kt/2.303$$

Where, C_0=Initial concentration of drug K=First order constant t=time The data obtained are plotted as log cumulative percentage drug remaining verses time, which yield a straight line with slop=K/2.303. This relationship can be use to describe the drug dissolved in Pharmaceutical dosage forms like those contained water soluble drugs in porous material (Costa et al., 2001; Ramteke et al., 2014).

2.17.4. Higuchi Model

Higuchi published the probably most famous and most often used mathematical equation to describe drug release from matrix system. This model is often applicable to the different geometrics and porous system.

The extended model is based on the following hypothesis.

- Initial concentration of drug in the matrix is much higher than the drug solubility.
- Diffusion of drug occurs only in one dimension (edge effect negligible).
- Drug particles much smaller than system thickness.
- Swelling of matrix and dissolution is negligible.
- Drug diffusivity constant.

  The basic equation of Higuchi model is…..

$$C = [D\,(2qt - Cs)\,Cst]^{1/2}$$

Where, C=total amount of drug release per unit area of the matrix [mg/cm^2] D=diffusion coefficient for the drug in the matrix [cm^2/hr] qt=total amount of drug in a unit volume of matrix

[mg/cm^3] Cs=dimensional solubility of drug in the polymer matrix [mg/cm3] t=time [hr] Data obtained were plotted as cumulative percentage of drug release verses square root of time.

By using this model dissolution of drug from several modified release dosage forms like some transdermal system and matrix tablet with water soluble drugs are studied (Higuchi., 1963; Siepmann et al., 2001; Grassi et al., 2005; Shoaib H.M et al., 2006).

2.17.5. Korsemeyer-Peppas Model

Korsemeyer et al (1983) derived a simple relationship which describes the release of drug from a polymeric system. To illustrate the mechanism of drug release, first 60 % of drug release data was fitted in Korsemeyer-Peppas model.

$$C_t/C\infty = kt^n$$

Where, $C_t/C\infty$=fraction of drug release at time "t". k=rate constant n = release exponent A modified form of this equation was developed to adjust the lag time (l) in the beginning of release of drug from the Pharmaceutical dosage form.

$$C_{(t-1)}/C\infty = a(t-l)^n$$

Where there is possibility of a burst effect, "b" this equation becomes.

$$C_t/C\infty = at^n + b$$

In the absence of lag time or burst effect l and b values would be zero and only at is used. This mathematical model also known as the power law has been used very frequently to describe the drug release from several different pharmaceutical modified release dosage forms.

There are several simultaneous processes considered in this model.

- Diffusion of water into the tablet.
- Swelling of tablet as water enters.
- Formation of gel.
- Diffusion of drug and filler out of the tablet.
- Dissolution of the polymer matrix.

Following assumptions were made in this model.

- The generic equation is applicable to small values of‘t’ or short terms and the portion of release curve, where Ct/C∞<0.6 should only use to determine the exponent n.
- Drug release in a one dimensional way.
- The ratio of system length to thickness should be at least.
- Plot made by log cumulative percentage drug release verses log time.

This model describes the drug release from several modified release dosage forms (Korsmeyer R.W et al., 1983; Riger P.L et al., 1987; Siepmann J et al., 2001).

2.17.6. Hixon-Crowell model

The model describes the release of dose from system, where there is change in surface area and diameter of particle or tablet. It is possible to derive an equation for a drug powder containing uniform size particles which expresses the rate of dissolution based on the cube root of the particles. The equation is as follows.

$$C_0^{1/3} - C_t^{1/3} = K_{HC}t$$

Where, C_t = amount of drug released in time t. C_0 = initial amount of drug in the tablet. K_{HC} = rate constant for Hixson-Crowell equation. When this model is used, it is considered the release rate is limited by the drug particles dissolution rate and not by the diffusion that might occur through the polymeric matrix. This model is used to describe the release profile keeping in mind the surface of the drug particles is diminishes during the dissolution.

Plot made in between cube root of drug percentage remaining in matrix verses time.

This expression is applied to Pharmaceutical dosage form such as tablet; where the dissolution occurs in planes which is parallel to drug surface if dimensions of the tablet diminish proportionality, in such a manner that the initial geometry form keep constant all the time (Hixson A.W et al., 1931; Chen S et al., 2007).