Chapter 1: Introduction

1. INTRODUCTION

1.1 Nanoparticles Drug Delivery Systems

Nanoparticles (NP) are a type of colloidal drug delivery system comprising particles with a size range from 10 to 1000 nm and are used to deliver the drugs or biomolecules. The word ‘nano’ is derived from Latin word, which means dwarf. Nano size refers to one thousand millionth of a particular unit thus nanometer is one thousand millionth of a metre (i.e. 1 nm = $10^{-9}$ m). (Jain et al., 2007) Generally, nanometric carriers also comprise sub-micron particles with size below 1000 nm. (Liu et al., 2008) They are drug carriers of natural, semisynthetic, and synthetic polymeric nature in the nanometer size range. Nanoparticles may or may not be are a collective name for nanospheres and nanocapsules as illustrated in Fig. 1. Nanospheres possess a matrix type structure, where drugs or biomolecules are absorbed or dissolved or entrapped within the polymeric carrier. Nanocapsules are vesicular system in which the drug or bioactive molecule forms a core, which is surrounded by a polymeric membrane. In this case, the active substances are usually dissolved in the inner core but may also be absorbed at their surface. (Reis et al., 2006) Conventional preparations like solution, suspension or emulsion suffer from certain limitations like higher dose requirements due to low bioavailability, often associated with first pass effect, intolerance and instability. Moreover, they are associated with fluctuations in plasma drug levels and do not provide sustained effect. Therefore, some novel carriers are the need of the hour, which meet the ideal requirements of drug delivery system. Nanoparticles may or may not be associated with other nanosize-related properties that differ significantly from those observed in fine particles or bulk materials. (Mudshinge et al., 2011)

![Fig1. Schematic representation of polymeric nanoparticles](Reis et al., 2006)
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The ideal requirements for designing nanoparticulate delivery system are the effective control particle size, surface properties, enhances solubility and release of pharmacologically active ingredients in order to achieve the site-specific delivery at predetermined rate. Although, liposomes have also been used as potential carriers with unique advantages including biocompatibility, targeting to site of action and reduction in toxicity or side effects. Their applications are limited due to inherent problems like low encapsulation efficiency, rapid leakage of drug and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. Polymeric nanoparticles offer stability of drugs/proteins and possess useful controlled release properties (Mohanraj et al., 2006).

Various methods had been utilized for preparing nanoparticles depending on the physicochemical properties of the polymer and the drug. Most of the methods involve the use of organic solvents, heat, sonication or vigorous agitation which may affect the stability of active pharmaceutical ingredients. Nanoparticles can also be formulated by utilizing electrostatic interaction between charged species. These polyelectrolyte complexes can be formulated by avoiding such stresses associated with other methods of preparation, therefore minimizing possible damage to drug during formulation. A variety of biodegradable polymers with diverse physicochemical properties are available to formulate nanoparticles. These polymers are either natural or synthetic. Natural polymeric carriers used for orally delivered nanoparticles include chitosan, dextran, gelatine, alginate, agar among which chitosan is the most popular one.

Chitosan is biocompatible, non-toxic and mucoadhesive derivative obtained from natural source. It is a widely available modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin obtained from shells of crustaceans like crabs, prawns etc. The physicochemical properties of chitosan are significantly influenced by its molecular weight and degree of deacetylation. The presence of reactive functional groups in chitosan provides great opportunity for chemical modification, which allows formulation of a wide range of derivatives possessing unique properties. Chitosan is soluble at lower pH although it has limited solubility at pH above 6.5. Derivatives of chitosan, synthesized by introducing alkyl groups to amine groups, e.g. quaternized derivatives of chitosan, are
permanently positively charged and possess better aqueous solubility. Chitosan is able to increase intestinal permeability by opening tight junctions. Chitosan can form polyelectrolyte complexes of approximately 200 to 400 nm. Overall, it is evident that Chitosan and its derivatives are useful carriers, owing to their biocompatible and biodegradable nature in addition to other physicochemical properties. Currently, dietary supplements of chitosan are tested in clinical trials to lower blood cholesterol but no clinical trials with chitosan nanoparticles are ongoing. Other polysaccharides like dextran, gelatine and alginate are also being investigated for medical applications due to their biocompatibility. (Ochekpe et al., 2009, Allemann et al., 1993)

Nanoparticles, prepared from polymers or macromolecules, are defined as particulate dispersions or solid particles with a size in the range of 10–1000 nm. They consist of macromolecular materials and can be used therapeutically as drug carriers, in which the active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated, or to which the active principle is adsorbed or attached. Depending upon the method of preparation, nanospheres or nanocapsules can be obtained. (Plapied et al., 2011) Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres consist of a dense polymeric matrix in which the drug is physically and uniformly dispersed. Polymeric nanoparticles present high stability when in contact with biological fluids and their nature allows controlled drug release. Nanoparticles have been proposed for a number of applications including biological markers, imaging, healthcare products, pharmaceuticals, drug-delivery systems as well as in detection, diagnosis and treatment of various types of diseases. They represent drug delivery systems suitable for most of the administration routes, even if a rapid recognition by the immune system limits their use as injectable carriers. Similarly, polymeric nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in drug targeting to particular organs/tissues, as carriers of DNA in gene therapy, and in their ability to deliver lipophilic drugs, proteins, peptides and genes through a per oral/mucosal route of administration(Irache et al., 2011)
Fig. 2 Types of biodegradable nanoparticles: nanocapsule, and nanosphere, where drug molecules are either entrapped inside the polymer matrix or adsorbed on the surface. (Kumari et al., 2010)

1.2 Rheumatoid arthritis
Rheumatoid arthritis (RA) is a chronic inflammatory disorder involving multiple joints in a symmetric pattern (Wheley et al., 2010). It is characterized by an inflammatory process that targets the synovium, ultimately leading to destruction of the articular cartilage, peri-articular bone erosion, and eventual alteration of joint integrity and function (Hughes et al., 2005). In addition to joint, systemic and extra-articular inflammation is also observed in many patients (Kreuter et al., 2007). The prevalence of RA in the general population has been estimated to be 0.8 % with a 2 to 3 fold higher incidence in women than in men. Although it can occur at any age, it is commonly diagnosed in the middle-aged population (40–60 years of age). In RA patients, the disease-associated disability often occurs early, with approximately 35% of adults reporting premature work cessation within 10 years of their diagnosis (Labhasetwar et al., 1997). Furthermore, studies have shown that there is a reduced life expectancy in patients with RA compared to the general population (Humphrey et al., 1997). Despite a dramatic increase in the lifespan in the general population, the lifespan of RA patients remains unchanged and research should be focussed in this area as there seems to be no respite from the sufferings associated with this disease (Kreuter et al., 1991). The most commonly used medications for treatment and management of the disease include: nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs) and disease-modifying antirheumatic drugs (DMARDs) (Oppenheim et al., 1981). The modern tools of molecular and cell biology have identified
many promising molecules and molecular pathways that can be targeted for immunologic and inflammatory disease intervention (Frohlich et al., 2012). These efforts have resulted in the development of many biological DMARDs (Yau et al., 2012). There is a current emphasis on the early diagnosis and treatment of RA with DMARDs and when combined with other medications, DMARDs have been shown to be effective in controlling the disease progression in many patients. (Costantino et al., 2011) Despite the success of the currently approved DMARDs, significant numbers of patients continue to experience joint inflammation and progressive deterioration in joint structure and function (Canal et al., 2011). This has led to an increasing interest in identifying new therapeutic approaches (Wang et al., 2011). As RA has no known cure, therapy is more focused on reducing symptoms and relieving pain. Anti-inflammatory drugs as glucocorticoids have been widely used but are associated to some serious systemic side effects. (Patel et al., 2012) Glucocorticoids can be delivered locally by an intra-articular way and rapidly disappear from the articular cavity. (Danhier et al., 2012) Therefore, different drug delivery systems have been developed in order to achieve sustained release of anti-inflammatory drugs. (Ensign et al., 2012) Furthermore, it has been reported that non-biocompatible particles may induce an undesirable effect called “crystal-induced pain”. (Fuentes et al., 2012) Because they are biodegradable, biocompatible and nano-sized, Chitosan nanocarriers might be well suited for developing an drug delivery system for nonsteroidal anti-inflammatory drugs. Chitosan nanoparticles directly injected inside the rat joint cavity were preferentially phagocytosed by macrophages. (Higaki et al., 2009)

1.3 Types of Nanoparticles

1.3.1 Polymeric Nanoparticles

Polymeric nanoparticles (PNPs) are prepared from a synthetic or semisynthetic polymeric block to increase the circulation half-life and to reduce phagocytic uptake and inactivation of the therapeutic moiety and can be used to deliver and target therapeutic agents. (Chen et al., 1998) They are formulated by incorporating biodegradable polymers in order to maximize tissue compatibility and minimize cytotoxicity. A number of polymers are approved by the U.S. Food and Drug Administration (FDA) for administration in human beings viz. polylactic acid (PLA), poly(glycolic acid) (PGA), PLGA, poly-ecaprolactone, and poly(methyl methacrylate). PLA and PLGA can easily be hydrolyzed into individual
monomers (lactic acid or glycolic acid), which are removed from the body via normal metabolic pathways. Methods of preparation of PNPs may be categorized two major classes: one deals with the polymerization of monomers (e.g., emulsion and dispersion polymerization), whereas the other essentially involves dispersion of polymers (e.g., salting out, emulsification-diffusion, and nanoprecipitation). It had been reported that higher entrapment efficiency in PNPs can be achieved by incorporation of drug during their preparation rather than adsorption on preformed nanoparticles. (Wong et al., 2010) Drug release takes place in polymeric nanoparticles through their simultaneous biodegradation, followed by desorption, diffusion, or erosion.

1.3.2 Solid Lipid Nanoparticles
Solid lipid nanoparticles (SLNs) are comparatively stable colloidal carrier system in which molten lipid is dispersed in an aqueous media containing surfactant by high-pressure homogenization or microemulsification. (Blasi et al., 2007) They are generally made up of a solid hydrophobic core containing the drug dissolved or dispersed. SLNs exhibit certain potential advantages over PNPs. They can have better brain uptake and exhibit the least toxicity due to the biodegradable nature of the carrier lipid. Smaller size (around 10 to 200 nm) and narrow size range (100 to 200 nm) allows them to cross tight endothelial cells of the blood brain barrier (BBB), thus escaping from the reticuloendothelial system (RES), and bypass the liver. (Mehnert et al., 2001) They have comparatively higher drug entrapment efficiency, render the drug more stable in their lipid matrix and provide sustained release of drug lasting up to several weeks. Their production can be scaled up with excellent reproducibility and therefore they can be easily transferred from labs to actual large scale production. (Muller et al., 2000)

1.3.3 Magnetic nanoparticles
Magnetically targeted nanoparticulate drug delivery systems involve binding of drug with magnetic nanoparticles (MNPs), such as oxidized iron (Fe) or magnetite. By virtue of their controllable sizes (ranging from 10 to 100 nm) and capacity of delivering the drug or biomolecules in the vicinity of a target site, they hold a lot of potential for targeted drug delivery especially in treatment of cancer as well as in diagnostics. For biomedical applications, magnetic carriers must be water based, biocompatible, nontoxic, and nonimmunogenic. (Underwood et al., 2012) Various magnetic carriers, which receive
external magnetic field, include nickel, cobalt, iron, and magnetite. Iron oxide is most commonly used due to its biodegradable nature, biocompatibility, super paramagnetic effects, and capacity to serve as a contrast agent in magnetic resonance imaging (MRI). Iron oxide particles are phagocytosed or endocytosed by the Kupffer cell in the RES of liver, spleen, lymph, and bone marrow. Once compartmentalized within the lysosomes of RES cells, they are broken down into ferritin and/or hemosiderin, which are antiferromagnetic forms of iron. (Kumar et al., 2011) The concentration of carriers at any specific location can be manipulated by calculation of capillary flow rate, vascular permeability, and hydrodynamic condition prevalent at the target site as well as pathophysiology of the individual. For therapeutic effect, MNPs are injected into the bloodstream and a high gradient magnetic field is generated outside the body so as to pull them out of biological stream and deliver the drug to a localized disease site. (Sun et al., 2008)

1.3.4 Metal and Inorganic Nanoparticles
Nanoparticles have also been prepared using various metals, such as gold (Au), copper (Cu), and silver (Ag) and inorganic carriers, like silica or alumina, among which gold nanoparticles are widely being invesigated due to their excellent optical and photoelectric properties. Moreover, gold nanoparticles exhibits some specific advantages, like inertness and nontoxicity, higher stability, ease of preparation, and possibility of bioconjugation and biomodification with thiol, disulfide, and amine functional groups. Its dispersion stability can be enhanced by conjugation with thiolated PEG. Gold nanoparticles are highly effective contrast agents in cancer diagnosis and photodermal cancer therapy. Further, they serve as a good vector for oligonucleotide, thiol-conjugated small interfering RNA (Si-RNA), insulin, and gene delivery. (Rica et al., 2012)

1.3.5 Quantum dots
Quantum dots (QDs) are colloidal semiconductor nanocrystals (up to 2 to 10 nm), composed of atoms from groups II–VI or III–V of the periodic table, having unique optical and fluorescent properties (Fig. 3). The most commonly used materials for preparation of quantum dots are cadmium selenide (CdSe), cadmium telluride (CdTe), and indium arsenide (InAs). Upon their interaction with photon, they get excited and emit energy in UV, visible, or near-infrared (IR) regions, which can be detected. Owing to their small size,
they can be used for the tagging of biological macromolecules, such as nucleoside and proteins. (Mishra et al., 2010)

**Fig.3 Fluorescent quantum dots** (Underwood et al., 2012)

### 1.3.6 Polymeric Micelles

Polymeric micelles (PMs) are nanosized core-shell structures formed by spontaneous self-assembly of individual amphiphilic di/tri-block co-polymers with hydrophobic core and hydrophilic surface shells or vice versa (Fig.4). (Liechty et al., 2012) They contain both hydrophilic and hydrophobic regions in their structure and serve as good carrier systems for poorly soluble drugs. Multifunctional polymeric micelles can be also be designed to facilitate simultaneous drug delivery and imaging. (Underwood et al., 2012) Their stability depends on strong cohesive force between drug and core polymer segments as well as cross-linking of the shell or core, which is performed by radical polymerization. Prolonged circulation and targeted delivery of PMs is possible by designing of environment-responsive polymeric micelles (pH, light, temperature, ultrasound, etc.).

**Fig. 4 Schematic representation of a self-assembled block copolymeric micelle** (Mishra et al., 2010)
1.3.7 Sterically Stabilized Micelles
Sterically stabilized micelles (SSMs), containing polyethylene glycol (PEGylated) and phospholipids (Phospholipid Micelles), have also been prepared as a safe, biocompatible nanocarriers for the delivery of poorly water-soluble as well as cytotoxic drugs like anticancer drugs. Camptothecin-containing SSMs (CPT-SSMs) has been prepared as a novel nanomedicine for parenteral administration, which showed higher solubilization potential, estimable stability, and less in vitro cytotoxicity. (Dhembre et al., 2011)

1.3.8 Colloidal Nano-liposomes
Liposomes are small artificial vesicles of globular shape composed of aqueous pores encapsulated with amphiphilic phospholipids and cholesterol bilayer (Fig.5). (Saraf et al., 2006) They are widely used owing to their ability to act as carrier for both hydrophilic as well as hydrophobic drugs, and better tissue biocompatibility along with the fact that their size can be suitably monitored. (Koo et al., 2005) Depending on size and number of phospholipid bilayers, liposomes had been classified into small unilamellar vesicles (SUVs; single lipid layer 25 to 50 nm in diameter), large unilamellar vesicles (LUVs; heterogeneous group of vesicles), and multilamellar vesicles (MLVs; several lipid layers separated from one another by a layer of aqueous solution). Liposomes have been widely investigated for the delivery of vaccine, toxoids, gene, anticancer, and anti-HIV drugs.

![Fig. 5 Schematic representation of sterically stabilized liposomes.](Mishra et al., 2010)

1.3.9 Hydrogel nanoparticles (Nanogels)
Hydrogel nanoparticles (NPs) (recently referred to as nanogels) as a family of nanoscale particulate materials, have recently attracted a lot of attention of scientists dealing with development of newer drug delivery systems. Interestingly, hydrogel nanoparticulate materials would demonstrate the features and characteristics of both hydrogels and NPs
simultaneously. Therefore, it seems that the pharmacy world will benefit from both the hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility of these particles and all the advantages of the NPs, mainly long life span in circulation and the possibility of being actively or passively targeted to the desired biophase, e.g. tumor sites. Different methods have been adopted to prepare NPs of hydrogel consistency. Besides the commonly used synthetic polymers, active research is now focused on the preparation of NPs using naturally occurring hydrophilic polymers and hydrocolloids. (Hamidi et al., 2008)

1.4 Advantages of Nanoparticles

- Improved stability i.e. long shelf life.
- Increased solubility of the drug.
- High carrier capacity or high drug entrapment efficiency.
- The drug can be incorporated without any chemical reaction, which is a prerequisite for preserving the drug activity. (Wu et al., 2011)
- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting.
- Targeted and controlled drug delivery is possible
- Increased therapeutic efficacy and reduction in side effects. (Gelperina et al., 2005)
- Drug and particle degradation characteristics can be easily modulated by proper selection of matrix constituents.
- Drug loading is relatively high
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
- Nanoparticles can be used for various routes of administration including oral, nasal, parenterals, intra-ocular etc. (Azarmi et al., 2008)
- They can be tailor-made to achieve both controlled drug release and disease specific localization by controlling the polymer characteristics and surface chemistry.
- Due to smaller size they are better suited for intravenous (i.v.) delivery without resulting in embolism.
- Nanoparticles are effective in sustaining the drugs thus allowing a more efficient
interaction with the receptors which are cytoplasmic. (Grama et al., 2011)

- Decrease the toxic side effects of the drug.
- Allow rapid formulation development. (Owens et al., 2006)
- Scale up to large scale production is feasible.

These systems in general can be used to provide targeted (cellular/tissue) delivery of drugs, to improve oral bioavailability, to sustain drug/gene effect in the target tissue, to solubilize drugs for intravascular delivery, and to improve the stability of therapeutic agents against enzymatic degradation (nucleases and proteases), especially of protein, peptide, and nucleic acids drugs.

### 1.5 Limitations of Nanoparticles

- Their small size and large surface area can lead to particle-particle aggregation.
- Making physical handling of nanoparticles difficult in liquid and dry forms.
- Small particles size and large surface area may result in limited drug loading and burst release.

These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available (Johnstone et al., 2011).

### 1.6 Effect of Characteristics of Nanoparticles on Drug Delivery

#### 1.6.1 Particle Size

Particle size and size distribution are the most important characteristics of nanoparticles, which impart certain characteristic properties to nanoparticles. These determine the in vivo distribution, biological fate, toxicity and the targeting ability of nanoparticulate systems. In addition, the formulation variables and processing techniques can also influence the drug loading, drug release and stability of nanoparticles. Many studies have demonstrated that nanoparticles of sub-micron size have generally, relatively higher intracellular uptake as compared to microparticles and become available to a wider range of biological targets due to their smaller size and relative mobility. Drug release in general is affected by particle size. Since, the smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface readily gets released into the dissolution media leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and lower contact area with dissolution medium results in their slower release. Smaller particles also have greater risk of aggregation of particles.
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during storage and transportation of nanoparticles dispersion and it is always a challenge to formulate nanoparticles with the smallest size possible with minimum particle aggregation and maximum stability. The particle size characterized by photon-correlation spectroscopy is usually verified by scanning or transmission electron microscopy SEM or TEM. (Acharya et al., 2011)

1.6.2. Nature of drug/additives

In addition to particle size, many other parameters particularly physicochemical properties of drug and other additives have been shown to affect the drug release profiles. The nature of the drug or other additives is obviously most important and log P, pKa and molecular weight of the drug are some of the important parameters. The acidic or basic nature of the additives as well as loading level in the case of therapeutic agents may markedly affect the degradation rate and release profile of drugs from nanoparticles. Basic compounds can catalyze ester linkage and thus accelerate polymer degradation causing burst release of drugs. On the other hand, appropriate amounts of basic compounds can neutralize carboxyl end groups and thus decrease the rate of degradation and drug release. The degree of drug loading and its physical state in nanoparticulate carrier is often correlated with the release profile, especially since the drug may be crystallized in the carrier matrix at high loadings. (Elzoghby et al., 2012)

1.6.3 Porosity

The porosity of the particles also affects the permeation of dissolution fluid in the interior part of nanoparticles followed by drug diffusion as well as rate of degradation of polymer. If a drug molecule is present within a network of micropores it will have to diffuse towards the surrounding media to be released outside. Movement of the drug within the porous media is highly restricted due to limited space available and is influenced by porosity, affinity with media, diffusivity of drug and tortuosity of matrix. (Yang et al., 2000)

1.6.4 Method of preparation

The method of preparation of nanoparticulate system also imparts certain attributes which may be suitably harnessed in order to modify and control the drug release. NPs preparation by emulsification technique is a complex process where organic solvent generates pores in the structure on evaporation which may then lead to a change in the microstructure of NPs and affect their release kinetics. Whereas, the nanoparticles prepared by polymer
degradation can significantly alter the microenvironment conditions. Furthermore, degradation products of polymer can crystallise within the core leading to altered porosities which may result in enhanced diffusion. Depending on their solubility and the respective micro environmental conditions, these degradation products subsequently dissolve and diffuse out of the device. At a critical time point, the polymeric structure of the system becomes unstable and leads to the breakdown of the macromolecular network. The nature of organic solvent used in formulation of nanoparticles also significantly affects the morphology of nanoparticles as well as rate of release of drug. For example, solubility of dichloromethane (DCM) in water is low and after formation of a primary emulsion, DCM gradually diffuses out of the nascent particles and its slower evaporation yields uniform spherical particles with a porous microstructure. Acetone, due to its high miscibility dissolves faster in the aqueous phase and hence irregular shaped particles are formed with lesser degree of predictability in terms of drug release. The organic phase may be used alone or in combination to form the organic phase where dispersion of the water soluble solvent would result in a decreased surface tension and size. Increase in water insoluble solvent would lead to further size reduction without pores. This would also lead to an increased drug loading and decreased rate of release. The nature of dissolution medium is extremely important particularly the pH, presence of surface active agents and presence or absence of lipid vesicles in it. As yet, there is no perfectly designed in vitro dissolution test for colloidal particles intended for potential administration and as a result, a wide variety of dissolution medium are being used. (Anton et al., 2008)

1.6.5 Physical state of drug and polymer

Physical state of the drug inside the nanoparticles significantly affects drug dissolution and drug diffusion from nanoparticles. An amorphous form of the drug may lead to rapid dissolution of the drug as drug molecules need not to overcome crystalline lattice energy barrier often associated with solid crystalline phase. Therefore, the amorphous substances have higher internal energy, larger free volume, and offers greater molecular mobility to individual drug molecules in comparison to the crystalline state. These properties are responsible for greater solubility and hence faster drug release associated with amorphous drug in nanoparticles. Small drug molecules can readily crystallize owing their large
surface charge density and consequently both crystalline and amorphous state can coexist in nanoparticulate system. The physical state of polymer is also instrumental in influencing drug release rate and amorphous or crystalline drug may be present in either amorphous or crystalline polymer. (Yang et al., 2000)

1.6.6 Drug Loading

A successful NPs system may be one which has a high loading capacity to reduce the quantity of the carrier required for administration of drug and successful formulation into a dosage form. Drug loading into NPs is achieved by either incorporating the drug at the time of NP production or by adsorbing the drug after the formation of NPs by incubating or impregnating them in the drug solution. Drug entrapment efficiency is usually higher with the incorporation method rather than by adsorption methods. (Veiseh et al., 2010) Zeta potential on the other hand varies depending on the charge of the incorporated compound and surface adsorption. A successful nano delivery system should have a high drug-loading capacity, thereby reducing the quantity of matrix materials for administration. Drug loading can be accomplished by two methods. The incorporation method requires the drug to be incorporated at the time of nanoparticles formulation. (Elzoghby et al., 2012) The adsorption/absorption methods call for absorption of the drug after nanoparticle formation; this is achieved by incubating the nano-carrier with a concentrated drug solution. Drug loading and entrapment efficiency depend on drug solubility in the excipient matrix material (solid polymer or liquid dispersion agent), which is related to the matrix composition, molecular weight, drug–polymer interactions, and the presence of end functional groups (i.e., ester or carboxyl) in either the drug or matrix. In addition, the macromolecules, drugs or protein encapsulated in nanoparticles show the greatest loading efficiency when they are loaded at or near their isoelectric point (pI). For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be very effective in increasing drug-loading and the drug release can be precisely monitored. (Singh et al., 2009)

1.7 Polymers used in Preparation of Nanoparticles

The polymers used in the preparation of polymeric nanoparticles can be divided into two broad categories.
1.7.1 Biodegradable Polymers

- Albumin
- Gelatin
- Alginic acid/alginites
- Chitosan and chitin derivatives
- Polylactic acid (PLA)
- Polyglycolic acid (PGA)
- Polylactide-co-glycolide (PLGA)
- Poly-e-caprolactone (PCL)
- Polylactide-co-caprolactone (PLC)
- Polyalkyl cyanoacrylates (Jeong et al., 1999, Tian et al., 2012)

1.7.2 Non-biodegradable Polymers

- Polymethyl vinyl ether/maleic anhydride
- Gantrez
- Polymethyl methacrylates (Eudragit)
- Polyamidoamines. (PAMAM) (Bonilla et al., 2012, Desai et al., 2011, Kotwal et al., 2007)

1.8 Methods of Preparation of Nanoparticles

Nanoparticles can be prepared using a wide variety of materials such as proteins, polysaccharides and synthetic and semisynthetic polymers as well as natural gums and hydrocolloids. The selection of ideal matrix forming material/ polymeric carrier is made after careful consideration of many factors such as:

- Size of nanoparticles required
- Inherent properties of the drug, e.g., pKa, aqueous solubility and stability
- Surface characteristics such as charge and permeability
- Degree of biodegradability, biocompatibility and toxicity
- Drug release profile desired
- Antigenicity of the final product.
- Method of preparation being used, etc

A number of methods have been reported for formulation of nanoparticles and can yield varying size and diverse properties attached with the formulated nanoparticles which are briefly described here:
1.8.1 Emulsion cross-linking
This method utilizes the reactive functional amine group of CS to cross-link with aldehyde groups of the cross-linking agent. In this method, water-in-oil (w/o) emulsion is prepared by emulsifying the CS aqueous solution in the oil phase. Aqueous droplets are stabilized with the help of suitable surfactant (Low HLB value). The stable emulsion is cross-linked by using an appropriate cross-linking agent such as glutaraldehyde, which results into hardening of the droplets. The resulting nanoparticles are separated by centrifugation, filtered and washed repeatedly with n-hexane followed by alcohol and then dried. By this method, size of the particles can be controlled by controlling the size of aqueous droplets. However, the particle size of final product depends upon the extent of cross-linking agent used while hardening, in addition to nature and concentration of surfactant along with speed of stirring during the formation of emulsion. This method is schematically represented in Fig.6. The emulsion cross-linking method has few drawbacks since it involves tedious procedures as well as use of harsh cross-linking agents, which might possibly induce chemical reactions with the active agent. Moreover, complete removal of the un-reacted crosslinking agent may be difficult in this process.

![Fig. 6: Schematic representation of preparation of chitosan nanoparticulate systems by emulsion cross-linking method. (Stolnik et al., 1995)](image)

1.8.2 Solvent evaporation method
In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the
hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil in water (o/w) emulsion. (Fig.7) After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. The particle size is found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication along with suitable surfactant and stabilizer may be employed. (Wei et al., 2009)

Fig.7 Schematic representation of the emulsification-evaporation technique. (Zohri et al., 2009)

1.8.3 Spontaneous Emulsification or Solvent Diffusion Method
Spontaneous emulsification or Solvent diffusion method is a modified version of solvent evaporation method. In this method, the oil phase consists of a water miscible solvent along with a small amount of the water immiscible organic solvent and due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both hydrophobic and hydrophilic drugs can be formulated into nanoparticles by solvent evaporation and solvent diffusion methods. In the case of hydrophilic drug, usually a multiple w/o/w emulsion is formulated with the drug dissolved in the internal aqueous phase. (Hans et al., 2002)

Emulsification/solvent diffusion (ESD) is based on the use of organic solvents, and then it was suitably modified in accordance with the salting-out technique. The polymeric carrier is dissolved in a water miscible solvent such as propylene carbonate and then is saturated with water to ensure the initial thermodynamic equilibrium between both the liquids. In order to induce the precipitation of the polymer and the consequent formation of
nanoparticles, it is necessary to facilitate the diffusion of the solvent of the dispersed phase by dilution with an excess of water, when the organic solvent is partly miscible with water or with another organic solvent in the case where organic solvent is immiscible with water. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, depending on the oil-to-polymer ratio. Finally, the solvent is removed by evaporation or filtration, depending on its boiling point. This technique offers several advantages, such as high encapsulation efficiencies (generally more than 70%), no requirement of homogenizers, high batch-to-batch reproducibility, easier scale-up, simplicity, and capability to yield narrow size distribution. Although there are certain limitations/ drawbacks as higher volume of water is to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, which may lower the encapsulation efficiency. As compared with some of the other techniques, this method is efficient in encapsulating lipophilic drugs.

Fig 8 Schematic illustration of the ESD technique (Reis et al., 2006)

1.8.4 Polymerization Method

In polymerization methods, monomers are polymerized with subsequent entrapment of drug particles to form nanoparticles or adsorbed on their surface. Drug is incorporated either by dissolving in the polymerization medium or by adsorption onto the nanoparticles after completion of polymerization. (Roney et al., 2005) The nanoparticle suspension is then purified to remove traces of free stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. (Fig.9) This technique has widely been utilized for formulating polybutylcyanoacrylate or poly(alkylcyanoacrylate) nanoparticles. The concentration of the surfactants and stabilizers affects the formation and stability of nanocapsule and their particle size depends on the nature of surfactant and stabilizer.
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1.8.5 Coacervation or Ionic Gelation Method

The use of ionic interaction between oppositely charged macromolecules to prepare CS nanoparticles has attracted much attention because the simplicity of process avoiding other stress conditions. In addition, reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking, has been applied to avoid the possible toxicity of reagents and other undesirable effects. Tripolyphosphate (TPP) is a polyanion, which can interact with the cationic CS by electrostatic forces. The preparations of TPP–CS complex by dropping CS droplets into a TPP solution have been explored for its potential pharmaceutical usage. In the ionic gelation method, CS is dissolved in aqueous acetic acid solution to obtain the cation of CS (Fig. 10), which is then added dropwise under constant stirring to polyanionic TPP solution. However, TPP/CS microparticles formed have poor mechanical strength thus, limiting their usage in drug delivery. (Nagarwal et al., 2009)

Fig. 9 Schematic representation of polymerization method for the production of nanoparticles. (Soppimath et al., 2001)

Fig.10. Schematic representation of the emulsification–internal gelation technique using alginate. (Reis et al., 2006)
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1.8.6 Complex Coacervation Methods
Complex coacervation is a spontaneous phase separation process involving two liquid phases in colloidal systems, which results by the interaction of two oppositely charged polyelectrolytes upon mixing in an aqueous solution. The process leads to formation of micrometric or nanometric colloidal particles, depending on substrates or process variables, such as pH, temperature, molecular weight, ionic strength, polyelectrolyte concentration, and so forth. The major drawbacks of this method are poor drug stability and lower drug loading efficiency, which, however, can be overcome by cross-linking of the complex by chemical reagents, such as glutaraldehyde.

![Diagram of complex coacervation](image)

Fig.11 Schematic representation of preparation of chitosan nanoparticles by coacervation/precipitation method. (Agnihotri et al., 2004)

1.8.6.1 Co-precipitation Method
Co-precipitation is a modified complex coacervation method for the preparation of nanosized core-shell particles having good dispersion stability of hydrophobic (poorly water-soluble) drugs. Drug loaded nanoparticles are stabilized by diethyl amino ethylcellulose (DEAE)-dextran (Ddex as water-soluble, positively charged resin). Ddex is used as a coating layer to coprecipitate with negatively charged drug. The method involves the precipitation of drug in a supersaturated solution, followed by deposition of Ddex onto the precipitated drug particles through electrostatic interactions. Transmission electron microscopy (TEM), atomic force microscopy (AFM), and zeta potential studies showed typical core-shell nanoparticles, with high encapsulation efficiency and good stability. (Hans et al., 2002)

1.8.6.2 Spray-drying
Spray-drying is a well-known technique to produce powders, granules or agglomerates...
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from the mixture of drug and excipient solutions as well as suspensions. The method is based on drying of atomized droplets in a stream of hot air and can be applied for formulation of nanoparticles. (Okuyama et al., 2006) In this method, CS is first dissolved in aqueous acetic acid solution, drug is then dissolved or dispersed in the solution along with a suitable cross-linking agent. This solution or dispersion is then atomized in a stream of hot air. (Wei et al., 2009) Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing particles as depicted in Fig. 12. Various process parameters are required to be carefully controlled in order to get the desired size of particles. Particle size depends upon the size of nozzle, spray flow rate, atomization pressure, inlet air temperature and extent of cross linking. (Elzobbi et al., 2012)

Fig. 12 Schematic representation of preparation of chitosan particulate systems by spray drying method (Agnihotri et al., 2004).

1.8.7 Salting-out Method

The salting-out method is widely used in the pharmaceutical industry owing to its high yield, purity, speed and simplicity of the operation. The method does not demand thermal treatment at any stage of sample processing and therefore, may be especially useful for the incorporation of thermolabile drugs. It is based on the phenomenon in which solubility of a non-electrolyte in water is decreased upon addition of an electrolyte. This method involves an emulsification step avoiding the use of surfactants and chlorinated solvents, where a water-soluble stabilizing polymer is added to a saturated solution of electrolyte (e.g., sodium chloride, magnesium acetate, or magnesium chloride) to obtain a viscous gel. Subsequently, polymer and drug are dissolved separately in an organic solvent. Most often,
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acetone is used as solvent because of its solubilizing properties and easier separation from aqueous solution upon salting-out with electrolytes. Addition of viscous gel into organic phase under continuous stirring causes salting out of the organic solvent, inducing formation of nanoparticles in organic-aqueous medium (Fig. 13). Finally, both solvent and electrolyte are eliminated by cross-flow filtration. (Muthu et al., 2009)

1.8.8 Nanoprecipitation Method

Nanoprecipitation, also known as solvent displacement method, is based on interfacial deposition of a polymer after displacement of a semi polar solvent miscible with water from a lipophilic solution. Rapid diffusion of the solvent into aqueous phase results in a decrease in the interfacial tension between the two phases, which increases the surface area and leads to formation of small droplets of organic solvent even without any mechanical stirring. However, it provides poor entrapment efficiency for water-soluble drugs. (Hans et al., 2002)

1.8.9 Supercritical Fluid Methods

Submicrometer-sized and nano-sized particles can also be prepared by using supercritical fluid (SCF) technology. A supercritical fluid can either be a liquid or gas and used above its thermodynamic critical point of temperature and pressure. Most commonly used SCFs are carbon dioxide (CO₂) and water. (Byrappa et al., 2008)

1.8.9.1 Rapid Expansion of Supercritical Solutions

Rapid expansion of supercritical solutions (RESS) is a useful technique for thermolabile drugs and is able to produce finely divided particles of nanometer size range with a precisely controlled size distribution (Fig 14). The process involves saturation of SCF with the drug and depressurization of the obtained solution through a heated nozzle into a low-pressure chamber to cause rapid nucleation of the drug yielding more uniform particle

Fig 13 Schematic of the salting-out technique (Reis et al., 2006)
size. As the solution is allowed to expand across a calibrated orifice, the density decreases gradually and the solute is precipitated as finely divided solid fibers or crystals. Expansion at high pressure keeps the density high and reduces the flow velocity of particles, thus providing the particles enough growth time for clustering and aggregation. Consequently, larger particles are produced at high expansion pressure, whereas, expansion into low pressure causes density to become low with higher velocity, so that both the flow time and density are less favorable for the growth of larger clusters. Besides, maintenance of homogeneous experimental conditions gives rise to controlled and uniform particle size distribution. Carbon dioxide has widely been used as SCF in most RESS techniques. For slightly soluble drugs, fluorinated hydrocarbons, like trifluoromethane (eg, CHF$_3$), can also be used owing to their higher polarity. (Mishra et al., 2010)

![Fig.14 Rapid expansion supercritical solution method](Mishra et al., 2010)

1.8.9.2 Supercritical Antisolvent Precipitation

Supercritical antisolvent precipitation (SAS) is now widely being investigated as an alternative to the liquid antisolvent precipitation (LAS) for producing micronized particles of some antibiotics.(Huerates et al., 2010) In the case of liquid antisolvent processing, removal of solvent is a cumbersome process, whereas SAS allows removal of the solvent under reduced pressure and results in appearance of sub micrometer-sized particles with narrow size distribution.(Amidi et al., 2010) Selection of proper solvent is a prerequisite as SAS technique is based on the use of two completely miscible liquid solvents; the drug or solute to be micronized should be soluble in the first solvent but not in the second one. (Nagarwal et al., 2009) Antisolvent addition can be carried out from the top or bottom
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of the precipitating chamber. (Rao et al., 2004) A faster diffusion of the solvent results in super saturation resulting in precipitation of particles in microsized form. Supercritical CO$_2$ is allowed to flow through the chamber until the end of precipitation in order to remove the remaining liquid solvent (Fig 15). Otherwise, the remaining liquid solvent may resolubilize the solutes during depressurization step affecting the product quality and stability (Date et al., 2004).

![Fig.15 Supercritical antisolvent precipitation method](Reverchon et al., 1995)

1.8.10 Osmosis Based Method

A novel osmosis based method had recently been proposed (Fig. 16) for the preparation of various natural- and synthetic Polymeric Nanoparticles. This method is based on the use of a physical barrier, particularly dialysis membrane or common semi-permeable membrane (SPM) that allow the passive transport of solvents in order to reduce the mixing of the polymer solution with a nonsolvent.

![Fig.16 Schematic representation of osmosis based method for preparation of polymer nanoparticles](Rao et al., 2011)
1.9 Applications of Chitosan Nanoparticles

Being a new area the diverse applications of nanoparticles fabricated for particular use are coming everyday and no area of research is being untouched by this technology. Pharmaceutical applications of Chitosan-based particulate systems are attracting pharmaceutical and biomedical, as a potential drug delivery devices. Some important applications are discussed below.

1.9.1. Colon targeted drug delivery

Chitosan is a promising polymer for colon drug delivery, since, it is biodegradable and possess mucoadhesive character. (Shukla et al., 2012) Chitosan is degraded by the colonic bacterial flora and therefore, dosage forms for colon targeted delivery can be easily fabricated. The pH-sensitive multicore microparticulate system containing CS microcores entrapped into enteric acrylic microspheres had been successfully developed (Ensign et al., 2012; Koo et al., 2005).

1.9.2. Mucosal delivery

Now a days, mucosal surfaces such as nasal, peroral and pulmonary are receiving a great deal of attention as alternative routes of systemic administration as well as for localized delivery of drugs. Chitosan has mucoadhesive properties and therefore, it is particularly useful in fabrication of Bioadhesive dosage forms for mucosal administration (ocular, nasal, buccal, gastro-enteric and vaginal-uterine therapy). Nasal mucosa has high permeability and easy access of drug to the absorption site. The particulate delivery to peroral mucosa is easily taken up by the Peyer’s patches of the gut associated lymphoid tissue (Takeuchi et al., 2001). Chitosan has been found to enhance the drug absorption through mucosa without damaging the biological system. Here, the mechanism of action of CS was suggested to be a combination of bioadhesion and a transient widening of the tight junctions between epithelial cells. The ability of insulin loaded CS nanoparticles to enhance the nasal absorption of insulin has been investigated in a conscious rabbit model. Chitosan nanoparticles enhanced the nasal absorption of insulin has been demonstrated using confocal laser scanning microscopy (Lai et al., 2009). The ability of CS microparticles to enhance both systemic and local immune responses against diphtheria toxoid (DT) vaccine after the oral and nasal administration in mice has also been
Investigated. In another study, mucoadhesive CS microparticles were prepared and characterized for size, zeta potential, morphology- and albumin-loading as well as release characteristics (Kreuter et al., 2005).

### 1.9.3. Cancer therapy

The rationale of using nanoparticles for tumor targeting is based on the fact that nanoparticles will be able to deliver higher dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles (Kedar et al., 2010). Moreover, nanoparticles will reduce the drug exposure of other tissues by limiting drug distribution to target organ as most of the anticancer drugs are cytotoxic in nature (Maeda et al., 2009).

A strategy most widely being investigated in cancer therapy is to formulate nanoparticles containing anti tumor drugs, with the aim to overcome non-cellular and cellular based mechanisms of resistance and to increase selectivity of drugs towards cancer cells while reducing their toxicity towards normal tissues (Hawkins et al., 2008). According to the process used for the preparation of the nanoparticles, nanospheres or nanocapsules can be obtained (Prakash et al., 2011). Unlike nanospheres (matrix systems in which the drug is dispersed throughout the particles), nanocapsules are vesicular systems in which the drug is confined to an aqueous or oily cavity surrounded by a single polymeric membrane (Serda et al., 2011). Nanocapsules may, thus, be considered as a ‘reservoir’ system. If designed appropriately, nanoparticles may act as a drug vehicle able to target tumor tissues or cells, to a certain extent, while protecting the drug from premature inactivation during its transport. Indeed, at the tumor level, the accumulation mechanism of intravenously injected nanoparticles relies on a passive diffusion or convection across the leaky, hyperpermeable tumor vasculature. The uptake can also result from a specific recognition in case of ligand coated nanoparticles (Brewer et al., 2011).

### 1.9.4. Gene delivery

Gene delivery had opened a new area of therapy has a lot of potential in prevention as well as mitigation in the area of genetic disorders and only future can reveal its actual application area. In case of gene delivery, the plasmid DNA is usually introduced into the target cells, which should get transcribed and the genetic information should ultimately be translated into the corresponding protein. To achieve the actual application, a number of
hurdles are to be overcome by the gene delivery system although many technological advancements have been made in the current decade. Transfection is affected through targeting the delivery system to target cell, transport through the cell membrane, uptake and degradation in the endolysosomes and intracellular trafficking of plasmid DNA to the nucleus. Chitosan being positively charged could easily interact electrostatically with the negatively charged DNA and forms polyelectrolyte complexes (Panyan et al. 2003). In these complexes, DNA becomes better protected against nuclease degradation due to attachment with CS leading to better transfection efficiency. DNA–CS nanoparticles have been investigated to understand the influence of several parameters on their preparation and stability invivo. The transfection efficiency of DNA-CS nanoparticles was cell-type dependent. The self-aggregates/DNA complex has been reported to be useful for transfer of genes into mammalian cells in vitro and chitosan has proved its applicability due to inherent properties in this area (Broichsitter et al., 2012). Several transfection studies has been carried out using chemically modified CS for delivery of nucleotides. Trimethyl CS oligomers were examined for their usefulness as DNA carriers. Chitosan and lactosylated CS carriers had also been investigated for their transfection efficiencies in vitro (Fuentes et al., 2012).

1.9.5. Topical delivery
Owing to good bioadhesive property and ability to sustain the release of drugs and medicaments, CS has also been used in topical delivery systems. Bioadhesive CS microspheres for topical sustained release of cetyl pyridinium chloride have been evaluated, which showed improved antimicrobial activity (Park et al., 2010).

1.9.6. Ocular delivery
Nanoparticles have shown promising results over the last 10 years in ophthalmology, providing protection of drug from chemical and enzymatic degradation, improved tolerance, increased corneal uptake, and longer intraocular half-life (Nagarwal et al., 2009). The first report on particulate systems for ocular delivery was in 1980 by Gurny and Taylor (Araujo et al., 2009). Subsequently, various types of nanoparticles were proposed to take advantage of prolonged residence time, as the short elimination half-life of ophthalmologic drugs was a major hurdle in ocular therapy (Zimmer et al., 1995). Cyclosporine A had also been nanoencapsulated in three important studies involving PCL, PACA, and chitosan to
evaluate the therapeutic efficacy of cyclosporine A–loaded nanoparticles. The nanoparticle approach has not yet yielded satisfactory results, because the precorneal clearance is still too rapid, although many researchers are working in this area (Diebold et al., 2010). Chitosan is the most promising carrier, as the therapeutic levels achieved in periocular tissues were satisfactory and it possess good tolerance; consequently it has widely being investigated for design and development of ocular drug formulations (Reis et al., 2006). Nanoparticulate technology holds a lot of potential for development as an ophthalmic drug delivery approach that not only enhance therapeutic efficacy of many drugs but can also enhance dosage form acceptability while providing sustained release in the ocular milieu. Particle size, particle size distribution, and stability constitute a major issue considered by formulation scientists when formulating dispersed systems, especially those intended for parenteral or ocular administration. Very small particles such as nanoparticles are well tolerated and possess adhesive properties, which could prolong the residence time of the drug in the cul-de-sac, prevent tear washout (due to tear dynamics), and increase ocular bioavailability. Other potential advantages of nanoscaled drug delivery systems in ocular therapy are the possibility of self-administration by patients as eye drops, no impairment of sight because of small dimensions of the delivery systems, protection against metabolic enzymes (such as peptidases and nucleases), possible uptake into corneal cells, prolonged drug release, reducing the need for repeated instillation or injection, targeting toward affected tissues, reducing possible side effects and reduced required dose (Akage et al., 2007, Diebold et al., 2010).

1.9.7 Therapeutics

Some recent applications of NP in therapeutics are discussed, possibly offering insights to the applications of NPs in therapeutics. The therapeutic applications of NPs are diverse, ranging from cancer therapeutics, antimicrobial actions, vaccine delivery, gene delivery and site-specific targeting to avoid the undesirable side effects of the current therapeutics. Many chemotherapeutic drugs such as carboplatin, paclitaxel, doxorubicin and etoposide, etc., have been successfully loaded onto NPs and these nanoparticulate systems are potent against various cancers as demonstrated by the studies of various research groups. (Vasir et al., 2007) In addition, multifunctional NPs with surface functionalized biomolecules are also being synthesized and serve as potential therapeutic agents. Functionalized NPs are
also being used for targeted gene silencing because these offer exciting prospects and have garnered the attention of researchers. Many NPs are also useful as therapeutics due to their antimicrobial properties (Danhier et al., 2012).

1.9.8 Diagnostics
The drive to understand biology and medicine at the molecular level with accurate quantification demands much of current advanced analytical systems and now a days diagnostics are reaping the recent advancements in nanotechnology and are becoming more and more important. Nanomaterials and nanotechnology combined with modern instrumentation have the potential the merger of these two has yielded many fruitful results for the mankind. This solution can be possible with the aid of a variety of nanomaterials for multiplex diagnostics and thus can offer sensitive, rapid and cost-effective solutions for the modern clinical laboratory. NPs are being increasingly applied to molecular diagnostics and several technologies are in development. NPs, such as gold (Au) NPs and quantum dots (QDs), are the most widely used but various other nanotechnological devices for manipulation at the nanoscale, as well as nanobiosensors, are also promising for potential clinical applications. (Underwood et al., 2012)

Semiconductor QDs are NPs with intense, stable fluorescence enabling the detection of tens to hundreds of cancer biomarkers in blood assays or on cancer tissue biopsies. They allow easier detection of cancer markers in biological specimens at pg/mL concentrations. Molecular diagnostics has significantly changed with the advent of AuNPs which promises increased sensitivity and specificity, multiplexing capability and short turnaround times. AuNP-based colorometric assays also show great potential in molecular diagnostics. The widespread use of AuNPs as labels in diagnostics and detection is due to a unique combination of chemical and physical properties that allow biological molecules to be detected at very low concentrations which could not have been detected by conventional assay methods. Aptamer-conjugated AuNPs has also become a powerful tool for point-of-care diagnostics and they can also be used for the collection and detection of multiple cancer cells. (Gaur et al., 2008)

1.9.9 Imaging
The development of the effective carrier system does not only mean the execution of delivery, but also the positive confirmation of the site-specific delivery of the drug, which
is made possible with the recent advances in medicad imaging techniques. Consequently, the ability to track the fate of any nanomedicine from the systemic to the subcellular level becomes essential. (Piotrowska et al., 2009) NPs can be successfully exploited to improve the utility of fluorescent markers for medical imaging and diagnostic purposes. (Solan et al., 2005) Although various fluorescent markers are widely being used in research and clinical diagnostic applications, current techniques have several disadvantages, such as the requirement of color-matched lasers, fluorescence bleaching and lack of discriminatory capacity of multiple dyes, etc. (Joshi et al., 2009) Fluorescent NPs can greatly overcome these problems and a major advancement toward clinical applicability is the use of NPs to obtain images of tumors and other tissues in vivo. (Jong et al., 2008) Recently, fluorescent silica NPs (FSNPs), which are a new class of engineered optical probes consisting of silica NPs loaded with fluorescent dye, have also created immense interest in cancer imaging. (Serda et al., 2011) The use of water-soluble, functionalized QDs that are highly stable against oxidation for biological and biomedical applications is currently one of the fastest-growing fields of nanotechnology. QDs manifest stable fluorescent properties, and also offer new prospects for studying live cells, in vivo imaging and diagnostics. Magnetic iron oxides NPs also have attracted extensive interest as novel contrast agents for biomedical imaging due to their capability of deep-tissue imaging associated with low toxicity. Dynamic magnetomotion of magnetic NPs (MNPs) detected with magnetomotive optical coherence tomography (MM-OCT) also offers a new methodology for contrast enhancement and therapeutic interventions along with molecular imaging. AuNPs are also widely being used for cellular imaging. Thus, different types of nanoparticulate systems can be efficiently used as in vitro and in vivo imaging agents for efficient diagnostics and therapeutics. (Parveen et al., 2012)

1.10 Stability of Nanoparticles

Generally, nanoparticles show a poor long-term stability due to different physical and chemical factors that may destabilize the system. In general, the stability of nanoparticles can broadly studied as two types, physical stability and chemical stability in order to clearly understand their fundamental aspects. (Wischke et al., 2008)

1.10.1. Physical stability

There are a number of factors that may affect the stability of nanoparticles. Generally, the
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colloidal suspension does not tend to separate because of the presence of Brownian motion and due to this zig zag movement the colloidal dispersions are homogenous. These submicron particles sediment so slowly that the effect is obliterated by the mixing tendencies of diffusion and convection. The suspended colloidal particles continuously change direction as a result of random collisions with the molecules of the dispersion media, other particles, and the walls of the containing vessel. As a result of thermal motion, colloidal particles diffuse from a region of high concentration to a region of lower concentration until the concentration is uniform throughout (homogenous). Gravitational forces, which cause particles to sediment, are nullified by the Brownian motion (diffusion forces), as they oppose one another. Colloids are of the size range at which the Brownian forces dominate the gravitational forces, so they tend to remain suspended. However due to aggregation of particles the particle size may increase and the gravitational forces may dominate and then sedimentation or creaming may be initiated causing physical instability. In order to avoid the aggregation phenomena, a suitable stabilizer can be used in the formulation. Nevertheless, the stability of colloidal nanoparticle suspensions may get disturbed after addition of other components. The adsorption of active molecules on the surface of nanoparticles may induce particle agglomeration, probably by displacing part of the steric stabilizing surfactant layer. Bridging flocculation may occur, if a solution of a high molecular weight polymer is added to a dilute colloidal dispersion. It may be attributed to the fact that the two ends of a polymer chain gets adsorbed on two separate particles and draw them together. Charge stabilized dispersions are coagulated by the adsorption of counter-ions in the electrical double layer, although oppositely charged particles are more effective. Coagulation also occurs when two oppositely charged dispersions are mixed. (Shegokar et al., 2011)

1.10.2 Chemical stability

The chemical stability of colloidal dispersion depends on their storage conditions (the temperature and the pH medium) and on the composition of the stored formulation including the type and molecular weight of the polymer used in preparing nanoparticles. Consequently, for each specific formulation, the corresponding stability study will have to be performed to assess the quality of the product. The chemical integrity of drugs entrapped in nanoparticles is another fundamental aspect of the overall stability evaluation
of these products. Since, most of the drugs have a pH-dependent degradation profile, the pH needs to be closely controlled. A number of cytotoxic drugs are light-sensitive. Hence, during the manufacturing procedure, exposure to light should also be minimized. However, since the final product usually consists of the drug incorporated within the bulk of a solid particle, light-induced degradation is less of a problem. (Wu et al., 2009) On the other hand, when studying the stability of colloidal carriers, it is important to analyze not only the particle size and polymer molecular weight, but also the eventual leakage of the drug from the formulation during storage. For example, during the encapsulation of strong hydrophobic drugs, precipitation in the external aqueous phase causes formation of nanocrystals that may be misinterpreted as nanoparticles. The nanocrystals, however, will become evident if the preparation is stored for several days or weeks to allow the crystallization nuclei to grow. In addition, it was observed that the presence of anionic surfactants in the dispersion causes a more rapid degradation of poly (D, L-lactide). Therefore, it could be expected that drugs with strong nucleophilic groups may catalyze the degradation of the polymer. In order to reduce this physical and chemical instability of nanoparticles in aqueous solution, freeze drying process is widely being employed. (Thorat et al., 2012)

1.10.2.1 Freeze-drying of nanoparticles
Freeze-dried of nanoparticles imparts certain desirable characteristics to nanoparticles which includes the preservation of the primary physical and chemical characteristics of the product (elegant cake appearance, faster reconstitution, an acceptable dispersion and low particle size distribution, maintaining the therapeutic efficacy of encapsulated drug) with an acceptable relative humidity and most importantly improved storage stability. For obtaining product with high quality, it is important to keep a precise control on the formulation steps, the freeze-drying process and the storage conditions.

1.10.2.1.1 Importance of the formulation
The ultimate target of the formulations scientist is to identify the right formulation conditions, to use right excipients in optimal quantities, and the right dosage form to maximize biological activity, safety, stability and marketability of a particular product with minimum toxic effects. If the formulation is required to be freeze-dried, it would be important to adapt the formulation, taking into account the thermophysical properties of
the nanoparticle suspensions. Many components of the nanoparticles formulation have a crucial effect on the stability and resistance of nanoparticles to the different stresses during freeze-drying, as the type and the concentration of cryoprotectant, the nature of surfactant, the chemical groups attached to the nanoparticles surface, or the polymer used to form the nanoparticles. For this reason, proper selection of all components of the nanoparticles formulation must be performed before the start of freeze-drying. (Torchilin et al., 2006)

1.10.2.1.2 Use of cryo and lyoprotectant

Freeze-drying may expose the formulations to many stresses that could destabilize colloidal suspension of nanoparticles, especially, the stress of freezing and dehydration. It is well known that during the freezing of a sample there is a phase separation into ice and cryo-concentrated solution resulting in faster agglomeration of particles. In the case of nanoparticles suspension, the cryo-concentrated phase is composed of nanoparticles and the other components of the formulation as free surfactants, buffers, and unloaded drugs. This high concentration of particulate system may induce aggregation and in some cases irreversible fusion of nanoparticles. Further, The crystallization of ice may exercise a mechanical stress on nanoparticles leading to their destabilization. For these reasons, special excipients must be added to the suspension of nanoparticles before freezing to protect these fragile systems. (Shegokar et al., 2011) These excipients are usually added in order to protect the product from freezing stress (cryoprotectant) or drying stress (lyoprotectant) leading to its better stability upon storage. The most popular cryoprotectants encountered in the literature for freeze-drying nanoparticles are sugars: trehalose, sucrose, glucose and mannitol. These sugars are known to vitrify at a specific temperature denoted $T_g'$. The immobilization of nanoparticles within a glassy matrix of cryoprotectant can prevent their aggregation and protect them against the mechanical stress caused by ice crystals. Generally, freezing must be carried out below $T_g'$ of a frozen amorphous sample or below Teu (eutectic crystallization temperature) which is the crystallization temperature of soluble component as a mixture with ice, if it is in a crystalline state in order to ensure total solidification of the sample. Trehalose seems to be a preferable cryoprotectant for biomolecules owing to its many advantages in comparison with the other sugars as: less hygroscopicity, an absence of internal hydrogen bounds which allows more flexible formation of hydrogen bonds with nanoparticles during
freeze-drying, very low chemical reactivity and finally, higher glass transition temperature \( T_g' \). The level of stabilization afforded by sugars generally depends on their concentrations. It has been proved that trehalose is more effective for stabilizing both comprotol (glycerol behenate) solid lipid nanoparticles and glycerol trilaurate SLN during freeze-drying at concentration 15%. Further, the weight ratio cryoprotectant nanoparticles is important for stabilizing nanoparticles. A complete redispersion of poly(lactide acid-co-ethylene oxide) nanoparticles after freeze-drying can be obtained when trehalose is added to the nanoparticles suspension at a weight ratio trehalose: nanoparticles (1:1). Some of cryoprotectants used in literature for the freeze-drying of Nanoparticles Glucose, Sucrose, Trehalose, Lactose, Mannitol, Sorbitol, Aerosil (colloidal silicon dioxide), Maltose, Poly(vinyl pyrrolidone), Fructose, Dextran, Glycerol, Poly(vinyl alcohol), Glycine, Hydroxypropyl-\( \beta \)-cyclodextrin, Gelatin during freeze-drying of polymer- DNA complex as gene delivery system. On the other hand, in some cases, increasing cryoprotectant concentration to a certain level may eventually reach a limit of stabilization and even results in destabilization of nanoparticles. For example, particle aggregation increased with higher glucose concentration during freeze-drying of cationically modified silica nanoparticles. The nanoparticles concentration has a crucial effect on the success of freeze-drying. This effect was investigated in the case of freeze-drying of poly(lactide acid)-poly(ethylene oxide) copolymer nanoparticles. It has been reported that regardless of the amount of lyoprotectant added (trehalose), the nanoparticles concentration in the suspension prior to freeze-drying plays a key role in the lyoprotective mechanism. Usually, a freeze-thawing study should be realized before freeze-drying to select the best cryoprotectant which is able to conserve the properties of nanoparticles. (Abdal et al., 2006)
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### Table 1. Commonly Used Excipients in freeze-drying of Pharmaceutical Products

<table>
<thead>
<tr>
<th>Type</th>
<th>Substance</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>Phosphate, Citrate, Histidine, Borate</td>
<td>Adjustment of pH changes during prefreezing and freeze drying</td>
</tr>
<tr>
<td>Tonicity adjusters</td>
<td>Mannitol, Sucrose, Glycerine, Glycerol and Sodium chloride</td>
<td>Yielding an isotonic solution and control osmotic pressure</td>
</tr>
<tr>
<td>Bulking agents</td>
<td>Hydroxy ethyl starch, Trehalose, Mannitol, Lactose and Glycerine</td>
<td>Provides bulk to the formulation especially when the conc. of product to freeze dry is very low to handle.</td>
</tr>
<tr>
<td>Collapse temperature modifiers</td>
<td>Dextran, Hydroxypropyl beta cyclodextrin, PEG</td>
<td>Increase collapse temp. of the product in order to get higher drying temp.</td>
</tr>
<tr>
<td>Stabilizer or cryoprotectents</td>
<td>Sucrose, Lactose, Glucose, Trehalose Glycerol, Mannitol, Sorbitol, Glycerine, PEG, PVP etc.</td>
<td>Protect the product during freeze-drying against the freezing and drying stresses</td>
</tr>
</tbody>
</table>

1.11 Physico-chemical characterization of Nanoparticles

It is very important to characterize the freeze-dried product and to investigate the conservation of the nanoparticle properties.

1.11.1 Structural characterization

Structural characterization plays an important role in determining various attributes of a nanoparticulate system like shape, size, surface morphology, spatial distribution, density, geometric feature etc. Scanning electron microscopy (SEM) produces the image down to length scales of 10 nm and provides valuable information regarding surface topology, structural arrangement, spatial distribution as well as surface morphology of nanoparticles. Transmission electron microscopy (TEM) and high resolution TEM are more powerful imaging tools than SEM and give more detailed geometrical features and information like crystal structure, quality, and orientation of nanoparticles along with varying density of the
phases involved. Moreover, scanning tunneling probe such as scanning tunneling microscope (STM), electrical field gradient microscopy (EFM), and scanning thermal microscopy, combined with atomic force microscopy (AFM) are also now being employed to illustrate structural, electronic, magnetic and thermal properties besides topographical properties of nanosystems. (Tan et al., 2010)

1.11.2 Particle Size Distribution
Particle size distribution and polydispersity index are one of the most important aspect of formulation of nanosystems, efforts are always made to achieve a system with narrow particle size distribution with lowest polydispersity index. Some techniques to determine the particle size distribution are dynamic light scattering techniques, microscopic techniques etc. The laser diffraction technique is used to detect microparticles or possible aggregates of drug nanoparticles. (Chasteingner et al., 2006)

1.11.3 Particle Charge / Zeta Potential
Zeta potential is the charge at particles mobile surface and is used to determine the degree of flocculation or deflocculation in nanosystems. Zeta potential measurement is carried out to optimize formulation parameters and to make predictions regarding the storage stability of the colloidal dispersion. Its value may be positive or negative depending on nature of, drug, polymer adsorbed ions. A sufficiently high zeta potential (positive or negative) indicates that the system shall be deflocculated as for aggregation particles have to overcome the electrostatic energy barrier.

1.11.4 Crystalline Status
Differential scanning calorimetry, X ray diffraction and other analytical methods are used to assess any possible changes brought about in the physical form, amorphous or crystalline structure and other polymorphic changes in the drug during formulation. The presence of different polymorphs can also be assessed by X ray diffractometer.

1.11.5 Toxicity Evaluation
Nanoparticles are also associated with some acute and long term toxicities determined in various animal models. Some important acute toxicities associated with nanosystem are enhanced endocytosis resulting in inflammation and granuloma formation; oxidative stress causing cell death due to free radical generation and altered and/or modified protein/gene structure resulting in immune responses. The long-term toxicities associated with
nanosystems are bioaccumulation, poor biodistribution and ultimate fate of nanosystem in body. (Jain et al., 2007)

1.11.6 Macroscopic aspect of freeze-dried product

A freeze-dried product is observed to assess of the final volume and the appearance of the cake. One of the desired characteristics of a freeze-dried pharmaceutical form is to yield an intact cake occupying the same volume as the original mass. An attentive microscopic examination of the freeze dried cake must be carried out in order to detect any shrinkage or collapse of the formulation. (Chasteigner et al., 1996)

1.11.7 Reconstitution time

To rehydrate the freeze-dried nanoparticles one must add the same volume of water lost after lyophilization. The time of reconstitution may be recorded. In general, freeze-dried product rehydrates immediately after the addition of water, but in some cases, a long reconstitution time could be obtained as in the case of collapsed formulations. Many methods could be used to achieve the re-suspension of freeze dried nanoparticles after the addition of water, as manual shaking, vortexing or sonication to ensure full re-suspension. Measurement of nanoparticles size and zeta potential after freeze-drying after reconstitution, nanoparticles size must be measured by photon correlation spectroscopy or another technique. The conservation of a nanoparticle diameter size after freeze-drying is considered as a good indication of a successful freeze-drying cycle. In general, the ratio of nanoparticles size after and before freeze-drying may be calculated. A value near from one indicates the conservation of nanoparticles size, whereas an important value of this ratio indicates the aggregation of nanoparticles. Furthermore, the index of polydispersity may be recorded after lyophilization. This index gives also an idea about the distribution of nanoparticles size and its value must be compared to the value before freeze-drying, to evaluate the conservation of nanoparticles distribution. The measurement of zeta potential is a good method to evaluate the state of nanoparticles surface and to detect any eventual modification after freeze-drying. Furthermore, it can be used to study the interaction between the cryoprotectant molecules and the nanoparticles surface. It has been found that the addition of 10% of sucrose to itraconazole loaded poly(ε-caprolactone) nanospheres suspension before freeze-drying decreased the negative surface charge from $-40.9 \text{ mV}$ to $-20.4 \text{ mV}$. The authors explain this by the fact that nanosphere surface being masked as a
result of hydrogen bonding between OH groups of the cryoprotectant agent and the surface of the nanospheres. After freeze-drying, the decrease in the negative surface charge is accentuated, showing a rearrangement of the surfactants (poloxamer) at the surface of the nanospheres, leading to a possible desorption of itraconazole molecules. (Abdelwahed et al., 2006)

1.11.8 Microscopic observation of freeze-dried product

The microscopic visualization of freeze-dried product is a direct way on the one hand to observe the microstructure of the freeze-dried matrix, on the other hand to prove the conservation of nanoparticles integrity and to observe whether any modification has occurred on their morphology. Many high resolution microscopic techniques are being used to observe the nanoparticle formulation after freeze-drying: transmission electron microscopy (TEM), cryogenic transmittance electron microscopy (cryo-TEM), atomic force microscopy (AFM), scanning electronic microscopy (SEM), environmental scanning electronic microscopy (ESEM). TEM was also used to observe freeze-dried itraconazole-loaded nanospheres and poly (ε-caprolactone) nanocapsules after reconstitution. It is clear from TEM image that nanocapsules were well conserved after freeze-drying using PVP as cryoprotectant. The polymeric membrane was intact around the oily cavity of nanocapsules. An amorphous matrix of PVP could be observed at the outer surface of nanocapsules. Furthermore, freeze-dried core shell nanoparticles have been imaged by cryogenic transmittance electron microscopy to verify the formation of core/shell nanoparticles. Freeze-dried cationically modified silica nanoparticles using 5% of trehalose as cryoprotectant could be observed by AFM. It was found from AFM images, that trehalose formed a matrix into which the nanoparticles were inter dispersed. ESEM imaging showed spherical mono disperse nanocapsules being well conserved after freeze-drying. ESEM offers the possibility to control the dehydration of sample by gradual reduction of pressure and temperature in the sample chamber. Such samples can be observed in a hydrated state without a complete drying which prevents the observation of individual nanocapsules. Further, This technique has the ability to image wet systems without prior sample preparation. Finally, ESEM is the best technique for observing of lyophilized nanocapsules in a hydrated state. The advantages of ESEM over SEM for observing colloidal particles with minimal perturbation are the possibility to observe
hydrated samples in their native state, without need of conductive coating of the samples and no need of the preparation of the samples. ESEM is the most adequate technique to observe nanoparticles in a hydrated state. (Veiseh et al., 2010)

1.11.9 Thermal analysis by differential scanning calorimetry (DSC)

During storage, freeze-dried nanoparticles included within a vitrified matrix of lyoprotectant must be stored at a temperature below the temperature of glass transition (Tg) of the dried formulation to prevent any shrinkage of the freeze-dried cake or any destabilization of included nanoparticles as a result of lyoprotectant crystallization. The temperature of glass transition may be determined by differential scanning calorimetry. This technique is very useful to study the interaction between the lyoprotectant and the nanoparticles. For example, in the case of solid lipid nanocapsules freeze-dried with trehalose, DSC study points out a complexation between lecithin (forming the shell of nanocapsules) and trehalose, reinforcing the stabilizing properties of lecithin. (Arora et al., 2012)

1.11.10 Drug content determination

The drug content in nanoparticles must be determined by an adequate analytical method measuring both free drug concentration as well as entrapped drug concentration and its value must be compared to that before freeze-drying to detect any leakage of drug from nanoparticles during freeze-drying. (Chasteingner et al., 2006)

1.11.11 Powder surface analysis

The elemental composition of the powder surface of freeze-dried nanocapsules can be analyzed by electron spectroscopy for chemical analysis (ESCA). This technique is based on the emission of electrons, in response to irradiation by photons of sufficient energy. These electrons are emitted at energies characteristics of the atoms from which they are emitted. ESCA has been reported to be used for studying the surface modification of nanoparticles, and the adsorption of proteins at the air/liquid interface during spray-drying and adsorption on the ice crystals surface in the freeze-dried product. Such studies have a significant importance especially in the case of freeze-drying of immuno-nanoparticles which have antibodies adsorbed at their surface. The adsorption of protein at the interface ice/liquid during the freezing can disrupt their native fold and results in surface induced denaturation of proteins. Surfactants may drop surface tension of protein solutions and
reduce the driving force of protein adsorption at the interface ice/liquid. Low concentrations of nonionic surfactants such as Tween 80 are often sufficient to prevent surface adsorption. (Abdull et al., 2006)

1.11.12 Study of water sorption and determination of residual moisture
The thermal and the structure properties of freeze dried nanoparticles are influenced by residual moisture present in the product. Residual moisture is determined by the water desorption process during secondary drying. Sorption isotherm of water study is realized in order to determine on the one hand the degree of hygroscopicity of the product and to assess the ease in secondary drying. In general, the easier the water adsorption, the easier water desorption. The content of residual moisture in freeze-dried nanoparticles can be determined by Karl Fischer titration or by other methods as the gravimetric method or the thermal gravimetric analysis (TGA). (Sunder et al., 2010)

1.12 Nanoparticles in vivo studies
Nanoencapsulation can significantly alter a drug's pharmacokinetics as well as pharmacodynamic properties. While free drug distributes in all tissues and organs, encapsulated drug distribution is affected by other characteristics of the nanoparticle formulation. However, for the encapsulated drug to deposit in tumour tissue via the EPR effect, it must remain associated with the carrier long enough, and the carrier itself must exhibit prolonged plasma residence time. It has been established that particle size distribution and surface properties can greatly affect the behaviour of the nanoparticulate drug after its IV administration. Typically, particles with mean diameters between 100 and 200 nm with a neutral hydrophilic polymer coating, such as PEG, exhibit stealth properties, thus resisting recognition by components of the immune system and exhibiting prolonged plasma circulation times in vivo. (Gaucher et al., 2010)

1.13 Nanoparticles available in National and International Market
Various types of drugs loaded polymeric nanoparticles are available in the Pharmaceutical market, some examples are shown in Table 2. (Ochekpe et al., 2009)
## Table 2. Marketed Preparations of Nanoparticles

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Brand Name</th>
<th>Active Ingredients</th>
<th>Indications</th>
<th>Name of Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adagen</td>
<td>Adenosine deaminase</td>
<td>Severe combined immune disease (SCID), Adenosine deaminase (ADA) enzyme deficiency</td>
<td>Ezon Pharmaceuticals inc., Bridgewater, NJ, USA</td>
</tr>
<tr>
<td>2</td>
<td>Oncaspar</td>
<td>PEG aspargase</td>
<td>Acute Lymphoblastic leukaemia</td>
<td>Enzon Pharmaceuticals inc., NJ, USA</td>
</tr>
<tr>
<td>3</td>
<td>Copaxone</td>
<td>Glatiramer Acetate</td>
<td>Relapsing-remittting multiple sclerosis</td>
<td>Teva pharmaceuticals, Tikva, Isreal</td>
</tr>
<tr>
<td>4</td>
<td>Macugen</td>
<td>Pegaptanib Sodium</td>
<td>All types of neovascular age-related macular degeneration</td>
<td>Nektar Therapeutics, San Carlos, CA, USA; OSI Pharmaceuticals, Melville, NY, USA</td>
</tr>
<tr>
<td>5</td>
<td>Pegasys</td>
<td>Peg-interferon alfa-2a</td>
<td>Hepatitis C</td>
<td>Nektar Therapeutics, CA, USA</td>
</tr>
<tr>
<td>6</td>
<td>Neulasta</td>
<td>Pegfilgrastim</td>
<td>Chemotherapy induced Neutopenia</td>
<td>Nektar Therapeutics, CA, USA; Amgen inc, Thousand Oaks, CA, USA</td>
</tr>
<tr>
<td>7</td>
<td>PEG-INTRON</td>
<td>PEG-interferon alfa-2b</td>
<td>Hepatitis C</td>
<td>Nektar therapeutics, CA, USA</td>
</tr>
<tr>
<td>8</td>
<td>Somavert</td>
<td>Pegvisomant</td>
<td>Acromegaly</td>
<td>Nektar Therapeutics, CA, USA</td>
</tr>
<tr>
<td>9</td>
<td>Protein (Albumin) Nanoparticles</td>
<td>Abraxane</td>
<td>Breast cancer</td>
<td>Abraxix bioscience, Losangles, CA, USA, Astra Zeneca, Landon, UK</td>
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</table>
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<table>
<thead>
<tr>
<th></th>
<th>Rapamune</th>
<th>Sirolimus</th>
<th>Immunosuppressant</th>
<th>Wyeth Pharmaceuticals</th>
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<td></td>
<td></td>
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<tr>
<td>11</td>
<td>Megace ES</td>
<td>Megestrol acetate</td>
<td>Treatment of anorexia, cachexia</td>
<td>Par Pharmaceuticals</td>
</tr>
<tr>
<td>12</td>
<td>Emend</td>
<td>Aprepitant</td>
<td>Antiemetic</td>
<td>Merck</td>
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<td>13</td>
<td>Tricor</td>
<td>Fenofibrate</td>
<td>Antihyperlipidemic agent</td>
<td>Abbott Laboratories</td>
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<tr>
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<td>Triglide</td>
<td>Fenofibrate</td>
<td>Antihyperlipidemic agent</td>
<td>Skye Pharma</td>
</tr>
<tr>
<td>15</td>
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<td>2-Methoxy estradiol</td>
<td>Estrozen metabolite</td>
<td>Entre Med Inc.</td>
</tr>
<tr>
<td>16</td>
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<td>Cyclosporine</td>
<td>Immunosuppressant</td>
<td>Novartis</td>
</tr>
<tr>
<td>17</td>
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<td>Cyclosporine</td>
<td>Immunosuppressant</td>
<td>Abbott Laboratories</td>
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<td>Ritonavir</td>
<td>Anti-retrovial</td>
<td>Abbott Laboratories</td>
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<td>Saquinavir</td>
<td>Anti-retrovial</td>
<td>Hoffman-La Roche</td>
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<tr>
<td>20</td>
<td>Feridex/Endorm</td>
<td>Iron Nanoparticles</td>
<td>Liver Tumor Imaging</td>
<td>Advance Magnetics(USA)</td>
</tr>
</tbody>
</table>
1.14 Drug and Polymer Profile

1.14.1 Drug Profile

Drug Name
Meloxicam

BCS Classification
BCS Class II

Official status
I.P., U.S.P., Martindale, Merck Index.

Category
Non Steroidal Anti-inflammatory Drug

Empirical formula
\( C_{14}H_{13}N_{3}O_{4}S_{2} \)

Chemical Structure

![Chemical Structure of Meloxicam]

IUPAC name
4-hydroxy-2-methyl-N-(5-methyl,2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide.

State
A pale yellow crystalline powder
Molecular Weight
351.401

Melting point
242-245 °C

Solubility
Soluble in Dimethyl formamide (DMF), slightly soluble in acetone, practically insoluble in water.

Dissociation constant (pKa)
3.02

Partition coefficient
4.08

Bioavailability
89% (After oral administration).

Dosage and Administration
7.5 – 15 mg daily orally.

Half-life
15-20 h

Mechanism of action
Meloxicam inhibits cyclooxygenase (COX-2), the enzyme responsible for converting arachidonic acid into prostaglandin (PG-H₂) the first step in the synthesis of prostaglandins, which are mediators of inflammation. Meloxicam has been shown, especially at its low therapeutic dose, selectively to inhibit COX-2 over COX-1.

Absorption
Meloxicam is well absorbed from the gastrointestinal tract, which is reflected by a high absolute bioavailability of 89% following oral administration. Following single dose administration of meloxicam, mean maximum plasma concentrations are achieved within
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5-6 hours for the tablets.

**Biotransformation**

Meloxicam undergoes extensive hepatic biotransformation.

**Protein binding**

Plasma protein binding is 99%.

**Therapeutic uses**

Meloxicam is used in the treatment of Osteoarthritis and Rheumatoid Arthritis.

**Toxicity**

As with other NSAIDs caution should be exercised when treating patients with a history of upper gastrointestinal disease and in patients receiving treatment with anticoagulants. Patients with gastrointestinal symptoms dosage regimen should be monitored.

**Adverse effects**

- Meloxicam tablets should not be given to patients who have developed signs of asthma, nasal polyps, angioedema or urticaria following the administration of aspirin or NSAIDs.
- Active or recent gastro-intestinal ulceration.
- Active Inflammatory Bowel Disease
- Severe hepatic insufficiency.
- Non-dialysed severe renal insufficiencies.

**Brand name**

Meloxicam is marketed under different brands names as Artaz tab, Ecwin, M-Cam tab, Meflam tab, Movac tab, Melocam, Muvik etc. ¹⁵

**Storage**

Preserve in well closed containers. Store at room temperature. (R-123,Sachan *et al.*, 2009)
1.14.2 Polymer Profile

1.14.2.1 Chitosan

Chemical name

Poly- β-(1, 4)-2-Amino-2-deoxy-D-glucose

Background

Chitosan is a natural linear biopolyamino saccharide obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose. Chitin is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as *aspergillus* and *mucor*. Chitin is a straight homopolymer composed of β-(1, 4)-linked N-acetyl-glucosamine units while chitosan comprise of copolymer of glucosamine and N-acetyl-glucosamine. Chitosan is a biodegradable, biocompatible, less toxic and mucoadhesive biopolymer. Its use as a pharmaceutical excipient is meanwhile well-established. It is a linear polyelectrolyte with reactive hydroxyl and amino groups (available for reaction or salt formation). Nitrogen in chitosan is mostly in form of 1°amine and gives amine reaction.

Chemical Structure

Chitosan is biocompatible with living tissues since it does not cause allergic reactions and rejection. It breaks down slowly to harmless products (amino sugars), which are completely absorbed by human body. Chitosan is a weak base and is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution (pH<6.5), which can convert the glucosamine units into a soluble form R-NH$_3^+$. It gets precipitated in alkaline solution and forms gels at lower pH. Commercially, chitosan is available in the form of dry flakes, solution and fine powder. It has an average molecular weight ranging between 3800 and 2,000,000 and is from 66 to 95% deacetylated.
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**Functional classification**
- Coating agent
- Disintegrant
- Film forming agent
- Mucoadhesive agent
- Tablet binder
- Viscosity increasing agent.

**Description**
Chitosan occurs as odourless, white to creamy white powder or flakes. Fiber formation is quite common during precipitation and may look like cotton.

**Alkalinity and acidity**
pH 4.0 – 6.0 (1% w/v aqueous sol.)

**Density**
1.35 – 1.40 g/cm$^3$

**Moisture content**
It is hygroscopic in nature and moisture content depends on temperature and relative humidity of surrounding air.

**Solubility**
It is sparingly soluble in water, practically insoluble in ethanol (95%). Chitosan dissolves readily in dilute and concentrated organic acid (except phosphoric and sulphuric acids). Upon dissolution amine groups of the polymer become protonated, resulting in formation of RNH$_3^+$.

**Stability and storage**
Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool and dry place (2-8 °C).

**Viscosity**
It acts as a pseudo-plastic material, exhibiting a decrease in viscosity with increasing rates of shear.

**Incompatibility**
Chitosan is incompatible with strong oxidizing agents.
Safety

Chitosan is generally regarded as non-toxic and non-irritant material. It is biocompatible with both healthy and infected skin. It has been shown to be biodegradable.(Kumar et al., 2000, Shukla et al., 2012, Shantha et al., 1995, Casettare et al., 2011)

1.14.2.2 Liquid Paraffin

Nonproprietary Names

BP : Liquid Paraffin
JP : Liquid Paraffin
PhEur : Paraffin, Liquid
USP : Mineral Oil

Synonyms

- Avatech
- Drakeol
- heavy mineral oil
- heavy liquid petrolatum
- liquid petrolatum
- paraffin oil
- paraffinum liquidum
- Sirius
- White mineral oil

Chemical Name

Mineral oil

Empirical Formula and Molecular Weight

Mineral oil is a mixture of refined liquid saturated aliphatic (C14–C18) and cyclic hydrocarbons obtained from petroleum

Functional Category

- Emollient
- lubricant
- oleaginous vehicle
- solvent
- vaccine adjuvant
Applications in Pharmaceutical Formulation or Technology

Mineral oil is used primarily as an excipient in topical pharmaceutical formulations, where its emollient properties are exploited as an ingredient in ointment bases. It is additionally used in oil-in-water emulsions, as a solvent, and as a lubricant in capsule and tablet formulations, and to a limited extent as a mold the preparation of microspheres and as a vaccine adjunct. Therapeutically, mineral oil has been used as a laxative. It is indigestible and thus has limited absorption. Mineral oil is used in ophthalmic formulations for its lubricant properties. It is also used in cosmetics and some food products.

Description

Mineral oil is a transparent, colorless, viscous oily liquid, without fluorescence in daylight. It is practically tasteless and odorless when cold, and has a faint odor of petroleum when heated.

Pharmacopeial specifications for mineral oil

Boiling point

\[ > 360°C \]

Flash point

210–224°C

Solubility

Practically insoluble in ethanol (95%), glycerine, and water; soluble in acetone, benzene, chloroform, carbon disulfide, ether, and petroleum ether. Miscible with volatile oils and fixed oils, with the exception of castor oil.

Viscosity (dynamic)

110–230 mPa s (110–230 cP) at 20°C

Stability and Storage Conditions

Mineral oil undergoes oxidation when exposed to heat and light. Oxidation begins with the formation of peroxides, exhibiting an ‘induction period’. Under ordinary conditions, the induction period may take months or years. However, once a trace of peroxide is formed, further oxidation is autocatalytic and proceeds very rapidly. Oxidation results in the formation of aldehydes and organic acids, which impart taste and odor. Stabilizers may be added to retard oxidation; butylated hydroxyanisole, butylated hydroxytoluene, and alpha tocopherol are the most commonly used antioxidants. Mineral oil may be sterilized by dry
heat. Mineral oil should be stored in an airtight container, protected from light, in a cool, dry place.

**Incompatibilities**

Incompatible with strong oxidizing agents.

**Method of Manufacture**

Mineral oil is obtained by distillation of petroleum. The lighter hydrocarbons are first removed by distillation and the residue is then redistilled between 330–390°C. The distillate is chilled and the solid fractions are removed by filtration. The filtrate is then further purified and decolorized by high-pressure hydrogenation or sulfuric acid treatment; the purified filtrate is then filtered through adsorbents. The liquid portion obtained is distilled and the portion boiling below 360°C is discarded. A suitable stabilizer may be added to the mineral oil.

**Safety**

Mineral oil is used as an excipients in a wide variety of pharmaceutical formulations. It is also used in cosmetics and in some food products. Therapeutically, mineral oil has been used in the treatment of constipation, as it acts as a lubricant and stool softener when taken orally. Daily doses of up to 45mL have been administered orally, while doses of up to 120mL have been used as an enema. However, excessive dosage of mineral oil, either orally or rectally, can result in anal seepage and irritation, and its oral use as a laxative is not considered desirable. Chronic oral consumption of mineral oil may impair the appetite and interfere with the absorption of fat-soluble vitamins. Prolonged use should be avoided. Mineral oil is absorbed to some extent when emulsified and can lead to granulomatous reactions. Similar reactions also occur upon injection of the oil. Injection may also cause vasospasm. The most serious adverse reaction to mineral oil is lipoid pneumonia caused by aspiration of the oil. Mineral oil can enter the bronchial tree without eliciting the cough reflex. With the reduction in the use of mineral oil in nasal formulations, the incidence of lipoid pneumonia has been greatly reduced. However, lipoid pneumonia has also been associated with the use of mineral oil-containing cosmetics and ophthalmic preparations. It is recommended that products containing mineral oil not be used in very young children, the elderly, or persons with debilitating illnesses. Given its widespread use in many topical products, mineral oil has been associated with few instances of allergic reactions. The
WHO has not specified an acceptable daily intake of mineral oil given the low concentration consumed in foods.

Handling Precautions
Observe precautions appropriate to the circumstances and quantity of material handled. Avoid inhalation of vapors and wear protective clothing to prevent skin contact. Mineral oil is combustible.

Regulatory Status
GRAS listed. Accepted in the UK for use in certain food applications. Included in the FDA Inactive Ingredients Database (dental preparations; IV injections; ophthalmic preparations; oral capsules and tablets; otic, topical, transdermal, and vaginal preparations). Included in nonparenteral medicines licensed in the UK.

Related Substances
Mineral oil and lanolin alcohols
Light mineral oil
Paraffin
Petrolatum (Neil et al., 2006)

1.14.2.3 Sodium Bicarbonate

Nonproprietary Names
BP : Sodium Bicarbonate
JP : Sodium Bicarbonate
PhEur : Sodium Hydrogen Carbonate
USP : Sodium Bicarbonate

Synonyms
- Baking soda
- E500
- Effer-Soda
- Monosodium carbonate
- Natrii hydrogenocarbonas
- Sal de Vichy
- Sodium acid carbonate
- Sodium hydrogen carbonate
**Chemical Name**  
Carbonic acid monosodium salt  

**Empirical Formula**  
NaHCO₃  

**Molecular Weight**  
84.01  

**Functional Category**  
- Alkalizing agent  
- Therapeutic agent.  

**Applications in Pharmaceutical Formulation or Technology**  
Sodium bicarbonate is generally used in pharmaceutical formulations as a source of carbon dioxide in effervescent tablets and granules. It is also widely used to produce or maintain an alkaline pH in a preparation. In effervescent tablets and granules, sodium bicarbonate is usually formulated with citric and/or tartaric acid. Combinations of citric and tartaric acid are often preferred in formulations as citric acid alone produces a sticky mixture that is difficult to granulate, while if tartaric acid is used alone, granules lose firmness. When the tablets or granules come into contact with water, a chemical reaction occurs, carbon dioxide is evolved, and the product disintegrates. Melt granulation in a fluidized bed dryer has been suggested as a one-step method for the manufacture of effervescent granules composed of anhydrous citric acid and sodium bicarbonate, for subsequent compression into tablets. Tablets may also be prepared with sodium bicarbonate alone since the acid of gastric fluid is sufficient to cause effervescence and disintegration. Sodium bicarbonate is also used in tablet formulations to buffer drug molecules that are weak acids, thereby increasing the rate of tablet dissolution and reducing gastric irritation. The effects of tablet binders, such as polyethylene glycols, microcrystalline cellulose, silicified microcrystalline cellulose, pregelatinized starch, and povidone, on the physical and mechanical properties of sodium bicarbonate tablets have also been investigated. Additionally, sodium bicarbonate is used in solutions as a buffering agent for erythromycin, lidocaine, local anesthetic solutions, and total parenteral nutrition (TPN) solutions. In some parenteral formulations, e.g. niacin, sodium bicarbonate is used to produce a sodium salt of the active ingredient that has enhanced solubility. Sodium bicarbonate has also been used
as a freeze-drying stabilizer and in toothpastes. Recently, sodium bicarbonate has been used as a gas-forming agent in alginate raft systems and in floating, controlled release oral dosage forms for a range of drugs. Tablet formulations containing sodium bicarbonate have been shown to increase the absorption of paracetamol and improve the stability of levothyroxine. Sodium bicarbonate has also been included in formulations of vaginal bioadhesive tablets and in carbon dioxide releasing suppositories. Therapeutically, sodium bicarbonate may be used as an antacid and as a source of the bicarbonate anion in the treatment of metabolic acidosis. Sodium bicarbonate may also be used as a component of oral rehydration salts and as a source of bicarbonate in dialysis fluids. It has also been suggested as a means of preventing radiocontrast-induced nephrotoxicity. Sodium bicarbonate is used in food products as an alkali or as a leavening agent, e.g. baking soda.

**Description**

Sodium bicarbonate occurs as an odorless, white, crystalline powder with a saline, slightly alkaline taste. The crystal structure is monoclinic prisms. Grades with different particle sizes, from a fine powder to free-flowing uniform granules, are commercially available.

**Acidity/alkalinity**

pH = 8.3 for a freshly prepared 0.1M aqueous solution at 25°C alkalinity increases on standing, agitation or heating.

**Density (bulk)**

0.869 g/cm³

**Density (tapped)**

1.369 g/cm³

**Density (true)**

2.173 g/cm³

**Freezing point depression**

0.381°C (1% w/v solution)

**Melting point**

270°C (with decomposition)

**Moisture content**

Below 80% relative humidity, the moisture content is less than 1% w/w. Above 85% relative humidity, sodium bicarbonate rapidly absorbs excessive amounts of water and
may start to decompose with loss of carbon dioxide.

**Stability and Storage Conditions**

When heated to about 50°C, sodium bicarbonate begins to dissociate into carbon dioxide, sodium carbonate and water. On heating to 250–300°C, for a short time, sodium bicarbonate is completely converted into anhydrous sodium carbonate. However, the process is both time- and temperature-dependent, with conversion 90% complete within 75 minutes at 93°C. The reaction proceeds via surface-controlled kinetics. When sodium bicarbonate crystals are heated for a short period of time, very fine needle-shaped crystals of anhydrous sodium carbonate are formed on the sodium bicarbonate surface. The effects of relative humidity and temperature on the moisture sorption and stability of sodium bicarbonate powder have been investigated. Sodium bicarbonate powder is stable below 76% relative humidity at 25°C and below 48% relative humidity at 40°C. At 54% relative humidity, the degree of pyrolytic decarboxylation of sodium bicarbonate should not exceed 4.5% in order to avoid detrimental effects on stability. At ambient temperatures, aqueous solutions slowly decompose with partial conversion into the carbonate; the decomposition is accelerated by agitation or heat. Aqueous solutions begin to break up into carbon dioxide and sodium carbonate at about 20°C, and completely on boiling. Aqueous solutions of sodium bicarbonate may be sterilized by filtration or autoclaving. To minimize decomposition of sodium bicarbonate by decarboxylation on autoclaving, carbon dioxide is passed through the solution in its final container, which is then hermetically sealed and autoclaved. The sealed container should not be opened for at least 2 hours after it has returned to ambient temperature, to allow time for the complete reformation of the bicarbonate from the carbonate produced during the heating process. Aqueous solutions of sodium bicarbonate stored in glass containers may develop deposits of small glass particles. Sediments of calcium carbonate with traces of magnesium or other metal carbonates have been found in injections sterilized by autoclaving. These are due to impurities in the bicarbonate or to extraction of calcium and magnesium ions from the glass container. Sedimentation may be retarded by the inclusion of 0.01–0.02% disodium edetate. Sodium bicarbonate is stable in dry air but slowly decomposes in moist air and should therefore be stored in a well-closed container in a cool, dry place.
Incompatibilities
Sodium bicarbonate reacts with acids, acidic salts, and many alkaloidal salts, with the evolution of carbon dioxide. Sodium bicarbonate can also intensify the darkening of salicylates. In powder mixtures, atmospheric moisture or water of crystallization from another ingredient is sufficient for sodium bicarbonate to react with compounds such as boric acid or alum. In liquid mixtures containing bismuth sub-nitrate, sodium bicarbonate reacts with the acid formed by hydrolysis of the bismuth salt. In solution, sodium bicarbonate has been reported to be incompatible with many drug substances such as ciprofloxacin, amiodarone, nicardipine, and levofloxacin.

Method of Manufacture
Sodium bicarbonate is manufactured either by passing carbon dioxide into a cold saturated solution of sodium carbonate or by the ammonia–soda (Solvay) process, in which first ammonia and then carbon dioxide is passed into a sodium chloride solution to precipitate sodium bicarbonate while the more soluble ammonium chloride remains in solution.

Safety
Sodium bicarbonate is used in a number of pharmaceutical formulations including injections and ophthalmic, otic, topical, and oral preparations. Sodium bicarbonate is metabolized to the sodium cation, which is eliminated from the body by renal excretion, and the bicarbonate anion, which becomes part of the body’s bicarbonate store. Any carbon dioxide formed is eliminated via the lungs. Administration of excessive amounts of sodium bicarbonate may thus disturb the body’s electrolyte balance, leading to metabolic alkalosis or possibly sodium overload with potentially serious consequences. The amount of sodium present in antacids and effervescent formulations has been sufficient to exacerbate chronic heart failure, especially in elderly patients. Orally ingested sodium bicarbonate neutralizes gastric acid with the evolution of carbon dioxide and may cause stomach cramps and flatulence. When used as an excipient, sodium bicarbonate is generally regarded as an essentially nontoxic and nonirritant material.

Handling Precautions
Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended.
**Chapter 1: Introduction**

**Regulatory Status**
Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Database (injections; ophthalmic preparations; oral capsules, solutions, and tablets). Included in parenteral (intravenous infusions and injections) and nonparenteral medicines (chewing gums; ear drops; eye lotions; oral capsules, chewable tablets, effervescent powders, effervescent tablets, granules, soluble tablets, orodispersible tablets, and tablets; suppositories and suspensions) licensed in the UK.

**Related Substances**
Potassium bicarbonate. (Indian Pharmacopoeia, 2010)

### 1.14.2.4 Dimethyl formamide

**Synonym**
DMF

**IUPAC Name**
\( \text{N,N-Dimethyl formamide} \)

**Molecular Formula**
\( \text{C}_3\text{H}_7\text{NO} \)

**Molecular Weight**
73.1

**Chemical Structure**

![Chemical Structure of Dimethyl Formamide](image)

**Description**
Dimethylformamide (DMF) is a colorless, high-boiling, mobile, polar liquid with a faint, characteristic odor. It does not decompose on distillation and is freely miscible with water, alcohols, ethers, ketones, esters, carbon disulfide and chlorinated and aromatic hydrocarbons. It is either immiscible or only partly miscible with aliphatic hydrocarbons. Even at elevated temperatures, aqueous solutions of DMF have very little tendency to hydrolyze. However the addition of acids or bases accelerate hydrolysis to formic acid and 1,3-dimethylamine. DMF is an aprotic solvent with a high dielectric constant.
Boiling point
153°C

Melting point
60.4°C

Solubility
Miscible with water and most common organic solvents

Vapour pressure
kPa at 20°C

Flash point
67°C, open cup

Safety
Dimethylformamide is a combustible liquid that may irritate the skin and eyes. Inhalation of vapors or mists may irritate the respiratory tract. DMF can be absorbed through the skin and can cause liver damage. Close attention must be paid to standard industrial hygiene measures.

General protective measures include: ensure work place is well ventilated · do not leave containers lying open storage containers must be grounded · personal protective equipment includes gloves, side shield, safety glasses or goggles, and aprons. Butyl rubber is one of the most protective materials; nitrile or neoprene gloves may be worn for short duration tasks. Always refer to the Material Safety Data Sheet (MSDS) for detailed information on health and safety.

Applications
The high solvent power of dimethylformamide can be ascribed to its molecular structure. DMF is an eminently suitable solvent for salts or compounds with a high molecular weight owing to the combined action of its small molecule, its high dielectric constant, its electron donor properties, and its ability to form complexes.

- solvents for plastics
- solvent for wire enamels
- selective absorbent for gases
- selective extractant
- solvent for electrolytes
DMF also offers tremendous scope as a feedstock for syntheses. An idea of its versatility in this respect is given by the following compounds:

- Aldehydes, e.g., introduction of the formyl group in aromatic or heterocyclic compounds in the Vilsmeier synthesis.
- DMF acetals
- amides
- amidines
- amine
- esters, e.g., esterification of carboxyl groups via DMF acetals
- heterocycles

**Storage & Handling**

Since DMF is hygroscopic, it must be stored under dry nitrogen if severe demands are imposed on the degree of anhydrazation. This is extremely important for preventing rust if DMF is stored in steel tanks. The vent lines should contain a suitable device for drying the air, e.g., silica gel dry cartridges. DMF for the production of acrylic fibers should be stored in aluminum tanks. Always refer to the Material Safety Data Sheet (MSDS) for detailed information on handling and disposal. (Furniss et al., 2003)

### 1.14.2.5 Polysorbate

**Synonyms**

Tween 80, Span 80, Alkest TW80

**IUPAC name**

Polyoxyethylene (20) sorbitan monooleate

**Molecular formula**

C_{64}H_{124}O_{26}
Chemical Structure

![Chemical Structure](image)

**Appearance**
Amber colored viscous water soluble liquid,

**Density**
1.06-1.09 g/mL, oily liquid

**Boiling point**
> 100°C

**Solubility**
Very soluble in water, soluble in ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene

**Hazards**
Irritant

**Application**
**Food Industry**
Polysorbate 80 is used as an emulsifier in foods, particularly in ice cream. Here, polysorbate is added to up to 0.5% (v/v) concentration and makes the ice cream smoother and easier to handle, as well as increasing its resistance to melting. Adding this substance prevents milk proteins from completely coating the fat droplets. This allows them to join together in chains and nets, which hold air in the mixture, and provide a firmer texture that holds its shape as the ice cream melts.

**Medical use**
Polysorbate 80 is an excipient that is used to stabilize aqueous formulations of medications for parenteral administration, and used as an emulsifier in the manufacture of the popular anti-arrhythmic amiodarone. It is also used as an excipient in some European and Canadian influenza vaccines. It is also used in the culture of Mycobacterium tuberculosis. (Neil et al., 2006)
1.14.2.6 Glutaraldehyde

Synonyms
Pentanedial, Glutardialdehyde, Glutaric acid dialdehyde, Glutaric aldehyde, Glutaric dialdehyde, 1,5-Pentanedia

IUPAC name
Pentane-1,5-dial

Appearance
A pungent colorless oily liquid

Molecular formula
C₅H₈O₂

Molecular Structure

\[
\text{H} - \text{C} - (\text{CH}_2)₃ - \text{C} - \text{H}
\]

Molecular weight
100.12 g/mol

Solubility
Soluble in water, alcohol, benzene

Density
1.06 g/mL

Melting point
-14 °C, 259 K, 7 °F

Boiling point
187 °C, 460 K, 369 °F

Applications
Glutaraldehyde is a chemical frequently used as a disinfectant and sterilizing agent against bacteria and viruses (2% solution), an embalming fluid and tissue fixative, a component of leather tanning solutions, and an intermediate in the production of certain sealants, resins, dyes, and electrical products. For commercial purposes, solutions of 99%, 50%, and 20% are available. Glutaraldehyde is also an atmospheric reaction product of cyclohexene. (Neil et al., 2006)
1.14.2.7 Petroleum Ether

**Synonyms**
Benzine

**Appearance**
Liquid

**Molecular Formula**
C\textsubscript{6}H\textsubscript{14}

**Molecular Structure**

\[
\text{ \hspace{1cm}}
\]

**Odor**
Slight

**Boiling Point**
90-100\(^{\circ}\)C

**Melting Point**
95 °C(lit.)

**Solubility**
Insoluble in cold water.

**Handling and Storage**

**Precautions**
Keep locked up. Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not breathe gas/fumes/vapor/spray. Avoid contact with eyes. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If you feel unwell, seek medical attention and show the label when possible.

**Storage**
Store in a segregated approved area. Keep container in a cool, well-ventilated area.

**Applications**
As a Pharmaceutical aids in various types of dosage forms. (Furniss et al., 2006)