Chapter I

CONCEPTS OF NANOPARTICLES AND NANOSCIENCE
Theoretical Characterization of Specific Fluorocarbon Nanoparticles – Drug Delivering Agents

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CONCEPTS OF NANOPARTICLES
AND NANOSCIENCE

1.1. DRUG DELIVERING PHENOMENON

Effective drug delivery has been the all time essential of human kind, which has attained a significant phase in the contemporary bio-medical era. The major aim of medicine has long been the early and accurate diagnosis of infection and to provide an efficient treatment without secondary effects. In conventional drug delivery systems such as oral ingestion, intravascular injection, etc. the medication is distributed throughout the body by the systematic blood circulation. This results in the increase of drug concentration in the affected as well as in the healthy parts of the body. As a consequence the maximum drug concentration cannot be achieved at the desired target site. In the present pharmaceutical science, the concept of site targeted drug delivery plays a vital role in making the drug action efficient on the diseased site.

The concept of site targeted drug delivery is, to deliver drug to the right place at right concentration for the right period of time. Targeted drug delivery is a method of delivering medication to a patient, in a manner, that increases the concentration of medication in the diseased parts of the body relative to others. The salient feature of the targeted drug delivery is to improve the therapeutic effectiveness of the drug while at the same time improving its safety.
Likely to be more important in the future is to construct an efficient drug carrier, as drugs become more complex and difficult to deliver. Nano sized drug delivering agents can overcome administration problems or it can be used to achieve improved targeting. Targeting will always involve the challenge of moving drug across biological membranes. The concept of drug delivery requires that the release of encapsulated drug be produced only at the diseased site with controllable rates. In order to achieve therapeutic levels of drug at the affected site without affecting healthy organs and tissues, it is important to recognize the physiological differences between the diseased and normal site. The phenomenon of delivering the drug is depicted in Fig. 1.

![Generalized Targeting Paradigm](image)

**Fig.1. Nanoparticles as a drug carrying agent [1]**

To ensure the proper or the maximum utility of drug, the drug life should be prolonged in the affected site to avoid the removal of drug. Controlled drug release should be achieved over an extended period of time
which retains the drug concentration within the therapeutic window as needed. An exciting potential solution is to encapsulate the drug in a biocompatible material, dissolving the drug in nano carriers (termed as nanosuspension /gels) that can be injected into the blood stream with the intention of delivering the drug to the required site. Hence to reach the pinnacle in drug therapy, development of new drugs alone is not sufficient yet a suitable drug carrier system has to be identified. Different methods of drug delivery are shown schematically in Fig. 2.

Fig.2. Drugs may be (a) attached to the membrane surrounding the microbubble, (b) drugs may be imbedded within the membrane itself, (c) materials, e.g., DNA, may be bound noncovalently to the surface of the microbubbles, (d) microbubbles might also be formulated to load the interior with drugs and gas, or (e) hydrophobic drugs can be incorporated into a layer of oily materials that forms a film around the microbubble, which is then surrounded by a stabilizing membrane [2]
1.2. NEED FOR DRUG DELIVERING CARRIERS

The drugs or the medical compounds have poor or low absorption at the present pH, this results in insufficient cellular uptake and rapid elimination which are the impediments for drug development. Hence a suitable pharmaceutical carrier, that is, a drug delivering agent is required. A drug delivering carrier can enhance a drug pharmacokinetic and cellular penetration. Obstacles arising from low drug solubility, degradation, fast clearance rate, non-specific toxicity and inability to cross biological barriers may be reduced by drug delivering agents. A drug delivering agent is required to be biocompatible with the process in the body as well as with the drug to be delivered. Of all the main features of a drug delivering agent is its size factor, since it has to travel efficiently inside the blood vessels of micro meter size. A successful delivering agent should have a long life in blood, allowing for their accumulation in pathological areas. The possible pathways for delivering a drug to specific cells by nano carriers are diffusion, particle fusion, internalization into cells, component exchange and convective flux or some combination of these mechanisms.

Drug delivering systems are essential to minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and a fraction of the drug has to be accumulated in the required zone. These carriers have to be slowly degradable, stimuli-reactive (eg. pH or temperature sensitive), and targeted. In this present arena, scientists are working on the
development of materials for controlled drug delivery. Based on their structure the different drug delivering agents are:

(i) nanoparticulate structures for encapsulation / dissolving drug in the agent
(ii) liposomes for passive or active targeting to sites of infection
(iii) biodegradable polymers for localized delivery of bioactive agents
(iv) water soluble polymers for increasing the half life of therapeutic proteins and reducing immune response
(v) linear polymers with side chains terminated in drugs

The few types of nano carriers which are used as drug delivering agent include micro bubbles, nanoparticles, micelles and liposomes. Theoretical analysis of these drug delivering systems are essential to improve their clinical translation of new generations of delivery systems; to understand potential alterations in mechanics of action of therapeutic agents when they are attached, complied or incorporated into biomaterials and to design new materials with improved properties.

1.3. NANOPARTICLES

Nanoparticles find their applications in variety of fields, few to be mentioned are drug delivery, cosmetics, printing ink, and in petroleum services. Nanometer-sized particles are in the same range of dimension as antibodies, membrane, receptors, nucleic acids and proteins. These bio-mimic features, together with their high surface to volume ratio and the possibility of
modulating their properties, make nanoparticles as a powerful tool for imaging, diagnosis and therapy. The physical and chemical properties like optical, magnetic, catalytic, thermodynamic and electrochemical, of nanoparticles are size dependent. Based on the constituents, nanoparticles are grouped as organic and inorganic nanoparticles [3]. Liposomes, dendrimers, carbon materials, perfluorocarbons, micelles, are examples of organic nanoparticles, whereas gold, silver, nickel, platinum and titanium oxide are examples of inorganic nanoparticles. The fundamental advantage of nanoparticles is their kinetic stability and rigid morphology. The characteristics that make the nanoparticle an important drug delivering agent are:

(i) improved solubility of poorly water-soluble drugs
(ii) possibility for modifications in their pharmacokinetics
(iii) increased drug half-life by reducing immunogenicity
(iv) increased specificity towards the target cell or tissue
(v) improved bioavailability
(vi) diminish drug metabolism and enable a more controllable release of therapeutic compound
(vii) deliver two or more drugs simultaneously for combination therapy (multifunctional nanoparticles)

In addition, nanoparticles act as contrast agent for medical imaging. Specific biological molecules can be imaged by administrating the nanoparticles at the systematic level. By reducing the possible side-effects, the
specificity and the image resolution of the diseased cells are increased. The demerits in optical imaging techniques while using organic fluorophores and fluorescence proteins gave a pathway to enter nanoparticles in this clinical diagnosis. Multifunctional nanoparticles are under trial where different functions are combined under a single construct. For example a core particle can be combined to a nanoparticle to identify its target and simultaneously the same particle can be modified with an imaging agent to monitor the drug transport process as shown in Fig. 3.

![Multifunctional nanoparticle](image)

**Fig. 3. Multifunctional nanoparticle**

Few merits of nanoparticles are:

(i) quantum dots are preferred in image diagnosis since it is resistant to photo bleaching, chemical and metabolic degradation

(ii) super paramagnetic iron oxide nanoparticles are used to increase the contrast in MRI applications, since it is limited by their insensitivity to low concentration of imaging agent

(iii) perfluorocarbon acts as an ultimate blood substitute
Stability of the nanoparticles was studied under stressed and accelerated conditions [4]. The results indicate that naked nanoparticles were not affected by major morphological changes under both stressful and accelerated storage conditions. Degradation of biodegradable nanoparticles is done by two mechanisms namely surface erosion and bulk erosion. Most of the time degradation of biodegradable nanoparticle is due to the combined effects of the two above mentioned mechanisms of which surface erosion occurs prior to bulk erosion. This shows that drugs are released initially by diffusion followed by degradation of nanoparticles. One of the most important strategies in establishing the drug delivering process is the size of the nanoparticles. When the particle size is reduced approximately to 100 nm, the uptake of these particles by non-targeted cells is minimized. Also the premature clearance of nanoparticle is less prone by minimizing its size. The fundamental advantage of nanoparticle is their kinetic stability and rigid morphology.

A thorough knowledge on size dependent properties of nanoparticles and formulating their potentials are essential to achieve the required purpose and need. Based on their morphology the generated nanoparticles are classified into polymeric nanospheres, and organic nanoparticles like liposomes, micelles, dentrimers, perfluorocarbon. The importances of these particles are discussed briefly.
1.3.1. Liposomes

Liposomes which are microscopic vesicles are spherical and composed of one or more phospholipid bi-layer shell surrounding discrete aqueous compartments. These were described as an efficient vesicle for the first time by Bangham in the middle of 1960s [5]. An aqueous volume enclosed by a lipid molecular membrane spontaneously forms a bilayer when these lipids are dispersed in aqueous media, giving rise to a population of vesicles known as liposomes. Their size could vary from tens of nanometer to tens of microns of diameter. Modifications of liposomes are possible so that they could entrap quantities of materials both within their aqueous compartment and within their membrane. Because of the similarity between the liposomes and the natural membranes, it acts as one of the best drug delivering agent. Liposomes which could be chemically modified may be exploited in areas such as drug targeting or immune modulation. The ability of liposomes to mimic the behaviour of natural membranes makes it to be degraded in the same pathway as the natural membranes do. This makes them a very safe and efficacious vehicle for medical applications. Alternatively liposomes can be composed of entirely artificial components chosen for their improved chemical properties. Its structure has been depicted in Fig.4.
Since 1970, liposomes have been widely investigated as drug carriers for improving the delivery of therapeutic agents to the specific sites in the body. Because of the multiple useful properties like biocompatibility, non-toxicity, bio-degradability, ease of preparation and composition or size control, liposomes have applications in medicine and biology including drug delivery. Since the architecture of liposome closely resemble that of biological cell it acts as an efficient drug carrier. Drug encapsulation within liposomes provides extended therapeutic response, because the liposomes have a slow – release ‘depot’ effect minimizing adverse effects. Liposomes which may have a size of the order of nanometer scale has a hydrophilic ‘head’ group and two hydrophobic tails leads the formation of phospholipid. They spontaneously arrange in heads up and tails down orientation. These vesicles can encapsulate water-soluble drugs in their aqueous spaces and lipid-soluble drugs within the membrane itself. Since liposomes are used to access both hydrophilic solute (inside their inner water cavity) and hydrophobic (through bi-layer
membranes), this container is able to be loaded with different bioactive compounds such as enzymes, hormones, vitamins and antibiotics. Liposomal incorporation protects drugs from spontaneous bio-degradation, thereby decreasing the number of repeated therapeutic doses [6].

Liposomes can be extended by connecting phospholipid sheets in a tail-to-tail array to form a concentric bi-layer membrane that encloses some of the water in an aqueous center. These multilayer shells are expected to act as a barrier to prevent oxidation and hydrolysis of the lipid molecules while at the same time, carrying specific vector molecules for addressing the delivery sites. Practically, small-diameter liposomes offer many advantages for drug delivery in terms of circulating stability and intracellular targeting with respect to large multilayer ones. The small size avoids the rapid clearance of colloidal nanometer devices by the metabolic system. This colloidal carrier of 100 nm to 200 nm size has the ability to pass through the tumors. Life of liposome could be increased when its head group is covalently linked to Poly Ethylene Glycol (PEG) since it shields the surface from protein adsorption [7].

Now-a-days studies are made on the accessibility and specificity of drug carriers to the target organs. Liposomes are best suited because of the ease of possible surface modification. Liposomes can interact with cells in many ways to cause liposomal components to become associated with those cells. Liposomes could deliver drugs by any one of these methods (i) by intermembrane transfer (i.e., transfer to occur with complete retention of the
contents of the liposome’s aqueous compartment), (ii) by contact-release (in which contact with the cell causes an increase in permeability of the liposome membrane which leads to release of water-soluble solutes in high concentration in the close vicinity of the cell membrane, through which these solutes may pass under certain circumstances), (iii) by adsorptions and (iv) by fusion. In addition to these methods few effective methods include site-selective delivery of liposomal contents. These liposomes could be temperature-sensitive, target-sensitive or pH-sensitive. The acceptance of liposomes into the cell has been depicted in Fig. 5.

![Fig. 5. Acceptance of liposome into cell](image)

Gregoriadis and Ryman [9] first reported liposomes as an advanced drug delivery vehicle since they are non-toxic, biodegradable and non-immunogenic. When a drug is associated with it, its pharmacokinetics is changed and lowers
its toxicity. Yet, liposomes which have hydrophilic flexible polymeric chains undergo substantial decline due to the metabolism process and subsequently its prolonged circulation time is minimized.

1.3.2. Micelles

Surfactant, which means ‘surface active agent’ are the molecules that are active at variety of surfaces, especially at air-liquid and liquid-liquid interfaces. Surfactant spontaneously associates to form a colloidal size object with 2-20 nm diameter. This colloidal-sized object is termed as micelle. Formation of micelles is mainly in aqueous solution though some are formed in non-aqueous solutions. Fig.6 depicts the spherical micelle of surfactant molecule in aqueous solution. The hydrophilic ionic head groups are exposed to the bulk aqueous solution while the hydrophobic hydrocarbon tail groups form the interior of the micelle. They have a tendency to change their interfacial properties dramatically, few to mention are surface tension, interfacial tension and surface diffusion and so on. The factors that govern the micelle structures include molecular structure of surfactant, concentration of surfactant, solvent polarity, dielectric constant of solvent, pH, size and balance of counter ion type and concentration of salt, temperature, pressure and so on. The evolution of micelle structures in aqueous solution runs through spherical, rod-shaped, wormlike and liquid crystal. The spherical structure of micelles has been shown in Fig.6.
The oldest application of this surfactant molecule is its detergency. By decreasing the interfacial tension at the oil-water interface by the adsorption of surfactant, the oil is detached from the solid substrates (glass, fiber, fabric etc.). Then the oil will be solubilized within the micelle in nearby solution, leaving the clean solid substrates behind. Micelles have the property of solubilizing the water-insoluble compounds and this is termed as micelle solubilization. Bi-layers formed by micelles can be used as vesicles with the size range of 20 nano meter to 50 micro meter in diameter and with the thickness of each single layer is limited to 3-5 nm. For each micelle there is a reverse micelle. The structure of the reverse micelle is that the head groups are at the center and the tail groups facing out.

**Fig. 6. Structure of micelles**

1.3.3. **Micro emulsions**

The potential of micro emulsions as drug delivering agent is greater when compared with micelles. Micro emulsions are liquid dispersions of water and oil. By adding a relatively large amount of a surfactant and co-surfactant these are made thermodynamically stable, homogenous and transparent. The
range of micro emulsion is 10-100 nm diameter. Schulman et al. [10], in 1959 first used the term “micro emulsions’ to describe a multiphase system consisting of water, oil, surfactant and alcohol, which forms a transparent solution. In late 1970’s and early 1980’s the recognized micro emulsion applications include detergency and lubrication. Together with these classical applications other discovered applications are catalysts, preparation of sub-micron particles, liquid-liquid extraction and especially drug delivering agent.

Versatile characteristics of micro emulsions include (i) their particle size less than 200 nm (ii) thermodynamically stable (iii) optically clear (iv) increased surface area and (v) high solubilizing capabilities [11].

Applications as drug delivering agent

When drugs are administered through intravenous route it is cleared more slowly than the coarse particle and hence have a longer residence time in the body. But the toxicity of the surfactant has to be minimized.

The benefits when micro emulsions are administered orally include increased absorption, improved clinical potency and decreased drug toxicity. In addition special features have been reported that the micro emulsions are ideal for delivering drugs as steroids, hormones and antibiotics.

For the treatment of eye diseases, drugs are essentially delivered topically. Micro emulsions have been investigated for ocular administration; to dissolve poorly soluble drugs to increase absorption and to attain prolong
release profile. Formation of micro emulsion stabilized by perfluorocarbon is intended for pulmonary delivery.

1.3.4. Polymeric nanoparticles

Polymeric nanoparticles are a broad term generally referring to fiber structure with diameter less than one micron. These are usually produced by electro-spinning. Polymer nanoparticles, also termed as nanospheres, nanocapsules are defined as nanometric colloidal carriers. These particles present high stability when in contact with biological fluids and their polymeric nature allows controlled drug release. Nanoparticles represented as drug delivering carrier are suitable for most of the administration routes, even if a rapid recognition by the immune system limits their use as injectable carriers. Based on the requirements nanospheres or nanocapsules are fabricated. Nanospheres consist of a dense polymeric matrix, in which drug can be dispersed. Nanocapsules present a liquid core surrounded by a polymeric shell. Polymeric nanoparticles are more stable and may be able to improve bioavailability, particularly for highly insoluble drugs by increasing surface area for dissolution which results in bio-adhesion [12].

Biodegradable polymeric nanoparticles are not only limited as drug delivering system but are also used for the fabrication of required materials in tissue engineering. Biocompatible polymers are prepared from aliphatic polyesters. These polymers do not require surgical removal after the
completion of drug release. Polymers could be made as more bio compatible by modifying the terminal groups located on the polymer surface as well as its structure. This modification helps the polymer nanoparticles to have long-life and thereby decreases the repetition of drug doses. In the case of nanopolymers being treated as a drug delivering agent the potential interactions between drugs (or active molecules to be encapsulated) and the polymers being formed must be categorized systematically. Polymeric nanoparticles are used to deliver drug when it is in their solid phase and also they are used as imaging agents [13]. Now-a-days new methods are obtained to encapsulate the drugs.

1.3.5. Microbubbles

A spherical void or a cavity filled by a gas constitutes microbubble. The microbubble is comprised of an outer shell, which can contain target ligands and gases within it. Since the constituted gas is of low density, microbubble is also considered as a particle with low density structure. Drugs can be encapsulated within the microbubbles, which can be targeted for drug release and it can reach the target site when it is enhanced by ultrasound. As a drug carrier should be sufficiently stable and have to circulate for a long enough period of time to reach the target site the stability of the microbubble should be considered. The stability of the microbubble depends on the gas in the bubble and the coating material surrounding it. Microbubbles comprised of perfluorocarbon gases, has a longer circulation time in blood. Microbubbles
can be used as therapeutic cavitation nuclei with ultrasound of frequency around 1MHz \cite{14}. It can be cavitated with ultrasound energy for site-specific local delivery of bioactive materials and for treatment of thrombosis. Microbubbles can be collapsed by ultrasound energy thereby releasing the entrapped material with very high velocity and they are termed as micro-jets. Any drug placed on the micro jets that produce the holes will be carried into the cells.

The technology of drug-filled microbubble is probably most advantageous to drugs that are highly active i.e., drugs that are highly active in milligram quantities. Also it is technically easier to develop microbubble carriers to deliver milligrams or micrograms rather than grams of drugs. Many drugs such as anti-cancer agents, peptides, bio-molecules and other agents which are highly active are amenable to deliver drug from microbubbles with the assistance of ultrasound. Ultrasound targeted microbubble destruction is a promising new method that could combine low invasiveness with possibly higher gene transfer efficiency as well as high organ specificity. Microbubbles are employed (eg. detection of tumors) in imaging the liver, cardiac and other organ systems. It can be used as a marker of blood flow to visualize changes in blood flow associated with therapy. Hence microbubble products have applications in diagnosis as well as in therapy. Commercially available perfluorocarbon entrapped microbubble contains a substantial population of nanobubbles.
1.3.6. **Nanoemulsions**

In general, emulsions are two phase mixtures of insoluble liquid phase surrounding discrete vesicles. The nano sized emulsions are used in delivering drug by carrying it in the non-aqueous liquid phase of the emulsion. Hydrocarbon or perfluorocarbon liquids are commonly employed in the dispersed phase and carry a hydrophobic drug for delivery.

Of all the available nano-structured materials, fine dispersed nanoemulsions are of great important because of their stability and compatibility. The main peculiarity of nanoemulsions are their greater stability as droplet suspensions, kinetic stability that last for months, stability against dilution and even against change of temperature (i.e. thermodynamically stable). Nanoemulsions are built by pure monomer droplets surrounded by the adsorbed and stabilizing surfactant.

1.3.7. **Need of perfluorocarbon**

![Fig.7. Perfluorocarbon as drug carrier](image)

Perfluorocarbons are biologically and chemically inert. They possess high gas dissolving properties. They are chains of eight to ten hydrocarbon
molecules where the hydrogen has been replaced by fluorine. Perfluorocarbon along with its payload is shown in Fig.7. Perfluorocarbons are not miscible with water and therefore have to be brought into an emulsion prior to use. Since perfluorocarbons are compounds derived from hydrocarbons by replacing hydrogen atoms by fluorine atoms, the carbon and fluorine atoms alone constitute Perfluorocarbons and few to mention are octafluoropropane, perfluorohexane and perfluorodecalin. Derivatives of perfluorocarbon are obtained by attaching some functional group with it. These derivatives are different from perfluorocarbons in their properties, applications and toxicity.

Perfluorocarbons are best drug delivering vehicles since they are chemically inert, thermally stable and non-toxic. In perfluorocarbon the carbon-fluorine bond is strong and it is structured with carbon as its backbone. They are non-flammable. Perfluorocarbon liquids are clear and colourless. Due to high molecular weight, it has high density. Other parameters like viscosity, surface tension and heats of vaporization are low due to very low intermolecular forces of perfluorocarbons in the liquid state.

Perfluorocarbons are chemically synthesized halogenated molecules that are completely inert but which dissolve large amounts of gases, including oxygen. The function of perfluorocarbon as an oxygen carrier is shown in Fig. 8. Perfluorocarbons are non-biological sources, cost effective and along the mentioned properties this tends to be an appropriate red blood cell substitute. Since they are immiscible with aqueous solution (such as plasma) it
must be emulsified before injecting into the blood stream. The levels of infused perfluorocarbons are lowered as they carry oxygen proportional to the inspired oxygen. The emulsions of perfluorocarbons are dissolved within the blood rather than being bound to hemoglobin of red blood corpuscles. Since the size of these artificial oxygen carriers is smaller than red blood corpuscles, it could diffuse and pass into narrower capillaries increasing local tissue oxygenation [15]. The first approved blood substitute was a perfluorocarbon based product called Fluosol DA20. But the product was withdrawn due to its limited success, complexity of use and side effects. The second generation perfluorocarbon based product was produced as a completely man-made powder, which could be stored indefinitely. To avoid adverse side-effects the dose of the product has been limited and its efficiency has to be improved. To improve its efficiency, its physical and chemical properties have to be studied in detail.

Fig. 8. Perfluorocarbon as oxygen carriers [14]
Acoustically active perfluorocarbon nanoemulsions and targeted microbubble with specific ligand can be developed for detecting disease at the molecular level. Compared to the microbubbles, having a mean size of around a micron, the perfluorocarbon nanoemulsions can be much smaller in size, say a mean diameter of about 200 nm. The smaller size of the nanoemulsions may increase the blood half-life of these agents relative to microbubble and may also improve the potential of the carrier for targeting the particles to the selected receptors. Perfluorocarbon materials, having a range of different boiling points, when mixed with halogenated compounds, their range expands further. This makes it possible to design a nanoemulsion that will undergo the phase transition from liquid to gaseous states at a range of required temperatures.

For gene delivery perfluorocarbon nanoemulsions (perfluorohexane) have interesting and potentially useful properties. It has been hypothesized that the low surface tension and low viscosity properties of the liquid perfluorocarbon improve the ability of these vehicles to fuse with cell membranes in gene delivery and also expected to improve dispersion of drugs in lung tissue following pulmonary administration. Gene delivery to the lungs by perfluorohexane is more effective than the commercially available liposomes [16].

Another application of perfluorocarbon is that it acts as an efficient gene carrier. Fig. 9 shows the gene carrying capacity of liquid perfluorocarbon. The
outer surface is stabilized by amphipathic lipid. Targeting ligands have been incorporated onto the head groups of the lipids. The genetic material is stabilized by cationic lipids. Electron microscopy studies have shown that the DNA is condensed as an electron-dense granule within the center of the nanoparticle. The diameter of these particles is about 100-200 nm.

Fig. 9. Liquid perfluorocarbon as a gene carrier [17]

Also the perfluorocarbon nanoemulsion (perfluoropentane) is in the liquid state at room temperature and they remain as liquid droplets after injection. Ultrasound with sufficient energy converts it to gas in turn increasing acoustic reactivity and potentially localized site-specific action of the particle. Perfluoroctylbromide (PFOB) has been tested as agents to improve dispersion of drugs and to improve aeration of lung tissues in patients. PFOB has also been tested for liquid breathing to deliver oxygen [18].

An attempt has been made in the present investigation to characterize these perfluorocarbons and to picturize their structures and properties.
1.4. ULTRASOUND ENHANCED DRUG DELIVERY

Formerly ultrasound was used only in diagnostic medicine. Now-a-days this finds a place in delivering nano carriers to the desired site because of their non-invasive nature and their focus-ability on the targeted tissue. The underlying mechanisms involve the formation of micropores in cell membranes due to cavitation and formation of micro jets by high amplitude oscillations. These oscillations are induced by ultrasound frequencies that are used in diagnostic echocardiography (about 1-2 MHz). Thus, no new ultrasonic devices are needed for this technique. Precise location to deliver drug can be obtained when an agent is enhanced by ultrasound, as ultrasound energy can be focused very tightly to well-defined regions. Theoretically, millimeter or sub-millimeter sized regions of tissue could be treated precisely with ultrasound and drug filled agents, as the wavelength of 1MHz ultrasound is of the order of millimeter size.

Like other waves ultrasonic waves can be focused, reflected and refracted through medium. These properties help us to have a control over ultrasound transmission and its directionality. Such site specific treatment is beneficial in drug delivery to localize the drug interactions with the target tissues, thereby sparing the body from adverse side effects. Depending upon the wavelength and the tissue type, ultrasound can penetrate deep into the human body, though muscles has fairly low attenuation, the bone and lung tissue have high attenuation. This attenuation may be minimized by lowering
the frequency of ultrasound, since they are directly proportional. And also when the applied frequency is decreased, the acoustic pressure threshold to produce cavitation decreases. Hence ultrasonic assisted drug and gene delivery from nano carriers to the target site has tremendous advantages. By increasing the shear stress on the nano carriers, with the aid of ultrasound, it releases drug from itself. Ultrasound enhanced liquid emulsions and solid nanoparticles are used to deliver genes and drugs with the non-invasive effect [19].

Drugs could be delivered efficiently either by ultrasound-controlled drug release or by ultrasound- enhanced drug delivery. Along with liposomes as nano carriers, ultrasound is used to deliver drug and gene in the treatment of cardiac disease, stroke and tumor therapy. With the help of ultrasound, drug transport is possible through cavitation process. Cavitation is done with the help of ultrasound triggered gas bubbles. But practically the blood and tissues environment are highly recalcitrant for this cavitations process unless they are exposed to high intensity waves of ultrasound. This leads to the risk of damaging the cell and tissues. To overcome this problem, ultrasound contrast agents can be used, which considerably decreases the threshold ultrasound intensity for cavitations. As an aliter to this cavitation process, a new process in the application of ultrasound is the contact facilitated drug delivery process. Ultrasound-facilitated nano carriers exhibit versatile features that include

- high drug and gene loading properties
- high local concentration and low systemic toxicity
• controlled release of drug with a single high amplitude ultrasonic pulse
  and a sustained release by a series of low amplitude pulses.
  Combination of these two types of releases are important in those cases
  where the drug concentration has to be raised to the therapeutic level
  and is maintained for a certain duration of time
• through cavitation process, drug or gene is transported into the cells or
tissues
• ultrasound reflectivity allows image-guided drug and gene delivery.

Ultrasound is one of the most common tools in medical imaging and
also in therapeutic applications. Sound travels more slowly in gas than it does
in liquid. Ultrasound energy can be reflected, transmitted or absorbed as it
passes through a biological medium. During the cavitation process, ultrasound
energy is concentrated within a domain around an agent. The frequency of
ultrasound is also important. Cavitation is generally more efficient at lower
frequencies of ultrasound. Benefits of ultrasound, compared to other
modalities include: real-time imaging, relatively short and efficient imaging
protocols, non-invasive with minimum patient discomfort and low operating
costs. Since the ultrasound can be applied across the skin and focused to a
given depth within the tissues, high intensity focused ultrasound, afford
advantages of being less invasive than the techniques such as radio frequency
ablation.
The blood-brain barrier can be reversibly opened without damaging the neurons using ultrasound applied across the intact skull to encapsulate microbubbles within the cerebral micro vasculature for delivery of both low and high molecular weight therapeutic compounds to the brain. Hence ultrasound plays a vital role in diagnosis and therapy.

1.5. NEED FOR CHARACTERIZATION

Nanoparticles have great scientific importance because they differ greatly in their properties from their own bulk materials. A bulk material should have constant physical properties regardless of its size, but at the nano-scale level this is often not the case. The change in the properties of the materials at the nano-scale is due to the increase in the surface area of the material. This facilitates them to react with other materials especially in the drug delivering process. Nanoparticles with high surface to volume ratio provide a tremendous driving force for diffusion, especially at the elevated temperatures.

There is a growing consensus that a complete and accurate particle characterization is an essential part of assessing nanoparticles in the day to-day applications. Proper characterization of test materials is important to ensure that results are reproducible, and also to provide the basis for understanding the properties of nanoparticles that determine their biological effects. Complete characterization of nanoparticles includes measurements such as size and its
distribution, shape and other morphological features (e.g. crystallinity, porosity, surface roughness), chemistry of the material, solubility, surface area, state of dispersion, surface chemistry and other physicochemical properties.

Development of nanoparticles must proceed in parallel with the assessment of the theoretical idea about the formation. It should be noted that each nanoparticle property, namely its small size, large surface area, chemical composition solubility and geometry, are directly related to its biological response. Consequently, it is imperative to characterize and analyze the physical and chemical properties of the nanoparticle under consideration to correlate them with the biological applications. It is understood that size does matter when particle’s bio-distribution is concerned. However, though the size determination of nanoparticles are done accurately with the help of Transmission Electron Microscope / Scanning Electron Microscope, the influence of other parameters in particle distribution still remain poorly understood and this is in part due to inadequate particle characterization. Several methods are available to characterize particles, especially in terms of size, but none of them are fully satisfactory. Here an attempt has been made to characterize the particle and hence to understand about their biological implications.

The most important feature of nanoparticles is their small size. There are many different parameters that influence the size of the particles during its synthesis procedure. Few parameters to be mentioned are their pH of the
medium, the stirring velocity and the period of the time exposed to ultrasound, temperature, etc. Along with these experimental parameters, the theoretical knowledge about the packing factor, segment diameter, pair potential and chemical potential of the nanoparticles, especially perfluorocarbons, play an important role in using it as an effective drug delivering agent. These parameters are determined for the seven different systems of perfluorocarbons in the following chapters and also the strength of particle interaction has been estimated with the aid of proposed new model. Also, to understand about the delivering process at the site, the non-linear behaviour of the perfluorocarbons is determined. To determine the suitability of the drug carrier for the process of ‘contact facilitated drug delivery process’ the knowledge about the above mentioned parameters are essential.
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