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Review article:

NANOENCAPSULATION FOR DRUG DELIVERY

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ABSTRACT

Nanoencapsulation of drug/small molecules in nanocarriers (NCs) is a very promising approach for development of nanomedicine. Modern drug encapsulation methods allow efficient loading of drug molecules inside the NCs thereby reducing systemic toxicity associated with drugs. Targeting of NCs can enhance the accumulation of nanonencapsulated drug at the diseased site. This article focussed on the synthesis methods, drug loading, drug release mechanism and cellular response of nanoencapsulated drugs on liposomes, micelles, carbon nanotubes, dendrimers, and magnetic NCs. Also the uses of these various NCs have been highlighted in the field of nanotechnology.

Keywords: nanocarriers, liposomes, micelles, dendrimers, carbon nanotube, magnetic

INTRODUCTION

Nanoencapsulation of therapeutic agents increases their efficacy, specificity and targeting ability (Soppimath et al., 2000). Nanocarriers (NCs) protect their payload from premature degradation in the biological environment, enhance bioavailability, and prolong presence in blood and cellular uptake (Kumari et al., 2010a, b). Many methods are reported in literature for the synthesis of NCs (Galindo-Rodriguez et al., 2004). Synthesis methods are chosen depending upon the chemical structure of therapeutic agent, type of application (Alonso, 2004) and time of retention inside the body (Peer et al., 2007). Different sized NCs can be synthesized by using different matrices (Dobrovolskaia and McNeil, 2007). Size and size distribution of NCs are important to determine their cellular uptake and penetration across biological barriers (Kumari and Yadav, 2011). Size and surface chemistry of NCs determine their *in vivo* performance (Suri et al., 2007). Drug release mechanisms are equally important from the drug-NC-formulation due to proposed application in drug delivery (Yoo et al., 1999). Release mechanism can also be modulated depending upon the nature of therapeutic agent and type of NCs (Yadav et al., 2013).

Conventional NCs are cleared from the body by mononuclear phagocytic system (MPS). MPS recognises NCs as foreign particles and rapidly removes from the systemic circulation (Storm et al., 1995). However, if prolonged presence in blood is required than surface of NCs are easily modified in order to prevent phagocytosis (Storm et al., 1995). Surface of NCs are also modified to increase their targeting ability and delivery of drug at target site. Surface modification of NCs is conducted either by tagging ligand (Weissleder et al., 2005) or hydrophilic polymers (Gref et al., 1995) on their surface. Surface charge is

another important parameter which affects cellular response of NCs (Verma and Stellacci, 2010). NCs with cationic charge are efficiently taken up by negatively charged cell membranes as compared to neutral or positively charged ones (Muñoz Javier et al., 2006).

Smart designing of NCs with respect to target site and route of administration will solve the problems faced by therapeutic agents. In the successive headings, we have discussed the effect of nanoencapsulation of various drugs on liposomes, micelles, carbon nanotubes, dendrimers, and magnetic NCs. This article further focused on the effect of various therapeutic agents upon encapsulation in different NCs, and their impacts on controlled release, surface characteristics and cellular response.

LIPOSOMES

Liposomes are spherical vesicles with a phospholipid bilayer and are extensively used in drug and gene delivery. Liposomes protect therapeutic agents from degradation, deliver it at target site and are versatile enough to allow tagging of small molecules for targeted delivery (Felnerova et al., 2004). Liposomes are synthesized by using cationic lipids, anionic lipids or neutral lipids depending upon the mode of use and drug to be encapsulated. Liposomes solely composed of charged lipids may not be suitable for drug delivery because they do not form charged vesicles that are capable of entrapping drug molecules (Shi et al., 2002).

Synthesis of liposomes

Many methods have been reported in the literature for the synthesis of liposomes. These are discussed here briefly.

Polycarbonate membrane extrusion method

In this method lipid dissolved in chloroform is dried into thin film. The dried lipid film is then added to buffer solution containing the drug molecule of interest. The lipid solution is sonicated, freeze dried and subjected to extrusion 10 times through 100 nm pore size polycarbonate membrane to form liposomes. Uniform sized liposomes are formed by this method (Shi et al., 2002).

High pressure homogenisation

Homogenous blend of lipids is prepared by dissolving them in organic solvents, shock freezing in liquid nitrogen and freeze drying the blend. Freeze dried lipid is then dissolved in PBS and subjected to high pressure homogenisation to form liposomes.

Reversed phase evaporation method

Lipids dissolved in mixture of chloroform and methanol is dried into thin film. Dried lipid film is then dissolved in diethyl ether aqueous phase and sonicated to form homogenous oil in water (o/w) emulsion. The organic solvent is then evaporated under vaccum (Gareipy et al., 2002).

Sonication method

Briefly, lipids dissolved in chloroform are dried into thin film and then suspended in tris-HCl buffer. The multilammelar vesicles produced are then sonicated in bath type sonicator to form unilammelar vesicles (Nakagawa et al., 2007).

Lipid film hydration sonication extrusion method

Lipid solution in organic solvent is dried into thin film. The dried lipid film is then hydrated in ammonium sulfate, sonicated in bath sonicator and sequentially extruded through polycarbonate membrane of desired pore size (Xiong et al., 2005).

Encapsulation of different types of drugs

Drugs are directly added to the lipid solution for the formation of drug loaded liposomes. Encapsulation efficiency is calculated indirectly by measuring the amount of drug in the supernatant by UV-Vis spectrophotometer and HPLC. Different types of drugs have been encapsulated on liposomes to increase their efficacy and specificity.

Anticancer molecule methotrexate was encapsulated in inner aqueous phase of lip-

osomes. More than 90 % of methotrexate was retained within liposome during storage of 24 hours at 4 ºC (Konigsberg et al., 1998). Another drug doxorubicin has been used in the treatment of cancers of bladder, stomach, ovaries, lung, thyroid and others. But its use has been found to be associated with toxicities like gastrointestinal myelosuppression, alopecia and mucositis. This was overcome by encapsulation of doxorubicin on liposomes that has increased retention time and reduced toxicity of doxorubicin (Kale et al., 2012). In another study doxorubicin was loaded on sterically stabilised liposomes. Administration of doxorubicin loaded in RGD grafted sterically stabilised liposomes has significantly retarded tumour growth (Xiong et al., 2005). N-butyldeoxynojirimycin is used for inhibition of tyrosinase in melanoma cells. N-butyldeoxynojirimycin was successfully loaded on pH sensitive liposomes made up of dioleoylphosphatidylethanolamine and cholesteryl hemisuccinate. pH sensitive liposomes have reduced the dosage required for tyrosinase inhibition by a factor of 1000 (Costin et al., 2002).

Ciprofloxacin was also successfully loaded on liposomes with 45 % loading efficiency. Release rate of encapsulated ciprofloxacin was modulated by addition of cysteine to the dithiobenzyl urethane linkage between the lipid and the PEG in liposomes (Karathanasis et al., 2005). The antifungal agent clotrimazole was successfully encapsulated in untradeformable liposomes. Liposome encapsulated clotrimazole has exhibited greater skin penetration than free clotrimazole (Maheshwari et al., 2012). Tretinoin has been used in treatment of various skin diseases like psoriasis, acne, epidermotropic and T-cell lymphomas. But the use of tretinoin as drug is limited due to low water solubility, skin irritation, and high instability in presence of air, light and heat. Therefore, tretinoin was encapsulated on liposomes. Negatively charged liposomes have strongly improved tretinoin retention in skin (Sinico et al., 2005).

Surface modification of liposomes

A major drawback of liposomes is their unspecific interaction with macromolecules and cellular surfaces resulting in their short half life. Conventional liposomes are rapidly cleared by macrophages of mononuclear system (MPS), particularly Kupffer cells in the liver and spleen macrophages. This drawback can be overcome by coating the surface of liposomes with hydrophilic polymers like PEG. Coating of PEG on the surface of liposomes creates a steric barrier (Kale et al., 2012). Targeting of liposomes to specific sites can be achieved by tagging ligands on the surface of liposomes. Monoclonal antibodies, ligands and peptide sequences can be attached to the surface of liposomes for targeted delivery (Felnerova et al., 2004).

Drug loading and release from liposomes

The drug is located in the interior part of liposomes. The efficient encapsulation of drug is essential requirement for the successful application of liposomes in drug delivery. Many encapsulation techniques are reported in literature which varies according to the type of drug and the type of liposomal formulation. Drug loading in liposomes can be achieved by passive and active loading. In passive loading dried lipid film is rehydrated in the presence of drug to give rise to drug loaded liposomes (Colletier et al., 2002). In active loading drug is loaded on readymade liposomes via concentration or pH transmembrane gradient (Abraham et al., 2004).

Release mechanism can be modulated by effective combination of functional head groups, lipid chains and linker groups in membrane components for controlled release of encapsulated material under specific conditions. Release mechanism in liposomes can occur by four mechanisms. First mechanism involves pH dependent neutralisation of charged functional groups of membrane components (Ellens et al., 1984). Second mechanism involves pH dependent hydrolysis of non charged components in-

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tegrated in membranes (Guo and Szoka, 2001). Third mechanism involves thiolysis of disulfide bonds present in membrane lipids. Thiolysis occurs by change of redox potential of surrounding environment, for example on moving from extracellular surface to cytoplasm (Zhang et al., 2004). Fourth mechanism uses temperature for release of drug. This mechanism is used by thermosensitive liposomes for release of drug (Lindner et al., 2004; Needham and Dewhirst, 2001).

Cellular response of liposomes

Cellular uptake of liposomes follows endocytic pathway. An important strategy is to attach a ligand on the surface of liposomes which will stick to its receptors on cell surface or in the extracellular matrix of the cell. Liposomes tagged with ligands undergo cellular uptake by receptor mediated endocytosis (Shao et al., 2006). In one study adhesion of plain, PEGylated liposomes and concanvalin grafted liposomes to model membranes was investigated. Plain liposomes exhibited unspecific adhesion to lipid membranes. The degree of membrane interaction was increased when plain liposomes were grafted with lectin (Bakowsky et al., 2008). N-acetylglucosamine-conjugated liposomes have exhibited greater interaction with cardiomyocytes *in vitro* (Aso et al., 2007). Human recombinant interleukin-2 grafted liposomes showed receptor mediated endocytosis in murine CTLL-2 T-cell line (Konigsberg et al., 1998).

POLYMERIC MICELLES

Polymeric micelles have attracted attention as a nanosized drug carrier due to small size and high structural stability (Yamamoto et al., 2007). Polymeric micelles are classified according to type of intermolecular forces driving the core segment from aqueous media. Three types of micelles are amphiphilic micelles formed by hydrophobic interactions, polyion complex micelles formed by electrostatic interactions and micelles stemming from metal ion complexation (Gaucher et al., 2005). Hydrophobic block of the copolymers form the core of the micelles. Many polymers that have been used as hydrophobic segment in polymeric micelles are poly(propylene glycol) (PPO, Pluronics®) (Marin et al., 2002), poly(aspartic acid) with chemically conjugated doxorubicin (PAsp(DOX) (Yokoyama et al.1998), poly(β-benzyl-L-aspartate) (PBLA) (Kwon et al., 1995), and polyesters such as poly(lactic acid) (PLA) (Liggins and Burt, 2002), poly(ε-caprolactone) (PCL) (Allen et al., 2000), and poly (trimethylenecarbonate) (PTMC) (Zhang et al., 2006). Core forming materials of the micelles have been found to affect stability, drug loading capacity and drug release profile. Micelles are formed when concentration of block copolymer is increased above a certain level known as critical micellar concentration (Rijcken et al., 2007).

Synthesis of micelles

Polymeric micelles are prepared by many methods. Choice of synthesis method depends on the nature of drug, and type of polymer used. Some of the commonly used techniques are as follow.

Solvent extraction technique

Solvent extraction technique is used for the preparation of polymeric micelles for high drug loading, good stability and extended release. In this method, preformed polymers are dissolved in acetone and added dropwise to double distilled water under stirring. Organic solvent is then removed after purging with dry nitrogen (Forrest et al., 2006a).

Dialysis method

Polymers dissolved in water miscible organic solvents are dialysed against water. Slow removal of organic solvent leads to micelle formation (Gaucher et al., 2005).

Solution casting method

Polymers dissolved in organic solvent are dried into thin film. The thin film is then rehydrated with heated aqueous sol-

vent to form micelles (Gaucher et al., 2005).

Encapsulation of different types of drugs

Many drugs have been encapsulated on micelles to enhance their solubility, and therapeutic activity. Doxorubicin was encapsulated on pluronic micelles. Doxorubicin encapsulation in micelles has reduced the systemic uptake of doxorubicin to normal cells by 2 fold as compared to free drug (Marin et al., 2002). Paclitaxel was successfully encapsulated in micelles made up of amphiphilic block copolymers composed of methoxy poly(ethylene glycol) (MPEG) and poly(ε-caprolactone) (PCL). Paclitaxel encapsulated in amphiphilic block copolymers have exhibited greater cytotoxicity to cancer cells documenting the significance of micelles (Park et al., 2005). Camptothecin was successfully incorporated into polymeric micelles formed from poly(ethylene glycol)-poly(benzyl aspartate) block copolymers. Camptothecin loaded in polymeric micelles have significantly inhibited the tumour growth after single *in vivo* injection. Also, camptothecin loaded in polymeric micelles showed greater retention in blood and higher accumulation in tumors as compared to free camptothecin (Kawano et al., 2006). Oxaliplatin was loaded on poly(ethylene glycol)-β-poly(glutamic acid) polymeric micelles. Oxaliplatin loaded in polymeric micelles showed enhanced antitumor activity (Cabral et al., 2007). β-lapachone is plant derived anticancer drug whose cytotoxic effect is significantly enhanced by NAD(P)H: quinone oxidoreductase 1. β-lapachone was encapsulated on PEG-PLA micelles. β-lapachone encapsulated in PEG-PLA micelles also showed slow and sustained release (Blanco et al., 2007).

Amphotericin B is a potent antibiotic used for fungal infections. Amphotericin B was successfully loaded on polymeric micelles of poly(ethylene oxide)-*block*–poly(*N*-hexyl-L-aspartamide). Amphotericin loaded in polymeric micelles showed sustained release (Adams et al., 2003). Geldanamycin was loaded on polymeric micelles of poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-b-PCL). Such loading of geldanamycin in polymeric micelles has significantly improved the aqueous solubility and efficacy (Forrest et al., 2006b). Andriamycin was loaded successfully on poly(L-histidine) poly(L-lactic acid) polymeric micelles. Andriamycin showed pH dependent release from polymeric micelles (Lee et al., 2003). Cyclosporin A is a potent immunosuppressive agent used primarily to reduce the incidence of graft rejection in recipients of transplanted organs. Cyclosporin A was loaded on methoxy poly(ethylene oxide)-β-poly(ε-caprolactone) micelles. Polymeric micelles showed slow and sustained release of cyclosporin A (Aliabadi et al., 2005). Rapamycin is lipophilic macrolide antibiotic with anti-fungal, immunosuppressive, and anti-tumor activities. Rapamycin was loaded on PEG-PCL polymeric micelles. Rapamycin showed slow and sustained release from PEG-PCL micelles (Forrest et al., 2006a).

Plasmid DNA containing bacterial LacZ gene was incorporated into poly(dimethylaminoethyl methacrylate (poly(butylmethacrylate) polymeric micelles. Polymeric micelles loaded with DNA were able to transfect cells without causing any toxicity (Funhoff et al., 2005).

Surface modification of micelles

Surface of micelles is modified for increasing circulation time in blood. Hydrophilic polymers are most commonly used polymer for surface modification of micelles. Among all, PEG is the most commonly used hydrophilic polymer for increasing persistence inside the blood. PEG forms a brush like corona projecting out from the surface of polymeric micelles (Gaucher et al., 2005). Hydrophilic PEG corona is important in preventing opsonin adsorption and clearance by mononuclear phagocytic system (Kwon, 2003). Stealth properties and half life of acetaldehyde

functionalised PEG-b-PDLLA micelles were enhanced by anchoring anionic peptidyl ligand on the surface of micelles which imparted negative charge to the micelles (Yamamoto et al., 2001).

Targeting ability can be imparted to micelles by attaching ligands, receptors or antibodies to the hydrophilic segment so that they interact with membrane receptors. Targeted micelles have exhibited greater cellular uptake and improved efficacy than unmodified counterparts (Gaucher et al., 2005).

Drug loading and release from micelles

Drugs can be incorporated into polymeric micelles by physical entrapment or chemical conjugation (Gaucher et al., 2005). Also, the drug can be loaded into the core of micelles with the help of organic solvents through o/w emulsion, dialysis and solid dispersion. Release of drug from polymeric micelles is affected by many factors such as the length of the core forming polymer segment, affinity between the drug and the core, and the amount of the loaded drug (Huh et al., 2005). The release of physically adsorbed drug from polymeric micelles is controlled by diffusion of drug from micellar core and the partition coefficient of the drug over micellar core and the aqueous phase.

Cellular response of micelles

Folate conjugated micelles showed greater uptake in MCF-7 cells through interaction with overexpressed folate receptors on the surface of cancer cells (Park et al., 2005). In another study folate conjugated poly (L-histidine)–poly (L-lactic acid) micelles were more effective in killing cancer cells (Lee et al., 2003). Poly(dimethylaminoethyl methacrylate), poly(butylmethacrylate) polymeric micelles were able to transfect COS-7 and OVCAR-3 cells with minor toxicity (Funhoff et al., 2005). Polymeric micelles of polyethyleneglycol/phosphatidyl-ethanolamine conjugates (PEG-PE) accumulated in the infarction zone with efficiency more than 8-fold higher as compared to a non damaged part of the heart muscle by EPR effect (Lukyanov et al., 2004).

CARBON NANOTUBE (CNT)

A carbon nanotube is like a sheet of graphite that is rolled into a cylinder, with distinctive hexagonal latticework making up the sheet. The carbon nanotubes (CNTs) are interesting material because of their size range in the nanoscale dimension and their wide applicability in the nanotechnology and various electronic devices (Frank et al., 1998; Kong et al., 1999), gas storage media (Liu et al., 1999), nanotweezers (Kim et al., 1999). The high aspect ratio, high conductivity and intrinsic strength of carbon-carbon sp^2 bond (Ijima, 1991) give them highest strength and conductivity composites to impart unique properties (Wong et al., 1997; Ajayan et al., 2000). Since the discovery of CNTs, a large number of efforts have been made worldwide to improve the preparation and the study of various mechanisms used in their formation. There are two main types of CNTs that can have high structural perfection. Single walled nanotubes (SWCNTs), consist of a single graphite sheet seamlessly wrapped into a cylindrical tube. Whereas multi walled nanotubes (MWCNTs), comprise an array of nanotubes one concentrically placed inside another like rings of a tree trunk (Qian et al., 2002).

Synthesis of CNT

Various methods reported for the formation of carbon nanotubes are arc discharge (Zeng et al., 1998), laser ablation (Ma et al., 2000), carbon monoxide disproportionation (Herreyre et al., 1995), chemical vapor deposition (CVD) (Benito et al., 1998), Hydrothermal method (Gogotsia et al., 2000).

Arc discharge method

Arc discharge method is the oldest method to synthesize CNTs. CNTs can be

synthesized using an arc discharge apparatus made up of stainless steel which is first evacuated and then filled with liquid N_2 or helium gas atmosphere. The pure graphite rod is made anode and doped with a suitable catalyst like Fe, Co, Ni or bimetallic catalyst like Y/Ni. Another graphite rod is made cathode. The arc is generated by a DC of 40–100 Amperes with maximum voltage of 50 V in a helium atmosphere at a pressure of 100–700 Torr. Under these experimental conditions the carbon material sublimating at the anode is deposited as a hard crust at the cathode. After the arc discharge, the carbonaceous material is collected from the cathode regions and characterized by SEM, TEM and Raman spectroscopy (Ijima, 1991).

The different sized SWCNT and MWCNT can be synthesized by varying the critical factors like metal catalyst used, range of direct current and voltage supplied and the pressure of helium gas or liquid N_2 and temperature gradient between the electrodes used in the experimental conditions.

Laser ablation method

In this process a carbon surface generally graphite is irradiated by a laser beam at a very high temperature under inert atmosphere in a particular reactor and a material is removed from the surface. Carbon is vaporized due to the heat produced by laser beam. The SWCNT and MWCNT both can be synthesized by this process. The metal catalysts are required to be added to the graphite target to synthesize SWCNTs. The laser beam is targeted on the carbon target containing 1.2 % catalyst with 98.8 % of graphite composite at 1200 °C in quartz tube furnace under the inert atmosphere of argon at high pressure of ~500 Torr to produce SWCNT. The diameter distribution of SWCNTs varies about 1.0-1.6 nm produced by this method (Guo et al., 1995).

The quantity, average length and diameter of CNTs depends on the parameters like laser wavelength, composition of catalyst used, temperature, pressure, type of inert gas used. The laser ablation process is much better than the arc discharge method. The 90 % purity of CNTs with a very narrow distribution of diameter can be obtained by laser ablation method.

CO disproportionation

Disproportionation reactions are those in which a single element is simultaneously oxidized and reduced. CO disproportionation reaction such as $2\ CO \rightarrow C + CO_2$ produces only C and CO_2 as a product. Since CO_2 is a stable gas and C is used as a pure source to obtain CNT. In this process, ferrocene and iron pentacarbonyl were used under CO or N_2 as a carrier gas at ambient pressure. Here CO reacts with iron pentacarbonyl to form SWCNT at a temperature between 1100 °C and 1300 °C with the flow direction upward. This process is called HiPCO (High pressure carbon monoxide reaction) (Moisala et al., 2006).

In another study the synergistic effect of Mo and Co on the catalyst performance for CNTs formation was analysed. The presence of both metals simultaneously at low Co: Mo ratio on the silica support was found to be effective as the catalyst. The extent of the interaction between Co and Mo is a function of Co: Mo ratio in the catalyst (Resasco et al., 2002). The characterization of CNTs was done by SEM, TEM, AFM and Raman spectroscopy.

Chemical vapor deposition (CVD)

This method is simple and is used for CNTs production with controlled growth direction on the substrate. The process involves the passing of a mixture of hydrocarbon gases (methane, ethylene or acetylene) through the tubular reactor in which the catalysts like Fe, Co, and Ni are deposited on the substrate (usually silica) at a temperature of 600 °-1200 °C at atmospheric pressure to decompose the hydrocarbon material (Kumar et al., 2010).

The silica is ideal substrate for growing self aligned CNT on large surfaces. The CNT formed as a result of thermal decomposition deposits on the substrate of catalyst. Silica substrate with oxide layer was

patterned with UV photolithography. Iron (Fe) as a catalyst is electron beam evacuated on this patterned substrate and ultrasonicated in acetone for 30 min. The substrate is then placed on quartz tube and sealed in quartz tube CVD furnace at temperature of 700 °-800 °C using methane, acetylene and hydrogen and then characterized (Turano and Ready, 2006).

Hydrothermal method

This method has many advantages as compared to CVD method since it is safe for environment, inexpensive, and uses lesser free energy for various equilibria. The formation of hydrothermal CNT occurs at relatively low temperature > 200 °C and low pressure 10-100 MPa. A low cost polymer polyethylene (PE) is used as a carbon source and placed in deionized water in Au capsule. The amount of water used can vary from 0-1.6 times the weight of PE. Ni catalyst is added to the capsule and heated in a reactor at a 700°-800 °C for 2-24 h at an initial pressure of 60-80 MPa and then pressure increased up to 100 MPa of water for a sufficient time period so as to allow complete thermal decomposition of PE (Gogotsia and Libera, 2000).

Encapsulation of different types of drugs on CNTs

Different kinds of drugs can be encapsulated into the hollow structure or inner cavities of CNTs to improve their efficacy. Folic acid (FA) and PEG was directly adsorbed on to the surface of CNT to make a water soluble and cancer cell targeting drug delivery system. The drug doxorubicin (DOX) was loaded onto the PEG-FA CNT surface at 149.3 ± 4.1 % loading efficiency. The drug delivery system released DOX at reduced pH value particular for the environment of cancerous cells and for lysosomes / endosomes. The released DOX will stop transcription leading to cell death (Niu et al., 2013). Paclitaxel (PTX) was conjugated to the PEGylated SWCNTs and upon conjugation their toxicity level to tumour cells was almost similarly as that of taxol under *in vivo* conditions. This has increased the blood circulation time of PTX that led to increased uptake of the drug by tumour cells (Liu et al., 2008).

The antitumour agent HCPT was covalently conjugated with MWNTs using diaminotriethylene glycol. MWNT-HCPT conjugated with 16 % drug loading was superior in its anti-tumour activity due to prolonged blood circulation time as compared to clinical formulations of HCPT. The fast release profile of drug HCPT was observed at pH = 5.0 than at pH = 7.4 within monitoring period of 128 h (Wu et al., 2009). Carboplatin, a therapeutic agent for cancer treatment was filled into opened CNT by a wet chemical method. The effect of this drug for cell proliferation and cell cytotoxicity was tested on human bladder cancer cell line EJ28. The tests have confirmed the anti cancer effect of the CNT filled with cisplatin (Hampel et al., 2008).

Surface modifications of CNT

CNTs are sterically bulky, π- conjugated ligands and electron deficient alkenes. SWCNTs can undergo reactions not only at the ends and defected sites, but along the side walls also. The unique mechanical and electronic properties can be changed in a determinate way by the controlled chemical functionalization (Banerjee et al., 2005). The CNTs synthesized by using all the above methods generally yield a sample that is a mixture of varied diameters and chirality of CNTs and often contaminated with metallic and amorphous impurities. The chemical processing of CNTs is necessary to separate them according to diameter and reactivity.

The interfacial interaction of MWCNTs can be improved with the matrix by attaching epoxy functional group to the nanotubes (Gojny et al., 2003). To improve the chemical compatibility of MWCNTs with specific polymers for application in nanotube-based polymer matrix composites, functionalized MWCNTs were silanized using a coupling agent, 3-aminopropyltriethoxysilane (3-

APTES) (Kathi and Rhee, 2008). A versatile method has been developed to selectively attach gold nanoparticles on CNTs with various modifications of the nanotubes to enhance the potential applications for the generation of electrical, optical, and sensor devices (Jiang et al., 2003).

Drug loading and release

There are two ways of drug loading to CNTs. In one case, drug molecule is incorporated into the nanoparticle at the time of formation of nanoformulation. While in other case, drug is loaded by adsorption method after the synthesis of nanoparticle (Singh and Lillard, 2009). Small molecules can be loaded by covalent conjugation method while other aromatic drug molecules can be loaded onto CNTs by non covalent supramolecular chemistry via π-π stacking (Liu et al., 2009). The drug release from CNTs can be controlled by varying the pH value and temperature.

Cellular response

The nature of the functional groups attached on the surface of CNTs plays a major role in deciding the mechanism of interaction with the cellular machinery. The CNT cellular uptake mechanism may also vary depending on the size of the CNTs, including endocytosis or passive diffusion. *In vitro* fibroblast response (Zhou et al., 2010) demonstrated that MC3T3-E1 cells strongly responded to surface nanotopography for PVA / halloysite nanotubes (HNTs) bionanocomposite films. The transparent bionanocomposite films favored to fibroblasts attachment and growth below 7.5 wt % of HNTs incorporated. The mechanism of cellular uptake by HeLa cells is that DOX/PEG-FA/SWCNTs firstly attach onto cell membrane of HeLa cells by folic acid (FA) targeting and enter into the lysosomes or endosomes by clathrin-mediated endocytosis. Then DOX is released in the acidic environment of lysosomes and migrates into nucleus to inhibit transcription by binding to DNA and induces cell death (Niu et al., 2013).

DENDRIMER

The dendrimer is a macromolecule which is highly branched, monodispersive, symmetric and spherical three dimensional polymeric molecules having a well defined molecular mass. The dendrimer chemistry was first discovered by Buhleier et al. (1978). In 1985, Tomalia and co-workers synthesized the first family of dendrimers. Newkome's group independently reported the synthesis of similar macromolecules and named them arborols (Newkome et al., 1985). The term dendrimer originated from the Greek word *dendron* (tree) and *meros* (part). The other synonyms of dendrimer are arborols and cascades. But the dendrimer is most acceptable term worldwide. The dendrimers provide a high degree of versatility and surface functionality. The functionality of these nanostructure dendrimers can be even improved by encapsulation or the conjugation of bioactive agents on the surface of dendrimers (Tomalia and Frechet, 2002). The dendrimer consists of three architectural components core, branches and surface moieties. Due to their multivalent and monodisperse character, dendrimers have stimulated wide interest in the field of chemistry and biology, especially in applications like drug delivery, gene therapy and chemotherapy.

Synthesis of dendrimers

The synthesis of dendrimer requires the selection of a suitable initiator which can be transformed into a core. Core reacts with a number of molecules in an iterative reaction sequence to form new generation of high molecular weight and branched dendrimers having specific molecular surface. Dendrimers are produced in iterative sequence of reaction steps where addition of each iteration produces a higher generation of dendrimer (Tomalia and Frechet, 2002). The synthesis method used for dendrimer preparation permits almost entire control

over the critical molecular design parameters such as size, shape, surface / interior chemistry, flexibility, and topology. The dendrimers can be synthesized by using following three different approaches (Crespo et al., 2005).

Divergent approach

In this method, dendrimer grows outwards from a multifunctional core molecule towards periphery. As a result of this growth, each new iterative reaction is characterized by the generation of an exponentially increasing number of functional groups on the periphery and double the molecular weight than the previous layer (Buhleier et al., 1978). The reaction steps can be repeated to increase the size of dendrimers as required. Many problems occur from side reactions and incomplete reactions of the end groups that lead to structural defects. To prevent side reactions and for completion of reactions, large amount of reagents are required. Therefore, there is difficulty in the purification of final product prepared through this method (Klajnert and Bryszewska, 2001).

Convergent approach

The convergent method forms a dendrimer from outside to inside just opposite to that of divergent approach. The dendrimer formation starts from the peripheral end and it progresses towards the core. It involves the synthesis of dendrimeric fragments followed by their subsequent addition to the core. With this method, it is easy to change the core group but difficult to change the end group. The convergent method has some advantages over the divergent method as it does not allow the formation of high generation of dendrimers due to the problems of steric hindrance between the reactions of end groups with the core molecules (Hawker and Frechet, 1990). It is relatively easier to purify the end product and the defects are minimized in the final structure (Crespo et al., 2005; Klajnert et al., 2001).

Double stage convergent approach

The synthesis of dendrimers by using either divergent or convergent approach requires significant number of steps and takes a lot of time. To overcome these problems, double stage convergent approach is the best approach for dendrimers synthesis. The double stage convergent approach is a conjugation of both divergent and convergent approach (Takanashi and Yamamoto, 2007). In this method, the building blocks are synthesized by divergent method followed by convergent dendrimer assembly. The preparation of higher generation dendrimers is not tedious as it reduces the number of steps for the synthesis and purification of end product. Moreover, it is easy to modify the building blocks during synthesis by divergent method and dendrimer assembly with other building blocks to enhance diversity (Crespo et al., 2005).

Encapsulation of different drugs on dendrimers

The drug molecules can be covalently attached to the periphery of the dendrimer to form dendrimer prodrugs. The second method is that the drug molecule is coordinated to the outer functional groups with ionic interactions. The third method describes that the dendrimer acts as a unimolecular micelle comprising a hydrophobic core and a polar surface by encapsulating a drug molecule through the formation of a dendrimer-drug (host–guest) interaction. The encapsulation ability of dendrimers increases with the increase in dendritic generation and with the increase in size and molecular weight of functional groups attached (Crampton and Simanek, 2007).

Doxorubicin (DOX) was conjugated to PAMAM dendrimers through amide or hydrazone (hyd). Both ‘light before’ and ‘light after’ photochemical internalization (PCI) strategies were used to improve the cytotoxicity of DOX on Ca9-22 cells. It was observed that ‘light after’ PCI treatment has enhanced the release of DOX

from the DOX-hyd-PAMAM dendrimer conjugate as compared to 'light before' PCI treatment which led to the accumulation of DOX in nucleus and caused more cell death. But both the PCI strategies were failed to improve the cytotoxic effect of DOX-amide- PAMAM dendrimer (Lai et al., 2007). Paclitaxel was encapsulated into the polymeric solution of poly(OEGMA), five-arm star poly(OEGMA) and poly-glycerol dendrimers (dendriPGs). The increased paclitaxel solubility was found in increasing order of DendriPGs, five arm star poly(OMEGA) and poly(OMEGA). Paclitaxel release study showed that all the paclitaxel was released from the dendrimeric solutions after 96h. Understanding the solubility mechanism by the use of polymers will help to develop better delivery system for poorly soluble drugs (Ooya et al., 2003). The anti-tumour drugs 10-hydroxy camptothecin (HCPT) and 7-butyl-10-aminocamptothecin (BACPT) were encapsulated into polyester dendrimers by solvent evaporation method. A 20-fold increase in water solubility of 10-HCPT was observed following encapsulation. The dendrimer encapsulation method has increased the drug potency and cell specific uptake of camptothecin by the human colon cancer cell lines HT29 (Morgan et al., 2006).

Flurbiprofen (FB) is a derivative of phenylpropionic acid having analgesic and anti-inflammatory activity. The drug is widely used in the treatment of rheumatoid arthritis. The use of PAMAM dendrimers for encapsulation and controlled release of FB has been reported (Asthana et al., 2005). *In vivo* studies showed the initial fast release of drug followed by the comparative slow release. The dendrimers were able to localize the drug at the site of inflammation and therefore drug showed its better pharmacological actions. The PAMAM dendrimers were also used as drug delivery vehicles to increase the transdermal permeation of idomethacin as the drug is hydrophobic and poorly soluble in aqueous media. The dendrimers act as carrier to transport the drug molecules in the skin in the solubilised form (Chauhan et al., 2003).

The drug molecule ibuprofen was encapsulated in PAMAM dendrimers generated with 3 and 4 $-NH_2$ functionality by solvent evaporation method. The dendrimers-ibuprofen complex entered in the lung epithelial carcinoma cells A549 rapidly. Pure ibuprofen was found to be entered into the cell in 3 hours but the dendrimers-ibuprofen complexes were entered into the cell in 1hour. So these results show that dendrimers can carry drug efficiently inside the cell (Kolhe et al., 2003).

Surface modifications of Dendrimers

Amine-terminated polyamidoamine (PAMAM) dendrimers were surface modified in a number of ways to reduce the toxicity of amine terminus, to increase the biocompatibility, to increase water solubility of the dendrimers so as to trigger the drug carrying efficiency to the target tissue (Kim et al., 2008). The ligand conjugated dendrimers serve for many biomedical applications as in drug delivery, anti-viral agents, development of various artificial proteins. The peptide and saccharide-dendrimer conjugates were prepared to increase efficacy (Chabre and Roy, 2008; Sebestik et al., 2011). The PAMAM dendrimers were surface modified with polyethylene glycol, L-arginine (Choi et al., 2004). Jevprasesphant et al. (2003) had proved that the PAMAM dendrimers modified with lauroyl moieties showed the reduced cytotoxicity and increased permeation when interacted with Caco-2 cells as compared to unmodified dendrimers.

Drug loading and release

Dendrimers are used as drug delivery vehicles bacause of relatively higher chemical and biological stability, efficacy, purity and long shelf life. The drug molecules can be loaded inside dendrimers by physical entrapement or chemical

conjugation. The drug molecule is released inside the cells by two different mechanisms. One method includes the *in vivo* break down of covalent bond between the drug and dendrimer which can be possible in the presence of suitable environment or the presence of enzymes. The second method for releasing the drug depends on changes in pH and temperature inside the cells (Mishra, 2011).

Cellular response

The increase in cellular uptake of drugs is dependent on the charge density and flexibility of the dendrimers (Morgan et al., 2006). The G-4 polypropylenimine dendrimers increased the cellular uptake of a 31 nucleotide triplex-forming oligonucleotide (ODN) by 14-fold in MDA-MB-231 breast cancer cells for delivery in prostate, breast and ovarian cancer cell lines as compared with control ODN uptake (Santhakumaran et al., 2004). Dendrimers enhanced availability and anti proliferative activity of intact ODN within cells up to 48 h or more. The SWCNT coated with PAMAM dendrimers reduced the cytotoxicity and improved the cellular uptake by MCF-7 cancer cell lines. The dendrimer coated SWCNT entered the cell cytoplasm by endocytosis but the uncoated SWCNT were unable to traverse the cell membranes (Pan et al., 2007).The dendrimers encapsulated with 10-hydroxy camptothecin enhanced the cellular uptake by 16 fold and also increased the retention time of the compound in cancer cell lines (Morgan et al., 2006).

MAGNETIC NANOPARTICLES (MNPS)

The magnetic nanoparticles (MNPs) are of great interest to the scientists from the last few decades due to their great magnetic properties for the use in various novel applications ranging from high density data storage to biomedical applications. The magnetic surface of MNPs allows their attachment to the functional molecules which then provide the magnetic behaviour to the target (Ahn et al., 2004). It helps to manipulate and transport the molecules to the desired target organ by controlling the magnetic field produced. Three functional parts of a MNP carrier are magnetic core, a surface coat and a functional outer coating (Vatta et al., 2006). The magnetic core consists of a supramagnetic molecule whose composition depends upon the application. The surface coating is required to provide steric repulsions, to enhance the stability and to prevent agglomeration of the particles (Charles et al., 1980). The functional outer coating may contain any ligand or the biologically active molecule for various biomedical applications (Hafeli, 2004).

Synthesis of MNPs

There are various methods for the synthesis of MNPs that include co-precipitation, thermal decomposition, microemulsion, hydrodermal synthesis, sonochemical, and sol-gel method. The most important technique to synthesize supramagnetic NPs is co-precipitation. The main challenge to synthesize the monodisperse MNPs of suitable size is to select the proper experimental conditions.

Co-precipitation method

Co-precipitation method generally deals with precipitation of substances generally soluble under the applied conditions. It is a facile and easy method for the synthesis of iron oxides (either Fe_3O_4 or γ-Fe_2O_3) from aqueous Fe^{2+}/Fe^{3+}salt solutions by adding a base at room temperature or at elevated temperature under inert conditions. Massart (1981) first time performed the controlled preparation of superparamagnetic iron oxide particles using alkaline precipitation of $FeCl_3$ and $FeCl_2$. The size, shape and composition of the MNPs depend on the type of salts used (e.g. chlorides, sulphates, nitrates), the ratio of Fe^{2+}/Fe^{3+}, the temperature of the reaction, pH and ionic strength of the media (Babes et al., 1999). The main benefit of this process is that a large amount of NPs can be synthesized. However, the control of particle size

distribution is limited, because only kinetic factors can control the growth of the crystal.

Thermal decomposition method

The MNPs of monodisperse type and small size can be synthesized by the thermal decomposition of organometallic precursors in high boiling solvents that contain the stabilizing surfactants. The ratios of precursors, surfactants, solvents, reaction temperatures, reaction times are significant parameters to control the size and shape of the synthesised MNPs. The halogen assisted formation of iron oxide nanocrystals was done and showed that both Cl^- and Br^- ions work on stabilizing 100 facets of spinal structured iron oxide nanocrystals (Xu et al., 2010).

In the literature, a general and very simple approach to synthesize the size and morphology controlled magnetic iron oxide nanocrystals is reported. The reaction system was based on thermal decomposition of the metal fatty acid salts like decanoic acid, lauric acid, myristic acid, palmitic acid, oleic acid, stearic acid and a hydrocarbon solvent such as octadecene (ODE), n-eicosane, tetracosane or a mixture of ODE and tetracosane. Using this approach, monodisperse Fe_3O_4 nanocrystals in a size range of 3-50 nm were synthesized. This method can also be used to synthesize metallic NPs (Jana et al., 2004).

Microemulsion

Microemulsions are clear, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant, and are frequently used in combination with a co-surfactant. The surfactant molecule lowers the interfacial tension between water and oil resulting in the formation of a transparent solution. There are three basic types of microemulsions; direct (oil dispersed in water, o/w), reversed (water dispersed in oil, w/o) and bicontinuous. The water-in-oil (w/o) microemulsion method has been widely used to synthesize uniform sized MNPs (Liu et al., 2000). The iron oxide MNP was synthesized by using sodium dodecylbenzenesulfonate (NaDBS) to form water-in-toluene reverse micelles. The average particle size of single crystalline MNPs was in the range of 4 to 15 nm.

Santra et al. (2001) reported a water-in-oil microemulsion method for the synthesis of uncoated and silica-coated iron oxide NPs. Triton X-100, Igepal CO-520, and Brij-97 as non-ionic surfactants and NH_4OH, NaOH as base were used for the preparation of microemulsions. Their effect on the particle size, crystallinity, and the magnetic properties was studied. Microemulsions can be used to synthesize monodisperse NPs with different morphology. The major disadvantages of this method are need of a large amount of solvent, low product yield, difficulty in scale-up and adverse effects of residual surfactants on the properties of MNPs (Faraji et al., 2010; Hasany et al., 2012).

Hydrothermal synthesis

Hydrothermal processing method for the synthesis of MNPs is also called solvothermal method. It comprises aqueous reactions usually carried out by using autoclaves at high pressure over 2000 psi and at high temperature of more than 200 °C. In this process, the reaction conditions of precursor material, solvent, temperature, pH and time have important effect on the product and phase purity of the nanoparticles (Chen and Xu, 1998). There are two main routes for the formation of ferrites via hydrothermal conditions: hydrolysis and oxidation and second is neutralisation of mixed metal hydroxides.

The synthesis of nickel ferrite NPs by using ethylene glycol as solvent and NaAc as electrostatic stabilizer has also been reported. The size of the NPs was easily controlled from 6 to 170 nm by adjusting the experimental parameters such as reaction duration, initial concentration of the reactants, amount of protective reagents and the type of acetates used. The major drawback of the conventional hydrothermal method is slow reaction kinetics at any given tempera-

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ture. However, this problem may be overcome by microwave heating (Wang et al., 2009).

Sonochemical method

The sonochemical method is being used to generate novel materials with unusual properties. Actually, the physiochemical effects of very high temperature generated hot spot by the rapid collapse of sonically created cavities allow the conversion of ferrous salts into MNPs. The superparamagnetic iron oxide NPs (SPIO) of high magnetization and crystallinity using a sonochemical method has been synthesized. The coated SPIO were dispersed in chitosan. The hydrodynamic diameter of the coated SPIO in the chitosan solution was found to be 65 nm (Kim et al., 2005).

Also, nanosized amorphous alloy powders of $Co_{20}Ni_{80}$ and $Co_{50}Ni_{50}$ were prepared by sonochemical decomposition of solutions of volatile organic precursors, $Co(NO)(CO)_3$ and $Ni(CO)_4$ in decalin, under an argon pressure of 100 to 150 kPa at 273 K. Magnetic measurements have indicated that the prepared amorphous CoNi alloy particles were superparamagnetic (Shafi et al., 1998).

Sol-gel method

The sol-gel process is based on the hydroxylation and condensation of molecular precursors like metal alkoxides in solution forming a "sol" of nanometric particles. The ''sol'' is then dried or termed as ''gelled'' by solvent removal or by chemical reaction to 3-D metal oxide network (Ismail, 2005). The crucial factors like solvent, temperature, nature and concentration of the salt precursors employed, catalyst, additives, pH, and agitation affect on kinetics, growth reactions, hydrolysis, condensation reactions and the structure and properties of the gel (Hasany et al., 2012).

Sol-gel method has been used to prepare a series of Fe_2O_3-SiO_2 nanocomposites (9-33 % Fe_2O_3 by wt) and submitted to thermal treatments in the temperature range of 300 °C - 900 °C. Synthesised nanocomposites were further characterized by XRD, TEM, EPR and magnetic susceptibility measurements (Cannas et al., 1998). A high-purity $La_{2/3}Ca_{1/3}MnO_3$ perovskite has also been produced via a simple sol-gel process by dissolving the appropriate inorganic salts in ethanol-acetic acid mixture without using any complexing agents (e.g., polyol or polyhydroxy acid, etc.) which is otherwise essentially used in the polymeric precursor routes (Mathur and Shen, 2002).

Encapsulation of different drugs inside MNPs

Various kinds of drugs have been encapsulated in MNPs for targeted delivery. The therapeutic drug molecules are either attached to the surface of MNPs or encapsulated within a nanocomposite mixture of a polymer and MNPs (Mody et al., 2013). The presence of various polymer or metal/non metal coating on the surface of MNPs provides better opportunity to attach various drugs for targeted delivery.

Under this category, DOX has been delivered to target tumour sites using G_2, G_3, G_4, and G_7 PAMAM dendrimer MNPs (DcMNPs). The maximum drug loading concentration was 400 µg/mL. Interestingly, G_4DcMNPs were found to be the most efficient drug delivery systems (Rouhollah et al., 2013). The anti tumour agent, paclitaxel was covalently conjugated to silica coated magnetic NPs (TXL-SiMNP). To check the anti tumour activity, *in vitro* evaluations of developed SiMNP-TXL and SiMNP as a control formulations of various concentrations was performed on human breast adenocarcinoma cells (MDA-MB-468 triple-negative) upto 5 days. The TXL loaded SiMNP formulations were reported cytotoxic against the tumour cells in a concentration dependent manner (Auzenne et al., 2013).

5-FU was encapsulated in magnetite / PLGA NPs for the magnetically controlled delivery of 5-FU to the cancer cells. The encapsulation efficiency of MNPs was 60 - 80 %. The *in vitro* release profiles of 5-

FU has indicated the initial fast release followed by a 7 day lag period. This has signified the potential viability of the magnetite / PLGA nanomaterials for effective drug delivery (Ashjari et al., 2012). Curcumin is a major polyphenolic pigment found in turmeric root, possessed antitumor and antioxidant activities. However, the efficacy and bioavailability of as such curcumin is very poor. Therefore, curcumin was encapsulated in a polymeric MNP that was synthesized by cross linking of polymers β-cyclodextrin with epichlorhydrin and magnetite as magnetic material. The *in-vitro* release of curcumin loaded MNPs showed initial release for first 24 hours followed by slow release up to 96 hours (Silambarasi et al., 2012).

Methotrexate (MTX), an anticancer drug was covalently bound with iron oxide supramagnetic NPs for targeted drug delivery to cancer cells having overexpressed folate receptors on the surface (Kohler et al., 2005). Treatment of developed MTX nanocomposite on MCF-7 and HeLa cells has shown their internalization at a high level into lysosome. Here in lysosome, MTX was released at low pH by enzymatic cleavage of peptide bond. These studies have documented that MNPs can be used as smart carrier for controlled and targeted drug delivery

Surface modification of MNPs

The MNPs are very sensitive to agglomeration and oxidation reactions due to their large surface area, and high magnetic dipole interaction. It is often desirable to protect the surface of MNPs with the use of stabilizers to reduce oxidation reactions and aggregation due to magnetic and electrical interactions. In addition, surface modifications also enhance the stability of MNPs enabling them more biocompatible. It allows for further functionalization of the nanoparticle surface to provide additional functionality to the magnetic core (Williams and Corr, 2013). Therefore, the surface of MNPs can be easily functionalized by coatings of polymers, silica, carbon, and metal.

The stability has been shown to be improved in biological media by the surface coating of MNPs with gum Arabic (GA). The GA-treated NPs were forming smaller agglomerates as compared to the untreated samples over 30h time period (Williams et al., 2006). The silica coated surface of MNPs was modified with (aminopropyl)-triethoxysilane (APTS) to enhance their potential in research and diagnostic applications (Bruce and Sen, 2005). The supramagnetic NPs were surface modified with PEG to increase their biocompatibility and cellular uptake. Such PEGylated NPs influence have been studied on human fibroblast cells (Gupta and Curtis, 2004).

Drug loading and release

MNPs serve as an efficient drug delivery system and therefore, offering a flexible drug loading and sustained therapeutic release of drug under the influence of external magnetic field. A drug molecule can be loaded into MNPs through different methods, including covalent binding, electrostatic interactions, adsorption or encapsulation within the pores of magnetic carrier (Mody et al., 2013). The encapsulation of drug inside MNPs is confirmed by UV-Vis spectroscopy, FTIR, and HPLC. Drug-loaded magnetic particles reach to the target site by active and passive mechanisms. Active mechanism is based on the MNP attachment to the ligands and attraction of these conjugates to the target-site proteins. Passive mechanism is based on the enhanced permeability retention effect (EPR) inside the blood vessels and target cells (Arruebo et al., 2007).

Cellular response

The MNPs are used as drug delivery system for tissue/cell specific delivery. The cellular response of a particular cell type for a drug is possible by coating the surface of MNPs with the suitable ligands that can bind to the receptors of the target organs

(Kim et al., 2006). The biotin conjugated lipid coated MNPs were surface functionalized by streptavidin fluorescein isothiocyanate (FITC) had shown uptake by HeLa cells and found to be localized in the lysosomal compartment by receptor mediated endocytosis (Becker et al., 2007). The uptake of silica coated MNPs containing rhodamine B isothiocyanate (RITC) into A549 human lung cancer cells is temperature dependent and occurs through energy dependent endocytosis by endosomes. The metabolic inhibitors and low temperature of 4 degrees have inhibited the cellular uptake of MNPs (Kim et al., 2006). The cellular uptake and cytotoxicity of pullulan coated MNPs as compared to uncoated MNPs were also investigated. The coated MNPs have reduced human fibroblast cell cytotoxicity and enhanced the cellular uptake by different routes distinct from the uncoated MNPs (Gupta and Gupta, 2005). Rodriguez et al. (2013) had coated carboxymethyl inulin on the surface of iron oxide NPs and tested their cytotoxicity towards various cancer cell lines. They observed satisfactory cellular uptake of coated MNPs by Caco-2 cell lines in a time and concentration dependent fashion.

CONCLUSIONS

NCs are important candidates for delivery of therapeutic agents important for pharmaceutical industry. They have advantages over conventional carriers. They can improve the efficacy and bioavailability of many therapeutic agents which are otherwise difficult to deliver. NCs will increase the half life and persistence of many therapeutic agents thus increasing their therapeutic index. Surface modified NCs with hydrophilic polymers or ligands will increase the targeting ability and specificity of many therapeutic agents. Surface modification of NCs offers way for increasing cellular uptake and binding. Coupling of small molecules on the surface of NCs also increases cellular uptake and interaction. By smartly designing NCs many therapeutic agents will enter into clinical trials. Current research is focussed on encapsulation, and targeted delivery of therapeutic agents. But more extensive studies are needed to establish cellular interactions and fate of therapeutic agent loaded NCs.

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