INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Review Article

NANOTECHNOLOGY: THE COMING REVOLUTION IN MODERN BIOLOGY AND MEDICINE

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Article Received on: 18/04/12 Revised on: 03/05/12 Approved for publication: 18/05/12

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ABSTRACT
Nanotechnology is a wide field that covers a variety of devices derived from engineering, physics, chemistry, and biology. The promising new field of nanotechnology, created up by rapid advances in life science and technology, gained countless new opportunities for modern medical science and disease treatment in human health care. Among the various researches in delivery of therapeutic and diagnostic agents for the diagnosis and treatment of a number of diseases. The development of nanopharmaceuticals achieved a lot of importance over first few years. The goal of this paper was aimed to discuss the different formulation techniques available and their characterization procedures. This review also incoporates the importance of nanotechnology in diagnosis and cure of several critical diseases like cancer and neurodegenerative diseases.
Keywords: Preparation, characterization, disease treatment and detection, current advancement

INTRODUCTION

Nano, a word originating from the Greek word for dwarf, is a prefix that has attracted major attention over the last decade. At the engineering faculties, scientists have for long been engaged in research related to nanotechnology. This area is defined by the ability to create and control objects on the 1–100 nm scale, with the goal to develop novel materials with specific properties and functions. Compared to the size of eukaryotic cells, nanometer-sized objects are several orders of magnitudes smaller. This has generated great expectations, from within the research community as well as from the society, in the novel area termed nanomedicine. This area is defined as the discipline where nanotechnological advances becomes integrated into medical applications. Whereas nanomedicine has the potential to shape, direct, and change future medical treatments in a revolutionary manner, the complexity of the human as an organism certainly makes nanomedicine a challenging area to engage in. Yet, this area is already showing great impact on biomedical research, primarily because novel experimental platforms are created for in vitro studies. As these platforms allow novel designs of the bio-experiments, questions that previously were impossible to solve due to lack of technologies can be addressed[1].

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale (one-billionth of a meter)[2,3]. In the past few years' nanotechnology has grown by leaps and bounds, and this multidisciplinary scientific field is undergoing explosive development[4-7]. It can prove to be a boon for human health care, because nanoscience and nanotechnologies have a huge potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger, lighter materials. Human health-care nanotechnology research can definitely result in immense health benefits. The genesis of nanotechnology can be traced to the promise of revolutionary advances across medicine, communications, genomics, and robotics. A complete list of the potential applications of nanotechnology is too vast and diverse to discuss in detail, but without doubt, one of the greatest values of nanotechnology will be in the development of new and effective medical treatments[2,8-11].

Over recent years, advancements in drug delivery have facilitated the targeting of specific tissues. With the advent of nanotechnology, these targeted tissues are now becoming specific organelles within individualized cells. Nanomedicine has blossomed into a billion-dollar industry because of these compounds' inherent ability to overcome solubility and stability issues, localize drug delivery, as well as to diagnose via in vivo imaging. Coupled with genomic tailoring, nanomedicine may soon spawn the much-anticipated individualized medicine. Upcoming innovations in nanomedicine may even generate multifunctional entities capable of simultaneously diagnosing, delivering therapeutic agents, and monitoring treatment. Several particle types and structures have been discovered. Noteworthy structures include polymeric micelles, dendrimers, quantum dots (QDs), and solid nanoparticles. Although these structures may promise endless opportunities, their safety should not be ignored. The reactivity of these tiny particles may be due to their large surface area in comparison to their overall mass. Semiconductor metals, such as colloidal gold and iron oxide crystals, are commonly used and have demonstrated toxicity. Additionally, increased pulmonary toxicity was noted with carbon nanotubes when compared to that of the carbon black and carbonyl iron particles seen in mice and rats[12,13]. Nanotechnology have emerged as one of the most promising field for research in pharmacy over the last few years known as Nanopharmaceuticals. A number of pharmaceutical industries have won approval from US Food and Drug Administration for the use and formulation of nanopharmaceuticals (Table I). More than thousand nanopharmaceutical patents have been issued by U.S. Patent and Trademark office during the period 1999 – 2008[14]. The overall goal of nanotechnology and the developed naopharmaceuticlas is, to diagnose as accurately and as early

as possible the disease as well as to treat them as effectively as possible without any side effects.

Table I. Few selected marketed nanopharmaceuticals

Drug	Formulation and route of administration	Brand name	Company	Therapeutic indication
Amphotericin B	Lipocomplex (intravenous infusion)	Amphocil	Sequus Pharmaceuticals	Serious fungal infections
Amphotericin B	Liposome (intravenous infusion)	Ambisome	NeXstar Pharmaceuticals	Serious fungal infections
Amphotericin B	Lipid complex (intravenous infusion)	Abelcet	The Liposome Company (Princeton, New Jersey)	Serious fungal infections
Paclitaxel	Albumin bound nanoparticles (intravenous injection)	Abraxane	American Biosciences (Blauvelt, New York)	Metastatic breast cancer
Sirolimus	Nanocrystal particles (oral)	Rapamune	Wyeth/ Elan (Madison, New Jersey)	Immunosuppressant in kidney transplant patients
Aprepitant, MK869	Nanocrystal particles (oral)	Emend	Merck/ Elan (Whitehouse Station, New Jersey)	For chemotherapy patient to delayed nausea and vomiting
Doxorubicin	Liposome (intravenous injection)	Doxil	Sequus Pharmaceuticals	Kaposi sarcoma in AIDS
Daunorubicin citrate	Liposome (intravenous)	DaunoXome	NeXstar Pharmaceuticals	Kaposi sarcoma in AIDS

PREPARATION OF NANOPARTICLES

Many methods have been developed for preparing nanoparticles; these methods can be classified into two main categories according to whether the formulation requires a polymerization reaction or is achieved directly from a macromolecule or preformed polymer[15]. The polymerization methods can be further classified into emulsion and interfacial polymerization, and there are two types of emulsion polymerization—organic and aqueous—depending on the continuous phase. Additionally, the interfacial condensation method will be further discussed later in this review. Nanoparticles can be also prepared directly from preformed synthetic or natural polymers and by desolvation of macromolecules. Recently these polymeric systems have been prepared by nebulization techniques. This review covers all these methods including their detailed procedures and technological advantages, as well as providing several examples of encapsulants that are entrapped into or adsorbed to these particles[16].

Nanoparticles obtained by polymerization of a monomer

Emulsion polymerization

Emulsion polymerization is one of the fastest methods for nanoparticle preparation and is readily scalable[17]. The method is classified into two categories, based on the use of an organic or aqueous continuous phase. The continuous organic phase methodology involves the dispersion of monomer into an emulsion or inverse microemulsion, or into a material in which the monomer is not soluble (nonsolvent). Polyacrylamide nanospheres were produced by this method[18,19]. This procedure has become less important, because it requires toxic organic solvents, surfactants, monomers and initiator, which are subsequently eliminated from the formed particles. As a result of the nonbiodegradable nature of this polymer as well as the difficult procedure, alternative approaches are of greater interest. Later, poly(methylmethacrylate) (PMMA), poly(ethylcyanoacrylate) (PECA), and poly(butylcyanoacrylate) nanoparticles were produced by dispersion via surfactants into solvents such as cyclohexane (ICH, class 2), n-pentane (ICH, class 3), and toluene (ICH, class 2) as the organic phase. Examples of drugs encapsulated with this system are triamcinolone fluorescein , pilocarpine , and timolol[20,21,22]. In the aqueous continuous phase the monomer is dissolved in a continuous phase that is usually an aqueous solution, and the surfactants or emulsifiers are not needed. The polymerization process can be initiated by different mechanisms. Initiation occurs when a monomer molecule dissolved in the continuous phase collides with an initiator molecule that might be an ion or a free radical. Alternatively, the monomer molecule can be transformed into an initiating radical by high-energy radiation, including g-radiation, or ultraviolet or strong visible light. Chain growth starts when initiated monomer ions or monomer radicals collide with other monomer molecules according to an anionic polymerization mechanism[23].

Interfacial polymerization

Cyanoacrylate monomer and drug were dissolved in a mixture of an oil and absolute ethanol[23]. This mixture was then slowly extruded through a needle into a well-stirred aqueous solution, with or without some ethanol (ICH, class 3) or acetone (ICH, class 3) containing surfactant. Nanocapsules are formed spontaneously by polymerization of cyanoacrylate after contact with initiating ions present in the water. The resulting colloidal suspension can be concentrated by evaporation under vacuum. PECA, poly(isobutylcyanoacrylate) and poly(isohexylcyanoacrylate) were used in production of nanoparticles by this process[24-28]. Examples of drugs encapsulated are insulin[25,29], calcitonin[19], octreotide[30], darodipine[31], indomethacin[32,33] and photoactivatable cytotoxic compounds used in photodynamic tumor therapy like phthalocyanines in an injectable vehicle[28]. An advantage of interfacial polymerization techniques is high-efficiency drug encapsulation (eg, insulin with 95%). In addition, the advantage of obtaining nanocapsules by this method is that the polymer is formed in situ, allowing the polymer membrane to follow the contours of the inner phase of an oil/water or water/oil emulsion[34].

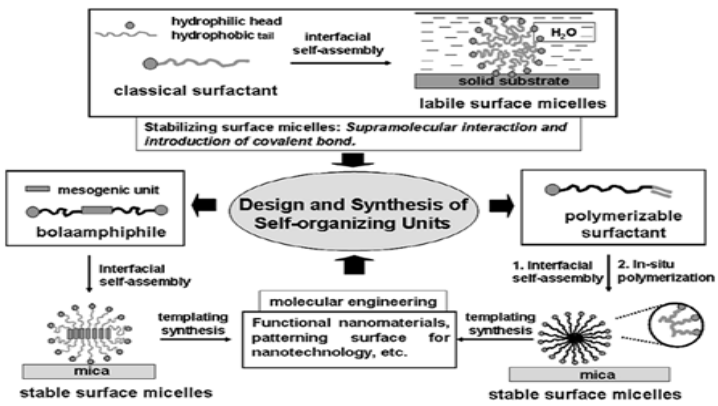

Fig.1 Schematic representation of interfacial polymerization

Interfacial polycondensation

Polymeric nanoparticles can be also prepared by the interfacial polycondensation of the lipophilic monomer, such as phtaloyldichloride and the hydrophilic monomer, diethylenetriamine, in the presence and absence of the surfactant[35].These nanoparticles were smaller than 500 nm. A modified interfacial polycondensation method was also developed. In this case, polyurethane polymer and poly (ether urethane) copolymers were chosen and successfully applied as drug carriers for a-tocopherol. Polyurethane and poly(ether urethane)–based nanocapsules were synthesized by interfacial reaction between two monomers[36].

Nanoparticles obtained from preformed polymers

With the exception of alkylcyanoacrylates and poly (dialkylmethylidene malonate), most of the monomers suitable for a micellar polymerization process in an aqueous phase lead to slowly biodegradable or nonbiodegradable polymers. In addition, residual molecules in the polymerization medium (monomer, oligomer, surfactant, etc.) can be more or less toxic, requiring meticulous purification of the colloidal material. To avoid these limitations, methods using preformed polymers instead of monomers have been proposed.

Emulsification/solvent evaporation

Emulsification-solvent evaporation involves two steps. The first step requires emulsification of the polymer solution into an aqueous phase (Fig. 2). During the second step polymer solvent is evaporated, inducing polymer precipitation as nanospheres. A polymer organic solution containing the dissolved drug is dispersed into nanodroplets, using a dispersing agent and high-energy homogenization , in a nonsolvent or suspension medium such as chloroform (ICH, class 2) or ethyl acetate (ICH, class 3)[37].

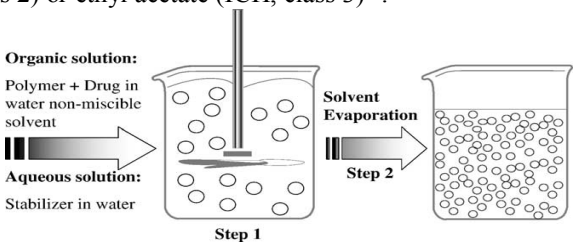

Fig. 2 Schematic representation of the emulsification-evaporation technique

The solvent is subsequently evaporated by increasing the temperature under pressure or by continuous stirring[38]. This method can only be applied to liposoluble drugs, and limitations are imposed by the scale-up of the high energy requirements in homogenization[39]. Frequently used polymers are Poly lactic acid (PLA)[39-40], Poly lactide co-glycolide (PLGA), ethylcellulose (EC), cellulose acetate phthalate, poly(E-caprolactone) (PCL), and poly(h-hydroxybutyrate) (PHB). Drugs or model drugs encapsulated were albumin, texanus toxoid, testosterone, loperamide, prazinquantel, cyclosporin A, nucleic acid, and indomethacin[40].

Solvent displacement and interfacial deposition

Solvent displacement and interfacial deposition are similar methods based on spontaneous emulsification of the organic internal phase containing the dissolved polymer into the aqueous external phase (Fig. 3). However, solvent displacement forms nanospheres or nanocapsules, whereas interfacial deposition forms only nanocapsules. Solvent displacement involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant[41].

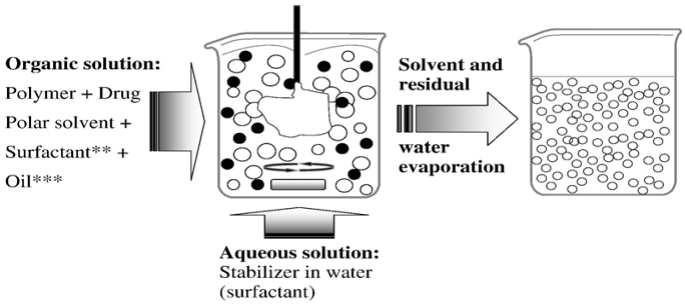

Fig. 3 Schematic representation of the solvent displacement technique. **Surfactant is optional. ***In interfacial deposition method, a fifth compound was introduced only on preparation of nanocapsules.

The polymer, generally PLA, is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension. The solvent displacement technique allows the preparation of nanocapsules when a small volume of nontoxic oil is incorporated in the organic phase. Considering the oil-based central cavities of the nanocapsules,high loading efficiencies are generally reported for lipophilic drugs when nanocapsules are prepared. This method has been applied to various polymeric materials such as PLA, PLGA, PCL[42], and poly(methyl vinyl ether-comaleic anhydride) (PVM/MA). Interfacial deposition is a process used for the production of nanocapsules; however, this is not a polymerization technique but an emulsification/solidification technique. In interfacial deposition, a fifth compound is introduced, of oil nature, miscible with the solvent of the polymer but immiscible with the mixture. The polymer deposits on the interface between the finely dispersed oil droplets and the aqueous phase, forming nanocapsules[42].

Emulsification/solvent diffusion

Emulsification/solvent diffusion (ESD) was proposed in the literature based on the use of organic solvents, and then it was adapted to the following salting-out procedure. The encapsulating polymer is dissolved in a partially watersoluble solvent such as propylene carbonate (ICH not given) and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. In fact, to produce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to promote the diffusion of the solvent of the dispersed phase by dilution with an excess of water when the organic solvent is partly miscible with water or with another organic solvent in the opposite case. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point. The procedure is illustrated in Fig. 4. This technique presents several advantages, such as high encapsulation efficiencies (generally N70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution. Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage

of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency. As with some of the other techniques, this one is efficient in encapsulating lipophilic drugs.

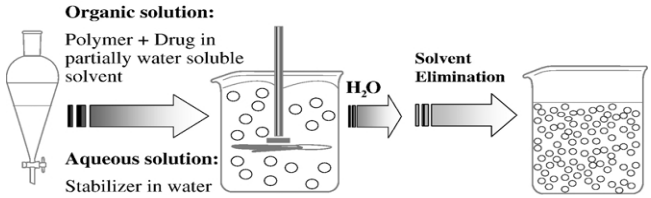

Fig. 4 Schematic illustration of the ESD technique

Several drug-loaded nanoparticles were produced by the ESD technique, including mesotetra(hydroxyphenyl) porphyrin-loaded PLGA (p-THPP) nanoparticles, doxorubicin-loaded PLGA nanoparticles, plasmid DNA-loaded PLA nanoparticles, coumarin-loaded PLA nanoparticles, indocyanine, cyclosporine (Cy-A)-loaded gelatin and cyclosporin (Cy-A)-loaded sodium glycolate nanoparticles[43].

Salting out with synthetic polymers

Salting -out is based on the separation of a watermiscible solvent from aqueous solution via a salting-out effect. The salting-out procedure can be considered as a modification of the emulsification/solvent diffusion. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non- electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose. This oil/water emulsion is diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. The selection of the saltingout agent is important, because it can play an important role in the encapsulation efficiency of the drug. Both the solvent and the salting-out agent are then eliminated by cross-flow filtration. This technique used in the preparation of PLA, poly-(methacrylic) acid, and EC nanospheres leads to high efficiency and is easily scaled up[41].The preparative steps of this procedure are described in Fig. 5.

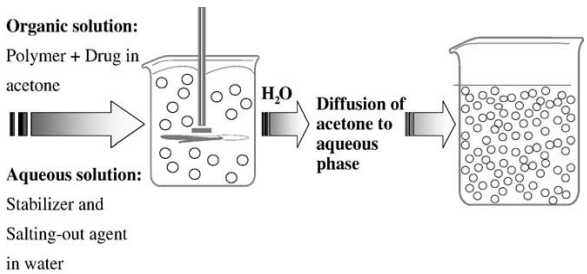

Fig. 5 Schematic of the salting-out technique

Production of nanoparticles from natural Macromolecules

Albumin nanoparticles produced in an external-oily emulsion

Two main methods are used in the preparation of albumin microspheres, characterized by the method of stabilization; thermal treatment at elevated temperatures (958–1708°C) or chemical treatment in vegetable oil, iso-octane emulsions, or aqueous medium. Other techniques involve slight modification of either of the two methods. In this case albumin nanospheres were formed by homogenizing the oil phase containing the albumin droplets and thermally stabilized by heating at 1758 to 1808C for 10 minutes. This mixture was cooled and diluted with ethyl ether to reduce the viscosity of the oil phase to permit separation by centrifugation. Heat treatment of albumin is applicable only to drug molecules that are not heat sensitive. For this reason, nanoparticles were produced emulsifying serum albumin aqueous solution in cottonseed oil at 258C, then denaturing the albumin by resuspending the particles in ether containing the cross-linking agents 2,3-butadiene or formaldehyde. The particles were stirred, isolated by centrifugation, and dried by lyophilization. Particles released the drug doxorubicin much faster than particles formed by heat treatment.

Gelatin nanoparticles produced in an external-oily emulsion

Emulsified gelatin solution droplets were hardened by cooling the emulsion below the gelation point in an ice bath, resulting in gelation of the gelatin droplets. Gelled nanodroplets were filtered, washed, and cross-linked with formaldehyde. The particle size ranged between 100 and 600 nm with a mean of 280 nm. This technique is applicable to heat-sensitive drugs; however, a number of drugs can be covalently bound to the gelatin by formaldehyde treatment, which constitutes a disadvantage. Another interesting system for drug delivery systems could be nanoparticulate carriers from bioacceptable macromolecules. For this reason, vegetable protein fractions termed gliadins have been chosen from wheat gluten, to efficiently encapsulate lipophilic substances such as a-tocopherol.

Alginate nanoparticles

Sodium alginate is a water-soluble polymer that gels in the presence of multivalent cations such as calcium. Alginate particles are usually produced by dropwise extrusion of sodium alginate solution into calcium chloride solution. Alginate particle size depends on the size of the initial extruded droplet. The smallest particles produced had a minimum size of 1 to 5 nm, obtained by air atomization. The preparation of alginate nanoparticles was first achieved in a diluted aqueous sodium alginate solution in which gelation was induced by the addition of a low concentration of calcium. This leads to the formation of invisible clusters of calcium alginate gels. In an additional advance, alginate particles have been produced by using a modified emulsification/internal gelation method as illustrated in Fig. 6. The preparation of alginate nanoparticles via this method does not require specialized equipment and can be performed at ambient temperature. The main difficulty of this method is the nanoparticle washing step to eliminate the residual oil droplets, but new strategies have been devised.

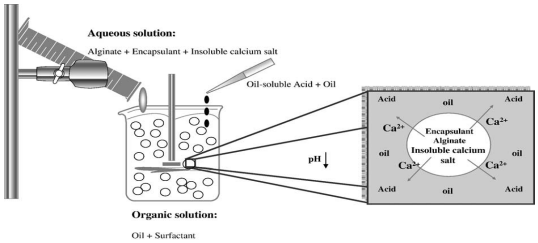

Fig. 6 Schematic representation of the emulsification–internal gelation technique using alginate

Chitosan nanoparticles

Chitosan nanoparticles have been developed to encapsulated proteins such as bovine serum albumin, tetanus and diphtheria toxoid[38], vaccines, anticancer agents[44], insulin, and nucleic acids. Chitosan considerably enhanced the absorption of peptides such as insulin and calcitonin across the nasal epithelium. The methods proposed to prepare chitosan nanoparticles are based on the spontaneous formation of complexes between chitosan and polyanions or the gelation of a chitosan solution dispersed in an oil emulsion. Chitosan nanoparticles obtained by formation of a spontaneous complex between chitosan and polyanions such as tripolyphosphate have small diameters (200–500 nm) and show a quasi spherical shape under transmission electron microscopy. Chitosan nanoparticles produced by a promoting gelation in an emulsification-based method as illustrated in Figure 7, results in a diameter of 400 nm. Compared with the previously described method, this technique has a major disadvantage of involving organic solvents during the isolation of the particles; these are difficult to remove and may cause toxicity.

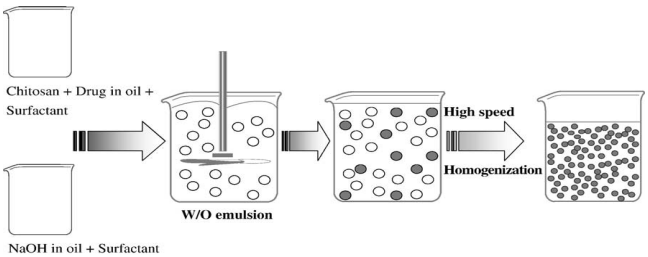

Fig.7 Schematic representation of chitosan nanoparticles preparation by the emulsification technique

Agarose nanoparticles

Agarose nanoparticles were developed for the administration of therapeutic proteins and peptides[45]. Agarose aqueous solution forms thermally reversible hydrogels while being cooled below the gelling temperature (31º–36ºC). Thermal gelation results from the formation of helicoidal structures responsible for a three-dimensional network in which large amounts of water can be entrapped. The hydrogel, being hydrophilic, inert, and biocompatible, forms a suitable matrix for proteins and peptides that can be entrapped in the gel during formation. Agarose nanoparticles were produced using an emulsion- based technology as illustrated in Figure 8. This methodology requires the preparation of an agarose solution in corn oil emulsion at 48º C. Peptides and proteins to be encapsulated are initially added to the agarose solution. The small size of the dispersed aqueous nanodroplets is achieved by homogenization. Gelation of agarose is then induced by diluting the emulsion with cold corn oil under agitation at 5ºC. The liquid nanodroplets then gel to protein-containing agarose hydrogel nanoparticles.

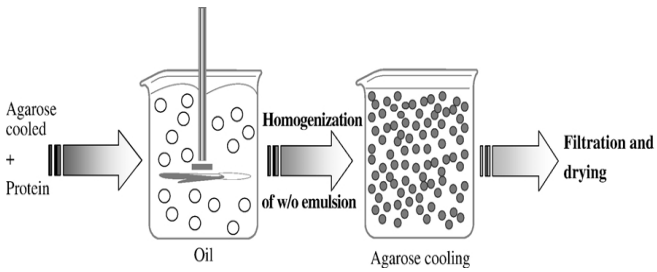

Fig. 8 Schematic illustration of agarose nanoparticles preparation by the emulsification technique

Nanoparticles produced by desolation of macromolecules

Another technology applicable to a wide range of polymers is based on desolvation by charge and pH changes, or by addition of a desolvating agent (ethanol or concentrated inorganic salt solutions). The main advantage is that this process does not require an increase in temperature and, therefore, may be useful when heat sensitive drugs are used. Nanoparticles were prepared using the process of reversible swelling of macromolecules using gelatin, human serum albumin, bovine serum albumin, and casein, as the macromolecular materials. This process offers the advantage of producing nanoparticles directly in aqueous suspension, but the use of potentially toxic compounds such as glutaraldehyde and desolvating agents requires subsequent purification[15]. Variations in nanoparticle production by the desolvation process were described, but unfortunately the yield is comparatively low. In the case of gelatin, different methods such as the two-step desolvation method have been applied to produce nanoparticles. Recent reports outline the important use of gelatin as drug delivery systems for DNA and cytostatics.

New techniques based on supercritical or compressed fluids

Some of the techniques described above are complex, and the products may often be characterized by high residual solvent content, low drug loading, drug degradation or denaturation, ineffective drug release, or unsuitable physical and morphological properties[46]. Techniques based on supercritical or compressed fluid can be an interesting tool for preparation of nanoparticulate and microparticulate products. In this technique, the drug and the polymer are solubilized in a supercritical fluid, and the solution is expanded through a nozzle. The supercritical fluid is evaporated in the spraying process, and the solute particles eventually precipitate. This technique is clean, because the precipitated solute is free of solvent. It also provides advantages such as suitable technological and biopharmaceutical properties and high quality. It has been demonstrated for numerous applications involving protein drug delivery systems. Protein drugs such as insulin were encapsulated in poly(ethylene glycol)/poly(l-lactide) (PEG/PLA) nanoparticles by this technique.

CHARACTERIZATION OF NANOPARTICLES

During the past decade, nanotechnology has become one of the fast growing fields in science and technology. Nanoparticles play key roles in all areas of nanotechnology, e.g., nano-material, nano-component and nano-fabrication. Characterization of various nanoparticles, from nano-spheres, nano-hydrogel, molecular associations, liposomes to quantum dots, is on the center stage in nanotechnology development. Recently, with more nanotechnologies being transferred from academic research to industry production, the objectives for characterization start to be steered towards quality control and environmental protection needs. The subjects for nanoparticles characterization are focused on particle size and particle surface charge determinations. For many nanoparticles, whether produced dry or wet, final applications are often in wet form. Analyzing nanoparticles in wet, concentrated slurry or diluted suspension therefore is the natural choice. However, small particles have huge specific surfaces; they may not be present in wet as individual particles. On the contrary, they often exist in aggregation or agglomeration. Therefore, one needs to disperse them into

individual particles if the analysis aims at obtaining primary particle information, or to maintain them as they are if one wants to study product stability or performance. To disperse nanoparticles into individual particles is not an easy task and the process can be complicated. Success of dispersion is sometimes difficult to justify. Much attention needs to be paid to sample preparation if correct result for dispersed particles is desired.

Size analysis and morphology

Dynamic light scattering (DLS) that utilizes time variation of scattered light from suspended particles under Brownian motion to obtain their hydrodynamic size distribution is the most popular technology in sizing nanoparticles. DLS has been used to measure macromolecules and small particles in dilute suspension since coherent light sources, i.e., commercial lasers, became available in the 1970s. The ISO Standard 13321 published in 1996 summarizes the common platform and achievement of the popular DLS technique, photon correlation spectroscopy (PCS) which uses photon–photon correlation function to analyze particles in suspension. To measure particles in concentrated suspensions, three techniques have been developed, i.e., frequency analysis (FA), photon cross-correlation function (PCCS), and back scattering (BS). Using these techniques, particle size measurement in suspension up to 10% by volume or even higher can be realized. The latter includes scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Freeze fracture techniques. The size evaluation of nanoparticles dispersion demonstrates better results with freeze fracturing microscopy and photon correlation spectroscopy as quantitative methods.

Zeta potential measurement

For small particles in liquid, there is no satisfactory technique to determine surface charge of the particles. The common practice is to determine the electric potential of a particle at a location away from the particle surface, somewhere in the diffuse layer. This location, related to particle movement in liquid, is called the slipping or shear plane. The potential measured at this plane is called zeta potential which is a very important parameter for colloids or nanoparticles in suspension. Its value is closely related to suspension stability and particle surface morphology. Therefore it is widely used in product stability studies and surface adsorption research. Among the three existing methods for zeta potential determination of suspended particles, viz., electrophoretic light scattering (ELS), acoustic and electro acoustic, due to its sensitivity, accuracy, and versatility, ELS is by far the best choice for many applications[47].

Specific surface

The specific surface area of freeze dried nanoparticle is generally determined with the help of Sorptometer. The equation given below can be used in the calculation of specific surface area.

$$A = 6 / \partial \times d$$

Where A is the specific surface area, ∂ is the density and d, is the diameter of the particle.

In most of the cases, the measured and calculated specific surface areas fairly compare while in some cases the residual surfactant could affect deviation in measured values. The surfactant coating apparently reduces the specific surface area.

Surface charge and electrophoretic mobility

The nature and intensity of surface charge of nanoparticles is very important as it determines their interaction with the biological environment as well as their electrostatic interaction with bioactive compounds. The surface charge of colloidal particles in nanoparticle can be determined by measuring the particle velocity in an electric field. Laser light scattering technique, i.e. Laser Doppler Anemometry or Velocimetry has become available as fast and high resolution technique for the determination of nanoparticle velocities.

Surface hydrophobicity

The surface hydrophobicity of nanoparticles has an important influence on the interaction of colloidal particles with the biological environment (e.g. Protein absorption and cell adhesion). The hydrophobicity and hydrophilicity collectively determine the bio fate of nanoparticle and their contents. Hydrophobicity regulates the extent and type of hydrophobic interactions of nanoparticulates with blood components. Several methods, including hydrophobic interaction chromatography, two phase partition, adsorption of hydrophobic fluorescent or radio labeled probes, and contact angle measurements have been adopted to evaluate surface hydrophobicity. Recently, several sophisticated methods of surface chemistry analysis have been used. For example, X ray photoelectron spectroscopy (XPS) permits the identification of specific chemical groups on the surface of nanoparticles.

Density

In addition to SEM, TEM following freeze fracturing could successfully be used in morphological investigation of nanoparticles. The interiors are continuous or some structural imperfection exists that provide an indication about the density distribution across the matrix. Some polymeric nanoparticles specially polycyanoacrylate and poly(methyl methacrylate) seem to have porous interior and they also exhibit more irregular and rough surface. The density of nanoparticle is determined with helium or air using a gas Pycnometer.

Determination of Drug Entrapment

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV-spectrophotometry. A standard calibration curve of concentration versus absorbance was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the coacervation process (W). Effectively, (W-w) will give the amount of drug entrapped in the pellet. Then percentage entrapment is given by

$$\frac{(W - w) \times 100}{W}$$

Molecular weight measurement of nanoparticles

Molecular weight of the polymer and its distribution in the matrix can be evaluated by gel permeation chromatography (GPC) using refrective index detector. The molecular weight of macromolecules on the polystyrene nanoparticles having surfaced grafted hydrophilic polymeric chains and correlated these parameters with a good water dispersibilty of the system.

Disease Treatment Through Nanotechnology

Nanotechnology in cancer therapy

As with any cancer therapy, the key issue is to achieve the desired concentration of therapeutic agent in tumor sites, thereby destroying cancerous cells while minimizing damage to normal cells. With this vision, it is imperative to create single agents with tremendous potential to make an important contribution in cancer prevention, detection and treatment. In this regard, several ligand-targeted therapeutic strategies, including immunotoxins, radioimmunotherapeutics and drug immunoconjugates, are being developed to overcome the problems associated with conventional chemotherapeutic drugs, thereby providing additional tools in the arsenal of cancer therapy. Although these conjugated agents have shown promising efficacy compared with conventional chemotherapy drugs, limitations in their delivery still remains a major problem. Recent advances suggest that nanotechnology (which involves the creation and manipulation of materials at nanoscale levels to create products that exhibit novel properties) will have a profound impact on disease prevention, diagnosis and treatment. Cancer nanotechnology is emerging as a new field of interdisciplinary research – cutting across the disciplines of biology, chemistry, engineering and medicine – and is expected to lead to major advances in cancer detection, diagnosis and treatment.

Nanotechnology in brain tumors

Due to their size ranging from 10 to 1000 nm (generally 50–300 nm), and like liposomes, they are unable to diffuse through the blood-brain barrier (BBB) to reach the brain parenchyma. Based on general parenteral formulation considerations and specific BBB features, Table 2 summarizes the ideal nanoparticle properties required for drug brain delivery. One particularly interesting application of nanoparticle could be the drug brain delivery, accompanied with the local sustained release, of the new large molecule therapeutics now available to treat the CNS: peptides, proteins, genes, antisense drugs. Due to their poor stability in biological fluids, rapid enzymatic degradation, unfavorable pharmacokinetic properties, and lack of diffusion toward the CNS, they may be advantageously formulated in brain-targeted protective nanocontainers. Compared with conventional drugs, they possess a high intrinsic pharmacological activity. The small dose requested for therapeutic efficiency could easily fit the loading capacity of nanoparticles and would not require the administration of large amount of potentially toxic nanoparticle excipients. Because of the large variety of the nanoparticles developed so far, this review will focus on nanoparticles investigated for brain delivery. Nanoparticles made of polybutylcyanoacrylate have been intensely investigated since the first papers in 1995 showing that when coated with the nonionic surfactant polysorbate 80 they permitted to deliver drugs to the brain.

Table II. Ideal properties of nanoparticles for drug brain delivery

1.	Nontoxic, biodegradable, and biocompatible
2.	Particle diameter < 100 nm
3.	Physical stability in blood (no aggregation)
4.	Avoidance of the MPS (no opsonization), prolonged blood circulation time
5.	BBB-targeted and brain delivery (receptor-mediated transcytosis across brain capillary endothelial cells)
6.	Scalable and cost-effective manufacturing process
7.	Amenable to small molecules, peptides, proteins, or nucleic acids
8.	Minimal nanoparticles excipients –induced drug alteration
9.	Possible modulation of drug release profiles

Nanotechnology in prevention of HIV/AIDS

The basic concept behind the use of nanotechnology-based systems for antiretroviral drug delivery is related with the modulation of pharmacokinetics of incorporated molecules. With this association, the properties that govern drug absorption, distribution, and elimination while in the human body are determined not by the drug properties, rather by the nanosystems physical–chemical properties, particularly surface exposed molecules and electric charge, and its size. General properties of nanosystems that favor their use in antiretroviral drug delivery are well known and include versatility (virtually all drugs may be encapsulated), good toxicity profile (depending on used excipients), possibility of drug-release modulation, high drug payloads, relative low cost, easiness to produce and possible scale-up to mass production scale. The use of nanoparticulate systems for antiretroviral drug delivery may be particularly advantageous for targeted delivery, namely to cells or organs that are directly implicated in HIV/AIDS[48]. This can be achieved either by passive or active targeting. Passive targeting is based in the inherent properties of different nanosystems, namely size, particle shape, and surface charge, which can modulate its bioavailability, biodistribution and/or targeting; in the case of active targeting, nanotechnology-based systems are conveniently modified, most commonly by surface attachment of specific ligands that are able to recognize target cells or sites, and/or escape bio elimination processes. One important limitation of many current antiretroviral drugs is their unavailability to circumvent efflux pumps (particularly P-glycoprotein) that are present, for instance, in the membrane of several HIV-target cells and BBB endothelium. Nanoparticulate systems' ability to escape these bioelimination processes is an added advantage in order to avoid this particular resistance mechanism to drug delivery, namely to the CNS. Thus, increasing the amount of available antiretroviral drugs and its residence time at target sites allow thinking about dose reduction and, consequently, simpler but improved regimens with less adverse effects and increased compliance.

Nanotechnology in Ocular drug delivery

The use of nanotechnology-based drug delivery systems like nanosuspensions, solid lipid nanoparticles and liposomes has led to the solution of various solubility-related problems of poorly soluble drugs, like dexamethasone, budenoside, gancyclovir and so on. Drugs can also be targeted to mononuclear phagocyte systems to allow regional specific delivery and minimize side effects in other organs. Besides this, depending on their particle charge, surface properties and relative hydrophobicity, nanoparticles can be designed to be successfully used in overcoming retinal barriers. In addition to these points, encapsulation of drugs in nanospheres, liposomes, and so on, can also provide protection for the drug and hence prolong exposure of the drug by controlled release. Nanotechnology-based drug delivery is also very efficient in crossing membrane barriers, such as the blood retinal barrier in the eye. The drug delivery systems based on nanotechnology may prove to be the best drug delivery tools for some chronic ocular diseases, in which frequent drug administration is necessary, for example in ophthalmic diseases like chronic cytomegalovirus retinitis (CMV).

Nanotechnological strategies for drug targeting in malaria therapy

The aim of using nanocarriers as drug delivery systems is to promote drug or vaccine protection against extracellular degradation, to improve selectivity in relation to the target, to reduce the frequency of administration and the duration of the treatment and to improve the pharmacokinetic profile of the drug. For the purposes of this review, the terms "nanosystems" or "nanocarriers" include all the drug carrier systems displaying sizes 1000 nm. The design of new nanocarriers should consider that in chemotherapy the plasma maximum concentration (Cmax) of a drug is proportional to its toxic effects and the efficacy is proportional to the area under the curve (AUC) of drug plasma concentration. Nanoparticulate drug delivery systems represent a promising approach for obtaining desirable drug like properties by altering the biopharmaceutics and pharmacokinetics property of the drug molecule. In general, long-circulating nanosystems are able to improve the AUC of the drugs and reduce the doses employed in chemotherapy, due to their enhanced selectivity. The most important property of a nanocarrier in the context of malaria is the ability to remain in the blood stream for a long period of time in order to improve the interaction with infected red blood cells (RBCs) and parasite membranes. Additional interesting properties are protection of instable drugs, cell-adhesion properties, and the ability to be surface-modified by conjugation of specific ligands[49]. It is noteworthy that, in the treatment of cerebral malaria, most of these potential benefits can be achieved by colloidal nanocarriers that fit intravenous administration. In uncomplicated malaria, the non-parenteral routes are preferred, but they reduce the spectrum of possibilities in terms of the use of drug nanocarriers. Many efforts have been made to meet the implement nanotechnologies in the context of malaria[49].

Nanotechnology and Parkinson disease therapy

Nanomaterials interact much more closely with cells thereby inducing changes in cell functions. For instance, carbon nanofibres promote neural growth. Materials investigated to date include: nanophase ceramics, metals, polymers, and composites. Nanoengineered scaffolds that support and promote neurite and axonal growth are highly promising. Spinal cord injury provides several examples of the potential applications of bioengineering with nanomaterials and nanostructures, particularly those concerning bridging and artificial substrates.

Current Advancement In Nanotechnology

Nanotechnology in gene delivery

Applications of nanotechnological tools in human gene therapy has been reviewed widely by Davis, who described nonviral vectors based on nanoparticles (usually 50-500 nm in size) that were already tested to transport plasmid DNA. He emphasized that nanotechnology in gene therapy would be applied to replace the currently used viral vectors by potentially less immunogenic nanosize gene carriers. So delivery of repaired genes or the replacements of incorrect genes are fields in which nanoscale objects could be introduced successfully. Nanoparticles and microparticles formulated using PLGA and PLA polymers are being investigated as a nonviral gene delivery system because of their sustained-release characteristics, biocompatibility, biodegradability, and ability to protect DNA from degradation in endolysosomes. Although PLGA/PLA nanoparticles are under extensive investigation for drug and protein delivery, their application as a gene expression vector is recent. Recently it has been demonstrated that rapid escape of nanoparticles takes place from the endolysosomal compartment to the cytoplasmic compartment following their intracellular uptake via an endocytotic process. The rapid escape of nanoparticles from the endolysosomal compartment could protect nanoparticles as well as the encapsulated DNA from the degradative environment of the endolysosomes.

Nanotechnology as a tool in imaging

Nanotechnology has a potential to transform the field of medicine, because it offers novel opportunities for sensing clinically relevant markers, molecular disease imaging, and tools for therapeutic intervention. Nanotechnologies already afford the possibility of intracellular imaging through attachment of quantum dots (QDs) or synthetic chromophores to selected molecules, for example proteins, or by the incorporation of naturally occurring fluorescent proteins that, with optical techniques such as confocal microscopy and correlation imaging, allow intracellular biochemical processes to be investigated directly. QDs are semiconductor nanocrystals with unique optical and electrical properties. Among others, one of their most valuable properties is their fluorescence spectrum, which renders them optimal fluorophores for biomedical imaging. Moreover, because of their quantum confinement of charge carriers in tiny spaces, QDs show some fascinating optimal properties, which are characterized as sharp and symmetrical emission spectra, high quantum yield, broad absorption spectra, good chemical and photo stability, and size-dependent emission wavelength tenability.

Nanotechnology in cardiac therapy

Nanotechnology-based tools can be effectively used to treat the cardiovascular diseases. These tools can be used in the areas of diagnosis, imaging, and tissue engineering. Miniaturized nanoscale sensors like QDs, nanocrystals, and nanobarcodes can sense and monitor biological signals such as the release of proteins or antibodies in response to cardiac or inflammatory events. Nanotechnology can also help in revealing the mechanisms involved in various cardiac diseases. It also helps in designing atomic-scale machines by imitating or incorporating biological systems at the molecular level. The use of these newly designed nanomachines can have a paradigm-shifting impact. Therefore, researchers have a great hope that nanotechnology-based localized drug therapy using sustained-release drug delivery systems could be more effective, because it can provide higher and prolonged drug levels in the target tissues without causing systemic toxicity. Nanotechnology could also have an impact in the diagnosis and treatment of unstable plaques and in the management of other cardiovascular problems like calcification of valves. Thus, nanotechnology could be an effective treatment modality to achieve localized and sustained arterial and cardiac drug therapy for the prevention of cardiovascular diseases.

Nanotechnology in dental care

Nanotechnology will have future medical applications in the field of nanodentistry. Nanodentistry will make it possible to maintain near-perfect oral health through the use of nanomaterials, biotechnology, and nanorobotics. Through this it will be possible to provide high-quality dental care to the millions of the world's population who currently receive no significant dental care. In the years to come it will be

possible through nanodentistry to induce local anesthesia. A colloidal suspension containing millions of active analgesic dental nanorobotic particles could be instilled on the patient's gingiva. These nanorobots, after contacting the surface of the crown or mucosa, reach the dentin by migrating into the gingival sulcus and pass painlessly to the target site. On reaching the dentin, the nanorobots enter dentinal tubule holes that are 1 to 4 Am in diameter and proceed toward the pulp, guided by a combination of chemical gradients, temperature differentials, and even positional navigation, all under the control of the onboard nano computer as directed by the dentist.

Nanotechnology in orthopedic applications

Nanostructure materials with sizes 1 to 100 nm can act as new and effective constituents of bone materials, because bone is also made up of nanosized organic and mineral phases. Several studies have reported improved osseointegration on nanostructure surfaces created from a wide range of chemistries including ceramics, metals, polymers, and composites. For instance, studies show that alumina nanometer fibers significantly stimulate osteoblast responses such as adhesion, alkaline phosphatase activity, and calcium deposition, when compared with conventional grain size alumina. Nanomaterials, nanopolymers, carbon nanofibers, nanotubes, and nanocomposites of ceramics will also lead to more efficient deposition of calcium-containing minerals on the implants. Recent studies have demonstrated that the adsorption and conformation of proteins that mediate specific osteoblast adhesion (such as fibronectin and vitronectin) are enhanced on nanospaced materials like 3D nanofibrous scaffolds[50]. It is also estimated that nanophase materials attract more proteins to their surface because of their altered surface energetics, brought about by a higher exposed surface area and altered electron distribution as compared with conventional materials.

CONCLUSION

Nanomedicine form a part of nanotechnology, and engineered nanostructures are achieving importance. Surface modifications of nanostructure are also under consideration. Nanopharmaceutic is widening its leading egde, with some current and capable approaches in drug delivery using carbon nanotubes, metallic nanoparticles and use for quantum dots. Targeted delivery is one of the attractions of nanopharmaceutics. Nanopharmaceuticals have a bright future in the delivery of therapeutic and diagnostic agents and in future years would result in a considerable development in the quality, efficacy and safety profile of drugs.

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