International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-4, 2013 ISSN No: 2277 - 7873

REVIEW ARTICLE

Recent Trends in Formulation and Applications of Nanoparticles

Ankita Chamoli*, Lakshmayya, Pranshu Tangri

GRD (PG) IMT, Dept. of Pharmacy, Rajpur Road, Dehradun-248001, Uttarakhand, India.

Manuscript No: IJPRS/V2/I4/00238, Received On: 19/12/2013, Accepted On: 25/12/2013

ABSTRACT

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to acavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. In this article we discuss about nanoparticles and its formulation and evaluation with current strategies.

KEYWORDS

Nanoparticles, Nanospheres, Nanocapsules, Particulate dispersions

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes.[1-4]

Apart from their basic structure, nanocapsules differ from nanospheres in their size and degree of polymerization. Nanocapsules are generally larger than nanospheres of same composition. Also the degree of polymerization is higher in nanocapsules as compared to nanospheres prepared by in-situ polymerization technique.[5]

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side

***Address for Correspondence:**
Ankita Chamoli
GRD(PG)IMT,
Dept. of Pharmacy, Rajpur Road,
Dehradun, Uttarakhand, India.
E-Mail Id: Ankitachamoli005@gmail.com

effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties.[6-7]

Advantages

Nanoparticles have several advantages over the other novel drug delivery systems, such as [5]

- Their size allows them to be administered intravenously via injection unlike other colloidal systems, which occlude both needles and capillaries.
- Due to their small size they can pass through the sinusoidal spaces in the bone marrow and spleen as compared to other systems like microspheres and liposomes. Thus they have a longer circulation time in the blood.
- Due to their larger surface area, nanoparticles have higher loading capacity.
- Nanoparticles can act as controlled release system depending on their polymeric composition.
- Nanoparticles have polymeric composition that renders them more stable than nanoemulsions and liposomes, which are fragile in nature.
- Nanoparticles also help to increase stability of drugs/proteins.
- Targeting moieties like monoclonal antibodies can be attached to nanoparticles to enhance their specificity.
- Nanoparticles are safe and effective in site specific and targeted drug delivery.

Applications

A list of some of the applications of nanoparticles to biology or medicine is given below:

- Fluorescent biological labels.[8-10]
- Drug and gene delivery.[11-12]
- Bio detection of pathogens.[13]
- Detection of proteins.[14]
- Probing of DNA structure.[15]
- Tissue engineering.[16-17]
- Tumour destruction via heating (hyperthermia).[18]
- Separation and purification of biological molecules and cells.[19]
- MRI contrast enhancement.[20]
- Phagokinetic studies.[21]

Other Promising Application Fields

Nanotechnology often brings together different disciplines and this interdisciplinary approach is expected to contribute to innovations that might solve many of today's challenges in the society. A selection of the applications involving nanoparticles that exist or show promises are presented here:

- Nanotechnology is already being used in commercial applications for bulk products, such as:
  - ✓ Sunscreens with increased transparency and
  - ✓ Cosmetics containing nanoparticles with the ability to target deeper into the body. The cosmetic companies have been active in using nanotechnology to improve their existing products and e.g. L'Oreal holds a very high number of nanotechnology patents.
- Also nanoparticles as fillers have been introduced in the composite materials with an enormous market. The nanoparticles change the material's properties as e.g. metal gets harder, ceramics get softer and mixtures like alloys may get harder up to a point where they get softer again. By introducing clay nanoparticles it is possible to make the materials stronger, lighter, more durable and often transparent. These have especially potentials in aerospace industry, packaging and in the car industry where they

already have been introduced in the GM Motors Safari and Chevrolet Astro vans.

- Other short-term uses includes solar energy collection (photovoltaics), medical diagnostic tools and sensors, flexible display technologies and e-paper, glues, paints and lubricants, various optical components, and new forms of computer memories and electronic circuit boards.
- There are smart textiles developed with the help of nanotechnology and in the long run textiles are expected to be able to change their physical properties according to the surrounding conditions, or even monitor vital signals. The introduction of nanoparticles in textiles can make it possible to produce very light and durable textiles with resistance against water, stains and wrinkling.
- Medical applications are one of the fields with the biggest expectations regarding human welfare. With the development of new materials and a combination of nanotechnology and biotechnology it could be possible to make artificial organs and implants through cell growth which could repair damaged nerve cells, replace damaged skin, tissue or bone.
- Another application field in medicine is drug-delivery where research is especially intensive on the possibility of manipulating nanoparticles to deliver drugs because nanoparticles can have a better solubility and absorption potential than bigger particles. The nanoparticles can carry the drug and perhaps release it in fine-tuned doses over a long time period to a targeted area, reducing the side-effects of the traditional drugs.
- Other applications researched are nanoparticles as bioremediation. Biological organisms that are used to clean up soil pollution face the problem that in the soil most pollutant are not bioavailable, but locked up with in pores in the soil structure. By using nanoparticles it may be possible to deliberately mobilize pollutants in a controlled manner so that they become bioavailable and ensuring that clean up organisms are not killed by a rapid release.

Recent Developments

Tissue Engineering

- Natural bone surface is quite often contains features that are about 100 nm across. If the surface of an artificial bone implant were left smooth, the body would try to reject it. Because of that smooth surface is likely to cause production of a fibrous tissue covering the surface of the implant. This layer reduces the bone-implant contact, which may result in loosening of the implant and further inflammation. It was demonstrated that by creating nano-sized features on the surface of the hip or knee prosthesis one could reduce the chances of rejection as well as to stimulate the production of osteoblasts. The osteoblasts are the cells responsible for the growth of the bone matrix and are found on the advancing surface of the developing bone.
- The effect was demonstrated with polymeric, ceramic and, more recently, metal materials. More than 90% of the human bone cells from suspension adhered to the nanostructured metal surface[22]but only 50% in the control sample. In the end this findings would allow to design a more durable and longer lasting hip or knee replacements and to reduce the chances of the implant getting loose.
- Titanium is a well-known bone repairing material widely used in orthopaedics and dentistry. It has a high fracture resistance, ductility and weight to strength ratio. Unfortunately, it suffers from the lack of bioactivity, as it does not support sell adhesion and growth well. Apatite coatings are known to be bioactive and to bond to the bone. Hence, several techniques were used in the past to produce an apetite coating on titanium. Those coatings suffer from thickness non-uniformity, poor adhesion and

low mechanical strength. In addition, a stable porous structure is required to support the nutrients transport through the cell growth.

- It was shown that using a biomimetic approach – a slow growth of nanostructured apatite film from the simulated body fluid – resulted in the formation of a strongly adherent, uniform nanoporous layer .[23]The layer was found to be built of 60 nm crystallites, and possess a stable nanoporous structure and bioactivity.
- A real bone is a nanocomposite material, composed of hydroxyapatite crystallites in the organic matrix, which is mainly composed of collagen. Thanks to that, the bone is mechanically tough and, at the same time, plastic, so it can recover from a mechanical damage. The actual nanoscale mechanism leading to this useful combination of properties is still debated.
- An artificial hybrid material was prepared from 15–18 nm ceramic nanoparticles and poly (methyl methacrylate) copolymer.[24] Using tribology approach, a viscoelastic behaviour (healing) of the human teeth was demonstrated. An investigated hybrid material, deposited as a coating on the tooth surface, improved scratch resistance as well as possessed a healing behaviour similar to that of the tooth.

Cancer Therapy

- Photodynamic cancer therapy is based on the destruction of the cancer cells by laser generated atomic oxygen, which is cytotoxic. A greater quantity of a special dye that is used to generate the atomic oxygen is taken in by the cancer cells when compared with a healthy tissue. Hence, only the cancer cells are destroyed then exposed to a laser radiation. Unfortunately, the remaining dye molecules migrate to the skin and the eyes and make the patient very sensitive to the daylight exposure. This effect can last for up to six weeks. To avoid this side effect; the hydrophobic version of the dye molecule was enclosed inside a porous nanoparticle.[25] The dye stayed trapped inside the Ormosil nanoparticle and did not spread to the other parts of the body. At the same time, its oxygen generating ability has not been affected and the pore size of about 1 nm freely allowed for the oxygen to diffuse out.

Multi Colour Optical Coding for Biological Assays [22]

- The ever increasing research in proteomics and genomic generates escalating number of sequence data and requires development of high throughput screening technologies. Realistically, various array technologies that are currently used in parallel analysis are likely to reach saturation when a number of array elements exceed several millions. A three-dimensional approach, based on optical "bar coding" of polymer particles in solution, is limited only by the number of unique tags one can reliably produce and detect.
- Single quantum dots of compound semiconductors were successfully used as a replacement of organic dyes in various bio-tagging applications.[26] This idea has been taken one step further by combining differently sized and hence having different fluorescent colours quantum dots, and combining them in polymeric microbeads.[17] A precise control of quantum dot ratios has been achieved. The selection of nanoparticles used in those experiments had 6 different colours as well as 10 intensities. It is enough to encode over 1 million combinations. The uniformity and reproducibility of beads was high letting for the bead identification accuracies of 99.99%.

Manipulation of Cells and Biomolecules [27]

- Functionalised magnetic nanoparticles have found many applications including cell separation and probing; these and other applications are discussed in a recent review.[28]Most of the magnetic particles studied so far are spherical, which somewhat

limits the possibilities to make these nanoparticles multifunctional. Alternative cylindrically shaped nanoparticles can be created by employing metal electrodeposition into nanoporous alumina template.[28] Depending on the properties of the template, nanocylinder radius can be selected in the range of 5 to 500 nm while their length can be as big as 60 μm. By sequentially depositing various thicknesses of different metals, the structure and the magnetic properties of individual cylinders can be tuned widely.

- As surface chemistry for functionalisation of metal surfaces is well developed, different ligands can be selectively attached to different segments. For example, porphyrins with thiol or carboxyl linkers were simultaneously attached to the gold or nickel segments respectively. Thus, it is possible to produce magnetic nanowires with spatially segregated fluorescent parts. In addition, because of the large aspect ratios, the residual magnetisation of these nanowires can be high. Hence, weaker magnetic field can be used to drive them. It has been shown that a self-assembly of magnetic nanowires in suspension can be controlled by weak external magnetic fields. This would potentially allow controlling cell assembly in different shapes and forms. Moreover, an external magnetic field can be combined with a lithographically defined magnetic pattern ("magnetic trapping").

Protein Detection [29]

- Proteins are the important part of the cell's language, machinery and structure, and understanding their functionalities is extremely important for further progress in human well being. Gold nanoparticles are widely used in immunohistochemistry to identify protein-protein interaction. However, the multiple simultaneous detection capabilities of this technique are fairly limited. Surface-enhanced Raman scattering spectroscopy is a well-established technique for detection and identification of single dye molecules. By combining both methods in a single nanoparticle probe one can drastically improve the multiplexing capabilities of protein probes. The group of Prof. Mirkin has designed a sophisticated multifunctional probe that is built around a 13 nm gold nanoparticle. The nanoparticles are coated with hydrophilic oligonucleotides containing a Raman dye at one end and terminally capped with a small molecule recognition element (e.g. biotin).
- Moreover, this molecule is catalytically active and will be coated with silver in the solution of Ag(I) and hydroquinone. After the probe is attached to a small molecule or an antigen it is designed to detect, the substrate is exposed to silver and hydroquinone solution. A silver-plating is happening close to the Raman dye, which allows for dye signature detection with a standard Raman microscope. Apart from being able to recognize small molecules this probe can be modified to contain antibodies on the surface to recognize proteins. When tested in the protein array format against both small molecules and proteins, the probe has shown no cross-reactivity.

Commercial Exploration

- There are companies that are involved in the development and commercialisation of nanomaterials in biological and medical applications .The majority of the companies are small recent spinouts of various research institutions. Although not exhausting, this is a representative selection reflecting current industrial trends. Most of the companies are developing pharmaceutical applications, mainly for drug delivery. Several companies exploit quantum size effects in semiconductor nanocrystals for tagging biomolecules, or use bio-conjugated gold nanoparticles for labelling various cellular parts. A number of companies are applying nano-ceramic materials to tissue engineering and orthopaedics.
- Most major and established pharmaceutical companies have internal research programs

on drug delivery that are on formulations or dispersions containing components down to nano sizes. Colloidal silver is widely used in anti-microbial formulations and dressings. The high reactivity of titania nanoparticles, either on their own or then illuminated with UV light, is also used for bactericidal purposes in filters. Enhanced catalytic properties of surfaces of nano-ceramics or those of noble metals like platinum are used to destruct dangerous toxins and other hazardous organic materials.

Method of Preparation

Preparation method for nanoparticles depends on [5]

- Physiochemical properties of drug.
- Physiochemical properties of polymer.
- Size of particle to be prepared.
- Type of system (matrix or reservoir) intended to be formed.

There are several techniques for the preparation of nanoparticles which can be broadly classified into two main categories:

- Polymerization of monomer.
- Dispersion of preformed polymer.

Polymerization Method

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles.[30-31] Nanocapsule formation and their particle size depends on the concentration of the surfactants and stabilizers used.[33]

Dispersion of Preformed Polymers

Dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA); poly (D,L-glycolide),PLG; poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA).[34-36] This technique can be used in various ways as described below.

Solvent Evaporation Method

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration.[37] In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.[38]

Spontaneous Emulsification or Solvent Diffusion method

This is a modified version of solvent evaporation method.[39] in this method; the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

Coacervation or Ionic Gelation Method

Much research has been focused on the preparation of nanoparticles using

biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation.[40-41]The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, adi-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanionsodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.

Production of Nanoparticles Using Supercritical Fluid Technology

Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe.[42] A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure.[42] SupercriticalCO2 (SC CO2) is the most widely used supercritical fluid because of its mild critical conditions (*T*c = 31.1 °C, *P*c = 73.8 bars), nontoxicity, non-flammability, and low price. The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid (SC CO2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles .[44]Thoteand Gupta (2005) reported the use of a modified SAS method for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose.[43] RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure. [42] Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles.[43] Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive.

Preparation of Drug Loaded Nanoparticles[44- 46]

Emulsion-Droplet Coalescence Method

Chitosan was dissolved in 1% acetic acid and 50 mg of drug in phosphate buffered saline. This solution was added to 10 ml of liquid paraffin containing 5%v/v tween 20. This mixture was stirred using a homogeniser 3 minutes to form water in oil (w/o) emulsion.

Similarly, another w/o emulsion consisting of Eudragit® S 100 in 3M sodium hydroxide solution was prepared.[47] Then these two emulsions were mixed and stirred using homogenizer. As a result of coalescence of the droplets, chitosan in the system was solidified to produce nanoparticles. Eudragit® S 100 producing second coating over chitosan nanoparticles.

The resultant drug loaded nanoparticles were centrifugated at 3000 rpm was 60 mts (REMI, India) and washed using ethanol and water, consecutively to remove the remaining surfactant and liquid paraffin. Later they were dried in air for 3 hour followed by hot air oven at 50° for 4 hour and stored in a dessicator.[48]

Ionic Cross Linking Method

Chitosan nanoparticles were prepared by ionic cross linking of chitosan solution with tripolyphosphate (TPP) anions. Chitosan was dissolved in aqueous solution of acetic acid (0.25v/v) at various concentrations such as 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml coded as batch number L1, L2, L3, L4 and L5 under magnetic stirring at room temperature, 5ml of 0.85% w/v TPP aqueous solution was added drop wise using syringe needle into 10 ml chitosan solution containing 10mg of drug. pH was adjusted to 6 by adding 0.1 N NaOH. The stirring was continued for about 30 min. The resultant nanoparticles suspensions were centrifuged at 12000x g for 30 min using C24 centrifuge. The formation of the particles was a result of the interaction between the positive groups of the TPP and the negatively charged amino groups of chitosan.[49-56]

Evaluation of Nanoparticles

Particle Size Analysis[57-58]

The particle size of the Nanoparticles was evaluated by Scanning Electron Microscope were ranging from 350 nm to 600 nm, particle size varies depending on the polymer load.

Determination of percentage of drug entrapment efficiency[59]

Prepared nanoparticle suspensions were centrifuged at 2000 rpm for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation. The amount of free drug in the supernatant was determined by the UV-Visible Spectrophotometer at 220 nm.

X-ray Diffraction Study

X-ray diffraction analysis was conducted using a XRD-6000 diffractometer. X-ray diffraction analysis was used to detect the crystallinity of the pure drug and the formulation. The powder was placed in an aluminium sample holder. Curadiation was generated at 30 mA and 40 kV. Samples were scanned at a range of 10° to 90°with scan speed of 10° min-1, as previously explained.[60]

Determination of Zeta Potential

The zeta potential of the drug-loaded chitosan nanoparticles was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell.[61-62] all the samples were measured in water at 25°C in triplicate.

In Vitro Drug Diffusion Studies Using Egg membranes

Permeation study with egg membrane was done according to the method reported byAnsari.[63] the egg shell was kept in concentrated HCl for 2 h. The separated membrane was attached to diffusion cell. Twenty mg of the drug was placed in the diffusion cell with 10 ml of phosphate buffer (pH 7.4). Fifty ml of phosphate buffer (pH7.4) was placed in the receptor compartment in 100 ml beaker. The assembly was then attached to magnetic stirrer. Samples were with drawn at specific time interval for 6 h and analyzed using UV-visible spectrophotometer at 220 nm.

CONCLUSION

There are now numerous preparation methods available for producing nanoparticles, and important technological advances have been achieved. Simple, safe, and reproducible techniques are now available to prepare drug-loaded nanospheres and nanocapsules. The foregoing show that nanoparticulate systems have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable drugs. The core of this system can enclose a variety of drugs, enzymes, genes and is characterized by a long circulation time due to the hydrophilic shell which prevents recognition by the reticular-endothelial system. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and particle engineering, is still required. Further advances are needed in order to turn the concept of nanoparticle technology into a realistic practical application as the next generation of drug delivery system.

Table 1: Overview of nanoparticles technology based products[64-65]

Brand	Generic name	Indication	Drug delivery company	Innovator	Status
Rapamune	Rapamycin, Sirolimus	Immunosuppressant	Elan Nanosystems	Wyeth	Marketed
Emend	Aprepitant	Anti-emetic	Elan Nanosystems	Merck & Co.	Marketed
Tricor	Fenofibrate	Hypercholesterolemia	Abbott Laboratories	Abbott Laboratories	Marketed
Megace ES	Megestrol	Anti-anorexic	Elan Nanosystems	Par Pharmaceuticals	Marketed
Triglide	Fenofibrate	Hypercholesterolemia	IDD-P Skyepharma	Sciele Pharma Inc.	Marketed
Avinza	Morphine Sulphate	Phychostimulant	Elan Nanosystems	King Pharmaceuticals	Marketed
Focalin	Dexmethyl-Phenidate HCl	Attention Deficit Hyperactivity Disorder (ADHD).	Elan Nanosystems	Novartis	Marketed
Ritalin	Methyl Phenidate HCl	CNS Stimulant	Elan Nanosystems	Novartis	Marketed
Zanaflex Capusules	Tizanidine HCl	Muscle Relaxant	Elan Nanosystems	Acorda	Marketed

REFERENCES

1. Langer R, “Biomaterials in drug delivery and tissue engineering: one laboratory's experience”, Accounts of Chemical Research, 2000, 33, 94-101.
2. Bhadra D, Bhadra S, Jain P, Jain NK, "Pegnology: a review of PEG-ylated systems", Pharmazie, 2002, 57, 5-29.
3. Kommareddy S, Tiwari SB, Amiji MM, “Long-circulating polymeric nano vectors for tumor-selective gene delivery”, Technology in Cancer Research and Treatment, 2005, 4, 615-625.
4. Lee M, Kim SW, “Polyethylene glycol-conjugated copolymers for plasmid DNA delivery”, Pharmaceutical Research, 2005, 22, 1-10.
5. Jain NK, “Introduction to novel drug delivery system”, 2010, 1, 134-137.
6. Vila A, Sanchez A, Tobio M, Calvo P, Alonso MJ, “Design of biodegradable particles for protein delivery”, Journal of Controlled Release, 2002, 78, 15-24.
7. Mu L, Feng SS, “A novel controlled release formulation for the anticancer drug paclitaxel (Taxol(R)), PLGA nanoparticles containing vitamin E TPGS”, Journal of Controlled Release, 2003, 86, 33-48.

8. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP**,** **"**Semiconductor nano crystals as fluorescent biological labels", Science,1998, 281, 2013-2016.
9. Chan WCW, Nie SM, "Quantum dot bioconjugates for ultrasensitive non isotopic detection", Science, 1998, 281, 2016-2018.
10. Wang S, Mamedova N, Kotov NA, Chen W, Studer J, "Antigen/antibody immune complex from CdTe nanoparticle bioconjugates", Nano Letters, 2002, 2, 817-822.
11. Mah C, Zolotukhin I, Fraites TJ, Dobson J, Batich C, Byrne BJ, "Microsphere-mediated delivery of recombinant AAV vectors in vitro and in vivo", Molecular Therapy, 2000, 1, S239.
12. Panatarotto D, Prtidos CD, Hoebeke J, Brown F, Kramer E, Briand JP, Muller S, Prato M, Bianco A, "Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses", Chemistry & Biology, 2003, 10, 961-966.
13. Edelstein RL, Tamanaha CR, Sheehan PE, Miller MM, Baselt DR, Whitman LJ, Colton RJ, "The BARC biosensor applied to the detection of biological warfare agents", Biosensors and Bioelectronics, 2000, 14, 805-813.
14. Nam JM, Thaxton CC, Mirkin CA, "Nanoparticles-based bio-bar codes for the ultrasensitive detection of proteins, Science, 2003, 301, 1884-1886.
15. Mahtab R, Rogers JP, Murphy CJ, Protein-sized quantum dot luminescence can distinguish between "straight", "bent", and "kinked" oligonucleotides, Journal of the American Chemical Society, 1995, 117, 9099-9100.
16. Ma J, Wong H, Kong LB, Peng KW, Biomimetic processing of nanocrystallite bioactive apatite coating on titanium, Nanotechnology, 2003, 14, 619-623.
17. De la Isla A, Brostow W, Bujard B, Estevez M, Rodriguez JR, Vargas S, Castano VM**,** Nanohybrid scratch resistant coating for teeth and bone viscoelasticity manifested in tribology, Materials Research Innovations, 2003, 7, 110-114.
18. Yoshida J, Kobayashi T, "Intracellular hyperthermia for cancer using magnetite cationic liposomes", Journal of Magnetism and Magnetic Materials, 1999, 194, 176-184.
19. Molday RS, MacKenzie D, "Immunospecific ferromagnetic iron dextran reagents for the labeling and magnetic separation of cells", Journal of Immunological Methods, 1982, 52, 353-367.
20. Weissleder R, Elizondo G, Wittenburg J, Rabito CA, Bengele HH, Josephson L, "Ultrasmall superparamagnetic iron oxide: characterization of a new class of contrast agents for MR imaging", Radiology, 175, 1990, 489-493.
21. Mah C, Zolotukhin I, Fraites TJ, Dobson J, Batich C, Byrne BJ, Microsphere-mediated delivery of recombinant AAV vectors in vitro and in vivo", Molecular Therapy, 2000, 1, S239
22. Panatarotto D, Partidos CD, Hoebeke J, Brown F, Kramer E, Briand JP, Muller S, Prato M, Bianco A, "Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses", Chemistry & Biology, 2003, 10, 961-966.
23. Edelstein RL, Tamanaha CR, Sheehan PE, Miller MM, Baselt DR, Whitman LJ, Colton RJ, "The BARC biosensor applied to the detection of biological warfare agents", Biosensors and Bioelectronics, 2000, 14, 805-813.
24. Nam JM, Thaxton CC, Mirkin CA, "Nanoparticles-based bio-bar codes for the ultrasensitive detection of proteins", Science, 2003, 301, 1884-1886.

25. Mahtab R, Rogers JP, Murphy CJ, "Protein-sized quantum dot luminescence can distinguish between "straight", "bent", and "kinked" oligonucleotides", Journal of American Chemical Society, 1995, 117, 9099-9100.

26. Ma J, Wong H, Kong LB, Peng KW, "Biomimetic processing of nanocrystallite bioactive apatite coating on titanium", Nanotechnology, 2003, 14, 619-623.

27. Yoshida J, Kobayashi T, "Intracellular hyperthermia for cancer using magnetite cationic liposomes, Journal of Magnetism and Magnetic Materials**,** 1999, 194, 176-184.

28. Molday RS, MacKenzie D, "Immunospecific ferromagnetic iron dextran reagents for the labeling and magnetic separation of cells", Journal of Immunological Methods, 1982, 52, 353-367.

29. Weissleder R, Elizondo G, Wittenburg J, Rabito CA, Bengele HH, Josephson L, "Ultrasmall superparamagnetic iron oxide: characterization of a new class of contrast agents for MR imaging", Radiology, 1990, 175, 489-493.

30. Zhang Q, Shen Z, Nagai T, "Prolonged hypoglycemic effect of insulin-loaded polybutylcyanoacrylate nanoparticles after pulmonary administration to normal rats", International Journal of Pharmaceutics, 2001, 218, 75-80.

31. Boudad H, Legrand P, Lebas G, Cheron M, Duchene D, Ponchel G, "Combined hydroxypropyl-[beta]- cyclodextrin and poly(alkylcyanoacrylate) nanoparticles intended for oral administration of saquinavir", International Journal of Pharmaceutics, 2001, 218, 113-124.

32. Puglisi G, Fresta M, Giammona G, Ventura CA, "Influence of the preparation conditions on poly (ethylcyanoacrylate) nanocapsule formation", International Journal of Pharmaceutics, 1995, 125, 283-287.

33. Kompella UB, Bandi N, Ayalasomayajula SP, "Poly (lactic acid) nanoparticles for sustained release of budesonide", Drug Delivery Technology, 2001, 1, 1-7.

34. Ravi MN, Bakowsky U, Lehr CM, "Preparation and characterization of cationic PLGA nanospheres as DNA carriers", Biomaterials, 2004, 25, 1771-1777.

35. Li YP, Pei YY, Zhou ZH, Zhang XY, Gu ZH, Ding J, Zhou JJ, Gao, XJ, "PEGylated polycyanoacrylate nanoparticles as tumor necrosis factor-[alpha] carriers", Journal of Controlled Release, 2001, 71, 287-296.

36. Kwon, HY, Lee JY, Choi SW, Jang Y, Kim JH, "Preparation of PLGA nanoparticles containing estrogen by emulsification-diffusion method", Colloids Surf. A: Physicochem. Eng. Aspects, 2001, 182, 123-130.

37. Zambaux M, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso M, Labrude P, Vigneron C, "Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by double emulsion method", Journal of Controlled Release, 1998, 50, 31-40.

38. Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y, "Preparation of biodegradable nanoparticles of water-soluble and insoluble drugs with D,Llactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior", Journal of Controlled Release, 1993, 25, 89-98.

39. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ, "Novel hydrophilic chitosan-polyethylene oxide nanoprticles as protein carriers", Journal of Applied Polymer Science, 1997, 63, 125-132.

40. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ, "Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines", Pharmaceutical Research, 1997, 14, 1431-1436.

41. Jung J, Perrut M, "Particle design using supercritical fluids: Literature and patent survey", Journal of Supercritical Fluids, 2001, 20, 179-219.

42. Thote AJ, Gupta RB, "Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release", Nanomedicine: Nanotech. Biology Medicine, 2005, 1, 85-90.

43. Sun Y, Mezian M, Pathak P, Qu L, "Polymeric nanoparticles from rapid expansion of supercritical fluid solution", Chemistry, 2005, 11, 1366-73.

44. Reverchon E, Adami R, "Nanomaterials and supercritical fluids", The Journal of Supercritical Fluids, 2006, 37, 1-22.

45. Lowman AM, "Biodegradable nanoparticles for drug delivery and targeting, Current Opinion in Solid State and Material Science", 2002, 319-327.

46. Scholes PD, Coombes AGA, Illum L, Davis SS, Vert M, Davies MC, "The preparation of sub-500 nm. Poly(lactide co-glycolide) microspheres for site specific drug delivery", Journal of Controlled Release, 1993, 25, 145-153.

47. Bodmeier R, Oh KH, Pramar Y, "Preparation and evaluation of drug containing chitosan beads", Drug Development and Industrial Pharmacy, 1989, 15, 1475-1494.

48. Conti B, Monden T, Genta I, Perugini P, Decarro C, Pavanetto F, "Microencapsulation of cetylpyridinium chloride with a bioadhesive polymer", Proc. Inl. Symp. Control, Release Bioact. Mater, 1998, 25, 822-823.

49. RR Punna, G Sindhura and RN Saha, "Design and study of lamivudine Oral Controlled Release Tablet", AAPS Pharm. Sci. Tech, 2007, 8, 1-9.

50. Jain S, Tiwari AK, Sapra B, Jain AK, "Formulation and evaluation of Ethosomes for Transdermal De-livery of Lamivudine", AAPS Pharm. Sci. Tech, 2007, 8, 1-8.

51. Tilak R, Bharadwaj MK, Roshan L, Anubha G, "Natural Gums and Modified as sustained release carriers", Drug Development and Industrial Pharmacy, 2000, 26, 1025-1038.

52. Yan W, Wuli Y, Changchun W, Jianhua H, Shoukuan F, "Chitosan nanopaticles as a novel delivery system for ammonium glycyrrhizinate", International Journal of Pharmaceutics, 2005, 295, 235-245.

53. Yesin A, Karine AB, Maria JC, Calvo BP, Nesli-han AP, Couvreur B, Yilmaz C, "Preparation and in vitro evaluation of chitosan nanoparticles containing a caspase inhibitor", International Journal of Pharmaceutics, 2005, 298, 378-383.

54. Zengshuan M, Meng LT, Lee YL, "Pharmacological activity of peroral chitosan insulin nanoparticles in diabetic rats", International Journal of Pharmaceutics, 2005, 293, 271-280.

55. Chen Y, Zhang G, Neilly J, Marsh K, Mawhin-neyana D, Sanzgiri Y, "Enhancing the bioavailability of ABT-963 using solid dispersion containing Pluronic F-68", International Journal of Pharmaceutics, 2004, 286, 69-80.

56. Mehta K, Kislalioglu S, Phuapradit W, Malick W, Shah N, "Multi-unit controlled release systems of nifedipine and Nifedipine: Pluronic F-68 Solid dispersions: characterization of release mechanisms," Drug Development and Industrial Pharmacy, 2002, 28, 275-285.

57. Krishna RSM, Shivakumar HG, Gowda DV, Banerjee, "Nanoparticles: A Novel Colloidal Drug Delivery System", Indian Journal of Pharmaceutical Education and Research, 2006, 40(1), 15-19.

58. Joseph NM, Sharma PK, Irchhaiya R, "Preparation and characterization of Modified Gelation Nanoparticles as Carriers

of Cytarabine", Asian Journal of Pharmaceutics, 2007, 1, 179.

59. Lamprecht A, Yamamoto H, Takeuchi H, Kawashima Y, "Observations in simultaneous microencapsulation of 5-fluorouracil and leucovorin for combined pH-dependent release", European Journal of Pharmaceutics and Biopharmaceutics, 2005, 59, 367-71.
60. Papadimitriou S, Bikiaris D, Avgoustakis K, Karavas E, Georgarakis M, "Chitosan nanoparticles loaded with dorzolamide and pramipexole", Carbohydrate Polymers, 2008, 73, 44-54.
61. Barratt G, "Characterization of colloidal drug carrier systems with zeta potential measurements", Pharmaceutical Technology Europe, 1999, 11, 25-32.
62. Lochmann D, Weyermann J, Georgens C, Prassl R, Zimmer A, "Albumin-protamine-oligonucleotide nanoparticles as a new antisense delivery system, Part 1: Physicochemical characterization", European Journal of Pharmaceutics and Biopharmaceutics, 2005, 59, 419-429.
63. Mehdi A, Maryam K, Monireh A, "The study of drug permeation through natural membrane", International Journal of Pharmaceutics, 2006, 327, 6-11.
64. Dubey R, "Pure Drug Nanosuspensions: Impact of nanosuspension technology on drug discovery & development", Drug Delivery Technology, 2006, 6(5), 65-71.
65. Ranjita S, Muller RH, "Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives", International Journal of Pharmaceutics, 2010, 399, 129-139.

