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PLGA Based Nanoparticle:Best tool for Treatment of Visceral Leishmaniasis

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ABSTRACT

Some of the natural macromolecules have been used to prepare NPs. These polymers include gelatin, alginate, chitosan and PLGA. They are hydrophilic natural polymers and have been used to synthesize biodegradable NPs by various method. Numerous techniques now exist for synthesizing different set of nanoparticles based on the type of drugs used, and the targeted organ and delivery mechanism selected. Depending upon the protocol of choice, the parameters can be tailored to create the best possible characteristics for the nanoparticles. In this manuscript we have reviewed a number of biodegradable nanoparticles currently in use, and the techniques of their preparation. We will also discuss advances in surface modifications, drug encapsulation and specific end applications of various types of NPs. Nanotechnology, although not a new concept, has gained significant momentum in recent years. Due to the recent advances in material science and nano-engineering in the last decade, the nanoparticles have become very attractive for their applications in the fields of biology and medicine. Nanostructured materials are materials with sizes in the 1-100 nm range, which demonstrate unique properties and functions due to their "size effect".Biodegradable nanoparticles (NPs) are gaining increased attention for their ability to serve as a viable carrier for site specific delivery of vaccines, genes, drugs and other biomolecules in the body.

Keyword: PLGA Nanoparticles, Method & Characterization, Visceral Leishmaniasis

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INTRODUCTION

Nanoparticles are the simplest form of structures with sizes in the nm range. In principle any collection of atoms bonded together with a structural radius of < 100 nm can be considered a nanoparticle.[1]

These can include, e.g., fullerens, metal clusters (agglomerates of metal atoms), large molecules, such as proteins, and even hydrogen-bonded assemblies of water molecules, which exist in water at ambient temperatures.[2]

Nanoparticles are very commonplace in nature - for instance proteins exist in almost all biological systems, metal-oxide Nanoparticles are easily produced, etc.[3]

WHAT IS NANOTECHNOLOGY ?

In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. It is further classified according to size: in terms of diameter, fine particles cover a range between 100 and 2500 nanometers, while ultrafine particles, on the other hand, are sized between 1 and 100 nanometers. Similar to ultrafine particles, nanoparticles are sized between 1 and 100 nanometers. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Although the size of most molecules would fit into the above outline, individual molecules are usually not referred to as nanoparticles. Nanoclusters have at least one dimension between 1 and 10 nanometers and a narrow size distribution. Nanopowders are agglomerates of ultrafine particles, nanoparticles, or nanoclusters. Nanometer-sized single crystals, or single-domain ultrafine particles, are often referred to as nanocrystals.[4-5]

Nanoparticles are sub nanosized colloidal structures composed of synthetic or semisynthetic polymers. Nanospheres are solid core spherical particulates which are nanometric in size.They contain drug embeded within the matrix or adsorbed on to the surface. Nanocapsules are vesicular system in which drug is essentially encapsulated with in the central volume surrounded by an embryonic polymeric sheath. In nanocrystals drug is mainly encapsulated in the solution system.[6]

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TYPES OF NANOPARTICLE

• Metal-based nanoparticles

• Lipid-based nanoparticles

• Polymer-based nanoparticles

•Biological nanoparticles

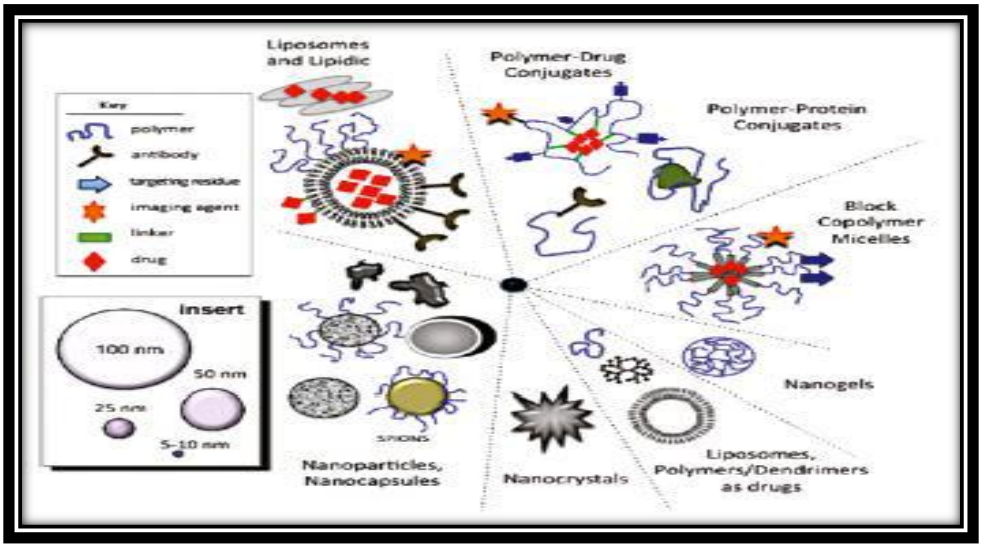


Figure 1 - Main classes of nanosystems used for drug delivery and targeting[1]

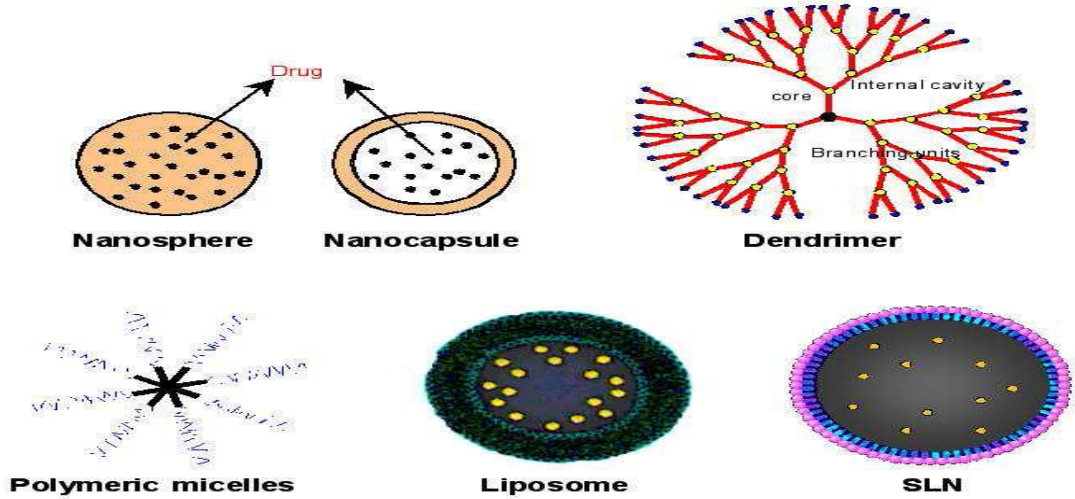


Figure 2 –Nanotechnology Based Formulation[1]

METHODS USED FOR THE PREPARATION OF NANOPARTICLES

The various methods are used for the preparation of Nanoparticles as follows:

SOLVENT EVAPORATION METHOD

In this technique the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate. The drug is dissolved or dispersed in the preformed polymer solution followed by emulsification of the mixture to form an oil/water (o/w) emulsion using an appropriate surfactant/emulsifying agents.[9]

Most commonly used surfactant/emulsifying agents for this purpose are gelatin and polyvinyl alcohol. After formation of a stable emulsion the organic solvent is evaporated by increasing the temperature or pressure along with continuous stirring of the solution. . shows a schematic representation of this method . Process parameters such as stabilizer and polymer concentration and stirring speed have a great influence on the particle size of the NPs formed.[10]

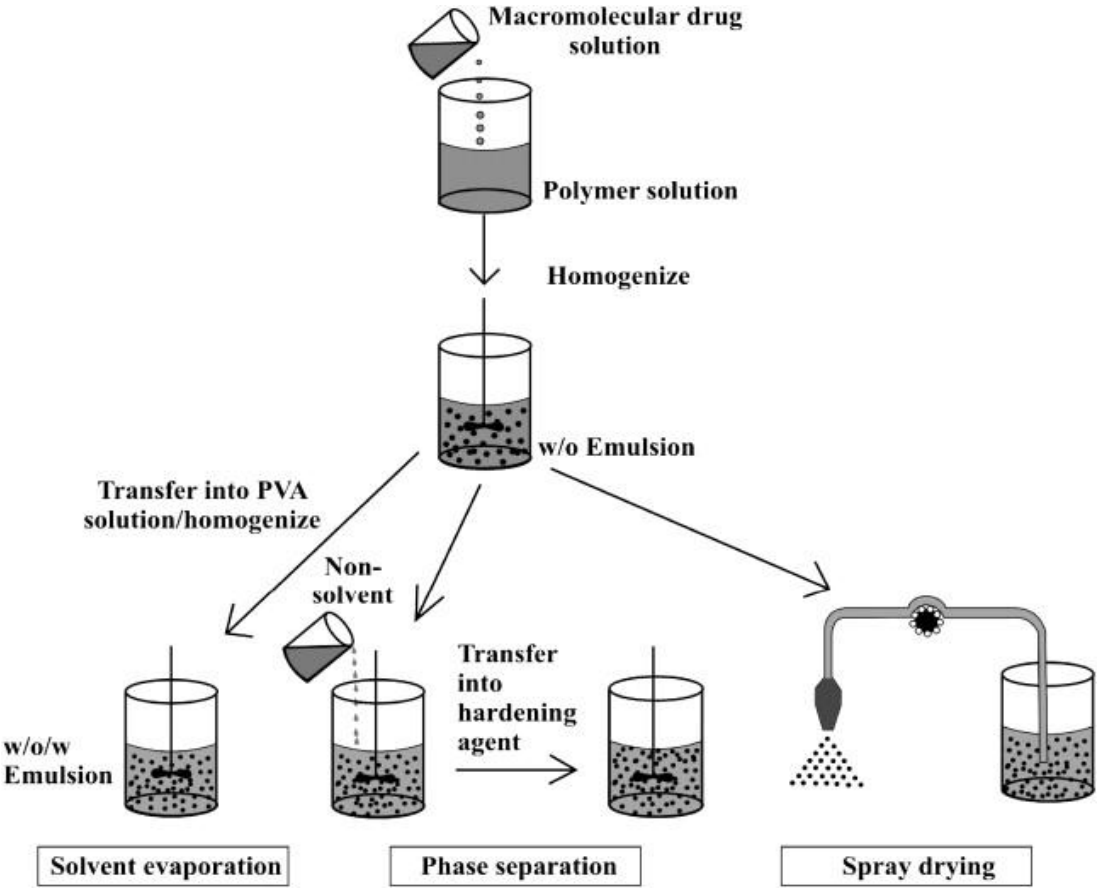


Figure 3 – Flow Diagram of Solvent Evaporation Method[1]

Spontaneous Emulsification/Solvent Diffusion Method

This is a modified solvent diffusion method where a water-miscible solvent such as acetone or methanol along with a water-insoluble organic solvent such as dichloromethane or chloroform are 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 smaller particles. As the concentration of water- soluble solvent increases, smaller particle sizes of NPs can be achieved.[11]

Nanoprecipitation Method

Typically, this method is used for hydrophobic drug entrapment, but it has been adapted for hydrophilic drugs as well. Polymers and drugs are dissolved in a polar, water-miscible solvent such as acetone, acetonitrile, ethanol, or methanol. The solution is then poured in a controlled

manner (i.e. drop-by-drop addition) into an aqueous solution with surfactant. Nanoparticles are formed instantaneously by rapid solvent diffusion. Finally, the solvent is removed under reduced pressure.[12]

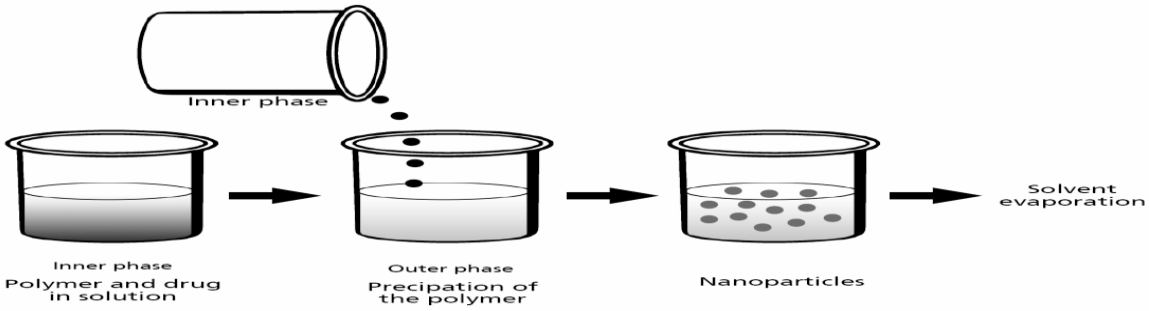


Figure 4 – Flow diagram of Nanoprecipitation Method[1]

Salting Out Method

In this method, the polymer is dissolved in the organic phase, which should be water-miscible, like acetone or tetrahydrofuran (THF). The organic phase is emulsified in an aqueous phase, under strong mechanical shear stress. The aqueous phase contains the emulsifier and a high concentration of salts which are not soluble in the organic phase.[12]

Typically, the salts used are 60% w/w of magnesium chloride hexahydrate or magnesium acetate tetrahydrate in 1:3 polymer to salt ratio . Contrary to the emulsion diffusion method, there is no diffusion of the solvent due to the presence of salts. The fast addition of pure water to the o/w emulsion under mild stirring reduces the ionic strength and leads to the migration of the water-soluble organic solvent to the aqueous phase inducing nanosphere formation. The final step is purification of nanoparticles by cross flow filtration or centrifugation to remove the salting out agent.[12]

Polymerization Methods

NPs are prepared from monomers that are polymerized to form NPs in an aqueous solution. Vaccines or drugs/therapeutic agents are incorporated in the NPs either by dissolving the drug in the polymerization medium or by adsorption/attachment of the drug onto the polymerized and fully formed NPs. The NPs suspension is then purified by removing stabilizers. The surfactants may be recycled for subsequent polymerization. This technique of NPs preparation has beenreported for making polybutylcyanoacrylate or poly-alkyl-cyanoacrylate NPs. The concentration of surfactant and the stabilizer determines the final size of the NPs formed.

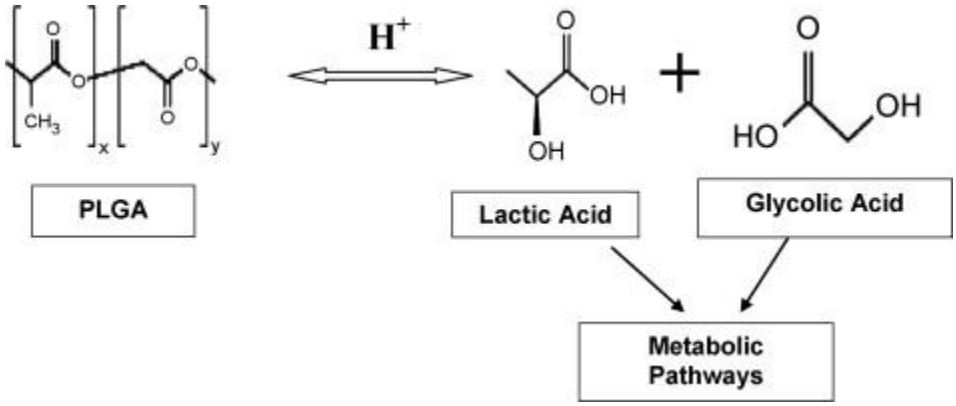
Ionic Gelation Method for Hydrophilic Polymers

Some of the natural macromolecules have been used to prepare NPs. These polymers include gelatin, alginate, chitosan and agarose.

They are hydrophilic natural polymers and have been used to synthesize biodegradable NPs by the ionic gelation method. This involves the transition of materials from liquid to gel due to ionic interaction at room temperature. An example of preparation of gelatin NPs includes hardening of the droplets of emulsified gelatin solution into gelatin NPs. The gelatin emulsion droplets are cooled below the gelation point in an ice bath leading to gelation of the droplets into gelatin NPs. Alginate NPs are reported to be produced by drop-by-drop extrusion of the sodium alginate solution into the calcium chloride solution . Sodium alginate is a water-soluble polymer that gels in the presence of multivalent cations such as calcium . [12-15]

Poly (Lactic-Co-Glycolic Acid) (PLGA)

PLGA has generated a huge interest on the development of nanocarriers due to its excellent biocompatibility, biodegradability and mechanical strength. This polymer is degraded in the body by hydrolytic cleavage of ester linkage with the production of two metabolite monomers, lactic acid and glycolic acid that are effectively metabolized in the body through the Krebs cycle.



Several factors influence the degradation of PLGA, including the method of preparation, the type of encapsulated drugs, intrinsic properties (molecular weight and copolymer composition), physicochemical parameters (pH, temperature and ionic strength) and site of implantation. The degradation time can go from months to years being that will be shorter for polymers with low molecular weight and composed by copolymers with higher glycolide content [13.17]

The degradation of PLGA nanoparticles can occur by two mechanisms, bulk erosion or surface erosion (Figure.3). The bulk erosion that is the main degradation pathway comprises three phases. Initially it is observed a significant decrease in the molecular weight of the polymer as a result of a random scission of ester bonds, that is followed by the formation of soluble monomeric and oligomeric products, being that the latter's in the final phase originate more soluble monomeric products, thus favoring the complete degradation of the polymer[18].

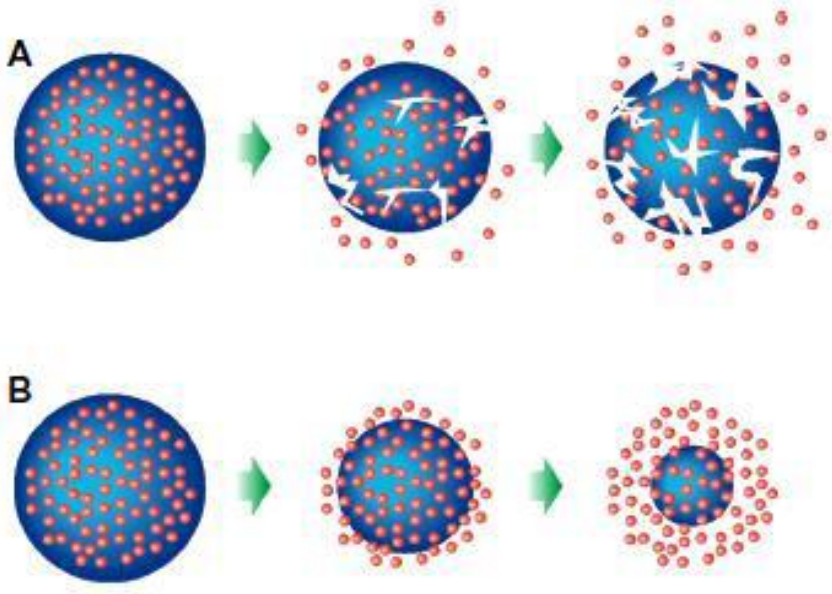


Figure 5 – Degradation mechanisms of PLGA nanoparticles

(A) bulk erosion and (B) surface erosion.[18]

Table 1 - Human pathogenic *Leishmania* species, clinical manifestations and geographical distribution

Species	Clinical manifestation	Geographical distribution
Old World, subgenus *Leishmania*		
L. donovani	VL; PKDL	Africa, India
L. infantum	VL; CL (rare)	Mediterranean, Asia, Sub-Saharan
L. tropica	CL; VL (rare)	Middle East, India, Mediterranean
L.major	CL	Africa, Middle East, India, China
L. aethiopica	CL; DCL	East Africa
New World, subgenus *Leishmania*		
L. chagasi *	VL; CL (rare)	Latin America
L. mexicana	CL; DCL	Central America
L. amazonensis	CL; DCL; MCL; VL (rare)	Central and SouthAmerica
New World, subgenus Viannia		
L.braziliensis	CL; MCL	South America
L. guyanensis	CL	South America
L. panamensis	CL	South America
L. peruviana	CL	Central and SouthAmerica

VL, visceral leishmaniasis; PKDL, post kala-azar dermal leishmaniasis; CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis.

*There are growing evidences suggesting that *L. infantum* and *L. chagasi* are the same species

LEISHMANIA PARASITE

History and taxonomy

William Leishman and Charles Donovan were the first ones to describe, separately but simultaneously in 1903, the protozoan parasite actually known as *Leishmania donovani* in the spleen from patients in India suffering from a life-threatening disease nowadays known as visceral leishmaniasis (VL).

Leishmania spp. is a diverse group of organisms that belongs to the order *Kinetoplastidae*, characterized by the presence of a kinetoplast in their members, and to *Trypanosomatidae* family. The *Leishmania* genus can be divided into two subgenera, *Leishmania (Leishmania)* spp. and *Leishmania (Viannia)* spp. (Table 1). These parasites are the causative agents of leishmaniasis, existing more than 20 species that can promote the development of the disease. They are transmitted by the bite of female sandflies, belonging to the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. [20,23]

Leishmania

It is expressed as a myriad of disease manifestations which depend upon parasite species, host responses, and poorly understood host-parasite-vector interactions. Leishmaniasis is one of the most important vector-borne diseases of humans. This parasitic disease can be caused by many species of *Leishmania*, most of which are zoonotic. Several species of *Leishmania* are capable of infecting humans and causing disease. Kala azar (Leishmaniasis) is a deadly disease caused by the parasitic protozoa *Leishmania donovani* and transmitted to humans by the bite of infected female sand fly, *Phlebotomus argentipes* [24,]

The disease forms of Leishmania are remarkably diverse. In most cases, the outcome is divided into three major clinical forms:

- Cutaneous leishmaniasis (CL),
- Mucocutaneous leishmaniasis (MCL), and
- Visceral leishmaniasis (VL).

Cutaneous Leishmaniasis (CL)

Cutaneous leishmaniasis, is primarily caused by *Leishmania major*, *L. tropica*, and *L. aethiopica* in the Old World or *L. Mexicana*, *L. amazonensis*, *L. braziliensis*, *L. panamanesis*, and *L. guyanensis* in the new world. It is the most common manifestation of the disease and is characterized by benign self-healing lesions that are generally painless and non-pruritic. The lesion represents a localized infection at the site of the sand fly bite. The infection spreads outward from this point due to the continuing cycles of replication within the macrophages.

About 1.5 million new cases of CL occur each year. It is endemic in more than 70 countries worldwide, and 90% of infections develop in Afghanistan, Pakistan, Syria, Saudi Arabia, Algeria, Iran, Brazil, and Peru [25]

Visceral Leishmaniasis(VL)

It is primarily caused by *L. donovani* and *L. Infantum* in the Old World and *L. chagasi* in the New World. It is the most serious form of the disease in which the parasites have migrated to vital organs and can be fatal if untreated. It is a generalized infection of the reticulo endothelial system (RES) involving the spleen, liver, bone marrow and lymph nodes. In contrast to CL, this post kala azar is easily cured with treatment. 0.5 Million new cases of visceral disease occur each year.[27]

Visceral leishmaniasis occurs in 65 countries; the majority (90%) of cases occur in agricultural areas and among the suburban poor of five countries: Bangladesh, India, Nepal, Sudan, and Brazil. [27]

Mucocutaneous Leishmaniasis (MCL)

MCL is mainly caused by *L. braziliensis* and occasionally by *L. panamensis* or *L. guyanensis*. MCL is characterized by metastasis of skin lesions to mucous tissues by lymphatic or hematogenous dissemination. Leishmania species are transmitted by a phlebotome sandfly vector. About 90% of the MCL cases occur in Bolivia, Brazil, and Peru . [27]

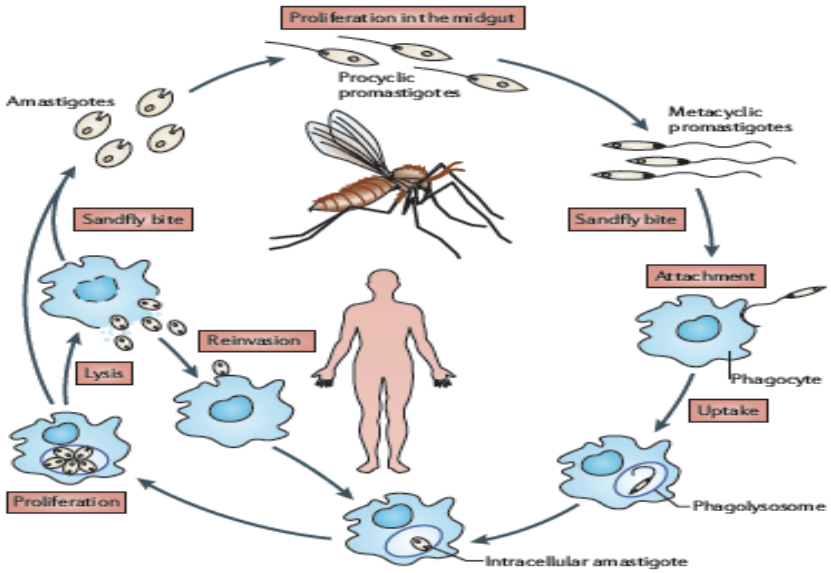
Life Cycle of Leishmania

Leishmania species are transmitted by a phlebotome sandfly vector. Most of the leishmaniases are zoonoses, although anthroponotic forms, in which humans are the sole reservoir, exist as well. Leishmania organisms have a relatively simple life cycle, characterized by two principal stages: the flagellated mobile promastigotes living in the gut of the sand fly vector and the immobile amastigotes with in phagolysosomal vesicles of the vertebrate host macrophages. Infected female sand flies transmit the disease by inoculating the promastigote form into the skin during their blood meal. In the vertebrate host, the parasites are phagocytosed by macrophages and dendritic cells in the dermis. After uptake and internalization of promastigotes into a phagosome, fusion with lysosomes proceeds as normal and the parasites survive in the phagolysosome.[1]

During this process, the promastigotes rapidly transform into amastigotes within 12–24 h and continue to grow and divide within the phagolysosomal compartment. When a sandfly takes a blood meal from an infected vertebrate host, it ingests amastigote-containing macrophages and monocytes.[27]

The amastigotes are released into the sand fly mid gut where they develop into flagellated promastigotes. These go through a process called metacyclogenesis, in which the dividing, noninfective procyclic form acquires virulence capabilities and is transformed into a non dividing, infective metacyclic form.[27]

The metacyclic promastigotes migrate into the pharynx and buccal cavity, ready for transmission during a next blood meal.[1-4].



Leishmania life cycle. [27,]

APPLICATIONS OF NANOPARTICULE DELIVERY SYSTEMS

1. NANOPARTICLES TO TARGET MACROPHAGES

Macrophages (Greek: big eaters, from macros “large” + phage in “eat”) are cells produced by the differentiation of monocytes in tissues. Human macrophages are about 21 micrometers (0.00083 inch) in diameter. Monocytes and macrophages are phagocytes. Macrophages function in both non-specific defense (innate immunity) as well as help initiate specific defense mechanisms (adaptive immunity) of vertebrate animals.[1]

Their role is to phagocytes (engulf and then digest) cellular debris and pathogens, either as stationary or as mobile cell. They also stimulate lymphocytes and other immune cells to respond to pathogens. They are specialized phagocytes cells that attack foreign substance, infectious microbes and cancer cells through destruction and including CD14, CD11b, F4/80 (mice)/EMRI (human), lysozyme M, MAC-1/MAC-3 and CD68 by flow cytometry or immunehistochemical staining. They move by action of amoeboid movement.[1]

PHAGOCYTOSIS OF PROMASTIGOTES BY MACROPHAGES IN VISCERAL LEISHMANIASIS (VL)

Phagocytosis is the process of engulfment of any foreign material by the macrophages. However, Leishmania parasite can resist the microbicidal activity of macrophages. Phagocytosis of promastigotes is comprised of two allied proceedings: i) attachment via low affinity, rapid kinetics interactions; and (ii) internalization following a high-affinity interaction. The uptake of promastigote by macrophages may occur by any of two mechanisms – zipper or coiling. In the zipper mechanism, the attachment of the parasite to a phagocyte receptor triggers the activation of more receptors from the contiguous membrane, forming a pseudopod, which advances along the parasite like a zipper, hence forcing the promastigotes into the phagosome. In the coiling mechanism various pseudopods are arranged asymmetrically, delivering the parasite to a cytoplasmic compartment where its survival becomes uncertain.[1]

2. TUMOR TARGETING USING NANOPARTICULATE DELIVERY SYSTEMS

The rationale of using nanoparticles for tumor targeting is based on 1) nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles; 2) nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ. [27,28]

3. LONG CIRCULATING NANOPARTICLES

To be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside MPS-rich organs. In the past decade, a great deal of work has been devoted to developing so-called “stealth” particles or PEGylated nanoparticles, which are invisible to macrophages or phagocyte.[28]

4. REVERSION OF MULTIDRUG RESISTANCE IN TUMOUR CELLS

Anticancer drugs, even if they are located in the tumour interstitium, can turn out to be of limited efficacy against numerous solid tumour types, because cancer cells are able to develop mechanisms of resistance 58. These mechanisms allow tumours to evade chemotherapy. Multidrug resistance (MDR) is one of the most serious problems in chemotherapy.[27,28]

MDR occurs mainly due to the over expression of the plasma membrane pglycoprotein (Pgp), which is capable of extruding various positively charged xenobiotics, including some anticancer drugs, out of cells. [27,28]

5. NANOPARTICLES FOR ORAL DELIVERY OF PEPTIDES AND PROTEINS

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is

limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation.[27,28]

6. TARGETING OF NANOPARTICLES TO EPITHELIAL CELLS IN THE GI TRACT USING LIGANDS

Targeting strategies to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Peyer's patches in the GI tract can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. The surface of enterocytes and M cells display cell-specific carbohydrates, which may serve as binding sites to colloidal drug carriers containing appropriate ligands. Certain glycoproteins and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism. Different lectins, such as bean lectin and tomato lectin, have been studied to enhance oral peptide adsorption.[27,28].

7. NANOPARTICLES FOR GENE DELIVERY

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system.[23,28]

8. NANOPARTICLES FOR DRUG DELIVERY INTO THE BRAIN

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems.[28]

It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps.[28]

NANOPARTICLES CHARACTERIZATION

The capability of nanoparticles to effectively target organs and tissues are influenced by morphological characteristics, particle size, surface charge and chemistry, and efficiency with which the drug is encapsulated and released. Thus, their use as drug delivery systems implies that a systematic characterization is made, in order to verify if their properties are the most suitable for pharmaceutical applications.[29]

1. MORPHOLOGY

Morphology of nanoparticles could be readily assessed by imaging techniques like scanning electron microscopy used mostly for surface characterization (shape, distribution and aggregation), transmission electron microscopy (TEM) used for shape, aggregation and internal details and, atomic force microscopy (AFM) used mostly for size and morphology.[29]

2. SIZE AND POLIDISPERSITY INDEX :

Size is a very important parameter in the characterization of nanoparticles that will be used as drug delivery systems, not only because it will influence the release profile and degradation rate of the nanoformulations, but also because it will determines their uptake by the cells of the mononuclear phagocytic system (MPS) and their biodistribution. Although different techniques based on different physical principles can be used to measure nanoparticles size, the one that is widely used is dynamic light scattering (DLS), also called photon correlation spectroscopy.[29]

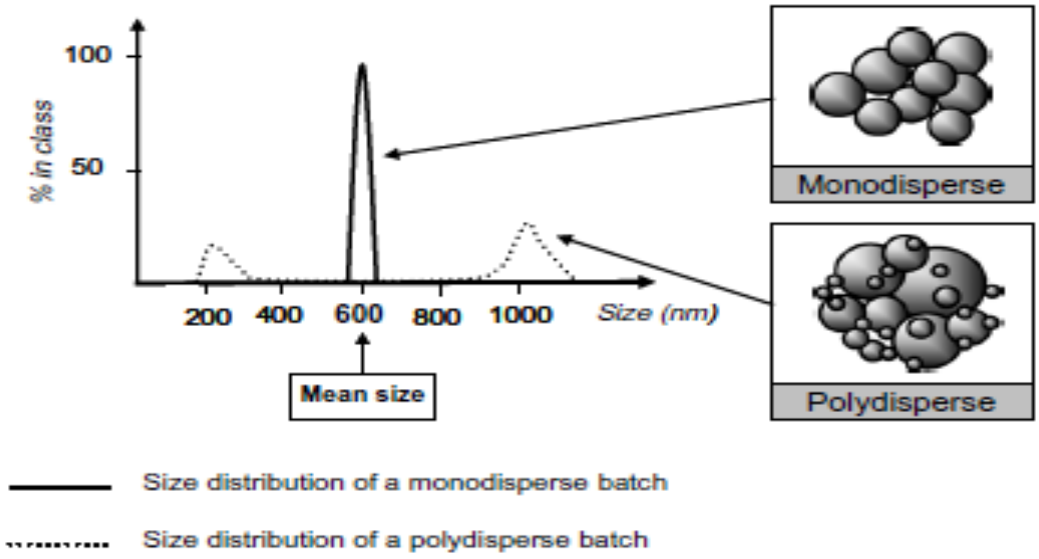


Figure 7 – Representation of the curves obtained for two nanoparticles batches of a monodisperse and polydisperse population after analysis by DLS. [1] .

3. SURFACE PROPERTIES

Zeta potential corresponds to the overall charge that the particles acquire in a particular medium, being an important parameter in the characterization of nanoparticles since it will determine if they will cluster in blood circulation or interact with oppositely charged cells membrane . Nanoparticles in a liquid suspension are surrounded by a liquid layer composed by two parts: an inner layer (Stern layer) where the ions are strongly bound to each other and with nanoparticles and an outer (diffuse) layer where they are not so firmly bound. Within the diffuse layer exists an imaginary boundary, in which ions and particles form a stable entity. The potential formed at this boundary surface is designated zeta potential and its determination is achieved through the

monitoring of the mobility of charged particles by application of an electrical potential. Zeta potential give us information on the stability of the particles in suspension, being that its value may be positive or negative depending on the nature of the polymer used and the occurrence or not of a surface modification. [30]

4. ENCAPSULATION EFFICIENCY AND DRUG LOADING PROFILE

When a drug is entrapped into the nanoparticles two parameters should be taken into consideration: encapsulation efficiency (E.E.) that corresponds to the percentage of drug retained in the nanoparticle matrix relatively to the total amount of drug initially used in the preparation process; and, drug loading which is the theoretical percentage of loaded amount of drug relatively to the total amount of polymer used in the preparation process.[30]

Ideally, nanoparticles should have high E.E. and a high drug loading in order to reduce the quantity of nanoformulation required for administration. Precise determination of drug loading is not easy because nanoparticles are colloidal systems and requires a previous separation of nanoparticles from non- encapsulated drug, normally by ultracentrifugation.[30]

5. MODIFICATION OF SURFACE PROPERTIES

One of the major issues in the use of polymeric nanoparticles as drug delivery systems is their hydrophobicity. After an intravenous administration, hydrophobic nanoparticles are rapidly recognized by the body as foreign and cleaned up from the systemic circulation by the MPS, ending up on liver, spleen or lymph nodes . If the goal is to use nanoparticles for the treatment of a condition in any of the previous referred organs the use of hydrophobic nanoparticles is the better choice, however if a sustained systemic circulation is required, in order to increase the probability for the nanoparticles reach their target, then the surface of hydrophobic nanoparticles must be modified to avoid the occurrence of phagocytosis.[31]

CONCLUSION

This study 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. The novel nanoformulations developed in the present work, through the functionalization of the surface of PLGA nanoparticles with mannose residues, constitutes a promising strategy for efficient delivery of AmB and seems to have potential as immunotherapeutic agents in the treatment of VL.

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