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An overview of biomolecule nanoparticles

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Abstract

Biomolecule-Nanoparticle structure contributes novel materials for incorporating the special electrical, mechanical or catalytic abilities of nano elements with biomolecule for the detection or biocatalytic functions. Considering the future problems and identifying various potential uses of the bioparticles are the future perspectives of this field are discussed. This review examines the categories of bio-nanoparticles, their methods of production, recent developments in biomolecule incorporation and the application in the field of pharmaceuticals, biosensors and bioelectronics as well as food and agriculture of biomolecular nanoparticles. Present review attempts to explain the significant developments in the incorporation of nanoparticles with biomolecules during the past five years and the applications of the biomolecule nanoparticle composites in the pharmaceutical sector and in diagnostic field as bioelectronics and biosensors.

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Introduction

Nanotechnology is a rapidly increasing sector which is concerned with the production, from manufacture to implementation, of products less than 1000 nm in thickness. It can be described as the science and engineering involved in designing, synthesizing, characterizing and applying materials and devices whose smallest functional organization is at the nanometer scale or one billionth of a meter in at least one dimension. Nanotechnology is widely used in all areas of life for more sophisticated technology (Silva, 2004). The reduction to nanoscale of the particles reflects major developments in physical, mechanical, electrical and optical features which are not seen in bulk materials, due to the small surface area to volume ratio than the nano materials. This development provides new medicinal possibilities for the drugs, which due to their limited performance cannot be used successfully as traditional formulations (Logothetidis S, 2006).

Biomolecules are the naturally occurring compounds in living organisms. They are primarily made of carbon, oxygen, sulphur and phosphorus and are quite large which contain several atoms bound covalently. Biomolecules include proteins, lipids, carbohydrates and nucleic acids. Biomolecules themselves are nanoscale components with embedded functional and structural information (Fan W *et al.,* 2016).Nature has given numerous biomolecules with its own distinctive characteristics, such as lipids, polysaccharides, nucleic acids and proteins which have made use of formulating nanoparticle. Nevertheless, the implementation of biological nanoparticles formulated from biomolecules has become increasingly important, as an substitute to chemical nanoparticles, in recent years. In addition to its other advantages, including ease of delivery and non-immunogenicity, the production of biocompatible and biodegradable nanoparticles is important (Mobasser S *et al.,* 2016). Biomolecules may also be designed to have specific compositions and features and can be coupled with various forms of nanoparticles, like metals and metal oxides, to balance the inherent properties of nanoparticles with the intrinsic characteristics of biomolecules and to create new biomolecule-nanoparticles combinations (George S, 2014).

In the last few decades, enormous advances have been made in the development and application of biomolecule-based nanomaterial's and nanostructures. The fundamental differences of the nanomaterial's and biomolecules propose that the incorporation of biomolecules into these nano-objects may lead to hybrid systems combining the characteristics of nanomaterial's with the natural acceptance and catalytic mechanisms of biomolecules (Yashveer S *et al.,* 2014). Many biomedical applications such as bio sensing, microbial separation, molecular imaging and anticancer treatment the nano-scale systems and substances (nanoparticles, nanowires, Nano fibers, nanotubes) have been studied due to their innovative characteristics and functions. Formulation of nanoparticles prevents degradation of sensitive drugs, denaturation in rough pH regions and also increases product exposing time by promoting formulation retention by bio adhesion (OV Salta, 2004).

Categories of biological nanoparticles

Biological nanoparticles are particles from biomolecules or organic compounds of a size varying from 10 nm to 1µm. They can be classified into four primary groups namely proteins, lipids, polysaccharides and nucleic acids (Mondal and Dr. Sumanta, 2018).

Proteins form under a category of natural compounds with specific properties and possible benefits for both biological and material fields utilized to prepare nanoparticles. Such features permits the numerous surface configurations, covalent drug binding possibilities for certain compounds such as drugs (Weber C *et al.,* 2000). In addition, they can be developed as gels, emulsions and dried particles, have improved stability in vivo as well as during storage, and are fairly easy to formulate with functional size distribution, making them the perfect resource for nanoparticle preparation. Protein nanoparticles may

be used for the pulmonary distribution of protein therapy or can be embedded into bio degradable polymer microspheres or nanospheres for controlled release or oral drug delivery. At present, intensive work focuses on the processing of nanoparticles utilizing proteins such as albumin, gelatin, collagen, gliadin, zein, ferritin, gliadin and legumin (Nitta S.K and Numata K, 2013).

Lipid-based nanoparticles had developed as a promising nanoparticle network and have been identified as one of the most important nanobiotechnologyencapsulant throughout the field (D. A. Groneberg *et al.,* 2006).They include, Nano emulsions, solid lipid nanoparticles, liposomes, and nanostructured lipid carriers (NLC). Solid lipid nanoparticles (SLNs) are a new encapsulation method developed originally in the pharmaceutical industry for the supplement of lipophilic bioactive compounds. It consists of crystallised nanoemulsions with a solid lipid-bioactive component mixture acting as dispersed phase. Over and above its increased encapsulation efficiency, lipid nanoparticles showed prolonged shelf life and ability to entrap the variable solubility compounds and have specific target (Tamjidi F *et al.,* 2013).

Polysaccharides, are the polymers of carbohydrates that are associated by glycosidic bonds and are derived from plants, animals and microorganism. For example; chitosan, alginate, pectin, insulin and dextran. Chitosan is considered to be potentially one of the most important polysaccharides of various forms because of its increased permeability. (Sahdev P. *et al.,* 2014) A wide number of biomolecules have been effectively incorporated in chitosan nanoparticles by the use of charge driven ionic interaction including proteins plasmid DNA and antigens as well as bioflavonoids. The high-density amino groups and mucoadhesive properties of Chitosan allows the simple chemical alteration and complexation of negatively charged compounds. The chitosan nanoparticles are generally produced by self-assembly of polyelectrolytes and ionotropic gelation methods and are operated in modified temperature with low pressure. (Jahanshahi M. and Babaei Z., 2008).

The nucleic acids sequences of DNA and RNA may also be formed with nanoparticles. Regardless of the versatility of their fundamental framework, these biomolecules may be built to construct three dimensional nano-scaffolds (Panigaj M. and Reiser J., 2016).In fact, nucleic acids have remarkable capacity to organize themselves into portable, compact structures by an identical test on the dimension, shape and composition of nanoparticles. Research on bio-based nanoparticles has shown that nanoparticles of DNA or RNA can be used as scaffolds and different biological and pharmaceutical substances, such as aptamers, fluorophores and oligonucleotides, can be used to label the specified efficiency (Thiel K.W. and Giangrande P.H., 2009).

Approaches to synthesize biological nanoparticles

The quality of nanoparticles with controlled properties like morphology, uniform size distribution, composition and high purity must be taken in to account and the method should also be simple, inexpensive and have a high throughput. A significant field of study in nanotechnology is the creation and development of biological nanoparticles with desirable properties and to enable these materials to use in specific environments, and will have an influence on nature and the human species (Friedman A.D. *et al.,* 2013).

A basic outline of the techniques for the synthesis of biological nanoparticles includes the two-step emulsification process, one-step process involving nano-precipitation, desolvation, and gelation, and the drying method, with a primary focus on drying processes.

Two Step Emulsification Process: Owing to developments in the technology and emulsifying systems, methods for preparing bio nanoparticles based on emulsifying strategies have developed in the past decades. This process involves formation of primary emulsion droplets where the emulsion

system can be a type of water in oil (w/o) or oil in water (o/w) or oil in oil (o/o) which will be based on the features of dispersed phase and dispersion medium. A complex system contains multiple emulsions such as oil in water in oil (o/w/o), water in oil in oil (w/o/o) and water in oil in water (w/o/w) are also developed. After the formation of primary emulsion, nanoparticles are synthesized by nanoprecipitation method (Sharma Nitin *et al.,* 2010; Kumar M *et al.,* 2019).Other methods like polymerization and ionic gelation are used for converting into nanoparticles. Emulsification method provides a high degree of encapsulation quality and reproductiveness for batch-to-batch process and have extensively narrow size distribution (Chaturvedi S.P. and Kumar V., 2012). This method is limited to the lipophilic drugs and the According to regulatory concern, the presence of the residual solvent is unacceptable at the final dispersion. Extreme washing procedures to remove solvent residues are therefore needed. (Sharma Nitin *et al.,* 2010; Kumar M *et al.,* 2019).

One Step Process: Nano-precipitation is a process of synthesizing nanoparticles depending on polymer interfacial deposition after a semi-polar solvent (miscible with water) has been removed from a lipophilic solution so it is called as the solvent displacement method ofr interfacial disposition method which is developed by Fessi *et al.,*Nanoparticles are formed in the non-solvent or aqueous phase by a rapid diffusion of the polymer solvent, such as acetone. Increased surface area has resulted from decrease in the interfacial tension between dispersed phase and dispersion medium (Hornig S *et al.,* 2009).This approach offers a easy and quick way to produce biological nanoparticles with enhanced reproducibility from natural polymers and peptides, also at low concentrations (Bilati U *et al.,* 2005).

In 1978 Marty *et al.,* developed a technique called desolvation or Coacervation is a thermodynamically guided self-assembly of proteins generated by the incorporation of desolvating compounds in the biomolecules solution where the molecules in the aqueous medium gets separated or coacervated. Electrostatic interaction plays a crucial role in this method in facilitating self-assembly of proteins. Usually, crosslinking reactions with glutaraldehyde and carbodiimide were performed to enhance nanoparticles 'stability and to avoid dissolution (Joachim Allouche., 2013).

Drying Method: Researchers had influenced by environmental concerns to find alternative methods to synthesize nanoparticles with the removal of organic solvents. Hence, the development of biological nanoparticles by drying method was seen as a successful alternative to the traditional methods to achieve this goal (Pakowski. Z, 2004). The dry formulation of bio nanoparticles provides additional stabilization fordegradation, enhanced shelf life and is simple to handle because of the nature of biological molecules that is often hard to achieve in liquid formulations.There are three key approaches to the development of biological nanoparticles by drying methods are spray drying, supercritical drying, freeze drying and electrospraying process, a currently evolving technique. (Pakowski. Z, 2007).

Spray drying: Spray drying is a well-recognized process, which results in solid nanoparticles by atomization of spray suspensions in to droplets and then drying of droplets (Okuyama K and Lenggoro I. 2003). When the drug delivery of the therapeutical proteins or medicines through a pulmonary route was discovered, this process of spray drying is used for drying biological compounds. The process involves atomization of the bimolecular solution in to a fine solid particles in the aid of drying it in the hot air and collected through a cyclone. However, because of the small size of the nanoparticles, the collection rate for particles produced by traditional spray drying method is very low. BÜCHI Labortechnik AG has recently developed a Nano spray dryer to improve the recovery of particles (Mehta P *et al.,* 2016).

Supercritical drying: Usage of supercritical fluid as a drying medium (anti-solvent) is utilized in

supercritical drying that provides the specific density and solvating capacity of a liquid but delivers in the form of gas. There are presently many super-critical technologies required for sub micrometer and nano particle scale manufacturing. (Sellers S.P. *et al.,* 2001).

Supercritical $CO_2$is usually used to formulate finely divided dried thermolabile drugs and pharmaceutical powders for rapid expansion of the supercritical solution (RESS). In this method, biological components are solubilized in supercritical CO_2 and then depressurize into ambient air via a nozzle. Due to the formation of the enhanced super saturation conditions it enables homogeneous nucleation and the biological solutes gets precipitated into well dispersed particles. (Peltonen L. *et al.,* 2010). While the process can be done in a solvent-free environment, many pharmaceutical components have a very low solubility in supercritical CO_2 which the led the way to development of |Solution Enhanced Dispersion by Supercritical Solution (SEDS). The solubility of pharmaceutical compounds in CO_2 was enhanced by co-solvents with this approach (Haggag Y.A. and Faheem A.M., 2015).

In order to overcome the poor solubility of the pharmaceutical compounds in supercritical CO_2 the Supercritical fluid antisolvent (SAS) technique is implemented by mixing a solute in fluid with compressed CO_2 in order to facilitate crystallization.Non-aqueous organic solvents namely dimethyl sulfoxide, is used in the SAS technique to improve the miscibility of solute compounds in CO_2 (Tabernero A. *et al.,* 2012).

Freeze Drying: This process comprises two phases, namely spray freezing and vacuum drying. The procedure consists of atomizing the fluid through the freezing agent (e.g. liquid N2), in which tiny droplets forming into a solid particles. Formed particles are then transferred after sufficient filtering into a vacuum freeze drying chamber from which the ice is warmed and sublimated. Nanoparticles are protected from agglomerating by the creation of a stable layer (Scherer G.W.1993).

Electrospraying: Electrospraying is an electro-hydrodynamic liquid atomization into standard sized droplets, under electrical influence. Gomez A. *et al.,* (1998). Had demonstrated the first attempt for the usage of electrospraying of protein nanoparticles. In order to test the potential for electrospraying to generate monodispersed protein particles with sustained biological activity, insulin was used as a model protein.

The study of electrosprayed insulin's receptor binding properties and the insulin regulation showed the similar findings, which suggest that electrospraying retains biological activity. Research on the development of active biomolecules particles using the electrospraying method was quickly established with these tests, particularly for the application of medicinal products. (Yurteri C.U. *et al.,* 2010). The important steps involved during the electrospraying process are:

Formation of charged droplets

Deflating of the droplets because of solvent elimination or evaporation.

Persistent disintegration of droplets to the formation of dry particles.

Particle aggregation or deposition. (Lenggoro I.W. *et al.,* 2002)

Integration of biomolecules with nanoparticle system

Many approaches for incorporation of biomolecules with NPs have primarily been established: 1) Biomolecules may be specifically functional as an unit for obtaining NPs;2) Biomolecules may be scattered across the pores or attached to the surface of NPs by physical interactions;3) Biomolecules possessing functional groups could be conjugated through covalent bonds; 4) Biomolecules may also be specifically immobilized by in situ encapsulation into the matrix of NPs (Jing Mu *et al.,* 2019).

Developmental strategies

Functionalization by covalent interactions: Biomolecules can be integrated with nano particles by covalent interaction through chemisorption or by the usage of a bifunctional linker of the biomolecules on the surface of compound. Chemisorption is defined as the complex chemical reaction between the thiol group of the biomolecules and the nanoparticles through the cysteine residue deposited on the biomolecular surface (Zhang Y. *et al.,* 2012). If there are no thiolated residues present in the active biomolecules, then the thiol group may be chemically incorporated into the outside portion of biomolecules with Traut's reagent (2-aminothiolane) to facilitate contact between biomolecules and NP (De M. *et al.,* 2008). Mejias S.H. *et al.,* (2016) have demonstrated the efficient assembly by thiol chemisorption of the protein cysteine residues of the ideal protein building block, consensus tetratricopeptide repeat (CTPR) on the surface of nanoparticles. Covalent linkage through bifunctional linkers provides a flexible means to conjugate the biomolecules. Bifunctional linker groups that include carboxy, amino and maleimide terminal anchor groups which have low molecular weight are widely utilized to link biomolecules to nanoparticles like CdSe / ZnS, CdS, Ag, ZnSand Au. Such anchor groups may be used to substitute for the stabilization of nanoparticles with weak adsorption molecules (Katz E. and Willner I., 2004).

Functionalization by non-covalent interactions:It is a physical technique that can be applied by interactions between electrostatics, hydrophobic and affinity for conjugation of biomolecules (Suryani S *et al.,* 2017). To require contact with biomolecules, biological nanoparticles could be developed to possess a definite charge. The use of cationic lipid nanoparticles adjusted with a coiled-coil protein that has charged arginine residues positively to allow contact with the negative SiRNA(Niemeyer C.M., 2001). Another instance is the production of a self-assembled nanocomplex based on fucoidan, a highly simple protein by utilizing the electrostatic reaction between the oppositely charged polysaccharides and the proteins (Lu K.Y. *et al.,* 2017). The powerful electrostatic interaction between the charged biomolecules and their host isn't always favoured in some circumstances. According to Lebre F. *et al.*, (2016), Strong bonding between the positive charged chitosan and negative charged DNA reported with low in vivo performance. This method permitted the intracellular release of DNA, thus improving the efficiency of the transfection (Rabbani P.S. *et al.,* 2017).

Puddu V. and Perry C.C. (2012) observed that the identification and adsorption of peptide sequence with specific charges on silica were responsible for the hydrophobic interactions in various pH environments. Researchers also demonstrated that the usage of biomolecules on nanoparticles can be modulated by changing surface and binding conditions, for example the bulk concentration of biomolecules and pH. If silica's surface charging is near to its zero charge, bioconjugation with peptide sequence with hydrophobic character is preferred (Suryani Saallah *et al.,* 2017).

Joye I.J. *et al.,* (2015) has reported that hydrophobic interaction may also be used to link the biomolecules with hydrophobic protein nanoparticles, such as gliadin. While comprehensive work on biomolecular interactions such as amino acids, proteins, and peptides with silica nanoparticles has shown that linking biomolecules to silica nanoparticles is predominantly guided by electrostatic interactions.

Entrapment of biomolecules in polymeric substances: Because of the unique properties of the polymeric nanoparticles, they are widely used as carriers for biomolecules. They can be co-polymerised, simple to synthesize and the surface of the polymer can be modified for the conjugation of biomolecules and can be developed in the form of Nanospheres of Nanocapsules (Menon J.U *et al.,* 2014). The complete mass of nanospheres and biomolecules may be added to the surface or incorporated into the material, while in nanocapsules, the biomolecules have been engineered to be enclosed within the core shell. In some instances bioactive materials can be adsorbed to

the capsule surface (Jawahar N. and Meyyanathan S., 2012).

Application of biomolecule- nanoparticle composites

Identification of bio macromolecule as external receptors by nanoparticles offers a possible route for regulating cellular and extracellular processes for various biological applications, such as inhibition of enzymes, modulation of transcription, sensing and transmission.

Pharmaceutical Applications: The Nanoparticles are suitable for loading several carriers like proteins, drugs, imaging agents, enzymes, genes and other therapeutic agents because of their wide surface area with a highly porous structure. In drug administration of cancer and other illness associated treatments, bio nanoparticles are widely utilized. Several experiments for cancer therapy have attempted to develop biomolecule-nanoparticle conjugates (Wen Yang *et al.,* 2019). Recent advances particularly in the drug-administration area of nano medicine contribute to the discovery of nanoparticle-based drug therapies for the diagnosis and treatment of diseases. The drug delivery mechanism based on nanoparticle allows a greater regulation of drug release in a targeted region, which hence decreases the frequency of administration and minimizes clinical adverse effects (Valo H., 2012).

The incorporation of nanoparticles into the biomolecule also allows the creation of three-dimensional scaffolds for use in osteogenesis. A remarkable impact on bone formation has been shown by particle morphology in which the spherical nanoparticles have the strong bone forming potential in comparison to other nanoparticles. "Safe" delivery offers gene therapy exposure and protein-based therapeutic solutions to these biomolecules (Yang G. *et al.,* 2017). Different types of medicines could be given in an advanced drug delivery system concurrently for the development of synergistic treatment for disorders. Binding medicines to nanoparticles with polysaccharides like albumin and gelatin that improve the antitumor role of the drugs. Protein-nanoparticles can carry drugs across the blood-brain barrier as a carrier for different types of therapies (Lee S.H. *et al.,* 2011; Huang Y.C. and Kuo T.H, 2016).

The delivery of anticancer drugs in the intracellular level had become significant challenge because of the acidification of drugs that takes place in endosomal compartment, vigorous changes in the pH takes place. To overcome this challenge mussel adhesive proteins (MAPs)-based iron (III)–3,4-dihydroxyphenylalanine (DOPA) nanoparticles have been synthesized. By utilizing pH related alterations in the stoichiometry of DOPA coordination, pH-responsive drug delivery system was achieved and has indicated successful cytotoxicity towards malignant growth cells. This system can be applied for the drug delivery of various controlled conditions (Kim BJ *et al.,* 2015). Bioelectronics and Biosensing: As NCP-based sensors are developed for the identification of biologically significant molecules, a wide variety of protein and nucleic acids with specific recognition capacities were developed because of flexible approaches to biomolecular immobilization on NCPs.

In the production of efficient biosensors and bioelectronic equipment for the diagnosis and identification of pathogens in the medical, food and agriculture industries, nanotechnology has widened the scope and introduced. Significant work is presently been performed on the use of hybridized molecules for the development of bioelectronic device and the modern bio-sensors (Yagati A.K. *et al.,* 2017). In bioanalytical instruments, nanomaterials were essential components because they clearly improve their sensitivity and detection limits in terms of their efficiency. One of the major interest in the diagnostic field is the Nanomaterial-based biosensors. Four types of nanoparticles namely carbon nanotubes, photonic crystals, gold nanoparticles and graphene have become prominent in biosensor research as there is a high demand for devices possessing enhanced selectivity and sensitivity, rapid response and with effective cost (Yang N *et al.,* 2015). For the monitoring of particular compounds in the food

industries and medical field Enzymes-based biosensors are now becoming more common. A modern glucose biosensor has been developed from hybrid nanoparticles of glucose oxidase (GOx), iron oxide and polypyrrole by immobilizing the inner shell of the particle on to the surface of magnetic carbon electron. This biosensor allowed high-selective glucose monitoring and fast detection with a low concentration limit and strong linear range (Yang Z. *et al.*, 2014). For the identification of phenolic compounds by aiding enzymes had utilized the enzyme-based biosensors (Rodriguez-Delgado M.M. *et al.,* 2015).

Conclusion

Different forms of biological nanoparticles and methods for synthesis were addressed in this study. The developments in the self-organizing of nanomaterials engineered biomolecules add new dimensions to the Nano biotechnology. Types of biological nanoparticles, fabrication methods and their approaches for synthesizing it were reviewed. However, in this rapidly developing sector, there are many more studies to be observed. The next generation of biological nanoparticles with different types of the functionalities, which will enhance the characteristics of the biological nanoparticles and expand their application into various areas, will be developed with recent technological advances and new developments.

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