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Design and Development of Biopolymer - Based Molecularly Imprinted Polymer Nanoparticles for Analytical Sensing Applications

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Abstract

This research on nanoparticles highlights the recent advancements in utilizing biopolymers, including proteins and polysaccharides, to revolutionize the development of biocompatible and environmentally degradable natural biological materials. The article provides insights into various fabrication methods, such as emulsification, desolvation, coacervation, and electrospray drying. Furthermore, it delves into the comprehensive characterization of nanoparticles, covering parameters like particle size, surface charge, morphology, stability, structure, cellular uptake, cytotoxicity, drug loading, and drug release, while also discussing the relevant measurement techniques. The review explores applications of these biopolymer-based nanoparticles in the fields of medicine and biotechnology and looks ahead to a promising future scope in this area.

Keywords: Nanoparticles, Biopolymers, Fabrication Methods, Characterization Parameters, Medicine and Biotechnology, Biocompatible Materials, Drug Delivery, Future Applications

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1. Introduction

Nanotechnology encompasses the realm of scientific inquiry and technological advancements at the scales of atoms, molecules, and macromolecules, enabling the deliberate manipulation and investigation of structures and devices within the nanometer range, typically spanning from 1 to 100 nm. The term "nanotechnology" is credited to its probable inception by Japanese scientist Taniguchi. This field represents a distinct branch of manufacturing where the precise dimensions at the nanoscale hold paramount significance.

Numerous researchers have underscored the importance of size, highlighted the advantages of nanoparticles when compared to larger microspheres (exceeding 1 μm). In particular, biological nanoparticles have emerged as a promising avenue for drug delivery systems, offering an alternative to traditional liposome technology. These nanoparticles address challenges related to the stability of vesicles in biological fluids and during storage.

In recent years, nanoparticle technology has played a pivotal role in enhancing the efficacy of drugs. Nanoparticles seamlessly integrate into colloidal drug delivery systems, presenting advantages like targeted drug delivery, modified distribution within the body, and improved cellular uptake. This, in turn, minimizes unwanted toxic side effects associated with free drugs. The accessibility of nanoparticles throughout the body allows for their circulation to various sites, facilitating systemic treatments.

Nanoparticles can be crafted from an array of materials, including proteins, polysaccharides, and synthetic polymers. The choice of materials hinges on several critical factors, encompassing the size and shape of the nanoparticles, surface charge, permeability, biodegradability, biocompatibility, cytotoxicity, and the desired drug loading and release profiles. This research expounds upon the latest advancements in protein and polysaccharide nanoparticles, exploring diverse fabrication methods, characterizations pertaining to size, surface charge, morphology, stability, structure, cellular uptake, cytotoxicity, drug loading, drug release, and their myriad applications in medicine and biotechnology.

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2. Biopolymer Nanoparticles

Initially, biopolymer nanoparticles were devised by combining albumin with non-biodegradable synthetic polymers such as polyacrylamide and poly(methylacrylate). However, the potential chronic toxicity risks arising from the intracellular or tissue accumulation of non-degradable polymers soon emerged as a significant drawback for the systemic administration of polyacrylamide and poly(methylacrylate) nanoparticles in human applications. Consequently, much attention shifted towards designing nanoparticles using synthetic biodegradable polymers like polyalkylcyanoacrylate, poly(lactic-co-glycolic acid), and polyanhydride.

Researchers explored the therapeutic potential of these biodegradable colloidal systems across various applications. While promising results were reported in the literature, these systems also raised concerns about potential toxicological issues. Another challenge encountered in the use of bionanoparticles for delivering hydrophilic molecules such as peptides, proteins, nucleic acids (including oligonucleotides and genes), which hold significant therapeutic potential, was their hydrophobic nature. The polymers comprising these nanoparticles were predominantly hydrophobic, posing difficulties in efficiently encapsulating and protecting these molecules against enzymatic degradation. Consequently, efforts have been made to develop nanoparticles using more hydrophilic and naturally occurring materials.

The demand for developing biodegradable nanoparticles (such as liposomes, virus-like particles (VLPs), and proteins) as effective drug delivery vehicles has been recognized for some time. This recognition stems from the advantages of nanoparticles in general, with biopolymer nanoparticles offering several specific benefits. These include their straightforward preparation from well-understood biodegradable polymers and their high stability in biological fluids and during storage. Nanoparticles crafted from biodegradable polymers like proteins and polysaccharides can serve as efficient drug delivery systems for achieving sustained, controlled, and targeted release, with the goal of enhancing therapeutic effects while minimizing the side effects of the formulated drugs.

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3. Protein Nanoparticles

The initial natural substance employed in the creation of nanoparticles was a combination of two proteins, namely albumin and gelatin. Colloidal systems relying on proteins hold great potential due to their biodegradability, low immunogenicity, and non-toxic nature. They exhibit increased stability both in living organisms (in vivo) and during storage. Moreover, they are relatively straightforward to produce, allowing for easy monitoring of size distribution, and can be readily scaled up in manufacturing. Furthermore, owing to the well-defined primary structure of proteins, protein-based nanoparticles present numerous opportunities for surface modification and the attachment of drugs through covalent bonding.

4. Albumin

Albumin is a remarkable protein found in blood plasma, known for its diverse functions and wide-ranging applications. Notably, albumin possesses biodegradable and biocompatible properties while eliciting minimal immune responses. Its primary role lies within the circulatory system, where it plays a crucial part in the transport, metabolism, and distribution of both foreign and intrinsic molecules. Furthermore, albumin serves as a vital extracellular antioxidant, offering protection against free radicals and harmful chemical agents. These distinctive characteristics have cemented albumin's integral role in drug therapy throughout history.

The literature supports the use of modified serum albumin for selective tumor detection and therapy, as well as a delivery tool for eliminating MYCOBACTERIUM TUBERCULOSIS through receptor-mediated drug delivery. The advent of the nanotechnology era has harnessed albumin's well-established properties, encompassing human serum albumin (HSA) and bovine serum albumin (BSA), for a multitude of purposes. These include employing albumin as carriers for nanoparticle drugs like antibodies, interferon gamma, and antiviral compounds, enhancing the therapeutic effects of anti-cancer drugs, and adapting it as a modified vehicle for drug delivery across the blood-brain barrier and into the central nervous system.

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5. Collagen

Collagen is a fundamental structural component found in vertebrates and stands as the most abundant protein in mammals, constituting approximately 20-30% of the total proteins in the body. It possesses a unique molecular structure, size, and amino acid sequence, which gives rise to the formation of triple-helix fibers. Collagen is highly regarded as a valuable biomaterial due to its outstanding biocompatibility, biodegradability, and widespread availability. Moreover, its capacity for various modifications has opened up numerous applications in the production of nanoparticles.

These modifications include the incorporation of other proteins like elastin, fibronectin, and glycosaminoglycans, resulting in enhanced physicochemical and biological properties. Additionally, the ability to control biodegradability and the subsequent release of ligands is achieved through the use of crosslinking agents such as glutaraldehyde, formaldehyde, ultraviolet, and gamma radiation. The biodegradable collagen-based nanoparticles exhibit excellent thermal stability, are easily sterilized, can be taken up by the reticuloendothelial system, and facilitate improved uptake of drug molecules by cells.

6. Gelatin

Gelatin is a naturally occurring water-soluble macromolecule formed through the process of collagen's heat dissolution and partial hydrolysis. There exist two distinct types of gelatin: Type-A gelatin is derived from collagen through acid treatment and has an isoelectric point (pI) falling within the range of 7.0 to 9.0, while Type-B gelatin is produced by subjecting collagen to alkaline hydrolysis, resulting in a pI between 4.8 and 5.0. Gelatin boasts a range of advantages when compared to synthetic polymers, including its non-irritating nature, biocompatibility, and biodegradability, making it a highly sought-after material for use as a carrier molecule. This natural macromolecule is non-toxic and non-carcinogenic, and it exhibits minimal immunogenicity and antigenicity. Furthermore, gelatin features numerous functional groups on its surface that facilitate chemical crosslinking and derivatization. These benefits have led to its widespread use in the synthesis of nanoparticles for drug delivery over the past three decades.

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7. Silk Proteins-Sericin and Fibroin Nanoparticles

Silk fibers are primarily made of fibroin and sericin where the structural protein, fibroin is enveloped by the gum-like sticky protein, sericin.

7.1 Fibroin

Fibroin, a hydrophobic glycoprotein and one of the fundamental proteins in silk, makes up more than 70% of the cocoon's composition. This protein, which is largely insoluble, is predominantly composed of the amino acids glycine, alanine, and serine (in a repeating pattern of -Gly-Ala-Gly-Ala-Gly-Ser-), leading to the development of antiparallel β-pleated sheets within the fibers. Fibroin exhibits a semi-crystalline structure, consisting of two distinct phases: a highly crystalline β-pleated sheet phase and a non-crystalline phase.

Silk fibroin possesses attributes such as histocompatibility, low immunogenicity, and non-toxicity. It can be processed into various forms, including gels, fibers, membranes, scaffolds, hydrogels, and nanoparticles. Silk fibroin matrices are characterized by high specific surface areas, substantial porosity, excellent biocompatibility, and the ability to biodegrade, making them exceptionally versatile materials with extensive applications in the fields of biomaterials and drug delivery.

Silk has a long history of use as suture material, spanning centuries, and is renowned as a biopolymer that elicits minimal foreign body responses. Furthermore, silk-based biomaterials exhibit high compatibility with various types of cells, facilitating cell growth and proliferation.

7.2 Sericin

Sericins, which are hydrophilic glycoproteins serving as an adhesive, constitute 20-30% of the cocoon. These proteins are soluble in hot water and consist of various polypeptides with weights ranging from 24 to 400 kDa. They have an unusually high serine content (40%) and significant amounts of glycine (16%). Sericin is composed of 35% β-sheet structure and 63% random coil, lacking any α-helical content, resulting in its partially unfolded state.

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Sericin nanoparticles, in addition to the general advantages of protein nanoparticles, offer specific benefits due to their inherent properties. These include antioxidant and antitumor properties, increased bioavailability of essential elements such as Zn, Mg, Fe, and Ca, and the ability to suppress coagulation when sulfated. Sericin is non-toxic to fibroblast cells, and the content of methionine and cysteine in silk sericin is crucial for promoting cell growth and collagen synthesis.

Water-soluble silk sericin does not induce an immune response and is biocompatible, similar to silk fibroin. A study examining the macrophage response to silk protein suggests that soluble sericin typically does not exhibit inflammatory activity. A recent study by Aramwit et al. has concluded that sericin promotes wound healing without causing inflammation. For a chronological overview of milestones in the fabrication and application of silk protein nanoparticles, please refer to Table 1.

Table 1

Milestones of silk protein nanoparticles, preparation, and application in chronological order.

Protein	Method of fabrication	Particle size (nm)	Remarks
Silk fibroin	Precipitation using water miscible protonic and polar aprotonic organic solvents	35–125	Globular insoluble particles well dispersed and stable in aqueous solution
	Precipitation using water miscible protonic and polar aprotonic organic solvents	50–120	Matrix for immobilization of L-asparaginase
	Microemulsion	167	Color dye doped silk fibroin nanoparticles
	Conjugated covalently with insulin using crosslinking reagent	40–120	Insulin—silk fibroin nanoparticles bioconjugates

	glutaraldehyde		
	capillary microdot technique	<100	Sustained and long-term therapeutic delivery of curcumin to breast cancer cells
	Desolvation	150–170	Cellular uptake and control release studies
Silk sericin	Conjugation of sericin with activated PEG	200–400	Overcomes its problem of instability in water and insolubility in organic solvents
	Sericin–PEG self- assembled through hydrophobic interactions	204	Self-assembled nanostructures for immobilization and drug delivery
	Sericin—poly methacrylate core-shell nanoparticles by graft copolymerizing technique	100–150	Potential biomedical application as delivery systems
	Self-assembled silk sericin/poloxamer nanoparticles	100–110	Nanocarriers of hydrophobic and hydrophilic drugs for targeted delivery
	Self-assembled silk sericin nanostructures	–	Fractal self-assembly of silk protein sericin

7.3 Keratin

Keratins are a family of structural proteins rich in cysteine, known for their exceptional mechanical strength due to numerous disulfide bonds. In a recent development, keratin has been harnessed to create ultrathin, transparent coatings via nanosuspensions for investigating

in vitro cell proliferation behavior. This innovative approach suggests that keratin nanosuspension coatings have the potential to serve as a cost-effective substitute for conventional materials like collagen or fibronectin in standard cultivation practices. Furthermore, with further exploration, keratin nanosuspensions could potentially find valuable applications in the field of tissue engineering.

7.4 Polysaccharide Nanoparticles

Nanoparticles derived from natural polysaccharides have been engineered to enhance the biocompatibility of materials that are potentially harmful to cells. This advancement is complemented by the ongoing development of innovative immobilization techniques for creating novel pharmaceutical formulations based on bionanoparticles. These polysaccharide-based nanoparticles have been specifically designed for the delivery of peptides, proteins, and nucleic acids.

7.5 Alginate

Alginate is a naturally occurring, water-soluble, unbranched linear polysaccharide derived from brown seaweed. It consists of two types of uronic acids, namely α-L-guluronic acid and β-D-mannuronic acid. These monomeric units are organized in three primary configurations: blocks of alternating guluronic and mannuronic residues, blocks solely composed of guluronic acids, and those consisting solely of mannuronic acids. Alginate is valued for its mucoadhesive properties, biocompatibility, and non-immunogenic characteristics, and it naturally dissolves and biodegrades under standard physiological conditions. The solubility of alginate in water is influenced by the presence of associated cations. Sodium alginate readily dissolves in water, while the presence of calcium induces gel formation. In addition to its interaction with calcium, alginate can also create complexes with polycations such as polyenimine (PEI), chitosan, or basic peptides like polylysine and polyarginine. The carboxylic groups stemming from the uronic acid provide alginate with negative charges. Chitosan imparts positive surface charge to nanoparticles, prolongs the contact time of active ingredients with epithelial surfaces, and enhances absorption via the paracellular transport pathway through tight junctions. Alginate micro and nanoparticles can be easily produced by initiating gelation with calcium ions. This property is harnessed for generating a pre-gel

containing very small aggregates of gel particles. Subsequently, an aqueous polycationic solution is added to form a polyelectrolyte complex coating. Poly-L-lysine (PLL), a cationic natural polymer, has been historically used in conjunction with alginates to prepare nanoparticles. However, it is worth noting that PLL can be toxic and provoke immunogenic responses when injected. More recently, chitosan (CS) has been identified as an alternative cationic polymer for this purpose. The chronological progression of milestones in the preparation and applications of alginate and its composite nanoparticles is detailed in Table 2.

Table 2

Milestones of alginate and its composite nanoparticles preparation and applications in chronological order.

Polymer	Method of fabrication	Particle size (nm)	Remarks
Alginate	Control of the gel formation of alginate by calcium ions	250–850	Evaluation for the drug- loading capacity with doxorubicin as a model drug
Alginate	Gelation in presence of calcium ions and further crosslinking with poly-L-lysine	–	Nanosponges are new antisense oligonucleotide carrier system for specific delivery to lungs, liver and spleen
Sodium alginate and BSA	Emulsion solidification method	166±34	Determination of the kinetic parameters of 5-FU sodium alginate-^{125}I BSA nanoparticles metabolism
Calcium alginate	Water-in-oil microemulsion followed by calcium crosslinking of glucoronic acid units	80	Examination of the nanoparticles for their potency as carriers for gene delivery

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Alginate and chitosan	Ionotropic pre-gelation of alginate with calcium chloride followed by complexation between alginate and chitosan	764–2209	Monitor the complexation of contrary charged polyelectrolytes as insulin nanoparticulate carriers
Alginate and chitosan	Induction of a pre-gel with calcium counters ions, followed by polyelectrolyte complex coating with chitosan	850±88	Development of an oral insulin delivery system having mild formulation conditions, high insulin entrapment efficiency for gastrointestinal release
Alginate and chitosan	Induction of a pre-gel with calcium counters ions, followed by polyelectrolyte complex coating with chitosan	750	*In vivo* evaluation of the pharmacological activity of the insulin loaded nanoparticles
Alginate	Gelation in presence of calcium ions and further crosslinking with Eudragit E-100	200–950	*In vitro* release study revealed sustained release of gliclazide from gliclazide loaded alginate nanoparticles
Alginate	Modified coacervation or ionotropic gelation method	205–572	Optimization of mucoadhesive nanoparticulate carrier systems for prolonged ocular delivery of the drug

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7.6 Chitosan

Chitosan ranks as the second most abundant naturally occurring polysaccharide. It is composed of a combination of β-(1-4)-linked D-glucosamine units (deacetylated) and N-acetyl-D-glucosamine units (acetylated). Chitosan is derived from the deacetylation process of chitin, which is obtained from the shells of crabs, shrimps, and krill. Commercially available chitosan typically undergoes deacetylation to varying degrees, ranging from 66% to 95%, and possesses an average molecular weight within the range of 3.8 to 2000 kDa. This biopolymer is characterized by its linear structure, hydrophilic nature, positive charge, and remarkable mucoadhesive properties. Its exceptional biocompatibility and biodegradability make it an ideal choice for the creation of microparticles and nanoparticles.

In vivo, chitosan undergoes degradation through the action of lysozyme. Additionally, the amino groups in the molecule provide it with a high charge density, making them readily available for various chemical reactions and the formation of salt complexes with acids. Chitosan exhibits solubility in a variety of acids and can also interact with polyions to produce complexes and gels. These unique properties are harnessed in the production of nanoparticles, either through the spontaneous formation of complexes between chitosan and polyions, such as DNA, or through the gelation of a chitosan solution dispersed in a water-in-oil emulsion. The chronological milestones in the fabrication and applications of chitosan and its composite nanoparticles are detailed in Table 3.

Table 3

Milestones of chitosan and its composite nanoparticle fabrication and applications in chronological order.

Protein	Method of fabrication	Particle size (nm)	Remarks
Chitosan	Complex coacervation technique	100–250	Encapsulate nucleic acids like plasmid DNA for gene delivery for efficient gene transfection

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Chitosan	Ionotropic gelation	213±3	Encapsulate anticancer agents like cationic anthracycline drug doxorubicin
Chitosan	Ionotropic gelation of Chitosan with TPP anions	200–1000	Encapsulate proteins such as bovine serum albumin, tetanus and diphtheria toxoid
Chitosan	AOT/n-hexane reverse micellar system	30–110	In bone imaging and targeting purpose
Low molecular weight chitosan	Ionotropic gelation of CS with TPP anions	350	Encapsulate vaccines
Chitosan	Complex coacervation technique	20–500	Encapsulate nucleic acids (DNA) and to improve the transfection efficiency *in vivo* and *in vitro*
Chitosan	Ionotropic gelation	145–172	Encapsulate insulin
Chitosan	Ionic gelation of chitosan with tripolyphosphate anions (TPP)	180–260	Novel delivery system for ammonium glycyrrhizinate
Chitosan–alginate	Water-in-oil reverse microemulsion	<100	Encapsulate plasmid DNA for gene delivery for efficient gene transfection
Chitosan	Ionic crosslinking of chitosan solution with TPP	100–200	Deliver cholinesterase inhibitor through the nasal mucosa to reach the brain for the treatment of

			neurodegenerative disease
Lauryl succinyl chitosan	Ionic crosslinking of chitosan solution with TPP	315–1090	Release of human insulin as the model protein drug and release kinetics in GI pH

8. Characterization of Nanoparticles

Nanoparticles are typically assessed based on their dimensions, shape, and surface charge using advanced microscopic methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). The average particle size, the distribution of sizes, and their electric charge all play a pivotal role in determining the physical stability and distribution of these nanoparticles within living organisms. Electron microscopy techniques are particularly valuable in revealing the overall structure of polymeric nanoparticles, which can impact their potential toxicity. Moreover, the surface charge of nanoparticles not only influences the physical stability and re-dispersion of polymer dispersions but also plays a critical role in their performance within living organisms.

9. Particle size

Nanoparticles find significant applications in drug delivery and drug targeting. The size of these particles plays a crucial role in drug release behavior. Smaller nanoparticles possess a larger surface area, resulting in more of the loaded drug being exposed to the particle surface, leading to faster drug release. In contrast, larger particles hinder drug diffusion, causing slower release. However, a drawback is that smaller particles tend to aggregate during the storage and transportation of nanoparticle dispersions. Therefore, a balance must be struck between achieving a small size and maximizing nanoparticle stability.

Nanoparticles also play a pivotal role in biosensors by enabling the immobilization of enzymes. Reducing the size of carrier materials generally enhances the efficiency of immobilized enzymes because smaller particles offer a larger surface area for enzyme attachment, reducing resistance to substrate diffusion. Moreover, physical characteristics of

nanoparticles, such as improved diffusion and particle mobility, can impact the catalytic activity of attached enzymes. Particle size can also affect polymer degradation. For example, in vitro studies have shown that the degradation rate of poly(lactic-co-glycolic acid) increases with increasing particle size.

Various tools are available for determining the size of nanoparticles, as discussed below.

9.1 Dynamic Light Scattering

The most widely adopted and swiftest approach for assessing particle size is Dynamic Light Scattering (DLS), also known as Photon-Correlation Spectroscopy (PCS). DLS is extensively utilized for determining the dimensions of nanoparticles in colloidal suspensions within the nano and submicron size ranges. In this technique, a monochromatic light source, typically a laser, is directed onto a solution containing spherical particles undergoing Brownian motion. As these particles move, they create a Doppler shift in the incident light, leading to a change in its wavelength. This wavelength alteration is directly correlated with the size of the particle. DLS enables the extraction of the size distribution of particles and provides insight into their motion within the medium by measuring the particle's diffusion coefficient using the autocorrelation function. This method offers several advantages: it is a rapid process, almost entirely automated, and requires minimal expertise. Additionally, it is cost-effective in terms of equipment and setup.

One notable advantage of employing dynamic scattering is its capacity to analyze samples comprising diverse species with varying molecular masses. This includes scenarios where one might need to detect and characterize minute quantities of larger mass species, often in concentrations as low as 0.01%. PCS, on the other hand, primarily provides information on the average particle size and the polydispersity index (PI), which signifies the range of particle sizes within the measured sample. Observations using PCS reveal that, at a given temperature, larger particles exhibit slower motion compared to their smaller counterparts.

10. Nanoparticle Tracking Analysis

Nanoparticle Tracking Analysis (NTA) is a modification of photon-correlation spectroscopy, innovatively developed by NanoSight Ltd. This technique is employed to assess the size distribution profile of minute particles within a liquid suspension. NTA operates in tandem

with an ultramicroscope, enabling the visualization of small particles as they undergo Brownian motion in the liquid. Advanced computer software is then utilized to meticulously track the movements of these particles, subsequently enabling the estimation of their hydrodynamic radius by applying the Stokes-Einstein equation. Importantly, NTA offers the advantage of minimal sample preparation, significantly reducing the time required to process each sample.

11. Particle Morphology

The physical and chemical properties of nanoparticles are significantly impacted by their size and morphology, which, in turn, affect their interactions with the environment and biological systems. Various techniques are available for analyzing the morphology of nanoparticles. Microscopic methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) are commonly employed. In addition to particle size and distribution analysis, these techniques can provide valuable information regarding other parameters like the shape and surface roughness of the nanoparticles.

12. Scanning Electron Microscope

To perform SEM characterization of nanoparticles, the initial step involves transforming the nanoparticle solution into a dry powder form. This dry powder is subsequently affixed to a sample holder and coated with a conductive metal, typically gold, through a sputter coater. Following this, a focused, fine electron beam is directed onto the sample, and the surface properties are assessed by detecting the secondary electrons emitted from the sample surface. It's crucial that the nanoparticles can endure the vacuum conditions, as exposure to the electron beam may potentially harm the polymer material. The average particle size determined via SEM is consistent with results obtained through dynamic light scattering.

12.1 Transmission Electron Microscope

TEM operates on different principle than SEM, yet it often brings same type of data. The sample preparation for TEM is complex and time consuming because of its requirement to be ultra thin for the electron transmittance. The nanoparticles dispersion is deposited onto support grids or films. To make nanoparticles withstand the instrument vacuum and facilitate

handling, they are fixed using either a negative staining material, such as phosphotungstic acid or derivatives, uranyl acetate, etc, or by plastic embedding. Alternate method is to expose the sample to liquid nitrogen temperatures after embedding in vitreous ice. The surface characteristics of the sample are obtained when a beam of electrons is transmitted through an ultra thin sample, interacting with the sample as it passes through.

12.2 Atomic force microscopy

Atomic Force Microscopy (AFM) stands as a versatile tool for the high-resolution characterization of a wide array of surfaces, including nanoparticles, at the atomic level. It ranks among the foremost types of scanning probe microscopes. A key strength of AFM lies in its capacity to image non-conductive specimens without necessitating any specialized treatment. This exceptional feature enables the examination of delicate nano and microstructures in the realms of biology and polymers. Furthermore, AFM demands minimal sample preparation and is amenable to operation under standard ambient conditions. The core methodology entails the use of a sharp probe, which is carefully scanned across the sample's surface. The tip-sample interactions are meticulously monitored and compiled to produce detailed images of the sample's surface.

13. Particle Stability

Colloidal stability is assessed by analyzing the zeta potential of nanoparticles, which serves as an indirect indicator of their surface charge. Zeta potential represents the electric potential difference between the outer Helmholtz plane and the shear plane at the particle's surface. The measurement of zeta potential is typically carried out using Laser Doppler Anemometry. This technique relies on assessing the particle's velocity through the shift in the interference fringe created by the intersection of two laser beams. The electrophoretic mobility is then converted into zeta potential values.

In most cases, colloidal particles exhibit negative zeta potential values within the range of approximately -100 to -5 mV. These negative surface charges play a crucial role in preventing the agglomeration of nanoparticles in polymer dispersions. They create strong electrostatic repulsion forces that enhance the stability of the nanoparticles.

Additionally, zeta potential can offer valuable insights into the composition of materials encapsulated within nanocapsules or those coated onto nanoparticle surfaces.

14. Particle structure

Analyzing structural changes in both the free protein sample and protein nanoparticles is crucial for gaining insights into the alterations occurring in the protein with regard to its conformation, folding, chemical bonding, and other characteristics during the nanoparticle synthesis process.

15. X-Ray Diffraction

X-ray diffraction (XRD) is a fundamental technique used to study the structural properties of crystalline materials. It provides valuable insights into various aspects of a material, including its atomic arrangement, crystallite size, and imperfections. XRD is also employed to analyze phase composition, crystallite dimensions and shapes, lattice distortions, composition variations, orientation, and the in situ development of nanoparticle structures.

Typically, the XRD pattern is generated by exposing the sample to X-rays, typically using a Copper Kα line source with a wavelength of 1.54 Å, and then scanning the diffraction pattern across a specific range of angles (2θ).

16. Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a valuable technique that complements X-ray Diffraction (XRD) in the structural analysis of proteins and other materials. What sets FTIR apart from crystallographic techniques is its ability to provide high-resolution structural information for proteins in solution, both spatially and temporally. Typically, minute quantities of lyophilized nanoparticles are used as samples for characterization.

The fundamental principle underlying FTIR spectroscopy is based on the vibrations of chemical bonds and bond groups, which occur at characteristic frequencies. When a molecule is exposed to infrared radiation, it absorbs energy at frequencies that are unique to that molecule. In FTIR analysis, a modulated infrared beam is directed onto the sample. The transmittance and reflectance of the sample at different frequencies are recorded, generating an IR absorption spectrum. This spectrum is then analyzed and compared to the known

spectral signatures stored in an FTIR library for the identification of the sample's constituents.

17. Cellular Uptake and Cytotoxicity

In addition to characterizing nanoparticles through various methods, it is crucial to assess their interaction with cells and their potential cytotoxicity, both in controlled laboratory settings (in vitro) and within living organisms (in vivo). Evaluating the cellular uptake of nanoparticles typically involves tagging the nanoparticles with fluorescent markers such as fluorescein isothiocyanate (FITC). Subsequently, these fluorescently labeled nanoparticles are incubated with cultured cells, and their internalization is visualized using a confocal laser scanning microscope.

To analyze cytotoxicity, researchers often perform an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. This assay is a commonly employed method for assessing the toxicity of biomaterials based on their impact on mitochondrial activity. The fundamental principle underlying this assay is the conversion of MTT from yellow to purple formazan in viable cells. Following this conversion, a solubilization solution (typically composed of either dimethyl sulfoxide, an acidified ethanol solution, or a solution containing the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product, forming a colored solution. The absorbance of this colored solution is then quantified at a specific wavelength (depending on the solvent used) using a spectrophotometer. The reduction of MTT to formazan only occurs when reductase enzymes are active, making this conversion a measure of viable cells.

18. Drug Loading and Drug Release

In addition to the aforementioned characterization, the evaluation of bionanoparticles can include an assessment of their drug loading and drug release properties. Drug loading in nanoparticles is typically described as the quantity of drug bound per unit mass of polymer, often expressed as moles of drug per milligram of polymer or milligrams of drug per milligram of polymer. Alternatively, it may be expressed as a percentage relative to the polymer mass. Analytical methods commonly used for this purpose include classical techniques such as UV spectroscopy or high-performance liquid chromatography (HPLC)

following procedures such as ultracentrifugation, ultrafiltration, gel filtration, or centrifugal ultrafiltration.

Encapsulation efficiency refers to the ratio of the amount of drug encapsulated or absorbed within the nanoparticles to the total (theoretical) amount of drug used in the context of the final drug delivery system involving nanoparticle dispersion. Quantification of encapsulation efficiency is typically accomplished using UV spectroscopy or HPLC.

Drug release assays share similarities with drug loading assays and involve assessing the release of the drug over a specified period to analyze the mechanism of drug release.

19. Conclusions

1. Nanoparticles can be synthesized from a diverse range of materials, including synthetic polymers, proteins, and polysaccharides. Biopolymeric nanomaterials offer several advantages over synthetic polymers due to their biodegradability, biocompatibility, and non-toxic nature.
2. Biopolymeric nanoparticles, composed of proteins and polysaccharides, are produced using four distinct methods: emulsification, desolvation, coacervation, and electrospray drying.
3. Characterization of biopolymeric nanoparticles typically involves assessing their size and morphology using techniques such as Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Atomic Force Microscopy (AFM). Additionally, their stability is measured through DLS and Zeta analyzer, while their structure is determined using X-ray Diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy. Evaluations for cytotoxicity and biocompatibility are also essential.
4. Biopolymeric nanoparticles have extensive applications as carriers for drug and vaccine delivery. These nanoparticles can encapsulate bioactive molecules, allowing them to target specific locations, such as tumors, for precise and effective delivery.

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19.1 Future Prospects:

1. There are several challenges that need to be addressed for the further advancement of biopolymer-based nanoparticles:
2. Enhancing the preparation methods for protein and polysaccharide-based nanostructures to achieve uniform dispersion and stability.
3. Continuing the development of properties and applications of protein and polysaccharide-based nanoparticles for drug delivery and therapeutic purposes.
4. Thoroughly evaluating the cytotoxicity, biodegradability, biocompatibility, and immune response of these biopolymeric nanoparticles to assess their potential for clinical drug delivery applications.
5. Scaling up the production of biopolymeric nanoparticles from the laboratory to industrial levels will require collaboration between biologists and chemical engineers.
6. Combining expertise in bioengineering, chemical modification, and nanomaterial sciences can enhance the pharmacokinetics and biodistribution of drug delivery systems designed using nano-particulate structures.

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