

A Review on Systematic Drug Utilizing Protein based Nanoparticles

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Abstract: *A chemical and bimolecular medication, including anti-cancer medication and therapeutic protein have been widely delivered by nanoparticles. Because of their safety, natural Biomolecules like protein are a desirable substitute for synthetic polymers, which are frequently utilizes in the creation of nanoparticles. Protein containing nanoparticles are particularly noteworthy due to their ability to target specific site. Nanoparticles and other particulate system have been employed as a physical method to modify and enhance the pharmacokinetics and pharmacodynamic characteristics of many kinds of medicinal molecules They have been employed in vivo to protect the drug entity in the systemic circulation, limit the drug access to certain areas , and transport the drug to the site of action steady and regulated rate. The creation of nanoparticles utilizing protein such as albumin, gelatin, gliadin and legumin is the subject of ongoing research nowadays. Protein nanoparticles have potential as oral and parenteral medication delivery vehicles. In recent years, it has become apparent that cancer nanomedicines reliance on synthetic nanoparticles as drug delivery systems has resulted in limited clinical outcomes. This is mostly due to a poor understanding of their “bio–nano” interactions. PNPs must be precisely constructed and designed in order to realize their full therapeutic potential. We describe the latest developments and uses of PNPs in cancer nanomedicine in this review.*

Keywords: Protein nanoparticles, biomolecules , medication, Drug delivery, nanomedicine

I. INTRODUCTION

The study of particles with sizes between 10⁻⁹ and 10⁻⁷ meters is known as nanotechnology. It is also the study of materials and technology whose composition and structure, because of their nanoscale size, show novel and drastically changed physical, chemical, and biological phenomena. Therefore, the manipulation of matter at the atomic level and the use of such materials by changing their physio-chemical properties and design can be characterized as nanotechnology. Nanoparticles carry the drug to a particular site or organ that aids in limiting the adverse effects and minimizes the dosage and dosage frequency of the therapeutic substance. Site-specific pharmacological effect at a therapeutically suitable pace and dosage is facilitated by the application of nanotechnology.[1] Furthermore, by shrinking the structural size from micrometers to nanometers, new protein vehicles with better delivery qualities can be created. It is commonly known that maintaining or boosting the bioavailability of bioactive substances (such as vitamins, nutraceuticals, and anti-inflammatories) in functional food items meant to support health and wellbeing is one of the food industry's concerns. However, only a small percentage of bioactive molecules remain available for oral administration due to harsh environmental conditions (such as gastric fluids, which have low pH, high concentrations of salts, and ionic strength) and their frequently low solubility during gastrointestinal digestion. This limits the activity and potential health benefits of bioactive compounds.[2] Nanoparticle research, technology, and production have all dramatically increased in recent years. These nanoscaled particles present chances for the creation of novel applications since their physio- chemical characteristics differ from those of bulk material. Numerous exposure scenarios come from the fact that some of these manufactured nanoparticles are currently in use in a wide range of industries, such as construction materials, food, clothing, personal care goods, information technology, and medicine. Determining the possible risks of nanoparticles to human health has therefore become crucial. In addition to eating, skin deposition, and injection for medicinal purposes, nanoparticles can enter the human body by inhalation. After entering the body by



inhalation, nanoparticles have the ability to go into the systemic circulation, reach a number of distant organs, and alter their function.[3]

Significant efforts are being undertaken to alter how drug formulations interact with various proteins, particularly immune system proteins, in order to maximize the effectiveness of nanomedicines. Drug-containing NPs have also been thought to be directed to the target tissue or tissues, such as tumor locations, by a variety of peptide sequences and proteins, such as cell- penetrating peptides, phage peptides, and antibodies [4]The use of nanoparticles as delivery vehicles for medications and bioactive chemicals is gaining traction Nanoparticles of proteins are comparatively simple to make, and because of their small size, they can enter tissues through capillaries or be absorbed by cells.[5]In instance, shielding the medication from renal clearance and enzymatic breakdown can increase its stability, activity, and half-life. Because protein nanoparticles are non-antigenic they can also be employed in a range of targeted therapeutics, including lung delivery , cancer therapy , tumor therapy , and vaccinations.[6]

For regulated and prolonged release, protein nanoparticles can be integrated with biodegradable polymers in the form of microsphere. As an alternative to chemically synthesized nanoparticles, biological nanoparticles made from biomolecules have garnered a lot of attention in recent years. This is because, in addition to the other benefits provided, such as non- immunogenicity and ease of availability, biocompatible and biodegradable nanoparticles must be developed.[7]Atoms and molecules function differently at this size and have a wide range of intriguing and unexpected applications. Studies in nanotechnology and nanoscience have grown quickly in recent years across a wide range of product categories. In areas where traditional methods can be at their limits, it offers chances for the development of materials, particularly those for medical uses. It is incorrect to think of nanotechnology as a singular method that only has an impact in particular domains. Despite being frequently referred to as the "tiny science," nanotechnology encompasses more than just extremely small objects and structures. Large surfaces and bulk materials frequently incorporate nanoscale characteristics. In order to create novel nanosized materials, nanotechnology is the design, manufacture, and use of materials at the atomic, molecular, and macromolecular scales.[8]

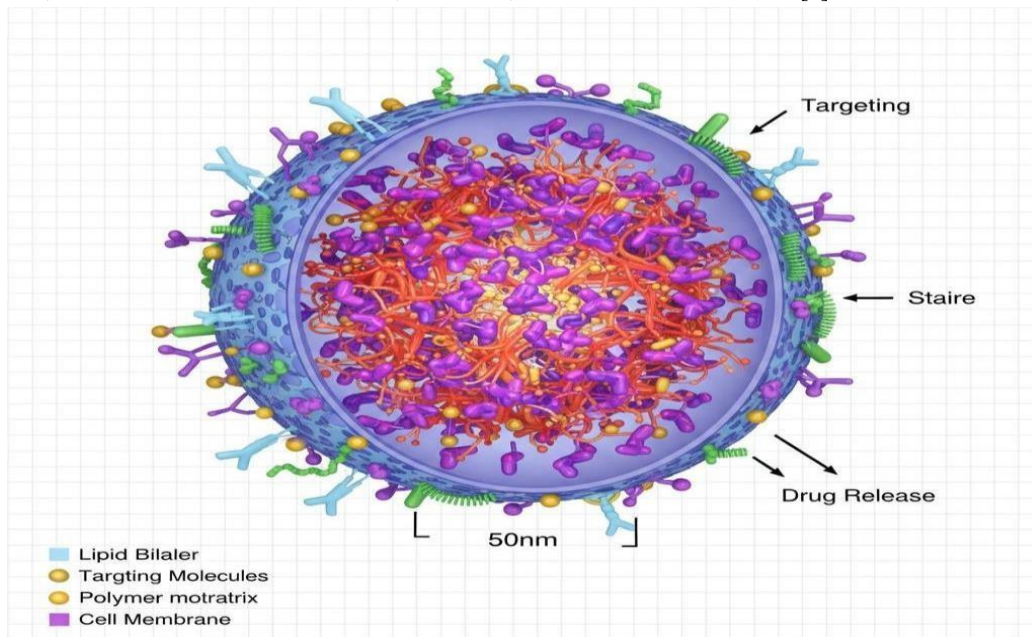


Fig 1: Nanoparticle for Drug Delivery System



Literature review:

□ Pathak et al,(2022). A review paper on “Nanoparticles for Drug Delivery System” The drug delivery nanoparticles, including polymeric, lipid-based, metallic, and protein nanoparticles, and emphasize their benefits, which include decreased toxicity, improved drug absorption, prolonged release, and the capacity to target particular tissues or cells. According to the article's conclusion, drug delivery methods based on nanoparticles have a great deal of promise for enhancing treatment results, particularly for chronic illnesses including cancer, infections, and inflammatory disorders.

□ Alok Mahor et al. (2014).“The Future of Drug Delivery Systems”. Albumin, gelatin, casein, silk fibroin, and legumin are recognized for their biodegradability, biocompatibility, non- toxicity, and ability to bind a wide range of drugs. According to the paper, protein nanoparticles can provide controlled and sustained medicine release, improve drug stability and solubility, and enable targeted administration to specified locations. Several preparation methods are reviewed, including desolvation, emulsification, coacervation, and nanoprecipitation, as well as factors that influence nanoparticle properties such as particle size, surface charge, and cross-linking.

□ Febrina Sandra, Anwar Sunna et al.(2019). “Developing Protein-based nanoparticles as Versatile Delivery Systems for Cancer Therapy and Imaging, Nanomaterials”.

The various proteins, including albumin, ferritin, elastin-like polypeptides, and virus-like particles, can be turned into nanoparticles for targeted medication delivery. These nanoparticles can encapsulate chemotherapeutic medications, increasing their solubility, stability, and tumor-targeting effectiveness while reducing side effects. The article also discusses their applications in diagnostic imaging, where PNPs can be modified with contrast agents or fluorescent markers to improve tumor detection.

□ Pratik M.Kedar,D.V.Derle et al.(2022) "Protein-Based Nanostructures: A Unique System as Drug Delivery Vehicles".

protein-based nanocarriers are biodegradable, biocompatible, and non-toxic, making them ideal for medical use. The numerous forms of protein nanostructures (such as albumin, gelatin, casein, and silk proteins) and how they can encapsulate medications, preserve them from degradation, and distribute them under controlled conditions.

□ Dr.M.Kannadasan et al,(2020) "Nanoparticle Drug Delivery Systems" nanoparticles enhance medicine solubility, stability, bioavailability, and targeted delivery while minimizing negative effects.

The paper examines many nanoparticle kinds, including polymeric nanoparticles, liposomes, dendrimers, metallic nanoparticles, and protein-based carriers. It focuses on popular preparation methods such as solvent evaporation, nanoprecipitation, emulsification, and ionic gelation. advantages, including controlled release, site-specific activity, and improved pharmacokinetics. Overall, the article highlights nanoparticle drug delivery systems as a promising platform for improved therapies, particularly in cancer and chronic disorders.

□ Dr. Pankaj M. Chaudhari and Mr. Vivek P. Rajkule et al .(2023). “A review on Nanoparticles -A Drug Delivery Systems”.

The many types of nanoparticles (polymeric, lipid-based, metallic, and protein nanoparticles) and their role in regulated and sustained medication release. It also emphasizes benefits such as fewer side effects, higher therapeutic efficacy, and the ability to deliver medications to targeted regions or cells. The review suggests that nanoparticles are attractive platforms for future medicine due to their versatility and precision in medication delivery.

□ Seyoung Hong and Dong Wook Choi et al.(2020). “Protein-Based Nanoparticles as Drug Delivery System”.

It Protein nanoparticles can efficiently load hydrophilic and hydrophobic medicines by utilizing binding sites, electrostatic interactions, and structural flexibility. The review covers essential preparation techniques such desolvation, emulsification, salting-out, thermal gelation, self- assembly, and nano-complexation. It also discusses how PNPs improve drug stability, solubility, controlled release, and targeted administration, making them beneficial in cancer therapy, imaging, vaccinatedevelopment, and gene delivery.



Advantages nanoparticles in drug Delivery system:

- Nanoparticles may be preserved for a long time and are non-toxic and biodegradable.
- Targeting ligands can be incorporated onto the surface of nanoparticles or magnetic guiding can be used to accomplish site-specific targeting for reducing medication accumulation in healthy tissues.[1]
- One of the most significant proteins in blood plasma, albumin serves a variety of vital physiological functions. Because the body contains large amounts of albumin, injecting large amounts of it into the body is either safe or has minimal negative consequences. [12]
- It is simple to modify the size and surface properties of nanoparticles to accomplish both passive and active medication targeting following parenteral injection.
- To boost the drug's therapeutic efficacy and decrease its side effects, they regulate and maintain the drug's release during transportation and at the site of localization, changing the drug's organ distribution and subsequent clearance.
- The selection of matrix constituents allows for easy modulation of controlled release and particle degradation characteristics. One crucial element in maintaining drug activity is the relatively high drug loading and the ability to absorb pharmaceuticals into systems without causing any chemical reactions.[3]

Disadvantages of nanoparticle drug delivery system:

- The majority of proteins, which are naturally occurring polymers, are heterogeneous mixes with varying molecular weights and sizes.
- Immunogenicity: varying degrees of immunogenicity are among the limitations of protein nanoparticles, as the human body has demonstrated an immunological response to foreign proteins. However, minimal immunological reaction was noted when albumin, gelatin, casein, and Zain nanoparticles were injected intravenously.
- Adding to the problems, nanotechnology is a very costly and time-consuming process that requires highly qualified engineers and staff. [12]
- The biotransformation of polymers results in the production of toxic metabolites with repeated dosage.
- Although polymeric nanoparticles disintegrate very slowly, they may cause systemic toxicity; dental implants may cause a hypersensitive reaction.[1]
- The development of appropriate and non-toxic linkers is one of the current study focuses in the field of protein nanoparticles because these interface molecules are frequently harmful. Moreover, hydrophobic plant proteins have demonstrated encouraging outcomes in the synthesis of protein nanocarriers with sustained release capabilities .

Role of Protein Based nanoparticles in immunotherapy and drug delivery system: Numerous critical bodily processes, including homeostasis, defense mechanisms, tissue healing, and the removal of dead cells, depend on the human immune system. Cells that are constantly moving throughout the body and screening every single cell make up our immune system. These cells look for malignant or dysfunctional cells as well as invasive infections. When defective cells are identified, they are promptly removed. Immune cells are categorized as either innate or adaptive, the two arms of the immune system. When a pathogen invades, innate immune cells are certain to react right away. [9] They have receptors that use molecular patterns to identify the pathogen. Following their phagocytosis of the pathogens, these cells generate substances that enable a prompt response to the pathogens. cancer remains a challenging disease to treat, making it one of the world's leading causes of death. It is the unchecked growth of abnormal cells. In addition to malignancies, classic cancer remedies like radiation, surgery, and chemotherapy are no longer effective due to poorly targeted medication accumulation, serious side effects, and drug resistance, which causes cancer degeneration and subsequent treatment failure. One of the best methods for treating cancer is immunotherapy. Utilizing a patient's immune system to fight cancer is known as immunotherapy. Immunotherapy can be categorized as either activation or suppression immunotherapy depending on whether it triggers or inhibits the immune response.



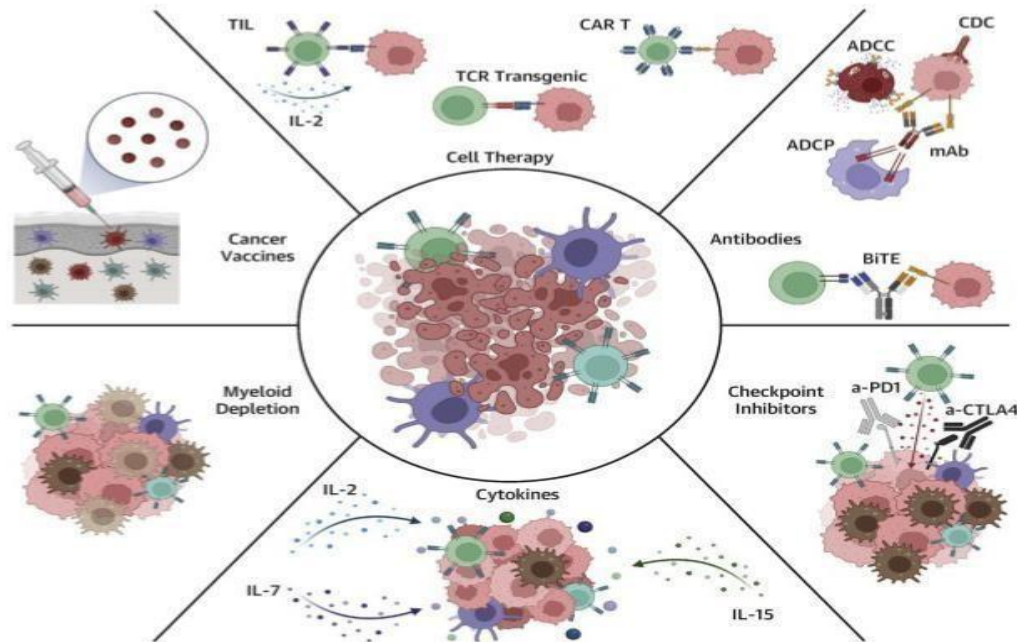


Fig 2 : Role of immunotherapy

Immunotherapy is a new cancer treatment that employs adoptive or monoclonal cell therapy. Although there are numerous forms of tumor immunotherapy, the majority of them primarily use T cells to achieve their anti-tumor effects. One type of immunosuppressive compounds is the immunological checkpoint. One of the best ways to increase T cell activation is to block immunological checkpoints. In recent years, it has also been a popular target for the development of anti-tumor drugs. The codelivery of an immune checkpoint inhibitor and photosensitizer was made possible by this effect. The in vivo findings demonstrated that medicines are released more quickly in acidic environments following in-situ injection of composite nanoparticles. Tumor growth was suppressed and T cell activation was elevated by the resulting ROS and tumor-associated antigens. This study increased the effectiveness of cancer treatment by combining PDT and immunotherapy.[10]

Types of protein based Nanoparticles:

1. Silk protein fibroin
2. Human serum albumin
3. Gliadin
4. Gelatin
5. Legumin
6. 30c19 protein derived from silkworm hymph
7. Lipoprotein
8. Ferritins

1. Silk Protein fibroin:

Heavy and light chains make up the semi-crystalline structure of fibroin. The heavy chain, which is composed of 12 large hydrophobic domains connected by 11 hydrophobic hydrophilic portions, is composed of 45% Gly, 30% Ala, and 12% Ser. While the hydrophilic section can be any amino acid sequence, each hydrophobic domain has a repeating sequence of Gly-Ala-Gly- Ala-Gly-Ser and many repeating sequences of Gly-X (X = Ala, Ser, Thr, Tyr, or Val).



Through van der Waals forces and intermolecular hydrogen bonds, primarily between Gly and Ala, the heavy chain creates stable antiparallel crystalline β -sheets. Silk fibroin has strong mechanical qualities and a high tensile strength because of this structure. A variety of amino acid compositions, including 15% Asp, 14% Ala, 11% Gly, 11% Ser, and trace amounts of cysteine, make up the light chain. In the end, the light chain contributes to fibroin flexibility by being less water-resistant and more hydrophilic. According to reports, silk fibroin has a molecular weight (MW) of 83 kDa and an isoelectric point (IEP) of pH 7 or lower; however, the size may differ based on the extraction procedure and treatment duration used.[11]

2. Human serum albumin:

The amount of crosslinking agent was found to be small (about 3 to 5 mg) at a protein concentration of 50 to 60 mg/mL. The creation of a solid yellow bulk and the size of the produced particles were deemed to be significant with the addition of salt to the albumin solution. It was established that sodium chloride and phosphate buffer were ineffective in creating albumin nanoparticles with a small particle size. It's crucial to remember that in this investigation, the crosslinking agent EDC was utilized instead of glutaraldehyde (GA). Furthermore, Langer et al. used the desolvation process to make HSA nanoparticles and verified that the particle size matched the production conditions [12] When examined at a low plasmid concentration level, nanoparticle-mediated transfection showed minimal cytotoxicity and no discernible difference in efficiency; however, employing a high plasmid concentration level led to an increase in transfection efficiency of up to 50%. HSA nanoparticles can also be employed as non-DNA carriers because they are hardly harmful. Redin et al., for instance, created HSA nanoparticles that contained the chemical medication bevacizumab, which is used to treat certain eye conditions and tumors.[6]

3. Gliadin:

Proteins make up the majority of the complex that is wheat gluten, which also contains carbohydrates. Among these proteins are gliadin and glutenin. 70% alcohol is used to isolate and detect these proteins. Glutenin has a molecular weight of 106 kDa and is an alcohol-insoluble protein. 70% of gluten is made up of gliadin, a group of proteins with a molecular weight between 25 and 100 kDa that are isolated from alcohol. Approximately 40% of these proteins' structure is made up of the amino acid glutamine. Due to the presence of hydrophobic amino acids and glutamine in its structure, gliadin can interact with the cell membrane through hydrophobic contacts while also forming many hydrogen bonds with the mucous layer of the mucosa. Because of this, gliadin nanoparticles have demonstrated promising results in the creation of oral formulations, particularly for the management of stomach disorders such as gastric ulcers.[13]

4. Gelatin:

Gelatin is a denatured protein that is obtained from the acidic hydrolysis of animal collagen. The food, cosmetics, and pharmaceutical sectors have long made use of this biomolecule. Because gelatin is denatured, it boosts the immune system. As a poly-ampholyte, gelatin contains a 1:1:1 ratio of hydrophobic and cationic active groups. As a result, the gelatin molecule contains 11% hydrophobic amino acids (leucine, isoleucine, methionine, and valine), 12% negative charge (glutamic and aspartic amino acids), and 13% positive charge (lysine and arginine amino acids). One of the key benefits of gelatin over polymers devoid of cell recognition and binding sites is this characteristic. This property is crucial, particularly when creating tailored drug delivery carriers and the potential to attach sizable amounts of drug to carriers. Gelatin's active groups enable a range of chemical alterations to be made to it directly or through the use of various linkers. A wide range of hydrophilic and hydrophobic medications, such as analgesics, numerous anti-cancer, anti-AIDS, anti-malarial, muscle relaxants, anti-inflammatory, and therapeutic medications, have been delivered by gelatin nanoparticles. Diabetes can be treated with topical eye medications, protein synthesis inhibitors, tissue plasminogen activators, gene delivery, and protein drug delivery.[13]



5. Legumin:

Legumin, a member of the 11S globulin protein family, is one of the main storage proteins found in soybean seeds (*Pisum sativum* L.). Legumin is composed of six subunits, has a molecular mass of 300–400 kDa, and is abundant in amino acids that contain sulfur. For the manufacture of legumin nanoparticles, the coacervation approach has been the most widely used technique. During the coacervation process, legumin becomes less soluble and undergoes phase separation, resulting in the formation of nanoparticles. In an effort to accomplish targeted distribution and prolonged release of the medication, Mirshahi et al. tried to create legumin colloidal delivery systems shaped like micro- and nanoparticles.[11] Near-neutral pH is ideal for producing coacervates with submicron sizes. The particles are between 250 and 300 nm in size and have a pH between 4.5 and 7. When kept in a pH-neutral environment, the particles demonstrated good stability. Although legumin-based nanoparticles exhibit minimal antigenicity, small size, and high durability, further optimization study is required to enhance the poor yield associated with these nanoparticles and establish their utility in biomedical applications.[12]

6. 30kc19 protein derived from silkworm hemolymph:

A collection of proteins with comparable structures that are extracted from the hemolymph of silkworms (*Bombyx mori*) is known as the 30K protein family (30Kc6, 30Kc12, 30Kc19, 30Kc21, and 30Kc23) [13] The desolvation process was used to create the nanoparticles, and why GA was then used to crosslink them. When 30Kc19 was the only material used to create the nanoparticles, they were too big and poorly shaped. Nevertheless, the drug activity was strong and the particle size was tiny when 30Kc19-HSA nanoparticles were made with 50 weight percent of 30Kc19 and HSA. The pH and the particle's concentration of 30Kc19 were inversely correlated. A high yield of 80–90% of the original protein was demonstrated by the loading capacity. Within 24 hours, 30 to 50% of β -gal was released using the 30Kc19 protein nanoparticles, and up to 60% continuous release was noted. Additionally, they used 30Kc19 and HSA to create nanoparticles that contained α -galactosidase (α -gal) and successfully transported them to cells. [12] Natural nanoparticles called lipoproteins move fats throughout the body [88]. Lipoproteins are appealing and adaptable delivery systems due to a number of characteristics. Although there are many different kinds of lipoprotein nanoparticles, they all share a common structure a coating of phospholipids covers the core, which is made up of triglycerides and cholesterol esters, with amphipathic apolipoproteins embedded. Based on their size and density, lipoproteins can be divided into a number of classes, including chylomicrons (80–1200 nm), high density lipoprotein (HDL; 7–13 nm), low density lipoprotein (LDL; 22–27 nm), intermediate density lipoprotein (IDL; 27–30 nm), and very low density lipoprotein (VLDL; 35–80 nm). [13]

7. Lipoprotein:

In the circulatory system, lipoproteins are important carriers of triglycerides, cholesterol, and other hydrophobic substances (Figure 1). Lipoproteins are naturally occurring nanoparticles that also transport several lipophilic substances, including hormone and enzyme inhibitors and fat-soluble vitamins. 9-11 Because of their reduced size, high capacity binding, low affinity, and extended half-life in the bloodstream, these also serve as drug delivery vehicles. Additionally, natural and reconstituted lipoprotein-based systems are used to transport therapeutic molecules and have a high high-drug payload¹² (Figure 2). 13. Lipoproteins are classified as chylomicron, very low density lipoprotein (VLDL), intermediate density lipoproteins (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) according to their density. These are utilized as a vehicle for the systemic delivery of medications, treatments, and imaging agents after being reconstituted with phospholipids and apolipoproteins. 17. These are very helpful for delivering siRNAs systemically in a focused and effective manner. 18. In addition to the HDL apolipoprotein HDL, apolipoprotein mediated solubilization of membrane lipids in vivo also produces discoidal high-density lipoproteins. Cholesterol esters are transported to peripheral cells via low-density lipoprotein (LDL). It has one main apolipoprotein, B-100, which enables LDL to attach to peripheral cell surface LDL receptors. It is then taken up by cells via receptor-mediated endocytosis. The endogenous cholesterol carrier is called LDL. Most cholesterol is



obtained as cholesterol ester through endocytosis mediated by the LDL receptor. The LDL particle resembles an oil droplet coated in a phospholipid membrane (Figure 3). Triglycerides (20%) and cholesterol esters (80%) make up the lipid core of Lipoprotein. Antitumor chemicals are delivered to cancer cells via low-density lipoprotein. Lipid-protein complexes that are biocompatible are also perfect for loading and delivering therapeutic and diagnostic substances for cancer. Clinical applications for both natural and synthetic/reconstituted lipoproteins are common, especially in chemotherapy and cancer imaging. Cardiovascular issues are brought on by lipoprotein density and cholesterol. Density also determines whether blood cholesterol levels are high or low, as well as triglyceridemia. Reverse cholesterol transport includes the transfer of lipids between lipoproteins. It occurs in both sub-organelles and the cytoplasm of cells. The mechanisms involved in sterol clearance are positively regulated by the lipoprotein free cholesterol ratio. By changing the availability of CE and TG to LTP at the lipoprotein surface, it modifies lipid transport.[14]

High-density lipoprotein

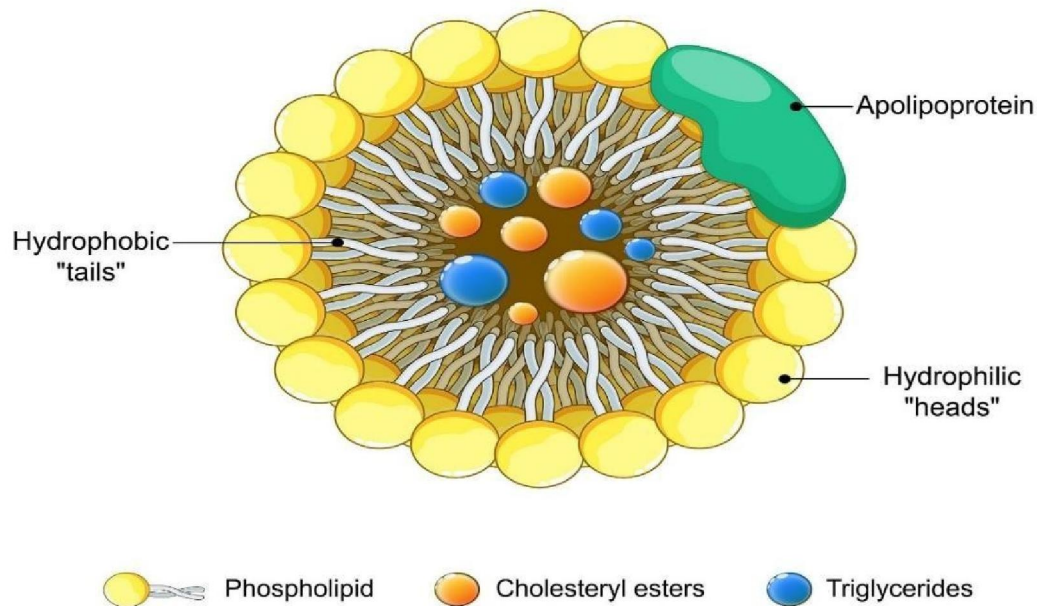


Fig 3:Lipoprotein of nanoparticles

8. Ferritin:

Microorganisms, plants, and mammals all contain ferritin, a protein that Laufberger initially identified in 1937 and which has the potential to store iron. It is a component, hollow, globular protein with a molecular weight of 474 kDa. Its spherical polypeptide shell (apoferritin) encircles a 6-nm inorganic core of hydrated iron oxide ferrihydrite. Mammalian cells contain two kinds of ferritin (H and L), which work in tandem with one another to facilitate iron absorption.[15] Ferritin nanoparticles are made up of twenty-four copies of the identical ferritin subunits that self-assemble. Because ferritin nanoparticles have both internal and exterior interfaces, their special structure makes drug



loading and targeting possible. While the interior portion of ferritin nanoparticles can be filled with different high affinity metals and small molecules, the outer surface can be chemically altered and a functional motif added .[14]]Ferritins are tiny, naturally occurring protein assemblies that are not virus-like. They are spherical particles with an internal cavity of 8 nm and an external diameter of 12 nm, formed by hollow globular complexes of 24 protein subunits. The particle naturally contains large amounts of iron, and channels in the protein shell allow the payload to be exchanged with the surroundings. Ferritin naturally targets the cell's ferritin receptor, which promotes cellular absorption. Ferritin is a useful drug delivery mechanism and cancer treatment .[15]

Method of preparation of Nanoparticles:

1. Emulsification method
2. Dissolvation method
3. Complex conservation method
4. Nano spray drying method
5. Self assembly

1. Emulsification method:

This procedure transforms an albumin aqueous solution into an emulsion in plant oil (cotton seed oil) and at room temperature. A homogenous emulsion can then be produced using a mechanical homogenizer that operates at a high speed. This approach would result in a high particle dispersion. Drop by drop, the aforementioned emulsion will be added to a large amount of oil that has been heated to more than 120°C. This process will cause the albumin to irreversibly degrade and the existing water to evaporate quickly. Nanoparticles will also form as a result of this process. depicts the aforementioned procedure. The suspension that was produced was placed in a cold, ice nanoparticles, and Gao and his colleagues improved it in 2010. depicts the aforementioned procedure. In order to demonstrate the impact of gelatin nanoparticle cellular uptake on the adhesion, morphology, and cytoskeleton organization of human fibroblasts, Synthesized cross-linked gelatin nanoparticles encapsulating a fluorescent marker molecule fluorescein isothiocyanate-dextran using emulsification method.[16]

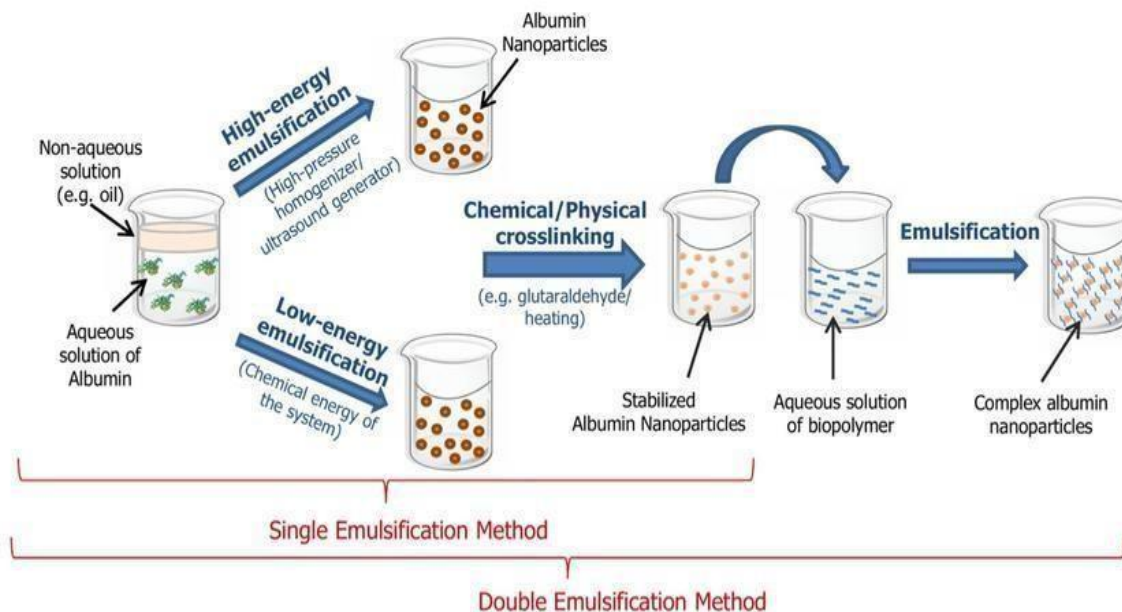


Fig 4 : Emulsification method
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2. Dissolution Method:

Marty and his colleagues (1978) proposed a novel approach, which was based on the gradual addition of a desolvation agent, such as alcohol or natural salts, to a protein solution. This component will alter the third structure of the protein. A protein clump will form once the desolvation process has reached a particular point. This polymerization clump crosslinking with a chemical component, glutaraldehyde, will produce nanoparticles in the subsequent stage. We must stop the system before the particles begin to gather if we want distributed nanoparticles rather than mass-formed ones. This desolvation factor will enhance the turbidity of the system. Particle accumulation will occur on its own when the turbidity of the system increases. The desolvation process produces protein nanoparticles that can change their size based on the circumstances [13] The primary determinants of particle size are temperature, PH, protein content, and the rate at which the desolvating agent is added. Smaller nanoparticles can be produced, namely, by high pH and low protein concentration. Although the desolvation approach does not directly involve the creation of nanoparticles, it is a self-assembly process since the desolvating chemical concentrates the protein by decreasing its solubility. The technique is commonly used to create albumin-based nanoparticles. Langer et al. used acetone and a desoluble agent for HSA receptors at pH values between 7 and 9 to create particles, which were then stabilized using GA as a bridging agent. [14]

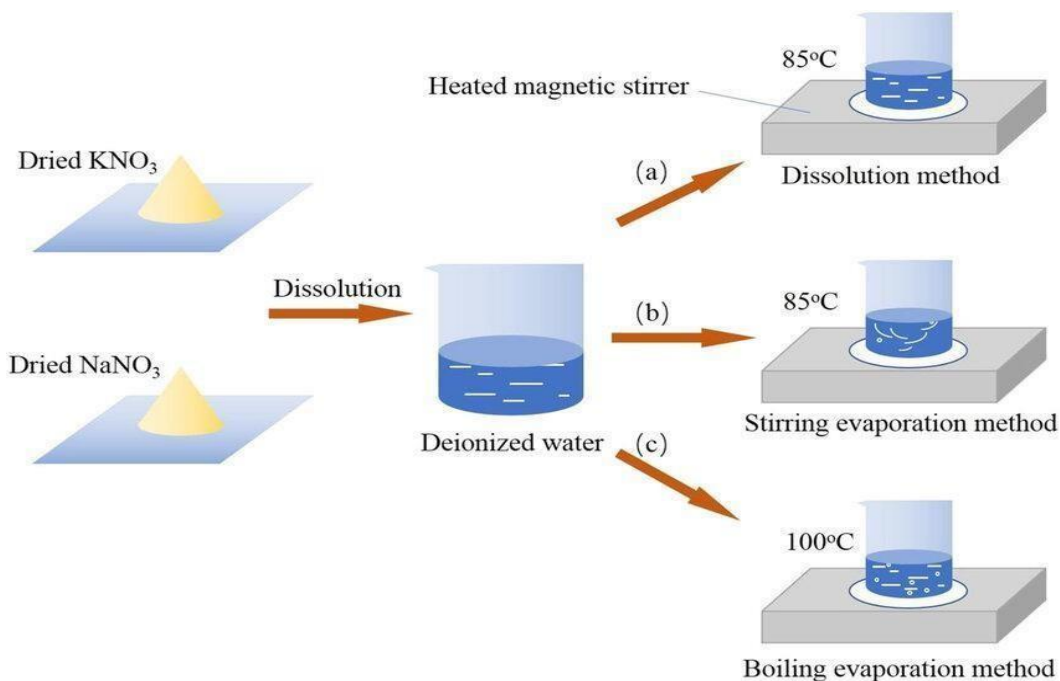


Fig 5: Dissolvation Method

3. Complex conservation method:

In general, this method works well for DNA trapping. Although proteins are amphoteric by nature, a pH shift can make them cationic or anionic. In this process, proteins were taken in an aqueous solution, and the particles with a positive charge rose higher, changing the pH. Next, a solution of salt and DNA was prepared and added to the previously mentioned aqueous protein solution. Complexes of proteins and DNA interact to create coacervation. A cross-linked, such as 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC), was added concurrently to produce cross-linked DNA-loaded protein nanoparticles. A different method for creating DNA complexes with cationized proteins exists. DNA was absorbed when chloramine was linked to the surface of gelatin nanoparticles. [17]



4. Nano spray Drying method:

One method for creating nanoparticles from a liquid sample is spray-drying. A nozzle sprays the liquid sample into a chamber, where heated carbon dioxide and nitrogen gases flow in the spray's direction. The electrodes at the bottom of the chamber are used to gather the nanoparticles. These electrodes cause the sprayed droplets to become electrostatically charged as they go toward the chamber's bottom. This one-step procedure offers a rapid and economical way to produce protein particles on a small scale. Because hydrophilic medicines can be encased in these spray-dried nanoparticles, spray-drying has one function in drug delivery systems. [18] Because the solvent evaporation helps preserve the temperature of the nanoparticle droplets, this method of fabricating nanoparticles is beneficial for samples that are heat-sensitive. Moreover, by adjusting variables like the nozzle's size and spraying speed, the user of this nanoparticle synthesis method can regulate the size of the particle that is created. [18]

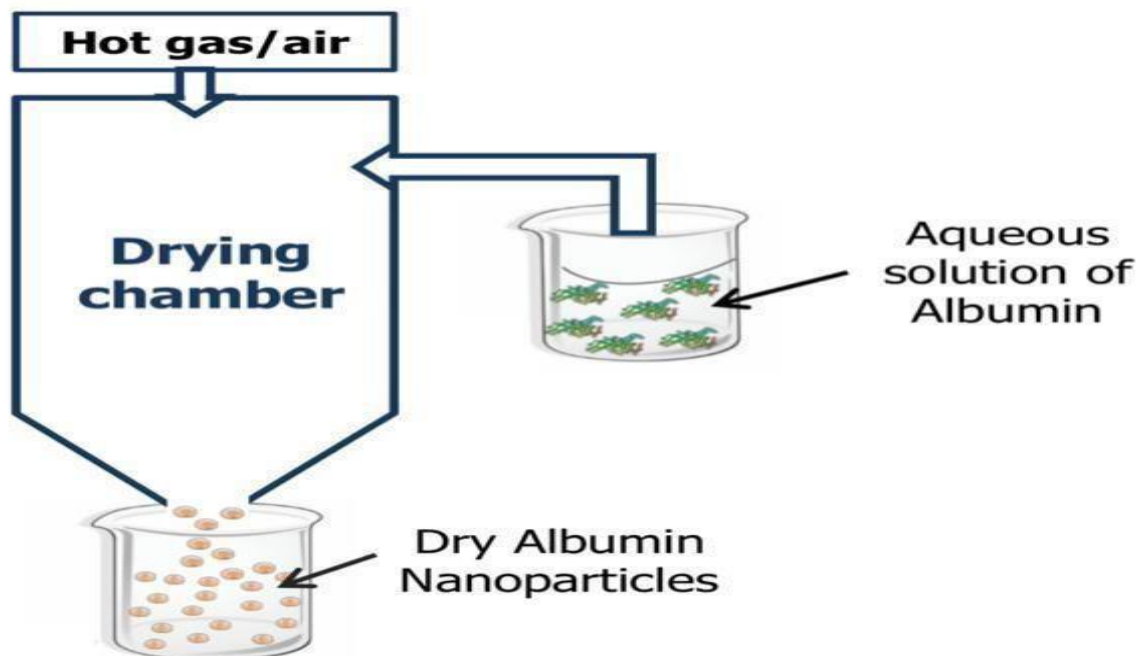


Fig 6 : Spray drying method

5. Self assembly method:

Nanosized aggregates may spontaneously form when individual protein chains dissolve in a solution that is higher than the critical micelle concentration (CMC) and the critical solution temperature (CMT). By creating a bridge between the chains, micelles can be kept stable during the solidification process. Albumin, a hydrophilic protein, can acquire an amphiphilic property by the process of hydrophobic alteration. Proteins that have undergone hydrophobic modification self-assemble into micelle nanoparticles when introduced to aqueous solutions. Additionally, hydrophobic cores can serve as a conduit for active substances. Gong et al. produced protein micelle nanoparticles and successfully created a core-shell nanomicelle by utilizing the unique interaction between the main amino group of albumin. [19]



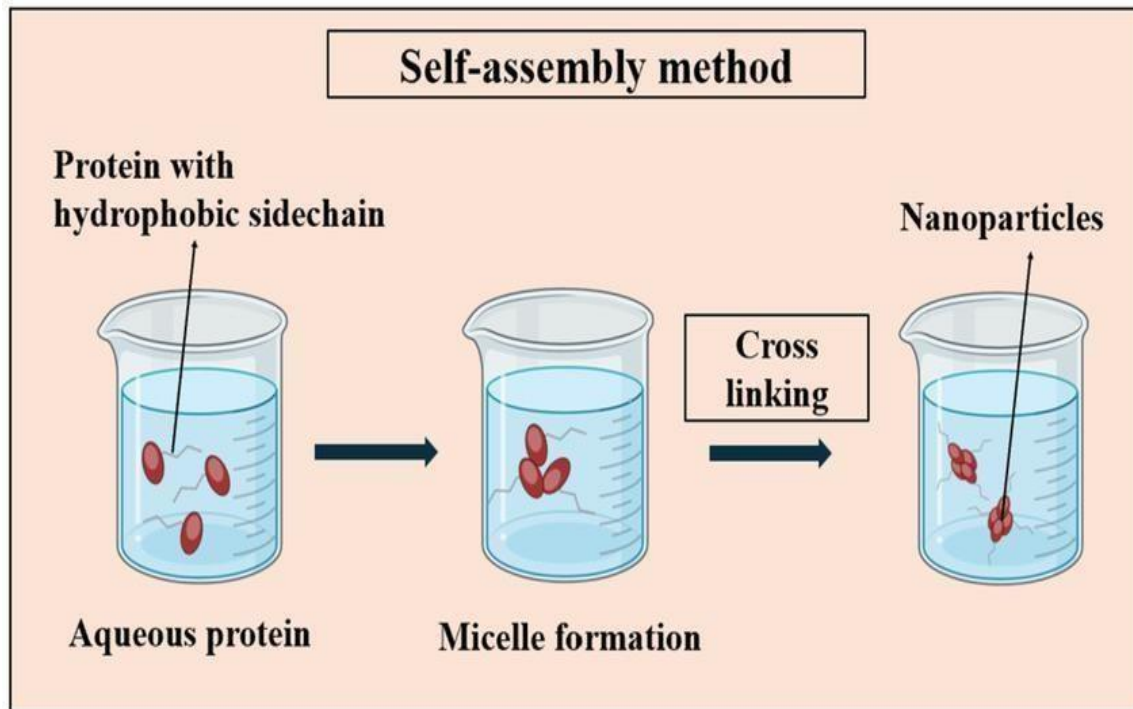


Fig 7: Self assembly method

Application of protein nanoparticles:

1. Tumor therapy:

Anti-Tumor Treatment According to certain research, albumin's extended half-life of approximately 19 days is linked to renal rescue mediated by the Megalin Cubilin receptor²²³ and its binding to endothelial and epithelial cell cycle neovascularized Fc receptors (FcRn). Through the intracellular sorting mechanism that guards against lysosomal degradation, FcRn maintains the 224 HSA level. Ginsenoside soap's pharmacokinetic behavior can be changed by polyethylene glycolated nanoparticle albumin binding, which can increase blood circulation time and decrease elimination. Reduced serum albumin levels, increased coagulation and Mitosis linked to high histone H4, severe cytokine storms, and inflammation in blood and lung tissue were all found in plasma samples from COVID-19 patients. In order to concurrently treat blood coagulation, Mitosis, and cytokine storms, albumin was selected as the ginsenoside delivery vehicle. By attaching to extracellular histones released by viral infections in individuals with severe COVID-19, albumin is thought to also prevent endothelial necrosis, pulmonary bleeding, and platelet aggregation. Ginsenosides contain steroid glycosides, which can inhibit.

2. Inflammatory therapy:

Polymorph nuclear neutrophils adhere to the lining of the circulatory system or vascular end to induce inflammatory disorders. The adhesion of polymorphonuclear neutrophils to the vascular endothelium or circulatory system lining, as well as the transmigration of the unchecked neutrophils, are the causes of inflammatory diseases. ²⁴². Therefore, using nanodrug delivery devices to target neutrophils is a viable therapeutic approach. ²⁴³ According to reports, albumin nanoparticles are transmigration of unregulated neutrophils and thallium. ²⁴². Therefore, using nanodrug delivery devices to target neutrophils is a viable therapeutic approach.^[20]



3. Medical and pharmaceutical:

The recent development in the field of nanomedicine particularly in the drug delivery application has led to the discovery of nanoparticle-based therapeutics for diagnosis and treatment of diseases such as cancer, diabetes, and allergy. The fundamental characteristics of nanoscale materials such as greater solubility and diffusivity have been shown to improve drug release characteristic and blood circulation half-life.

4. Biosensor and bioelectronics:

In the fields of medicine, food, and agriculture, nanotechnology has expanded options and added a new dimension to the design of potent biosensors and bioelectronic devices for disease diagnosis and contamination detection. The use of biological molecules hybridized with nanoparticles for the creation of innovative biosensors and bioelectronic systems is currently the focus of significant research efforts created an ultrasensitive biosensor for the detection of epithelial tumor marker by immobilizing hairpin oligonucleotide (HO) and horseradish peroxidase (HRP) on AuNPs. The multimodal signal amplification technique offered by thAuNP-HRP conjugate may allow for quick detection and improved detection sensitivity over a broad linear range.[21]

5. Nanoparticle of gene delivery:

Gene delivery using nanoparticles: In order to trigger an immune response, polynucleotide vaccines transfer genes encoding pertinent antigens to host cells where they are produced. This produces the antigenic protein close to professional antigen- presenting cells. Because intracellular protein synthesis, as opposed to extracellular deposition, stimulates both arms of the immune system, such vaccines generate both humoral and cell-mediated immunity.[22]

6. Nanoparticle of Lactoferrin:

Lactoferrin nanoparticles (LF-NPs) are a cutting-edge nanotechnology platform that uses lactoferrin's inherent bioactivity to provide strong antibacterial and anti- inflammatory effects.[23] A glycoprotein that is naturally present in milk and other physiological fluids, lactoferrin has the amazing capacity to stop the growth of a wide range of pathogens, including fungus and bacteria. Lactoferrin's therapeutic potential is significantly increased by incorporating it into nanoparticulate systems, which enables targeted distribution and better stability in biological contexts. This strategy has demonstrated potential in treating infections and inflammatory conditions, especially in situations where traditional therapies have drawbacks such systemic toxicity or resistance.[24]

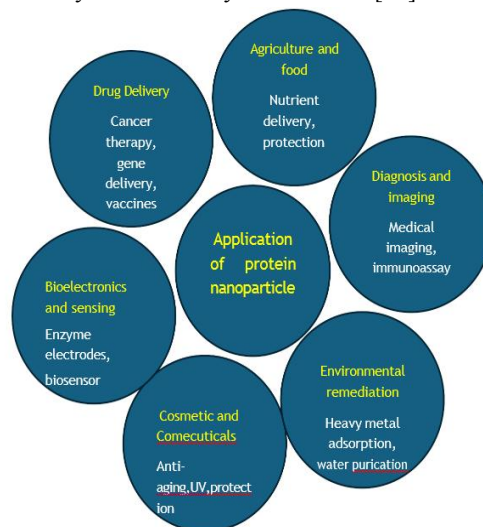


Fig 8: Application of protein Nanoparticle



Nanoparticle Limitation:

1. In liquid and dry forms can be challenging to physically handle due to their small size and vast surface area, which can cause particle aggregation. Furthermore, limited drug loading and burst release are easily caused by small particle sizes and large surface areas. Before nanoparticles may be used in therapeutic settings or made commercially available, these practical issues must be resolved.[25]
2. can be challenging to physically handle nanoparticles in liquid and dry forms due to their small size and huge surface area, which can cause particle aggregation.
3. Limited drug loading and burst release are easily caused by small particle sizes and large surface areas.[26]

Nanoparticle properties for drug delivery:

Important characteristics of nanoparticles for medication delivery Two main categories can be used to classify the delivery of pharmacologic drugs formed as NPs (i.e., those designed for local versus systemic administration). When administered locally, the NP formulation is supposed to serve as a "depot," maintaining the drug's active form while maintaining a local concentration, typically in a hostile environment. Additionally, the NP may function as a controlled-release formulation in which the medication release rate is precisely regulated by the NP's characteristics. When administered systemically, the NP formulation is anticipated to avoid the first clearance processes in circulation and ideally localize at the drug's site of action. Particle size and circulation time Long-circulating carriers are more likely to reach their target and leading to a therapeutic benefit. Greater NPs are more likely to filter in or settle in circulation. capillary beds, and it is widely acknowledged that the NPs should not exceed 200 nm for this reason. [27]. Physicochemical properties of protein Nanoparticle:

Properties	Description
Biocompatibility	Natural protein
Targeted drug delivery	Antibodies, peptide, ligand
Stability of biological systems	Enzymatic degradation
Biodegradability	Not toxic drug release
Solubility enhancement	Poorly soluble drug
Tunable size surface	Size can be controlled (10-200)

FDA Approval of protein nano-drug for medical purposes:

The FDA has approved 50 Nano pharmaceuticals since 1995, and they are now accessible for clinical usage. Nano-drugs are often given orally or intravenously. Liposome nanoparticles, polymer nanoparticles, micelle nanoparticles, nanocrystal nanoparticles, inorganic nanoparticles (metals/metal oxides and other inorganic nanomaterials), and protein nanoparticles are among the FDA-approved nano-drugs that are accessible for clinical use. The therapy of cancer is one of the many uses for which these nano-drugs have received approval. Between 2001 and 2005, there was a high number of FDA approvals for nanomedicines; but, following the 2008 financial crisis, there was a sharp decline in R&D funding. It should be mentioned that out of the 50 nanodrug., only two protein nanoparticles have received FDA approval. One is Abraxane (Celgene), a paclitaxel nanoparticle that is albumin-bound and used to treat pancreatic, breast, and non-small cell lung cancer. Abraxane's enhanced solubility and targeted administration to tumors are its advantages. The second is Ontak (Eisai), a Denileukin difitox nanoparticle, which is used to treat cutaneous T-cell lymphoma. It is a designed protein that combines L-2 with diphtheria toxin. Ontak's lysosomal escape property and focused T-cell specificity are its advantages. The fact that albumin has its own endocytosis pathway regulated by albumin receptor gp60 situated at the caveolae.may be the cause of the FDA-approved list of nanomedicines only include two protein nanodrugs.To effectively transfer albumin nanoparticles loaded with medicines into target cells, more ligands are needed for cells that lack or have few caveolae. The medicines' non-specific distribution to non-targeted cells with albumin receptors may be another factor.[25]



Fifty nano-pharmaceuticals have been approved by the FDA since 1995 and are currently being used in clinical settings [1]. Nanodrug are often given orally or intravenously. Liposome nanoparticles, polymer nanoparticles, micelle nanoparticles, nanocrystal nanoparticles, inorganic nanoparticles (metals/metal oxides and other inorganic nanomaterials), and protein nanoparticles are among the FDA-approved nano-drugs that can be used in clinical settings. Numerous uses, including the treatment of cancer, have been authorized for these nano-drugs [2]. Between 2001 and 2005, the FDA approved a large number of nano-drugs; but, with the 2008 financial crisis and a decline in R&D funding, this number significantly decreased. [28] One is the albumin-bound paclitaxel nanoparticle Abraxane (Celgene), which is used to treat pancreatic cancer, NSCLC, and breast cancer. Abraxane's enhanced solubility and targeted tumor delivery are its advantages. The second is Ontak (Eisai), a Denileukin diftitox nanoparticle (an synthetic protein that combines L-2 and diphtheria toxin) used to treat cutaneous T-cell lymphoma. Ontak's lysosomal escape property and focused T-cell specificity are its advantages.

The fact that albumin has its own endocytosis route, mediated by albumin receptor gp60, situated at caveolae, may be the reason why there are only two protein nano-drugs on the FDA-approved list of nanomedicines [29]

Example of FDA approval drug:

Drug	Description Nanoparticles	Indications
Oncaspar®	Protein enzyme (asparaginase) PEGylated	Acute lymphoblastic leukemia in children (asparaginase) therapy
Asparlas®	Conjugated with PEG for extended half life	Acute lymphoblastic leukemia
Tagraxofusp®	A fusion of protein (interleukin -3 fused to diphtheria toxin)	Blastic plasma cystoid dendritic cell neoplasm

Mechanism of drug Delivery using Nanoparticles:

After entering the bloodstream, nanoparticles adsorb different plasma proteins such albumin, fibrinogen, and apolipoprotein to create a protein corona. The stability and longevity of nanoparticles can be greatly impacted by the properties of this protein corona. The characteristics of the nanoparticle's surface, including its size, shape, and charge, determine the type of protein corona. [30]. Nanoparticles from the blood can either enter the target organ or be eliminated via the kidneys, liver, or lymphatic system, among other processes. Nanoparticles with a diameter of less than 10 nm are usually eliminated by the kidneys. Macrophages and other phagocytic cells eliminate nanoparticles with a diameter of about 50 nm. It is now known that a diameter of 100 nm is ideal for extended blood circulation. In addition to diameter, a number of different tactics can be used to avoid the blood's enzymatic proteins' clearance mechanisms. PEG-ylation, or coating with polyethylene glycol, is one such method. [31]



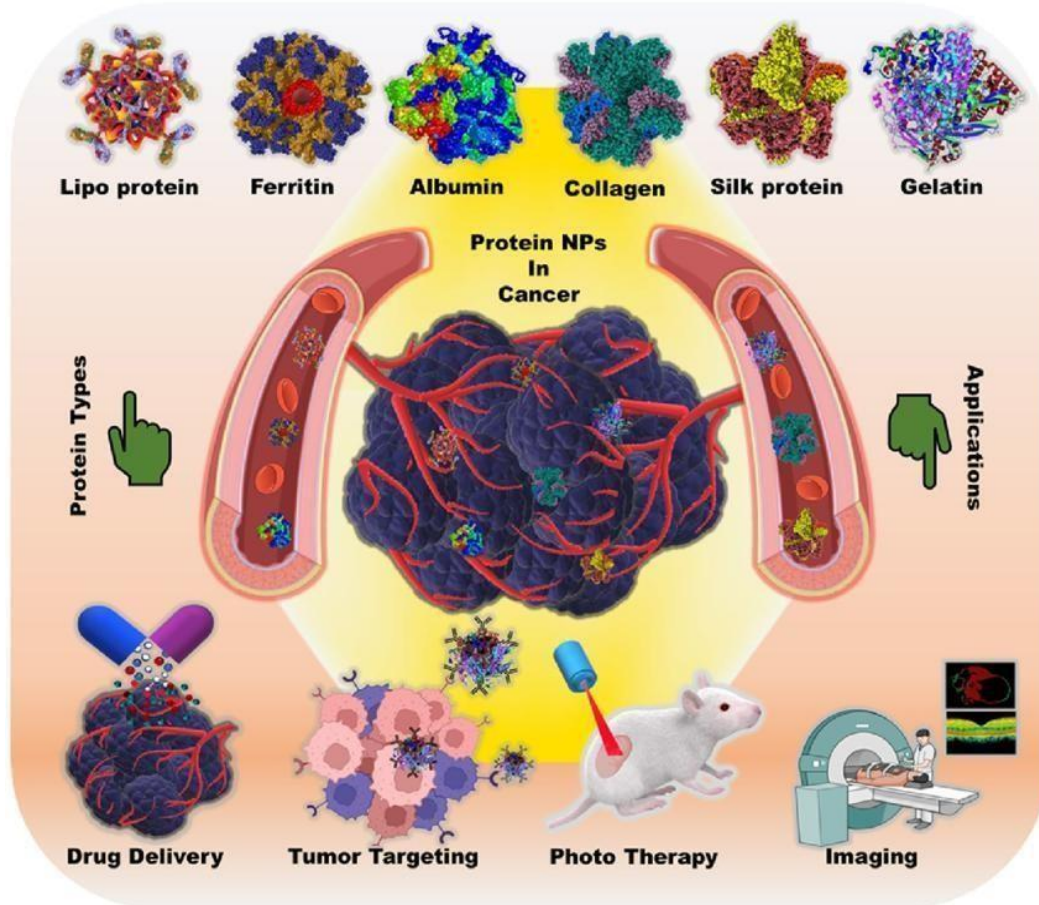


Fig 9: Mechanism of Nanoparticles .

Degradation of the nanoparticles is essential for releasing the medicine that has been encapsulated once they have reached the target tissue or cell. The molecular weight and component content of the nanoparticle have an impact on the degradation process. For example, the molecular weight and functionalization of the polymer components affect the degradation of PLGA-based polymeric nanoparticles. Sixty Kinetic control of medication distribution and controlled-release characteristics can be obtained by comprehending and adjusting these parameters.

Approved or clinical protein based Nanoparticle drug:

Drug Name	Protein Used	Uses
Abraxane®	Human serum albumin	Used to breast, lungs, pancreatic cancer.
Aldoxorubicin®	Human serum albumin	Used to cancer therapy (clinical trials).
Nabrapamycin®	Albumin	Albumin-bound rapamycin formulation for cancer and cardiovascular disease.
Levemir®	Insulin	Long acting insulin that forms self assembled nanoparticle in the body.
Pegasus®	Interferon protein	Protein conjugated to PEG forming nanoparticle like complexes for hepatitis B and C

Drug Name Protein Used Uses



Future Direction:

- Clinical translation: Conducting clinical trials to evaluate the safety and efficacy of protein-based nanoparticles in humans.
- Scalability and reproducibility: Developing scalable and reproducible methods for the production of protein-based nanoparticles.
- Targeting and specificity: Improving the targeting and specificity of protein-based nanoparticles to enhance therapeutic outcomes.
- Combination therapy: Exploring the use of protein-based nanoparticles in combination with other therapies, such as immunotherapy and gene therapy.

II. CONCLUSION

Protein-based nanoparticles have emerged as a promising platform for systematic drug delivery, offering several advantages over traditional delivery systems. These biodegradable and biocompatible nanoparticles can be engineered to target specific cells or tissues, reducing toxicity and improving efficacy. The versatility of protein-based nanoparticles allows for the delivery of a wide range of therapeutic agents, including small molecules, nucleic acids, and proteins. Recent advances in nanotechnology and protein engineering have led to the development of various protein-based nanoparticles, such as albumin-based nanoparticles, gelatin-based nanoparticles, and silk-based nanoparticles. These nanoparticles have shown great potential in preclinical studies, demonstrating improved pharmacokinetics, biodistribution, and therapeutic outcomes. However, several challenges need to be addressed to translate protein-based nanoparticles from bench to bedside, including scalability, reproducibility, and regulatory issues. Further research is needed to optimize the design and formulation of protein-based nanoparticles, as well as to evaluate their safety and efficacy in clinical trials.

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