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Enzymosomes: Recent Emergence of Novel Delivery System for Targeted Therapeutic Applications

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ABSTRACT

The goal of the research is to create enzymosomes as a new medication delivery method with site-specific activity. Enzymosomes make use of an enzyme's particular nature, which is to bind to a specific substrate at a regulated pace and catalyze the product manufacturing step. Enzymosomes are formed when an enzyme is covalently attached to the surface of liposomes/lipid vesicles. To produce enzymosomes with tailored activity, enzymes are linked via acylation, direct conjugation, physical adsorption, and encapsulating techniques. Such new drug delivery systems demonstrate effective drug release while also reducing the negative side effects of traditional treatment techniques, resulting in an improvement in long-term disease therapy. They are a potential alternative to gout therapy, antiplatelet activities, and other traditional treatments. Enzymosomes are recently developed supramolecular vesicular delivery systems that may improve drug targeting, physicochemical characteristics, and therefore bioavailability in pharmaceutics. It demonstrates that medicines with a narrow precision have positive effects because directing these treatments to their site of action enhances their overall pharmacodynamics and pharmacokinetic profile. It also reduces changes in normal enzymatic activity, improving half-life and achieving enzyme activity on specific locations like malignant cells.

Keywords: Novel drug delivery systems, Enzymosomes, Drug carriers, Preparation, Characterization, Applications

I. INTRODUCTION

A new approach to medication delivery is a focused system in which entities are guided to particular areas of action. Novel drug delivery systems (NDDS) are being used in a variety of sectors, including pharmaceutical, food, and cosmetics. It primarily achieves and overcomes the constraint of traditional drug delivery methods, namely, delivering the full active moiety of the medication to the site of action. It also transports the medication via the body's appropriate pathways at a pace determined by the body's needs. Most medicines with bioavailability issues, as well as cells and genetic engineering, diagnostic tests, and immunological methods, have turned to vesicular carriers as the current vehicle of choice. Vesicular drug delivery aids in the maintenance of drug release at a preset pace, reducing the risk of drug toxicity and therefore being useful in the treatment of ocular disorders. Such vesicles have a user-friendly shape and can hold both hydrophilic and lipophilic medicines. Most carriers have ordered concentric congregates of lipid bilayers in which different locations are used to encapsulate hydrophilic and lipophilic medicines, eventually resulting in amphiphilic nature. Bingham first announced the vesicles in 1965, referring to them as Bingham bodies. By spatial induction of drugs surrounding the sick organ or tissue, particularly by chemical derivatization, the new carriers localize their effect. Various medicines for glaucoma, ulcerative colitis, colon disorders, NSAIDS, and insulin-like therapies have been shown to have increased bioavailability and duration of effect in people in studies. Vesicular drug distribution lowers treatment costs and improves pharmacodynamics, particularly for poorly wetting medicines. It may be a watershed moment in traditional chemotherapy techniques, when medication penetration and cell permeability were severely restricted. The method employs a successful route for medication delivery to the infection site while minimizing side effects. Lipoidal and non-lipoidal carriers are two types of vesicular drug delivery systems based on their composition. Sphingosomes, transferosomes, liposomes, emulsomes, and virosomes are lipoidal carriers, while aquasomes, niosomes, and bilosomes are non-lipoidal carriers. Lipid particles (including low and high-density lipoprotein),

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nanoparticles, colloidal transfer systems, and polymeric micelles, as well as other pharmacological carriers such as cellular macromolecules, are emerging as effective instruments for targeted drug administration [1].

CELLS TO VESICLES

"Somes" is a kind of biological cellular structure that includes internal organs, tissues, nucleic acid, and genetic resources for reproduction and replication. In mammalian cells, the cell membrane that encompasses the cell's outer portions is a phospholipid bilayer. The physiological nature is mostly lipophilic due to the two-layered arrangement of lipid molecules. As a result of their similarities to the cells that border every organ, lipoidal medicines and proteins may readily penetrate the body's membranous barriers. A vesicle is a collection of supramolecular lipid molecules that resembles a cell membrane. Vesicles are secretory transport vesicles that are released inside our cells. Endocytosis allows cells to ingest exogenous vesicles, which are then broken down into smaller pieces by lysosomal enzymes. The focus of the review is on the use of these vesicles for targeted and site-specific medication delivery of both hydrophilic and lipophilic medicines. Enzymes are proteins that fold into various forms to allow smaller molecules to fit within them. Enzymes are biological catalysts that operate on a substrate molecule to achieve their goal. Every cell requires an enzyme to convert these reactants to product molecules in order to carry out a reaction at a significant pace. Because enzymes are very specific and selective, they are primarily responsible for determining the cell's metabolic route. Enzymes are site-specific and interact with the substrate through a lock-and-key mechanism. The reaction response is determined by the degree to which the enzyme binds to the substrate. Combining enzyme specificity with the idea of vesicular drug administration, this selectivity of enzymes is utilized [2].

ENZYMOSOMES

Enzymosomes (**Figure 1**) is a novel targeted vesicular drug delivery method that is presently under development. Enzymosomes are essentially enzymes with a specific catalytic function for a substrate that is integrated into cell-like structures with a strong lipid backdrop. They produce freshly designed liposomes in which the enzymes are covalently attached to the lipid molecules' surfaces. The liposomes were designed in this way to create a suitable microenvironment for the enzymes inside them to be disabled. Liposomes are micro-sized vesicles with an aqueous environment surrounding a lipid bilayer. The hydrophilic medicines may be dissolved in the internal aqueous compartment, whereas the lipophilic drugs are integrated into the phospholipid-cholesterol lipid bilayer membrane. Lipid-based drug delivery systems have unique properties such as lowering the volume of distribution, interrupting drug clearance, and altering drug distribution with increased capillary permeability towards infected tissues while reducing toxicity in normal tissues, proving to be an effective nanoscale drug delivery system for clinical use.

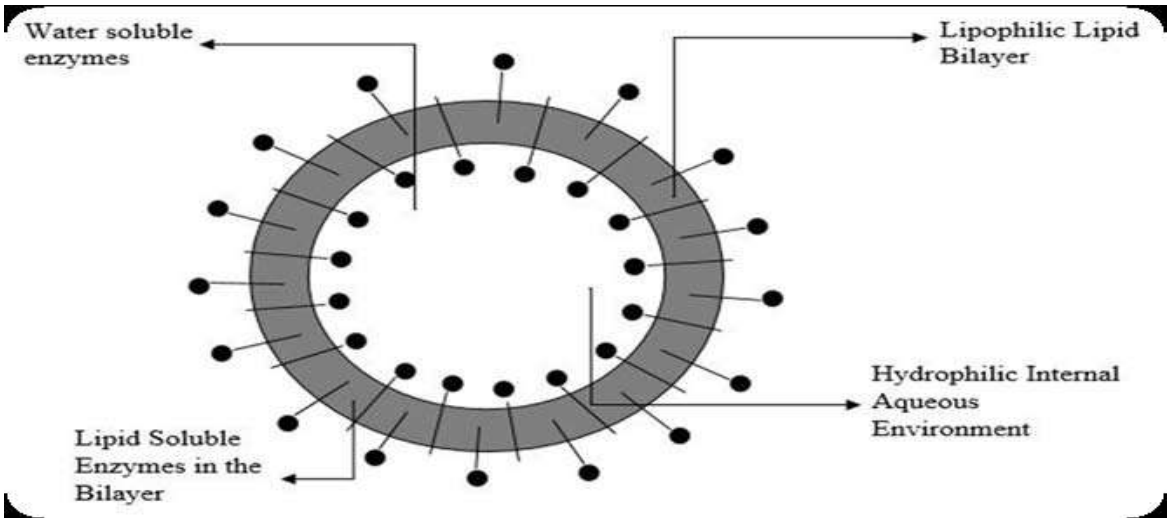

Figure 1. Structure of Enzymosomes.

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Enzymes are involved in catalysis, site-specific pharmacological activity, and the activation of prodrugs (**Table 1**), among other things. However, because of their limited lipid membrane penetrability, if an enzyme is encased on the liposome's surface, the enzyme's breakdown and transmutations are reduced, increasing its half-life and focused activity. Enzymosomes are a new vesicular rate-directed drug delivery technology that delivers the active form of the drug to the site of action while quickly degrading it for simple absorption. Enzymes may be bound to the liposome surface in two ways: by connecting functional hydrophobic compartments with the enzyme, such as long-chain fatty acids, or by associating the enzyme with the liposome layer's phospholipids. By transporting the therapeutic drug to the intended tissue receptors, particularly those found on an organ or system, the tailored method allows for increased therapeutic effectiveness and fewer adverse effects. For the coated drug molecule, such modified vesicles have improved solubility, stability, and therapeutic index. Due to their lipophilicity, these lipid nanocarriers serve as natural attractants of BBB, making them useful in the treatment of CNS diseases including epilepsy and convulsions. The therapeutic enzymes are delivered through polymeric carriers such as liposomes and lipoplexes, with the attachment of enzymes to exposed regions of liposomes showing the best results. A drug-loaded vesicular delivery system produces accurate results at the site of infection or inflammation, with little drug toxicity and side effects, making it helpful for centrally acting medicines that must pass through the BBB, which is critical to the brain's homeostatic function. By lowering the purchasing cost, it also helps to improve medication bioavailability, or the smallest quantity of drug concentration accessible for systemic circulation. When evaluated in vitro and in vivo, the covalently bonded enzyme and liposome will have minimal changes in enzyme activity, and the enzyme loaded in a vesicle will maintain its structural integrity and enzymatic function. Advances in enzymosome design have a wide range of applications, including the creation of novel recombinant proteins, biotechnological products, and so on [3].

Table 1. Enzymosomes-based prodrug activation systems.

Antigen/Antibody	Active drug	Enzyme	Prodrug
MAb BW431/26/Carcinoembryonic Antigen (CEA)	Etoposide	Alkaline phosphatase	Etoposide phosphate
MAb L6 (against a carbohydrate antigen on human carcinomas)	Doxorubicin Melphalan	Penicillin amidase	Doxorubicin-phenoxyacetamide
MAbs W14A and SBIO/chorionic onadotropin	Benzoic acid mustards	Carboxypeptidase G2	Benzoic acid mustards-glutamic acid
MAb 323/A3 (against a pan-carcinoma membrane glycoprotein)	Epirubicin	β-Glucuronidase	Epirubicin-glucoronide
MAb KS1/4/UCLA-P3 human lung adenocarcinoma	Methotrexate	Carboxypeptidase A	Methotrexate-alanine

ADVANTAGES OF ENZYMOSOMES

• Reduces harmful medication exposure to vulnerable tissues.
• Improved encapsulation and stability.
• Improvements in pharmacokinetics (Increase half-life, reduction in elimination).
• For active drug targeting, it combines with site-specific ligands.
• Efficacy and therapeutic index have improved.
• Biodegradable.
Both Systemic and non-systemic given dosages are non-immunogenic.
• Electrical characteristics may be changed on the fly.
• It is non-toxic.
• Biodegradable to the fullest extent possible.
• The restraining impact of the site.

DISADVANTAGES OF ENZYMOSOMES

• Because liposomes are classified as nanotherapeutics, their manufacturing costs are usually expensive.
• The component phospholipids in lipid vesicular structures are susceptible to oxidation and hydrolysis.
• It has poor solubility and a short half-life, which lowers bioavailability.

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• The enclosed drug molecule or molecules undergo fusion and leaking.

LIPID-BASED DRUG CARRIERS

Oral, parenteral, and topical dosage forms are all made using lipid-based chemical systems, which is a widely recognized technique. Such systems' safety and effectiveness have become their hallmark, making them appealing for formulations, diagnostics, and vaccine preparations. It is tailored to a variety of needs by taking into account cost, toxicity from therapies like ultrasound, effectiveness, and stability, as well as illness indications. The advantages of such systems are extensive:

• Improved bioavailability and fewer plasma concentration diversifications.
• Superior characterization and adaptation of lipophilic excipients.
• Ability to identify important affairs for technology transfer and scale-up manufacturing.

Liposome-based vesicular carriers, lipid particle systems, and emulsions are all examples of vesicular systems. Modified liposomes, marinosomes, phytosomes, and transferosomes may all be carried in liposome carriers. The lipid particulate system refers to biocompatible lipid microparticles and lipid nanoparticles that have been developed in recent years. Due to controlled drug release, a solid grid of stable lipids, physicochemical compatibility, and the preservation of the active component from degradation, they are beneficial. Solid-lipid nanoparticles, lipid drug conjugates, and other nanostructure lipid carriers are newly discovered improved drug delivery methods that outperform polymer nanoparticles in terms of cytotoxicity. Many studies have been conducted on semisolid solid lipid nanoparticles for topical drug administration, with typical semisolid to dispersion mixes of the systems being used. Pickering emulsions, nanoemulsions, SLNs, and self-emulsifying delivery methods are all emulsions. The essential characteristics of enzymosomes are the preservation of enzymatic activity and the structural integrity of vesicles, which are achieved via ligating enzymes with lipid carrier molecules [4].

PHARMACOKINETICS

Data gathered at various time periods after the administration of nanomedicine in animal models allows for a comprehensive pharmacokinetic and pharmacodynamic study of the nano-agent in vivo. WinNonlin data management, a statistics, modeling, and visualization program for PK data analysis, is used to conduct PK/PD evaluations. Drug concentration over time, Area Under the Curve (AUC), elimination half-life, clearance, and time of maximum concentration are all parameters that are usually reported for a particular Nanomedicine [5].

CHARACTERIZATION

A proper physicochemical characterization of the final formulation is critical, but it is not a simple job owing to the tiny size, complexity, and-in most cases-heterogeneity of dispersions. The physicochemical state, size and size distribution, surface characteristics of nanoparticles, shape of nanoparticles, existence of extra colloidal structure (e.g., owing to an excess of stabilizer), and drug localization are the primary areas of interest (nanoparticle core, interface additional structures, precipitated drug, etc) [6].

Size and zeta potential

The most essential characteristics of nanoparticles are their size and surface qualities, since these are the primary determinants of the drug carrier system's efficacy in vivo. Photon Correlation Spectroscopy (PCS) is the most common method for determining the size of colloidal particles, but methods based on static light scattering (laser diffraction with suitable instrumentation for sub-micron range) can provide additional information about particles in the micro-range and size distribution. The Asymmetrical Flow Field-Flow Fraction (A4F) in combination with multi-angle light scattering is another technique that isn't often utilized. Due to the separation of the nanoparticles before size measurement, this technique can provide precise information on the size distribution.

Photon Correlation Spectroscopy (PCS)

Photon correlation spectroscopy, also known as Dynamic Light Scattering (DLS) or Quasi-Elastic Light Scattering (QELS), is a reliable and fast technique for determining colloidal size (between 5 nm and 1 μm). PCS measures the intensity variations of scattered light caused by particle motion as a function of time. The particle diameter may be estimated using the Stoles-Einstein equation and the diffusion coefficient of the particles in the measuring fluid.

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Laser diffraction with sub-micron equipment

In addition to PCS, the most popular method for determining the size of SLN is laser diffraction, in which the intensity of scattered light is measured as a function of angle (Static light scattering). Traditional laser diffraction (Fraunhofer diffraction) can only be used to measure particles that are bigger than the wavelengths of the laser light being utilized (normally 633 nm).

Asymmetrical Flow Field-Flow Fraction (A4F)

For size measurements, asymmetric flow field-flow fractionation was employed. The variations in particle shapes were blamed for the disparities in size and elution profiles. Formulations are more likely to be maintained by cross-flow than spherical emulsion droplets due to their anisometric, platelet-like structure. This technique seems to be extremely promising as an extra size determination method, especially in terms of colloidal structure separation and detection.

Zeta potential

Information on the surface charge of nanoparticles may be obtained through zeta potential studies. Due to enhanced particle repulsion by electrostatic forces, a sufficiently high zeta potential increases the stability of electrostatically stabilized nanoparticles. However, for formulations stabilized using polymers that result in steric stabilization, this criterion cannot be rigorously followed.

STABILITY STUDIES

The thermal stability of free catalase is proportional to its concentration. The greater the enzyme concentration, from 0.25 mg/ml to 5.0 mg/ml, the better is the heat stability. This implies that the deactivation of the enzyme is dominated by its dissociation into its subunits. Catalase at the maximum dosage, 16 mg/ml, on the other hand, is less stable than catalase at 5.0 mg/ml. The development of irreversible intermolecular aggregates among the conformationally changed enzyme molecules is aided by the 16 mg/ml of catalase. Importantly, the thermal stability of liposomal catalase (CAL100-III) at a concentration of 16 mg/ml is significantly greater than that of the free enzyme at the same concentration. This is because the contact of the inner surface of the liposome membrane with the encapsulated enzyme molecules prevents the development of enzyme aggregates in the liposomal aqueous phase. As a result, the liposome-encapsulated catalase molecules' structure and activity are stabilized by the functional carrier liposomal system [7].

REGULATORY PERSPECTIVES

Significant advancements in many fields have increased the design and development of engineered nanosystems for disease detection, prevention, and therapy. This has been used to create goods that are both efficient and safe. Even if some of those nanoproducts have successfully entered the market, part of the scientific community has yet to fully grasp the consensus underlying the nanomedicines-related regulatory requirements, and thus academia and pharmaceutical companies may face significant challenges during the research and development life cycle of these medicinal products. However, significant progress has been achieved in recent years, indicating regulatory bodies' awareness of unique characteristics associated with nanosystems-based medications. As a result, significant problems have progressively challenged the regulatory environment for those new medicinal nanosystems, providing a chance to offer unambiguous direction for their development.

APPLICATIONS OF ENZYMOSOMES

Enzymosomes are a kind of lipid nanoparticulate drug delivery system made up mainly of phospholipids organized in a bilayer shape that can hold any chemical, regardless of solubility, electric charge, or molecular weight, and therefore enhance GIT absorption and oral bioavailability. Enzymosomes can be loaded onto lipid-based nanocarriers such as liposomes and solid-lipid nanoparticles, inorganic nanocarriers such as gold nanoparticles and magnetic nanoparticles, polymeric nanocarriers such as nanogels and micelles, and protein-mediated nanocarriers such as super positively charged proteins, among other materials. Since the cell membrane has been utilized as the target for therapeutic intervention, one guaranteeing a current collection of medicines without DNA interaction exists inside ether and alkylphospholipids. These were shown to be very beneficial in clinical trials for the treatment of metastases, breast cancer, anti-inflammatory activity, and other conditions [8].

Preparation of enzymosomes of surface-exposed superoxide dismutase

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Although directly binding enzymes to the lipids of liposomes is a difficult task, it is now employed as a compelling method in the treatment of immune-mediated illnesses or antibodies. SOD is an example of a direct conjugated therapeutic enzyme (Super Oxide Dismutase). Cu, Zn-superoxide dismutase (SOD) has been identified as an inherent defence mechanism that reduces the teratogenicity of harmful free radicals. It causes the harmful superoxide radical anion O_2^{2-} to dismutate into O_2 and H_2O_2, disrupting a variety of metabolic inflammatory pathways triggered by the free radical. SOD seems to be a potential alternative to traditional anti-inflammatory treatments, since it avoids the adverse effects of non-steroidal anti-inflammatory medications. Due to its limited properties, short half-life in the circulation, and low penetration into cells, the enzyme was not therapeutically acceptable. Many researches were conducted to improve the substrate on which the enzyme was loaded with an improved half-life and liposomal incorporation efficiency, as well as clinical trials in particular risk categories such as obesity, Type-2 diabetes, and so on. Targeting protein to cell-penetrating peptides (CPPs) or protein transduction domains is the most common use for intracellular SOD delivery to date (PTDs). Regardless of the practical benefits of enzyme transduction technology, the primary focus of this strategy is on the ineffective egress from the endosome to the cytosol, resulting in CPP-tagged payloads being trapped in intracellular vesicles. Proteins are essential for biological functions, and current encapsulation methods have made it possible to regulate the distribution of these peptides, allowing for their potential application in the treatment of a variety of illnesses. The driving electrostatic force is used to create spontaneous binding using the physical adsorption technique. Nanoparticles may be gathered from a variety of materials and arranged into desirable geometries and combinations, gaining valuable functions and characteristics in the process. Conventional chemotherapy treatments include several side effects, as well as the inability to reach the disease's core. As a result, nano-sized polymeric carriers can deliver drugs to cells selectively and precisely (**Figure 2**) [9].

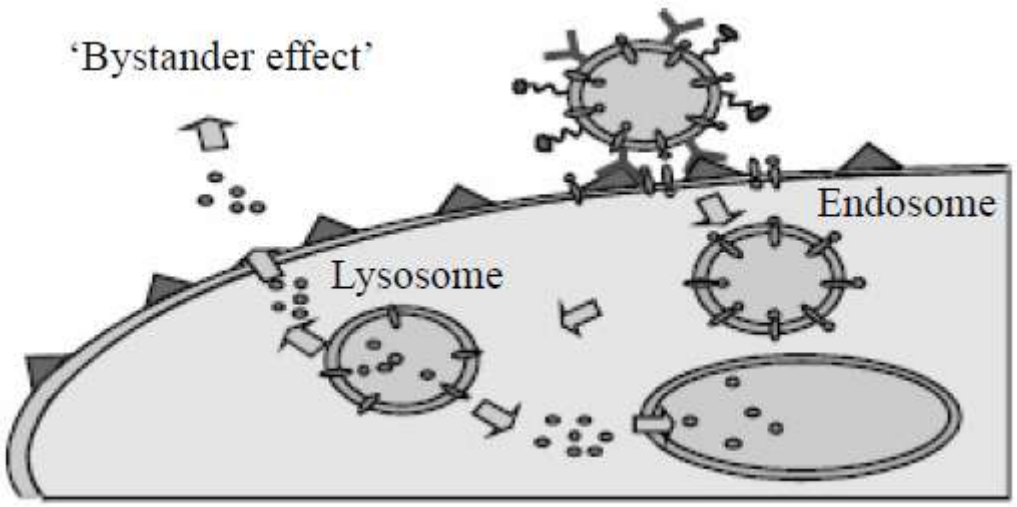

Figure 2. Bystander killing effect.

Construction of nanocarriers

Polymer colloids that include nanocarriers are produced using techniques such as carbon nanotubes and cross-linked nanogel matrices. The biological circumstances as well as the intermolecular force of attraction influence how productive these systems are. A nanocarrier must be able to transport medicines to the active site without deactivation, the drug must be released according to kinetic rules, the drug must be stable after administration, and the drug must be actively delivered with site-specificity (**Figure 3**). Chemical ligation, either covalent or non-covalent, was used to modify the molecules. Another option was for proteins and nanocarriers to self-assemble.

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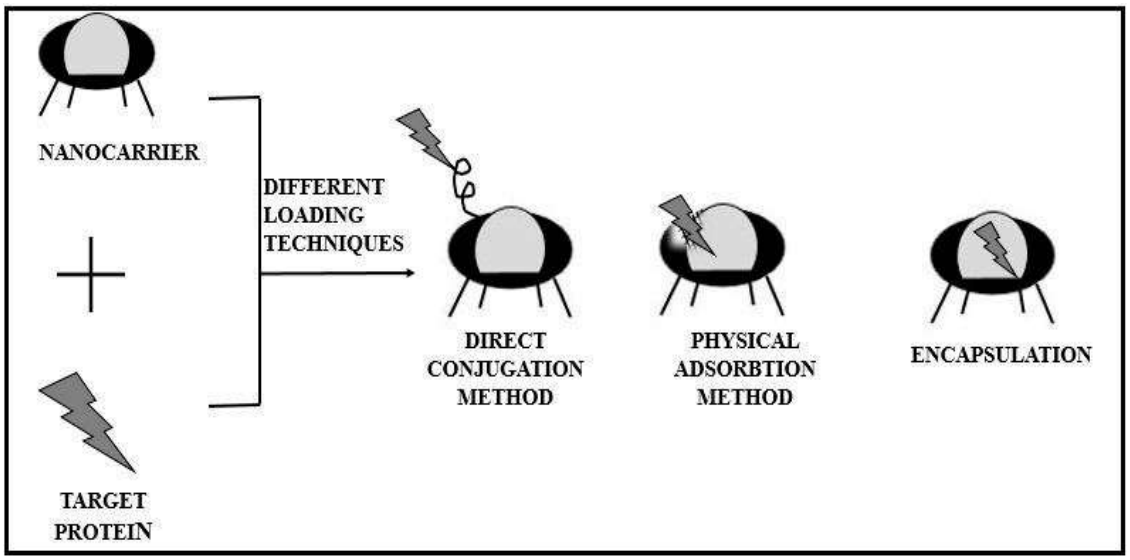

Figure 3. Methods for preparing protein/nanocarrier composites.

The driving electrostatic force is used to create spontaneous binding using the physical adsorption technique. Nanoparticles may be gathered from a variety of materials and arranged into desirable geometries and combinations, gaining valuable functions and characteristics in the process. Conventional chemotherapy treatments include several side effects, as well as the inability to reach the disease's core. As a result, nano-sized polymeric carriers can deliver drugs to cells selectively and precisely.

Because the release of the enzyme from the liposomes at the site of inflammation may not be required to provide therapeutic action, releasing SOD across the liposomal surface is thought to be more helpful than encapsulation of the enzyme in liposomes. The technique of acylation by covalent attachment of palmitic acid to q-NH_2 groups of SOD was used to achieve surface localization of SOD on enzymosomes (Ac-SOD). The researchers hypothesized that the method would produce a more hydrophobic enzyme with a higher affinity for liposomal bilayers. We also hoped to direct SOD to inflammatory areas by exposing it to the exterior liposomal surface. Liposomes were produced with lengthy circulation duration for this study in order to achieve localization at inflammatory sites and serve as an effective intracellular drug delivery mechanism. Long-circulating liposomes (LCL) have been shown to preferentially cluster in inflammation sites after i.v. treatment in these studies. This selective localization may be due to an inflammatory reaction that causes increased capillary permeability in a specific area, enabling liposomes to pass through. As a result, LCL offers promising possibilities for delivering Ac-SOD to specific locations. PEG-coated LCL (PEG liposomes) and non-PEG-liposomes containing stearyl amine was used to integrate SOD and Ac-SOD (SA-liposomes). Because enzymes are vulnerable to physical and chemical stress, such as heat treatment, they are protected inside lipid bilayers, where they retain their natural conformation. Incorporation efficiency, zeta potential, preservation of enzymatic activity, and externally exposed enzyme activity were all measured in both liposomal formulations. The research into creating and improving SOD enzymosomes was primarily done to extend the period that human blood circulated and concentrate the enzyme at the target location, allowing the enzyme to remain perfect inside the lipid framework. Therapeutic enzymes within the hydrophilic range are maintained or encapsulated inside the produced vesicles' inner watery region. A liposome that has catalytic activity in its intact state, that is, before it is degraded. The enzymes could be bound to liposomes by two approaches:-

- By reacting with the enzyme's hydrophobic anchoring molecules, such as long-chain fatty acids.
- By first connecting the enzyme to the phospholipid bilayer components of the liposome bilayer.

The enzyme is assimilated to the liposomal membrane in the first stage, and the docking

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between the liposome bilayer and the coupling reaction with the liposomal surface occurs in the second. Both the processes are having a difference in:

• The enzyme-liposome complex's stability.
• The number of enzyme molecules entrapped/displayed in the outer lipid bilayer.
• The nature of modified enzymosomes, which may dock to a variety of molecules, including phospholipids, long-chain fatty acids, and polymer-like compounds linked to phospholipids. The technique of production is usually selected based on the many therapeutic requirements that need enzyme administration mediated by enzymosomes.

The ligation of enzymes to the interior hydrophilic environment, i.e. native enzyme happens, as well as to the lipid bilayer membrane of liposomes via Ac-enzyme, is a critical problem. The enzyme in the interiors of the liposomes is not accessible for reaction while the liposomes are intact. However, when enzymes are directly linked to the lipid membrane, they are ready to catalyze even before the liposomes are destroyed. The process of acylation (adding of an acyl functional group) happens during the unification of hydrophilic enzyme to the acyl residual chains of lipids, culminating in the creation of Ac-enzyme. As a consequence, the enzyme is diverted from a hydrophilic to a hydrophobic microenvironment. The length and quantity of fatty chains attached to the enzyme's surface influence the degree of hydrophobicity achieved. The conservancy of the changed enzyme's other properties must be assessed utilizing standard conjugation methods. When the active site of the enzyme is inhibited by the substrate during conjugation, the enzyme L-asparginase, which is utilized to treat acute lymphoblastic leukemia, withholds 100% of its catalytic activity. Different methods are utilized to add a fine dosage of Ac-enzyme to the liposomal structure and enable appropriate enzyme release. Ac-enzyme is partially inserted into lipid bilayers or hidden inside the hydrophobic lipid vesicular matrix. The electrostatic interactions between the charges associated with enzymes play a major role in the attachment of Ac-enzyme to the lipid bilayer membrane. The ratio between catalytic values calculated during the intact life span vs disturbed enzymosomes is used to evaluate the efficiency of the Ac-enzyme integrated into liposomes. The size of the vesicle, content, ionic charge, and other perfect features of liposomes are extremely helpful during the creation of enzymosomes [10].

Acylated-SOD Liposomes

In general, SOD enzymosomes were formed as a homogeneous film by dispersing an aqueous solution containing SOD, and the non-bounded enzyme was then extruded using the ultracentrifugation separation technique. The produced enzymosomes were characterized by Gaspar MM et al's study effort, which used several criteria to guarantee that the enzymosomes were uniform in their properties. The different parameters included where;

• Dynamic light scattering was used to determine the average particle size (diameter) of liposomes.
• After disrupting liposomes with Triton X-100 and sodium dodecyl sulfate, protein coupling to liposomes was evaluated (SDS).
• Lipids and free amino acid groups were determined.
• Colorimetric test for phospholipids.
• Ac-SOD and SOD formulations were tested for enzymatic activity.
• The capacity of enzymes to slow down the autoxidation of epinephrine to adrenochrome was tested.
• To measure the overall enzyme activity inside the SOD or Ac-SOD enzymosome, a series of dilutions were first prepared to achieve a final protein concentration.
• The enzyme's enzymatic activity was evaluated when it was exposed to the external surface.
• The zeta potential was investigated.
• The strength of the electric field and the angle of dispersion were discovered.
• The thermotropic behavior of enzymosome membrane phospholipids was discovered.

Innovative methods for analyzing Ac-SOD and determining whether it was superior to ordinary SOD and other pharmaceutical preparations were given as the outcomes of different studies in chemical modifications of SOD:

• When compared to free SOD, the surpassing characteristics for Ac-SOD revealed a greater affinity towards the hydrophobic region of the liposomal bilayer.
• The effectiveness of Ac-SOD incorporation in SA-containing enzymosomes was compared to PEG-liposomes, which had a lower initial (protein/lipoprotein) ratio due to competition between Ac-SOD and cholesterol for phospholipid inclusion.
• The various electrostatic interactions indicated that positively charged SA-liposomes were

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beneficial because they decreased lipid-protein charged interactions owing to PEG at the lipid surface.

As a result, Ac-SOD demonstrated significant activity for the integrated enzyme, which operates independently of the rate and extent of enzyme release, resulting in a unique mode of action. Thus, the completed study shows that the proposed enzymosome has considerable potential, since the PEG-enzymosome transformed the substrate even in the presence of surface PEG chains. As a result, it was not a barrier, and the enzyme's release was not a need for dismutation activity at the inflamed location. If the Ac-SOD enzymosome could be made with circular micro-reservoirs, it would be much more than enough for expressing the enzyme's activity without interruption and for a long-term release pattern. Peptides and polymers undergo similar changes, forming a significant drug targeting route. As a result, the Ac-enzymosome may be a replacement therapeutic agent for rheumatoid arthritis with a strong effect and longer-flowing active particles for reperfusion diseases. SOD's affinity for negatively charged lipid molecules is a useful tool for investigating membrane structure and dynamics, and it accounts for at least part of its capacity to shield lipid membranes from oxygen-induced damage [11].

Designing of immuno-enzymosomes having enhanced enzyme targeting capability and cell binding properties

Because of the insufficiency of differentiation between normal and malignant cells, the effectiveness of conventional anticancer medicines for chemotherapy treatment is limited. After examining the research on immuno-enzymosomes, it was discovered that they may be utilized to target enzymes for site-specific activation of anticancer prodrugs. The value of developing a single well-represented liposomal system that can bind to a range of specific ligands is considerable. These techniques allow enzymes to be hidden inside tiny packet-like structures and then released when they reach the action site. When combined with immunoliposomes, the enzyme beta-glucuronidase, which was capable of activating anthracycline glucuronide prodrugs, was said to be effective against ovarian cancer cells (OVCAR-3). By cleaning the commercially available enzyme beta-glucuronidase (GUS), an immune-enzymosome formulation with a 2-fold increase in enzyme-specific activity when incubated with ovarian cancer cells may be created. The concept entails fusing a cell-specific antibody with a (liposome) immunoliposome encapsulating the chemotherapeutic agent, allowing for selective drug delivery and cell-specific cytotoxicity. As a consequence, novel therapeutic protein compounds with low immunogenicity are created. The antibody-enzyme complex was given, followed by an infusion of a non-toxic prodrug after the complex had bound to malignant cells and had been removed from the blood and tissues. The enzyme's activity targets the prodrug, which is transformed into an active cytotoxic compound inside the confluence of tumor cells, resulting in its selective eradication (**Figure 4**).

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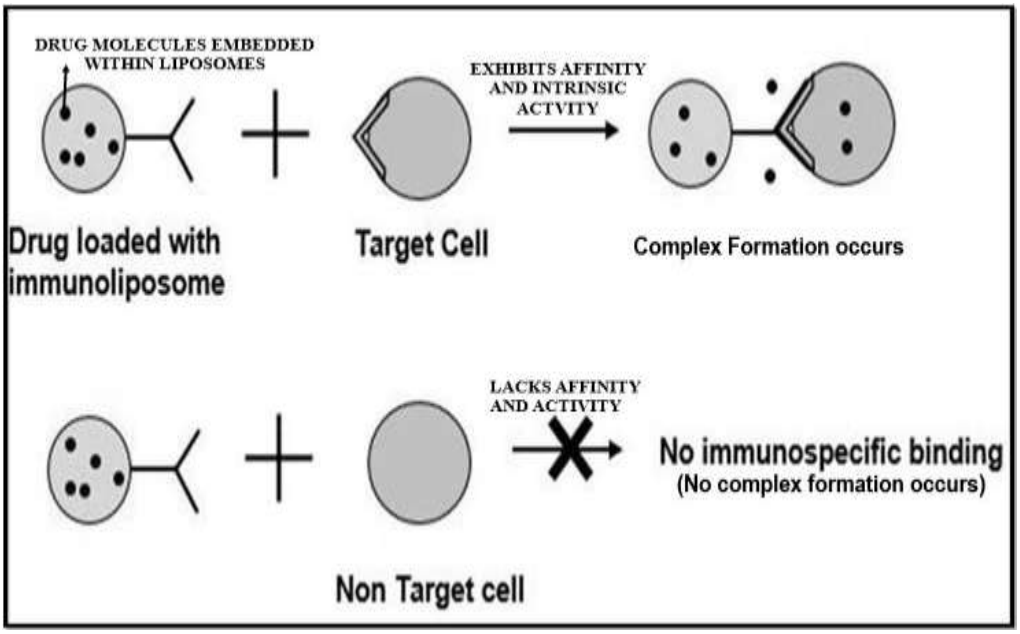

Figure 4. Specificity of immuno-enzymosomes to target cells.

Advantages

• More than one enzyme moiety could be admitted in one targeted carrier system.

• Enhanced enzyme density at the cancer cell surface, thus providing efficient conversion of the prodrug.

• The enzyme GUS was preferred due to its superiority over other enzymes, which were present localized intracellularly.

• They cause limited activation of hydrophilic glucuronide drugs since they have only low penetration. Thus, lowering the immunogenicity problem.

Because of its bulky steric hindrance, the GUS enzyme may occasionally interfere with cell coupling. However, investigations have shown that just raising the enzyme density on the surface may result in significant enzymatic targeting. The electrosome was employed in research by Szczupak et al. as a tool for the release and activity of a cascade of enzymes, overcoming the disadvantage of a low number of enzymes on the surface. The GUS was purified first, and then Fab' fragments were created. The enzymosome or immuno-liposome was created using this method, and then it was characterized.

For the most part, the evaluation of research activity was done to highlight some significant achievements in the area of nanoscience that may be utilized as a springboard for creating effective medication delivery systems for severe illnesses. The study's primary goals were to maintain maximal enzyme density in the cell environment while also quantitatively improving the enzyme without causing it to aggregate by experiencing further conformational changes, which affects the enzyme's effectiveness and selectivity. Purification of commercially available GUS enhanced its enzymatic activity twofold as the experiment progressed. Thus, reducing enzyme density up to the level of a steric barrier for interacting with a target antigen may be a viable approach for generating immuno-enzymosomes with maximal enzyme targeting capabilities. The immune-enzymosomes were most often used in the detection and treatment of ovarian carcinomas. Even in the early stages, cancer cells have the potential to move to the peritoneal cavity. Surgical excision of the tumorous mass, followed by chemotherapy, is performed on a regular basis. As a result, for a complete cure with the total removal of leftover debris that might otherwise cause additional harm to normal cells. The findings show that by injecting the immuno-enzymosomes prepared intravenously (i.p.) for antibody–directed enzyme prodrug treatment, the peritoneally administered drug may readily reach the cells where the enzyme needs to attach (**Figure 5**) [12].

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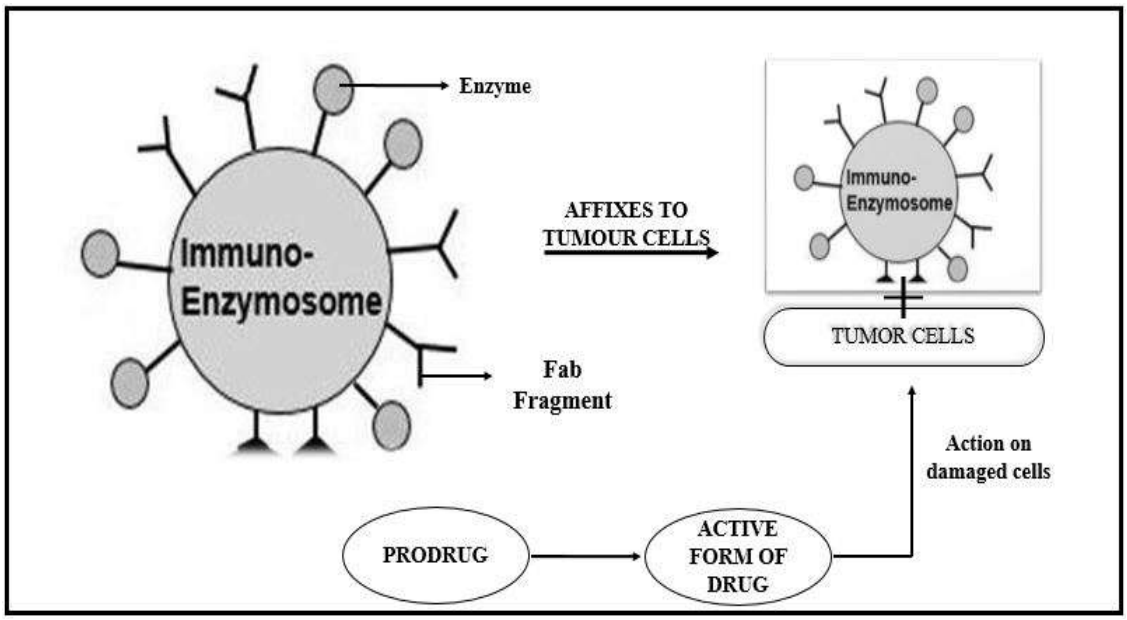

Figure 5. Enzyme bound drug targeting tumor cells.

Production alkaline enzymosomes loaded with Bacillus fastidious

Tan Q et al. conducted research to assess the potential of a new alkaline enzymosome for delivering the enzyme Uricase from Bacillus fastidious (UBF), as well as to enhance its biochemical activity, therapeutic usage, and pharmacological properties. Uricase has a typical physiological function of catalyzing uric acid via an oxidation process to decrease plasma uric acid levels. Studies have shown that UBF loaded in new alkaline enzymosomes (ESUBFs) required less time than free UBF to reduce plasma uric acid concentration to normal levels from higher levels. Their findings revealed that ESUBFs transport UBF with ease, boosting its total area under the curve (AUC), half-life, and catalytic activity while also changing its biochemical and pharmacological properties. As a result, ESUBFs may be a good second-line treatment for gout and hyperuricemia. The added benefit of increased in vivo uricolytic activity may have significant therapeutic implications since the clinical dose provided as well as the adverse effects generated during the usual dosage of UBF formulation could be greatly decreased via conjugation. The creation of new alkaline UBF enzymosomes was profitable created to enhance the pharmacological, biological, and biochemical characteristics of the enzyme uricase from B. fastidious, and exploited for hyperuricemia effects. The oral administration of uricase enzyme was shown to decrease uric acid levels in a hyperuricemia rat model by Szczurek et al. Uricase enzyme had certain benefits, such as increased selectivity and efficient, rapid reactivity, but it also had some drawbacks, such as a short in vivo half-life and restricted therapeutic use. The primary goal of encapsulating an enzyme in an enzymosome is to control its distribution while still maintaining its molecular structure. The catalytic activity of UBF loaded in new alkaline enzymosomes (ESUBFs) in vitro was nearly three times that of free UBF at the optimal pH, according to the study. In the research model, ESUBFs had a substantially larger area under the plasma concentration (AUC) and a longer circulation half-life ($t_{1/2}$) than free UBF after intravenous (i. v.) injection, comparable to findings by Xiong H et al. The preliminary security evaluation actively shown that ESUBFs were acceptable when administered through the parenteral mode of administration. It is indicated that they might be utilized in an emergency to treat enzyme deficiency disorders. The systematic research trends show that alkaline enzymosomes' encapsulated biochemical and pharmacological characteristics have been optimized. As a result, enzymosomes may be a better option for the formulation of UBF and ESUBFs, which may be a better option for treating hyperuricemia and gout [13].

Antiplatelet activity of CD 39 enzymosomes

The body's endothelial cells contain the enzyme CD39/NTPDase-1, which is expressed on

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the cell's opening side. It possesses the physiological capacity to quickly digest ATP and ADP, as well as AMP, while reducing platelet sensitivity to the main agonists. As a result, there was an impetus to investigate therapeutic anti-platelet 'enzymosomes' formulations including CD39 embedded inside liposome lipid bilayers. Initially, CD39 enzymatic activity was optimized, which seems to be reliant on either of its transmembrane domains being expressed. A yeast expression system was used as a model to produce full-length human CD39, which was purified and reconstituted inside an appropriate lipid vesicle. The dephosphorylation and production of ADP and ATP were used to evaluate the catalytic effectiveness of detergent-solubilized CD39 as well as when it was reconstituted inside a lipid membrane. Platelet aggregometry was used to determine the efficacy of CD39-containing lipid vesicles to suppress platelet activation caused by ADP, collagen, and thrombin in vitro. The efficacy and therapeutic application of intravenously given CD39 enzymosomes in reducing platelet consumption and mortality were investigated using a mouse model of thromboplastin-induced thromboembolism. The restoration of human CD39 in lipid vesicles resulted in a roughly one-order decrease in the Km value, as well as an increase in both ADPase and ATPase catalytic efficiency. Platelet activation by ADP, collagen, or thrombin was efficiently suppressed by CD39 lipid vesicles, which effectively prevented platelet aggregation and generated a platelet disaggregation response when platelets were stimulated. Treatment with CD39 lipid vesicles significantly reduced the decrease in platelet counts induced by thromboplastin, according to research. When compared to its solubilized counterpart, incorporating the enzyme into a lipid bilayer substantially increased CD39 enzyme activity. As a result, investigations showed that CD39 enzymosome therapy reduced platelet consumption and mortality in an animal model. CD39 enzymosomes produced in this manner may be a helpful therapeutic alternative for supplementing other anti-platelet treatments that cause platelet thrombus formation [14].

Generation of streptavidin-liposomal conjugates for targeted ligand-specific applications

Streptavidin, a tetrameric biotin-binding protein isolated from Streptomyces avidinii, has a low level of nonspecific binding in immunohistochemistry. As a result, it's a must-have in a variety of detecting systems. It is a member of the avidin family of antimicrobial proteins, and its particular interaction with biotin molecules makes it helpful in nonradioactive detection methods. Biotin is a vitamin that is needed by live cells for a variety of biological activities, including cell development. The biotin tag was formerly utilized to aid the process of affinity purification of molecules utilizing immobilized biotin-binding protein when biotin was added to a molecule. Thus, the avidin-biotin interaction is used in ELISA, immunohistochemistry (IHC), cell-surface labeling, and fluorescence-activated cell sorting (FACS), among other applications. Streptavidin linked to liposome results in a well-characterized protein-liposome conjugation under ideal circumstances. The resultant targeted vesicle system has greater activity in relation to its size and binds to biotinylated targeting ligands more securely. In recent years, there has been increased interest in combining natural circulating cells with enzymes for medication delivery. Streptavidin is non-covalently linked to biotin, a vitamin, and phosphatidylethanolamine, a phospholipid family, in the research. Because of its main function in dissipating the negative charge generated by anionic membrane phospholipids, the phospholipid was selected. The sample was prepared using the extrusion technique, which included dissolving the lipid combination in a solvent, coating it over a tube, and drying it to a thin film using a stream of suitable gas under a high vacuum. The biotinylation process involves the covalent attachment of biotin to a protein or nucleic acid. Biotin in conjunction with streptavidin, a member of the avidin family, has a high affinity and quick action, making it useful for separating biotinylated molecules of interest in various areas of biotechnology. The liposome vesicles were treated with streptavidin to achieve binding, and optimum coupling effectiveness was achieved when a consistent ratio of streptavidin to lipid molecules was maintained. Indirect targeting methods make use of the excellent communication between streptavidin and biotin-containing lipid molecules. Other pharmacological discoveries, such as photoaffinity, are utilized as additional methods for molecular interactions and binding site targets through light activation. The covalent binding of streptavidin to two lipid derivatives containing thiol groups to start the reaction was another technique explored in research. The presence of

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long-spacing reactive arms improved the cross-connection of maleimide derivatives of lipids in the study described in the reference paper. The enzyme is then attached to these liposomes and tailored to take advantage of its capacity to conjugate in a particular way with membrane-linked antigens, resulting in a variety of nanocarriers with useful physicochemical properties for drug administration. The biotinated antibody was combined with enzyme-linked liposomes, which has a broad range of uses in vivo and in vitro. The preservation of hydrophilic medicines and fluorescent groups in water-loving compartments of targeted liposomes for cell surface action is aided by such applications. Small-size conjugates have a longer half-life and therefore maintain their capacity and activity longer in the plasma, altering the severity of the infection and acute reactions [15].

Enzymosome offers a small bioenvironment by covalently immobilizing or coupling enzymes to the surface of liposomes. To the tumor cell, a targeted medication delivery mechanism is used. Enzyme-containing prodrugs conjugates are useful and valuable for targeting cells in tumor-like disorders. In certain instances, administering medicines or prodrugs to the body through blood circulation may result in some pharmacological activity failure. In addition, administering these medicines directly to target cells is difficult. That is why, by combining enzyme conjugates with prodrugs (**Figure 6**), it is possible to reduce the failure of target cell treatment while also increasing the utilization of complete medicine through appropriate enzyme activity. As a result, in the coming years, such enzyme-containing life-threatening drugs may be developed for the treatment of cancerous diseases.

FUTURE PERSPECTIVES

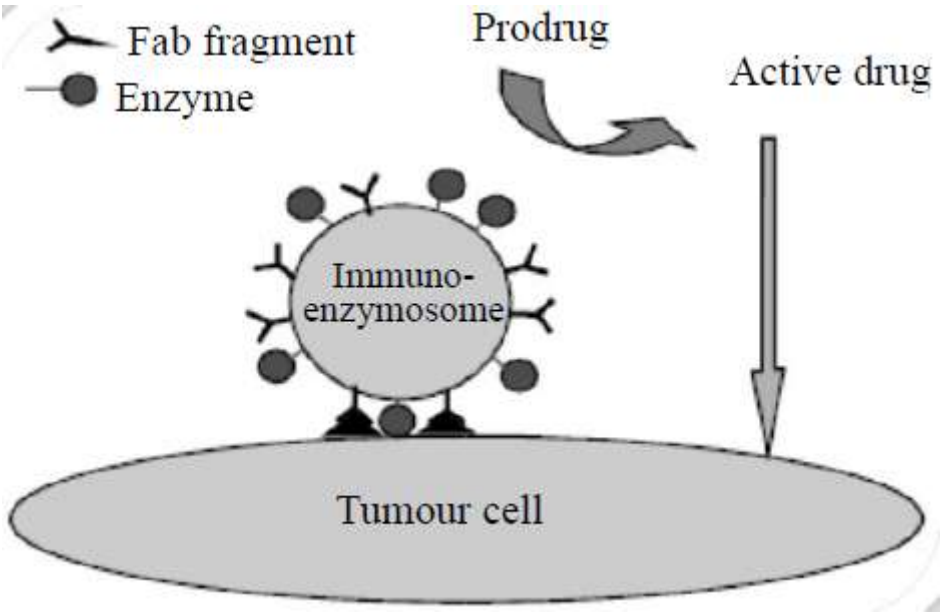

Figure 6. Prodrug converted into an active drug by enzymes.

Enzymosomes are specially designed lipid structures that provide a favorable microenvironment for the enzyme to be sequestered inside lipid structures or covalently linked to the lipids' exterior surfaces. Its main use is to make penetration easier as well as to deliver targeted drugs to tumorous cellular locations. These vesicular systems provide a great deal of versatility in medication development, allowing for the elimination of a variety of adverse effects and bioavailability issues. The use of nanosystems to transport medicines to the CNS across the BBB is a potential lead for improving treatment approaches for many types of cancers. The technique delivers various prodrugs, as well as charged proteins and complexes, to the brain, raising the drug concentration linearly. Despite the fact that various carrier nuclei have drawbacks such as oxidative stress damage, they play an important role in medication selection and targeting, as well as bringing a fresh origin to traditional pharmacological treatments. The newest creative developments in targeted medication delivery include biotinylation and pegylation. For prolonged

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drug release and cellular targeting, a variety of lipoidal and non-lipoidal vesicular carrier nanoparticles are used. Enzymosomes, like ethosomes, transferosomes, pharmacosomes, virosomes, and non-lipoidal ones like niosomes, aquasomes, and others, are lipid bio carriers. Various investigations have shown that each carrier is effective in its activity. PEG-SOD enzymosomes were shown to have an acceptable effect in CA Valeetal's tests, indicating that they may be utilized therapeutically. When opposed to free supplements, liposomes are now becoming clinically recognized since they keep toxicity to a minimum and provide increased effectiveness. When compared to free medication, Nair et al. demonstrate that emulsomes are proven carriers for loading low water-soluble medicines and therefore variable bioavailability. Enzymosomes may therefore serve as a reliable carrier nucleus in the development of a new generation of adaptable drug design systems in the future [16].

II. CONCLUSION

The various strategically authorized techniques are being used to improve the better uses of carrier-mediated protein delivery. The therapeutic enzymes are incorporated into carriers of the polymeric nature of lipid vesicles' hydrophilic region and hydrophobized forms assimilated to lipid bilayers of vesicles. Nothing in the preceding list can completely maintain the therapeutic protein's action. Aside from therapeutic enzymes, another way of strategy is its connection to the outer surface of liposomes, through which scientific know-how for an antibody with drug production was created. Enzymosomes were formed when the enzyme was complexed with lipid nature carriers. The preservation of enzymatic activity and the restriction of structural integrity are two attractive features of enzymosomes. The idea of encapsulating the medication in lipid vesicles to allow for more precise drug targeting to the appropriate tissue terminal is generally recognized. Different deformable and fatty, stiff supramolecular vesicular constructions are clearly capable of delivering medicines for targeted administration of various bioactives. Several studies have been conducted in recent years to accomplish the objective, with the goal of improving targeting and reducing dosage frequency. These may be synthesized in semi-liquid or liquid drug delivery systems with a matched lipid composition to achieve these goals. From a topical level, the new vesicular systems actively showed their therapeutic potential to genetic levels. Using supramolecular chemistry concepts, an enzymosome, and a new vesicular system may acquire the characteristics described.

CONFLICTS OF INTEREST

No conflict of interest is declared.

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REFERENCES

[1]. Vingerhoeds MH, Haisma HJ, Belliot SO, Smit RH, Crommelin DJ, Storm G. Immunoliposomes as enzyme-carriers (immuno-enzymosomes) for antibody-directed enzyme prodrug therapy (ADEPT): optimization of prodrug activating capacity. Pharm Res. 1996;13(4):604-10.

[2]. Vale CA, Corvo ML, Martins LC, Marques CR, Storm G, Cruz ME, Martins MB. Construction of Enzymosomes: Optimization of coupling parameters. Nanotech. 2006;6:7-11.

[3]. Corvo ML, Marinho HS, Marcelino P, Lopes RM, Vale CA, Marques CR, Martins LC, Laverman P, Storm G, Martins MB. Superoxide dismutase enzymosomes: carrier capacity optimization, in vivo behaviour and therapeutic activity. Pharm Res. 2015;32(1):91-102.

[4]. Hundekar YR, Nanjwade BK, Mohamied AS, Idris NF, Srichana T. Nanomedicine to tumor by enzymosomes. J Nanotechnol Nanomed Nanobiotechnol. 2015;2:004.

[5]. Gaspar MM, Martins MB, Corvo ML, Cruz ME. Design and characterization of enzymosomes with surface-exposed superoxide dismutase. Biochimica Biophysica Acta. 2003;1609(2):211-7.

[6]. Tan Q, Zhang J, Wang N, Li X, Xiong H, Teng Y, He D, Wu J, Zhao C, Yin H, Zhang L. Uricase from Bacillus fastidious loaded in alkaline enzymosomes: enhanced biochemical and pharmacological characteristics in hypouricemic rats. Eur J Pharm Biopharm. 2012;82(1):43-8.

[7]. Vingerhoeds M, Haisma HJ, Van Muijen M, Van de Rijt RB, Crommelin DJ, Storm G. A

new application for liposomes in cancer therapy: Immunoliposomes bearing enzymes (immuno-enzymosomes) for site-specific activation of prodrugs. FEBS Lett. 1993;336(3):485-90.

[8]. Tan QY, Zhang JQ, Wang N, Yang H, Li X, Xiong HR, Wu JY, Zhao CJ, Wang H, Yin HF. Improved biological properties and hypouricemic effects of uricase from Candida utilis loaded in novel alkaline enzymosomes. Int J Nanomed. 2012;7:3929-38.

[9]. Shefrin S, Sreelaxmi CS, Vijayan V, Nair SC. Enzymosomes: a rising effectual tool for targeted drug delivery system. Int J Appl Pharm. 2017;9(6):1-9.

[10]. Gaspar MM, Boerman OC, Laverman P, Corvo ML, Storm G, Cruz ME. Enzymosomes with surface-exposed superoxide dismutase: in vivo behaviour and therapeutic activity in a model of adjuvant arthritis. J Contr Rel. 2007;117(2):186-95.

[11]. Chaikof EL, Haller CA, Cui W, Wen J, Robson SC. CD39 enzymosomes inhibit platelet activation in vitro and in vivo. J Surg Res. 2006;130(2):234-5.

[12]. Skólmowska M, Kmieć M. Antioxidant enzymosomes - properties and application antioxidant enzymosomes - properties and application. Postepy Hig Med Dosw. 2011;65:640-4.

[13]. Fonseca MJ, Haisma HJ, Klaassen S, Vingerhoeds MH, Storm G. Design of immuno-enzymosomes with maximum enzyme targeting capability: effect of the enzyme density on the enzyme targeting capability and cell binding properties. Biochimica Biophysica Acta. 1999;1419(2):272-82.

[14]. Zhou Y, Zhang M, He D, Hu X, Xiong H, Wu J, Zhu B, Zhang J. Uricase alkaline enzymosomes with enhanced stabilities and anti-hyperuricemia effects induced by favorable microenvironmental changes. Sci Rep. 2016;6 (1):1-5.

[15]. Storm G, Vingerhoeds MH, Crommelin DJ, Haisma HJ. Immunoliposomes bearing enzymes (immuno-enzymosomes) for site-specific activation of anticancer prodrugs. Adv Drug Deliv Rev. 1997;24(2-3):225-31.

[16]. Marcelino P, Marinho HS, Campos MC, Neves AR, Real C, Fontes FS, Carvalho A, Feio G, Martins MB, Corvo ML. Therapeutic activity of superoxide dismutase-containing enzymosomes on rat liver ischaemia-reperfusion injury followed by magnetic resonance microscopy. Eur J Pharm Sci. 2017;109:464-71.