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Enzymosomes: Novel Targeted Enzyme Delivery System

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ABSTRACT

The purpose of the study is to develop enzymosomes as a novel site-specific medicine delivery technique. Enzymosomes take use of an enzyme's unique properties, which include the ability to bind to a specific substrate at a controlled rate and catalyse the product synthesis process. When an enzyme is covalently bonded to the surface of liposomes/lipid vesicles, enzymosomes are generated. Enzymes are connected via acylation, direct conjugation, physical adsorption, and encapsulation strategies to create enzymosomes with customized activity. Such innovative drug delivery systems exhibit effective drug release while decreasing the unfavorable side effects of previous treatment methods, leading in better long-term illness therapy. They might be a viable option to gout treatment, antiplatelet therapy, and other conventional therapies. Enzymosomes are supramolecular vesicular delivery methods that have recently been created and may enhance medication targeting, physicochemical properties, and hence bioavailability in pharmaceuticals. It illustrates that drugs with a narrow precision have good benefits because targeting their site of action improves their overall pharmacodynamic and pharmacokinetic profile. It also improves half-life and achieves enzyme activity on particular places, such as malignant cells, by reducing alterations in normal enzymatic activity.

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

INTRODUCTION

A targeted system in which entities are led to specific regions of activity is a novel way to medicine administration. NDDS are employed in a range of industries, including pharmaceuticals, food, and cosmetics. It accomplishes and overcomes the limitation of standard drug delivery systems, namely, delivering the whole active moiety of the treatment to the site of action. It also carries the drug via the right channels in the body at a rate regulated by the body's requirements. Vesicular carriers are the current vehicle of choice for most drugs with bioavailability concerns, as well as cells and genetic engineering, diagnostic testing, and immunological approaches. Vesicular drug delivery helps to keep medication release at a constant rate, lowering the risk of toxicity and making it effective in the treatment of ocular illnesses. These vesicles are easy to utilise and can store both hydrophilic and lipophilic medications. The majority of carriers feature arranged concentric congregates of lipid bilayers in which various sites are employed to encapsulate hydrophilic and

lipophilic medications, resulting in an amphiphilic character. The vesicles were initially disclosed in 1965 by Bingham, who referred to them as Bingham bodies. The novel carriers localise their action via spatial induction of pharmaceuticals near the ill organ or tissue, notably by chemical derivatization. In investigations, many drugs for glaucoma, ulcerative colitis, colon diseases, NSAIDS, and insulin-like therapy were shown to have enhanced bioavailability and effect duration. Vesicular medication distribution reduces treatment costs and enhances pharmacodynamics, especially for drugs that are poorly absorbed. It might mark a turning point in classical chemotherapy, when drug penetration and cell permeability were severely limited. The procedure uses a proven mechanism for delivering treatment to the infection location while limiting adverse effects. Based on their composition, lipoidal and non-lipoidal carriers are two kinds of vesicular drug delivery methods. Lipoidal carriers include sphingosomes, transferosomes, liposomes, emulsomes, and virosomes, whereas non-lipoidal carriers include aquasomes, niosomes, and bilosomes. Lipid particles (including low-

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ISSN: 2231-2188 (Print)

ISSN: 2231-685X (Online)

Received: 10.10.2017

Revised: 15.11.2017

Accepted: 25.12.2017

Published: 05.04.2019

and high-density lipoprotein), nanoparticles, colloidal transfer systems, and polymeric micelles, as well as other pharmacological carriers such cellular macromolecules, are proving to be useful tools for targeted drug delivery¹.

CELLS TO VESICLES

Internal organs, tissues, nucleic acid, and genetic resources for reproduction and replication make up “somes,” a kind of biological cellular structure. The cell membrane that surrounds the cell’s outer sections in mammalian cells is a phospholipid bilayer. Because of the two-layered structure of lipid molecules, physiological nature is largely lipophilic. Lipoidal medications and proteins may easily permeate the body’s membranous barriers due to their similarities to the cells that surround every organ. A vesicle is a cell membrane-like collection of supramolecular lipid molecules. Vesicles are intracellular secretory transport vesicles. Endocytosis permits cells to take in exogenous vesicles, which lysosomal enzymes subsequently break down into smaller pieces. The review focuses on the utilisation of these vesicles for targeted and site-specific drug delivery of both hydrophilic and lipophilic drugs. Enzymes are proteins that fold into different shapes to accommodate smaller molecules. Enzymes are biological catalysts that accomplish their goals by interacting with a substrate molecule. In order to carry out a reaction at a substantial rate, every cell needs an enzyme to convert these reactants to product molecules. Enzymes are principally responsible for selecting the cell’s metabolic pathway since they are exceedingly particular and selective. Enzymes are site-specific and have a lock-and-key interaction with the substrate. The degree to which the enzyme binds to the substrate determines the reaction response. This selectivity of enzymes is used by combining enzyme specificity with the notion of vesicular drug delivery².

ENZYMOSOMES

Enzymosomes (Figure 1) are a new kind of tailored vesicular drug delivery system currently under development. Enzymosomes are enzymes that are incorporated into cell-like structures with a strong lipid background and have a specialized catalytic function for a substrate. They make newly designed liposomes with enzymes covalently linked to the surfaces of the lipid molecules. The liposomes were created in this fashion to establish an appropriate milieu for the enzymes within to be inhibited. Liposomes are micro-sized vesicles containing a lipid bilayer surrounded by an aqueous environment. The lipophilic pharmaceuticals are incorporated into the phospholipid-cholesterol lipid bilayer membrane, while the hydrophilic medicines may be dissolved in the interior watery compartment. Lipid-based drug

delivery systems have unique properties such as lowering drug distribution volume, interrupting drug clearance, and altering drug distribution with increased capillary permeability towards infected tissues while reducing toxicity in healthy tissues, proving to be an effective nanoscale drug delivery system for clinical use.

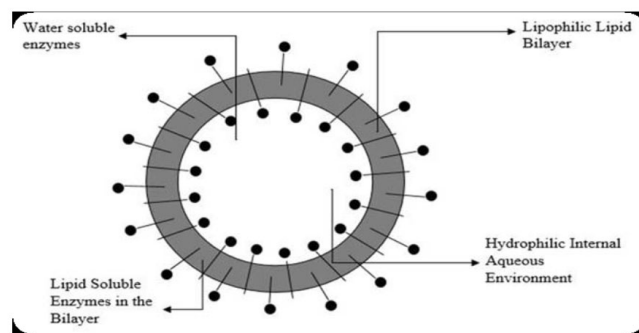


Figure 1: Structure of Enzymosomes.

Catalysis, site-specific pharmacological action, and the activation of prodrugs are only a few of the functions of enzymes (Table 1). If an enzyme is encased on the liposome’s surface, however, because of their restricted lipid membrane penetrability, the enzyme’s breakdown and transmutations are decreased, enhancing its half-life and concentrated activity. Enzymosomes are a novel kind of vesicular rate-directed drug delivery system that delivers the active form of the drug to the site of action while rapidly degrades it for easy absorption. Enzymes may be attached to the liposome surface in two ways: by linking functional hydrophobic compartments with the enzyme, such as long-chain fatty acids, or by associating the enzyme with the phospholipids in the liposome layer. The customized technique provides for better therapeutic efficacy and fewer unwanted effects by conveying the therapeutic medicine to the desired tissue receptors, especially those situated on an organ or system. Such modified vesicles increased the solubility, stability, and therapeutic index of the coated medicinal molecule. These lipid nanocarriers function as natural attractants of BBB due to their lipophilicity, making them effective in the treatment of CNS illnesses like as epilepsy and convulsions. The therapeutic enzymes are supplied through polymeric carriers such as liposomes and lipoplexes, with the optimum outcomes achieved by attaching enzymes to exposed portions of liposomes. A drug-loaded vesicular delivery system delivers precise results at the site of infection or inflammation with little drug toxicity and side effects, making it useful for centrally acting drugs that must pass through the BBB, which is crucial to the brain’s homeostatic function. It also helps to enhance medicine bioavailability, or the least amount of drug concentration accessible for systemic circulation, by decreasing the purchase cost. The covalently linked enzyme and liposome will have minimal changes in enzyme activity

when tested *in vitro* and *in vivo*, and the enzyme placed in a vesicle will preserve its structural integrity and enzymatic function. Enzymosome design advances have a broad

variety of applications, including the development of new recombinant proteins and biotechnological products³.

Table 1: Enzymosomes-based prodrug activation systems.

Antigen/Antibody	Active drug	Enzyme	Prodrug
MAB BW _{431/26} /Carcinoembryonic Antigen (CEA)	Etoposide	Alkaline phosphatase	Etoposide phosphate
MAB L6 (against a carbohydrate antigen on human carcinomas)	Doxorubicin Melphalan	Penicillin amidase	Doxorubicin-phenoxyacetamide
MAbs W _{14A} and SBIO/chorionic onadotropin	Benzoic acid mustards	Carboxypeptidase G ₂	Benzoic acid mustards-glutamic acid
MAB 323/A ₃ (against a pan-carcinoma membrane glycoprotein)	Epirubicin	β-Glucuronidase	Epirubicin-glucoronide
MAB KS _{1/4} /UCLA-P ₃ human lung adeno-carcinoma	Methotrexate	Carboxypeptidase A	Methotrexate-alanine

ADVANTAGES OF ENZYMOSOMES

- Protects sensitive tissues from dangerous pharmaceutical exposure.
- Encapsulation and stability have been improved.
- Pharmacokinetics improvements (Increase half-life, reduction in elimination).
- It combines with site-specific ligands for active drug targeting.
- The therapeutic index and efficacy have both improved.
- The material is biodegradable.

Both Systemic and non-systemic given dosages are non-immunogenic.

- On-the-fly changes to electrical properties are possible.
- It is a non-toxic substance.
- To the greatest degree practicable, biodegradable.
- The site's restricting effect.

DISADVANTAGES OF ENZYMOSOMES

- Because liposomes are categorized as nanotherapeutics, they are often costly to produce.
- The phospholipids that make up lipid vesicular structures are prone to oxidation and hydrolysis.
- It is poorly soluble and has a short half-life, lowering bioavailability.
- The medication molecule or molecules that are encapsulated fusion and leak.

LIPID-BASED DRUG CARRIERS

Lipid-based chemical systems are used to make oral, parenteral, and topical dosage forms, which is a well-known approach. Safety and efficacy have become hallmarks of

such systems, making them desirable for formulations, diagnostics, and vaccine preparations. It is customised to meet a range of demands by considering cost, toxicity from treatments such as ultrasound, efficacy, and stability, as well as sickness indications. The advantages of such systems are extensive:

- Improved bioavailability and fewer variations in plasma concentration.
- Lipophilic excipients are better characterized and adapted.
- Ability to recognize critical issues in technology transfer and industrial scale-up.

Vesicular systems include liposome-based vesicular carriers, lipid particle systems, and emulsions. Liposome carriers may carry modified liposomes, marinosomes, phytosomes, and transferosomes. Biocompatible lipid microparticles and lipid nanoparticles that have been produced in recent years are referred to as the lipid particulate system. They are helpful because they allow for regulated drug release, a solid grid of stable lipids, physicochemical compatibility, and the protection of the active component from degradation. Improved drug delivery strategies such as solid-lipid nanoparticles, lipid drug conjugates, and other nanostructure lipid carriers have recently been identified that surpass polymer nanoparticles in terms of cytotoxicity. Many investigations on semisolid solid lipid nanoparticles for topical drug delivery have been undertaken, with typical semisolid to dispersion systems being employed. Emulsions include Pickering emulsions, nanoemulsions, SLNs, and self-emulsifying delivery techniques. The preservation of enzymatic activity and the structural integrity of vesicles are crucial aspects of enzymosomes, which are accomplished via ligating enzymes with lipid carrier molecules⁴.

PHARMACOKINETICS

Data collected at different time intervals following nanomedicine delivery in animal models allows for a full pharmacokinetic and pharmacodynamic analysis of the nano-agent *in vivo*. To perform PK/PD assessments, WinNonlin data management, a statistics, modelling, and visualisation application for PK data analysis, is employed. The concentration of a drug over time, the Area Under the Curve (AUC), the elimination half-life, clearance, and the time of maximum concentration are all metrics that are often reported for a Nanomedicine⁵.

CHARACTERIZATION

Because of the small size, complexity, and-in most cases-heterogeneity of dispersions, appropriate physicochemical characterisation of the final formulation is crucial. The primary areas of interest (nanoparticle core, interface additional structures, precipitated drug, etc.) are the physicochemical state, size and size distribution, surface characteristics of nanoparticles, shape of nanoparticles, existence of extra colloidal structure (e.g., due to an excess of stabiliser), and drug localization⁶.

Size and zeta potential

The size and surface properties of nanoparticles are the most important aspects since they are the key determinants of the drug carrier system's effectiveness *in vivo*. The most common method for determining the size of colloidal particles is photon correlation spectroscopy (PCS), but methods based on static light scattering (laser diffraction with sub-micron range instrumentation) can provide additional information about particles in the micro-range and size distribution. Another approach that isn't typically used is the Asymmetrical Flow Field-Flow Fraction (A4F) in conjunction with multi-angle light scattering. This approach may offer exact information on the size distribution since the nanoparticles are separated before being measured.

Photon Correlation Spectroscopy (PCS)

Photon correlation spectroscopy, also known as Dynamic Light Scattering (DLS) or Quasi-Elastic Light Scattering (QELS), is a quick and accurate way to determine colloidal size (between 5 nm and 1 μ m). PCS is a time-dependent measurement of the intensity fluctuations of scattered light generated by particle motion. The Stokes-Einstein equation and the diffusion coefficient of the particles in the measurement fluid may be used to determine particle diameter.

Laser diffraction with sub-micron equipment

Laser diffraction, which measures the intensity of scattered light as a function of angle, is the most used technique

for estimating the size of SLN in addition to PCS (Static light scattering). Traditional laser diffraction (Fraunhofer diffraction) can only measure particles larger than the wavelengths of the laser light employed (normally 633 nm).

Asymmetrical Flow Field-Flow Fraction (A4F)

Asymmetric flow field-flow fractionation was used to measure size. The variances in size and elution profiles were attributed to differences in particle-shapes. Because of their anisometric, platelet-like shape, cross-flow is more likely to preserve formulations than spherical emulsion droplets. This methodology seems to have a lot of promise as a means for determining additional sizes, particularly in terms of colloidal structure separation and detection.

Zeta potential

Zeta potential investigations may provide information on the surface charge of nanoparticles. A sufficiently high zeta potential enhances the stability of electrostatically stabilized nanoparticles by increasing particle repulsion via electrostatic forces. This requirement, however, cannot be strictly followed for formulations stabilized with polymers that result in steric stabilization.

STABILITY STUDIES

Free catalase's thermal stability is related to its concentration. The stronger the heat stability, the higher the enzyme content, from 0.25 mg/ml to 5.0 mg/ml. This means that the dissociation of the enzyme into its subunits is the most important factor in its deactivation. Catalase at its highest concentration, 16 mg/ml, is less stable than catalase at 5.0 mg/ml. The 16 mg/ml of catalase aids the creation of irreversible intermolecular clumps among the conformationally altered enzyme molecules. Importantly, at a dosage of 16 mg/ml, the thermal stability of liposomal catalase (CAL100-III) is much higher than that of the free enzyme. This is because in the liposomal aqueous phase, the interaction of the inner surface of the liposome membrane with the encapsulated enzyme molecules precludes the formation of enzyme aggregates. As a consequence, the functional carrier liposomal system stabilizes the structure and activity of the liposome-encapsulated catalase molecules⁷.

REGULATORY PERSPECTIVES

Engineered nanosystems for illness diagnosis, prevention, and treatment have grown in popularity as a result of significant developments in a variety of sectors. This has been used to the development of things that are both efficient and safe. Even if some of those nanoproducts have made it to market, a portion of the scientific community has yet

to 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, in recent years, substantial progress has been made, demonstrating that regulatory authorities are aware of the specific qualities associated with nanosystems-based drugs. As a consequence, substantial issues have arisen in the regulatory environment for those novel medicinal nanosystems, presenting an opportunity to provide clear guidance for their development.

APPLICATIONS OF ENZYMOSOMES

Enzymosomes are a kind of lipid nanoparticulate drug delivery system comprised mostly of phospholipids structured in a bilayer form that can contain any chemical, independent of solubility, electric charge, or molecular weight, and hence improve GIT absorption and oral bioavailability. Enzymosomes can be loaded onto lipid-based nanocarriers like liposomes and solid-lipid nanoparticles, inorganic nanocarriers like gold nanoparticles and magnetic nanoparticles, polymeric nanocarriers like nanogels and micelles, and protein-mediated nanocarriers like super positively charged proteins, among others. Since the cell membrane has been used as a target for therapeutic intervention, ether and alkylphospholipids provide a current collection of pharmaceuticals that do not interact with DNA. Clinical investigations have demonstrated that they are effective in the treatment of metastases, breast cancer, anti-inflammatory action, and other disorders ⁸.

Preparation of enzymosomes of surface-exposed superoxide dismutase

Despite the difficulty of directly attaching enzymes to the lipids of liposomes, it is presently used as a convincing strategy in the treatment of immune-mediated diseases or antibodies. SOD (Super Oxide Dismutase) is an example of a therapeutic enzyme that has been directly conjugated. Cu, Zn-superoxide dismutase (SOD) has been discovered to be a natural defense mechanism that decreases the teratogenicity of damaging free radicals. It causes the damaging superoxide radical anion O_2^{2-} to dismutate into O_2 and H_2O_2 , interrupting a number of metabolic inflammatory pathways that the free radical has started. Because it avoids the side effects of non-steroidal anti-inflammatory medicines, SOD seems to be a viable alternative to standard anti-inflammatory therapy. The enzyme was not clinically acceptable due to its restricted characteristics, short circulation half-life, and minimal cell penetration. Many studies were undertaken to enhance the substrate on which the enzyme was loaded, as well as clinical trials in specific risk categories such as obesity, Type-2 diabetes, and so on. To far, the most prevalent method for

intracellular SOD administration has been to target protein to cell-penetrating peptides (CPPs) or protein transduction domains (PTDs). Regardless of the practical advantages of enzyme transduction technology, the major emphasis of this technique is on inefficient egress from the endosome to the cytosol, which results in CPP-tagged payloads being trapped in intracellular vesicles. Proteins are required for biological processes, and modern encapsulation techniques have made it feasible to control the distribution of these peptides, potentially enabling for their use in the treatment of a range of diseases. Using the physical adsorption approach, the driving electrostatic force is employed to generate spontaneous binding. Nanoparticles may be collected from a variety of materials and organised into desired geometries and combinations, acquiring useful functions and properties in the process. Chemotherapy treatments have a number of drawbacks, including the inability to reach the disease's core. As a consequence, nano-sized polymeric carriers may selectively and accurately deliver medications to cells (Figure 2) [9].

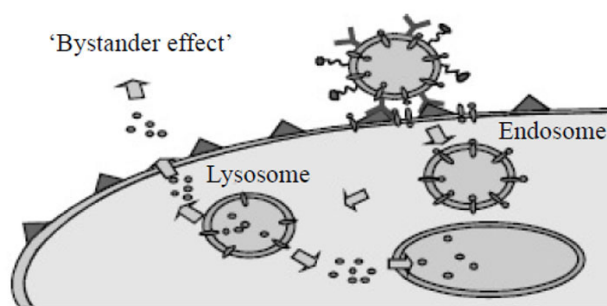


Figure 2: Bystander killing effect.

Construction of nanocarriers

Carbon nanotubes and cross-linked nanogel matrices are used to create polymer colloids that incorporate nanocarriers. The productivity of these systems is influenced by biological factors as well as the intermolecular force of attraction. A nanocarrier must be able to carry medications to the active site without deactivating them, the drug must be released according to kinetic laws, the drug must be stable after administration, and the drug must be given actively and with site-specificity (Figure 3). The molecules were modified through chemical ligation, either covalent or non-covalent. Self-assembly of proteins and nanocarriers was another possibility.

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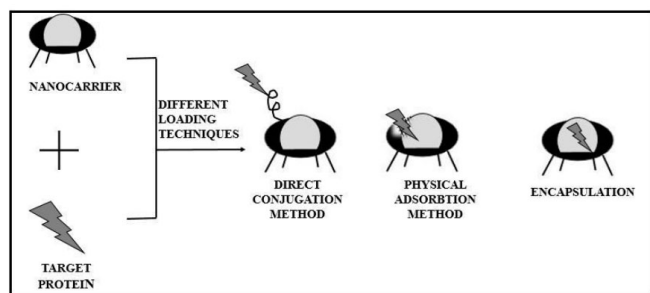


Figure 3: Methods for preparing protein/nanocarrier composites.

Because the enzyme's release from liposomes at the site of inflammation may not be necessary for therapeutic activity, it's suggested that releasing SOD across the liposomal surface is more beneficial than encapsulation of the enzyme in liposomes. To produce surface localization of SOD on enzymosomes, the approach of acylation by covalent attachment of palmitic acid to q-NH₂ groups of SOD was applied (Ac-SOD). The researchers predicted that using this strategy would result in a more hydrophobic enzyme with a stronger affinity for liposomal bilayers. By exposing SOD to the outside liposomal surface, we intended to route it to inflammatory regions. In order to establish localization to inflammatory areas and function as an efficient intracellular drug delivery mechanism, liposomes with a long circulation lifetime were created for this investigation. In these researches, long-circulating liposomes (LCL) were shown to preferentially cluster in inflammatory areas following i.v. therapy. This selective localization might be due to an inflammatory response that increases capillary permeability in a particular location, allowing liposomes to flow through. As a consequence, LCL has excellent prospects for delivering Ac-SOD to precise sites. SOD and Ac-SOD were integrated using PEG-coated LCL (PEG liposomes) and non-PEG-liposomes containing stearyl amine (SA-liposomes). Enzymes are protected within lipid bilayers, where they maintain their native conformation, since they are subject to physical and chemical stress, such as heat treatment. In liposomal formulations, incorporation efficiency, zeta potential, enzymatic activity preservation, and externally exposed enzyme activity were all assessed. The goal of developing and refining SOD enzymosomes was to increase the time that human blood flowed and concentrate the enzyme at the target area, enabling the enzyme to stay in pristine shape inside the lipid framework. Therapeutic enzymes in the hydrophilic range are kept or encapsulated inside the inner watery area of the generated vesicles. In its intact condition, a liposome that possesses catalytic activity before it is destroyed. The enzymes could be bound to liposomes

by initially linking the enzyme to the phospholipid bilayer components of the liposome bilayer by interacting with the enzyme's hydrophobic anchoring molecules, such as long-chain fatty acids.

In the first step, the enzyme is assimilated to the liposomal membrane, whereas the second stage involves docking between the liposome bilayer and the coupling reaction with the liposomal surface. Both the processes are having a difference in:

- The number of enzyme molecules entrapped/displayed in the outer lipid bilayer, as well as the stability of the enzyme-liposome combination.
- The nature of modified enzymosomes, which may dock to phospholipids, long-chain fatty acids, and polymer-like substances connected to phospholipids, among other molecules.
- The method of manufacture is generally chosen based on the several therapeutic needs that necessitate enzyme delivery through enzymosomes.

A key difficulty is the ligation of enzymes to the interior hydrophilic environment, i.e. native enzyme, as well as to the lipid bilayer membrane of liposomes through Ac-enzyme. While the liposomes remain intact, the enzyme in the interiors is not available for reaction. Enzymes that are directly attached to the lipid membrane, on the other hand, are ready to catalyse even before the liposomes are destroyed. During the unification of hydrophilic enzyme to the acyl residual chains of lipids, the process of acylation (adding an acyl functional group) occurs, resulting in the formation of Ac-enzyme. As a result, the enzyme's microenvironment shifts from hydrophilic to hydrophobic. The degree of hydrophobicity obtained is influenced by the length and number of fatty chains linked to the enzyme's surface. Standard conjugation procedures must be used to test the stability of the modified enzyme's other features. The enzyme *L*-asparaginase, which is used to treat acute lymphoblastic leukemia, withholds 100% of its catalytic activity when the active site of the enzyme is blocked by the substrate during conjugation. To add a fine dose of Ac-enzyme to the liposomal structure and permit optimum enzyme release, many approaches are used. Ac-enzyme is buried within the hydrophobic lipid vesicular matrix or partly inserted into lipid bilayers. The attachment of Ac-enzyme to the lipid bilayer membrane is largely dependent on electrostatic interactions between the charges associated with enzymes. The efficiency of the Ac-enzyme incorporated into liposomes is measured using the ratio between catalytic values computed during the intact life span versus disturbed enzymosomes. During the production of enzymosomes, the size of the vesicle, content, ionic charge, and other ideal properties of liposomes are incredibly useful¹⁰.

Acylated-SOD Liposomes

SOD enzymosomes were created by distributing an aqueous solution containing SOD into a homogenous film, and the non-bounded enzyme was subsequently extruded using an ultracentrifugation separation process. Gaspar et al's research effort characterised the created enzymosomes, using numerous criteria to ensure that the enzymosomes were uniform in their features. The different parameters included where;

- The average particle size (diameter) of liposomes was determined using dynamic light scattering.
- Protein coupling to liposomes was assessed when liposomes were disrupted using Triton X-100 and sodium dodecyl sulphate (SDS).
- Free amino acid groups and lipids were determined.
- Phospholipids colorimetric test
- The enzymatic activity of Ac-SOD and SOD formulations was investigated.
- Enzymes were examined for their ability to slow down the autoxidation of epinephrine to adrenochrome.
- A series of dilutions were initially made to attain a final protein concentration before measuring overall enzyme activity within the SOD or Ac-SOD enzymosome.
- When the enzyme was exposed to an external surface, its enzymatic activity was measured.
- Researchers looked at the zeta potential.
- The electric field's strength and angle of dispersion were found.
- Enzymosome membrane phospholipids' thermotropic activity was revealed.

Different investigations in chemical modifications of SOD yielded innovative ways for assessing Ac-SOD and deciding if it was superior to regular SOD and other medicinal preparations:

- The efficacy 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, and had a lower initial (protein/lipoprotein) ratio due to competition between Ac-SOD and cholesterol for phospholipid inclusion.
- The electrostatic interactions revealed that positively charged SA-liposomes were helpful because PEG at the lipid surface reduced lipid-protein charged interactions.

As a consequence, Ac-SOD showed substantial activity for the integrated enzyme, which functions independently of enzyme release rate and extent, resulting in a novel

mechanism of action. Since a result, the completed investigation demonstrates that the suggested enzymosome has significant potential, as the PEG-enzymosome altered the substrate even when surface PEG chains were present. As a consequence, there was no barrier, and the release of the enzyme did not need dismutation activity at the inflamed site. If the Ac-SOD enzymosome could be built with circular micro-reservoirs that would be enough for expressing the enzyme's activity without interruption and a long-term release pattern. Peptides and polymers go through comparable transformations, producing a useful drug targeting pathway. As a consequence, the Ac-enzymosome might be used as a rheumatoid arthritis replacement therapy with a powerful effect and longer-flowing active particles for reperfusion illnesses. The affinity of SOD for negatively charged lipid molecules is a helpful tool for studying membrane structure and dynamics, and it accounts for at least some of its ability to protect lipid membranes from oxygen-induced damage ¹¹.

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

The efficiency of standard anticancer drugs for chemotherapy treatment is restricted due to a lack of distinction between normal and malignant cells. After reviewing the study on immuno-enzymosomes, it was determined that they might be used to target enzymes for anticancer prodrug site-specific activation. The importance of creating a single well-represented liposomal system that can bind to a variety of different ligands is enormous. Enzymes may be concealed within small packet-like structures and then released when they reach the action site using these strategies. The enzyme beta-glucuronidase, which can activate anthracycline glucuronide prodrugs, was shown to be effective against ovarian cancer cells when coupled with immunoliposomes (OVCA-3). When incubated with ovarian cancer cells, an immune-enzymosome formulation with a 2-fold increase in enzyme-specific activity may be generated by cleaning the commercially available enzyme beta-glucuronidase (GUS). The idea is to combine a cell-specific antibody with a (liposome) immunoliposome that encapsulates the chemotherapeutic agent, allowing for targeted drug delivery and cytotoxicity. As a result, new therapeutic protein molecules with reduced immunogenicity are being developed. After the antibody-enzyme combination had attached to malignant cells and been cleared from the blood and tissues, the patient was given an infusion of a non-toxic prodrug. The enzyme's action is directed towards the prodrug, which is converted into an active cytotoxic substance within the tumour cells' confluence, resulting in its selective elimination (**Figure 4**).

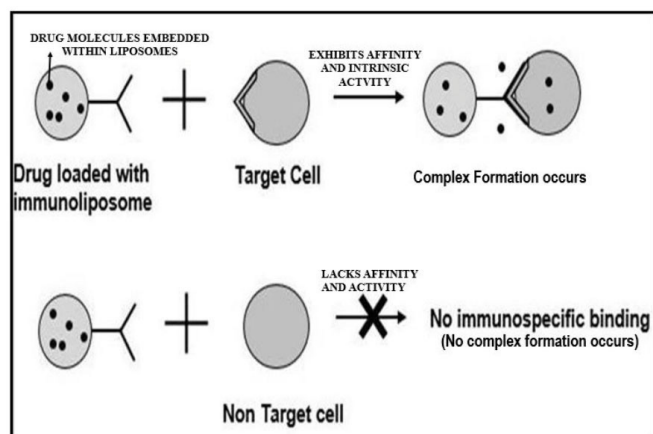


Figure 4: Specificity of immuno-enzymosomes to target cells.

Advantages

- In a single targeted carrier system, more than one enzyme moiety might be accepted.
- Increased enzyme density at the cancer cell surface, allowing for effective prodrug conversion.
- The enzyme GUS was chosen above other intracellular enzymes because of its excellence.
- Because of their modest penetration, they produce limited activation of hydrophilic glucuronide medicines. As a result, the immunogenicity issue is reduced.

The GUS enzyme may sometimes interfere with cell coupling due to its substantial steric hindrance. However, research has shown that just increasing the density of enzymes on the surface may result in considerable enzymatic targeting. Szczupak et al. used the electrosome as a tool for releasing and activating a cascade of enzymes, overcoming the drawback of a small number of enzymes on the surface. The GUS was refined initially, followed by the creation of Fab' fragments. This approach was used to make the enzymosome or immuno-liposome, which was subsequently described.

The majority of the research effort was evaluated in order to highlight certain notable discoveries in the field of nanoscience that may be used as a springboard for developing effective medicine delivery systems for serious diseases. The study's key objectives were to keep the enzyme at its maximum density in the cell environment while also quantitatively enhancing it without causing it to aggregate by undergoing more conformational changes, which would reduce the enzyme's efficacy and selectivity. As the experiment continued, purification of commercially available GUS increased its enzymatic activity by threefold. As a result, lowering enzyme density to the level of a steric barrier for interacting with a target antigen might be a realistic strategy for creating immuno-enzymosomes with the best enzyme targeting capabilities. Ovarian carcinomas were most often detected and treated using immune-enzymosomes. Cancer cells have the ability to migrate to the peritoneal cavity

even in the early stages. On a regular basis, the tumorous mass is surgically removed, followed by treatment. As a consequence, for a full cure with the complete elimination of any remaining debris that may otherwise injure normal cells. The results reveal that injecting immuno-enzymosomes made intravenously (i.p.) for antibody-directed enzyme prodrug therapy allows the peritoneally delivered drug to reach the cells where the enzyme needs to bind (Figure 5)¹².

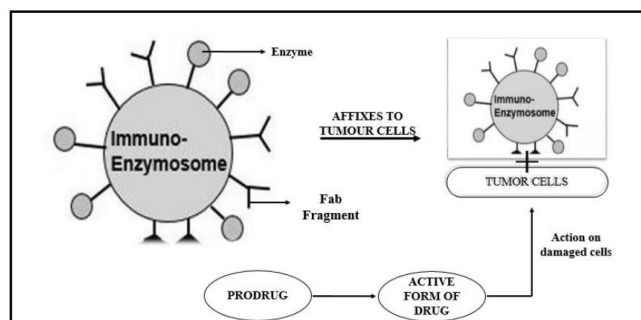


Figure 5: Enzyme bound drug targeting tumor cells.

Production alkaline enzymosomes loaded with *Bacillus fastidiosus*

Tan Q et al. investigated the possibility of a novel alkaline enzymosome for delivering the *Bacillus fastidiosus* enzyme Uricase (UBF), as well as improving its biochemical activity, therapeutic use, and pharmacological features. Uricase performs a typical physiological role by catalysing the oxidation of uric acid to lower plasma uric acid levels. According to studies, UBF loaded in new alkaline enzymosomes (ESUBFs) took less time to lower plasma uric acid concentrations to normal levels from greater levels than free UBF. Their results demonstrated that ESUBFs efficiently transport UBF, increasing its AUC, half-life, and catalytic activity while also altering its biochemical and pharmacological characteristics. As a consequence, ESUBFs might be an effective second-line therapy for gout and hyperuricemia. The extra advantage of improved *in vivo* uricolytic activity might have important therapeutic ramifications, since the clinical dose delivered as well as the side effects created during standard UBF usage could be significantly reduced by conjugation. It was profitable to generate novel alkaline UBF enzymosomes to improve the pharmacological, biological, and biochemical properties of the enzyme uricase from *B. fastidiosus*, which was then exploited for hyperuricemia effects. Szczurek et al. found that giving uricase enzyme to rats reduced uric acid levels in a hyperuricemia rat model. Uricase enzyme had certain advantages, such as enhanced selectivity and efficient, quick reactivity, but it also had some disadvantages, such as a short *in vivo* half-life and therapeutic usage limitations. The basic purpose of encapsulating an enzyme in an enzymosome is to retain its molecular structure while controlling its

distribution. According to the research, the catalytic activity of UBF loaded in novel alkaline enzymosomes (ESUBFs) was about three times that of free UBF at the appropriate pH *in vitro*. After intravenous (i.v.) injection, ESUBFs exhibited a significantly greater area under the plasma concentration (AUC) and a longer circulation half-life ($t_{1/2}$) than free UBF, similar to studies by Xiong H et al. The preliminary security assessment actively shown that ESUBFs were acceptable when delivered through parenteral route. They might be used in an emergency to treat enzyme deficiency illnesses, according to the researchers. The encapsulating biochemical and pharmacological features of alkaline enzymosomes have been improved, according to systematic research trends. As a consequence, enzymosomes could be a better alternative for making UBF and ESUBFs, which might be a better option for treating hyperuricemia and gout¹³.

Antiplatelet activity of CD 39 enzymosomes

The enzyme CD39/NTPDase-1, which is expressed on the cell's opening side, is found in endothelial cells throughout the body. It has the physiological ability to digest ATP, ADP, and AMP fast while lowering platelet sensitivity to the major agonists. As a consequence, research into therapeutic anti-platelet 'enzymosomes' formulations containing CD39 embedded within liposome lipid bilayers was prompted. Initially, CD39 enzymatic activity was optimised, which seems to be dependent on the expression of either of its transmembrane domains. Full-length human CD39 was isolated and reconstituted within an appropriate lipid vesicle using a yeast production system as a model. The catalytic efficacy of detergent-solubilized CD39 as well as when it was reconstituted within a lipid membrane was assessed using the dephosphorylation and generation of ADP and ATP. The efficiency of CD39-containing lipid vesicles to inhibit platelet activation induced by ADP, collagen, and thrombin *in vitro* was determined using platelet aggregometry. Using a mouse model of thromboplastin-induced thromboembolism, the effectiveness and therapeutic use of intravenously delivered CD39 enzymosomes in lowering platelet consumption and mortality were studied. The restoration of human CD39 in lipid vesicles resulted in a one-order drop in the Km value, as well as an increase in the catalytic efficiency of both ADPase and ATPase. CD39 lipid vesicles significantly reduced platelet activation by ADP, collagen, or thrombin, preventing platelet aggregation and generating a platelet disaggregation response when platelets were activated. According to studies, treatment with CD39 lipid vesicles dramatically decreased the decline in platelet counts caused by thromboplastin. Incorporating the enzyme into a lipid bilayer significantly improved CD39 enzyme activity as compared to its solubilized cousin. As a consequence, studies in an animal model revealed that CD39 enzymosome treatment decreased platelet consumption and death. CD39 enzymosomes made this way might be a useful therapeutic

adjunct to current anti-platelet therapies that promote platelet thrombus formation¹⁴.

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

In immunohistochemistry, Streptavidin, a tetrameric biotin-binding protein isolated from *Streptomyces avidinii*, displays a low amount of nonspecific binding. As a consequence, it's a need in a wide range of detection systems. It belongs to the avidin family of antimicrobial proteins, and its unique interaction with biotin molecules makes it useful for nonradioactive detection. Biotin is a vitamin that is required for a range of biological functions, including cell growth, in living cells. When biotin was added to a molecule, the biotin tag was previously used to enhance the affinity purification process using immobilized biotin-binding protein. In ELISA, immunohistochemistry (IHC), cell-surface labeling, and fluorescence-activated cell sorting (FACS), among other applications, the avidin-biotin interaction is exploited. Under optimum conditions, streptavidin coupled to liposome results in a well-characterized protein-liposome conjugation. The resulting targeted vesicle system is more active in proportion to its size and more firmly binds to biotinylated targeting ligands. Combining natural circulating cells with enzymes for medicine delivery has gained popularity in recent years. In the study, streptavidin was shown to be non-covalently connected to biotin, a vitamin, and phosphatidylethanolamine, a phospholipid family. The phospholipid was chosen for its primary role of dissipating the negative charge created by anionic membrane phospholipids. The sample was made utilizing the extrusion approach, which included dissolving the lipid mixture in a solvent, spreading it over a tube, and drying it to a thin film under high vacuum using a stream of appropriate gas. Biotinylation is the process of attaching biotin to a protein or nucleic acid in a covalent manner. Biotin has a strong affinity and rapid action when combined with streptavidin, a member of the avidin family, making it beneficial for isolating biotinylated molecules of interest in numerous fields of biotechnology. To establish binding, the liposome vesicles were treated with streptavidin, and the best coupling results were obtained when the ratio of streptavidin to lipid molecules was kept constant. The efficient communication between streptavidin and biotin-containing lipid molecules is used in indirect targeting approaches. Through light activation, other pharmacological findings, such as photoaffinity, are used as additional ways for molecular interactions and binding site targets. Another strategy studied was the covalent binding of streptavidin to two lipid derivatives containing thiol groups to initiate the reaction. In the investigation detailed in the reference publication, the presence of long-spacing reactive arms increased the cross-connection of maleimide derivatives of lipids. The enzyme is then connected to these liposomes and

modified to take advantage of its ability to conjugate with membrane-linked antigens in a specific fashion, resulting in a range of nanocarriers with relevant physicochemical features for drug delivery. *In vivo* and *in vitro*, the biotinylated antibody was coupled with enzyme-linked liposomes, which offers a wide variety of applications. Such uses help in the storage of hydrophilic medications and fluorescent groups in water-loving compartments of targeted liposomes for cell surface action. Smaller conjugates have a longer half-life and, as a result, keep their capacity and activity in the plasma for longer, changing the severity of infection and acute responses¹⁵.

FUTURE PERSPECTIVES

By covalently immobilising or attaching enzymes to the surface of liposomes, the enzymosome provides a tiny bioenvironment. A tailored medicine delivery system is employed to reach the tumour cell. Conjugates of enzyme-containing prodrugs are beneficial for targeting cells in tumor-like diseases. In rare cases, delivering medications or prodrugs to the body via the bloodstream might cause pharmacological activity failure. Furthermore, directly giving these drugs to specific cells is tricky. As a result, combining enzyme conjugates with prodrugs (**Figure 6**) may minimise target cell treatment failure while simultaneously boosting full medication use via proper enzyme activity. As a consequence, life-threatening enzyme-containing medications may be produced for the treatment of malignant disorders in the future years.

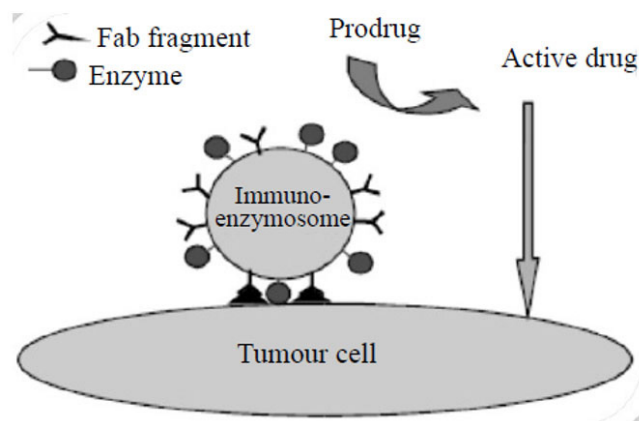


Figure 6: Prodrug converted into an active drug by enzymes.

Enzymosomes are specifically engineered lipid structures that create a conducive milieu for enzymes to be isolated within or covalently bonded to the lipids' external surfaces. Its primary function is to facilitate penetration and to deliver targeted medications to tumorous cellular sites. These vesicular systems provide a lot of flexibility in drug development,

allowing for the removal of a wide range of side effects and bioavailability concerns. The utilization of nanosystems to carry drugs over the BBB to the CNS might lead to better treatment options for a variety of malignancies. The method transports different prodrugs, as well as charged proteins and complexes, to the brain, resulting in a linear increase in drug concentration. Despite the fact that diverse carrier nuclei have downsides such as oxidative stress damage, they play an essential role in drug selection and targeting, as well as giving existing pharmacological therapies a new lease of life. Biotinylation and pegylation are two of the most recent breakthroughs in targeted medicine administration. A variety of lipoidal and non-lipoidal vesicular carrier nanoparticles are utilised for sustained drug release and cellular targeting. Enzymosomes, such as ethosomes, transferosomes, pharmacosomes, and virosomes, are lipid bio carriers, as are non-lipoidal ones such as niosomes, aquasomes, and others. Several studies have proven that each carrier is successful at what they do. In Valetal's testing, PEG-SOD enzymosomes were demonstrated to have a satisfactory impact, suggesting that they might be used therapeutically. Liposomes, as opposed to free supplements, are increasingly being acknowledged in the medical community because they reduce toxicity and boost efficacy. Nair et al. show that emulsomes are proven carriers for loading low water-soluble medications with variable bioavailability when compared to free medication. Enzymosomes might therefore be used as a trustworthy carrier nucleus in the future creation of a new generation of flexible drug design systems¹⁶.

CONCLUSION

To enhance the utilization of carrier-mediated protein delivery, numerous strategically permitted strategies are being employed. The therapeutic enzymes are integrated into carriers of the polymeric nature of the hydrophilic portion of lipid vesicles, as well as hydrophobized forms that are assimilated to lipid bilayers of vesicles. Nothing in the previous list is capable of fully maintaining the therapeutic protein's function. Aside from therapeutic enzymes, another technique is its linkage to the outer surface of liposomes, which has resulted in the development of scientific know-how for antibody drug manufacture. When an enzyme was complexed with lipid nature carriers, enzymosomes were created. Enzymosomes have two appealing properties: they preserve enzymatic activity while restricting structural integrity. The concept of encapsulating medicine in lipid vesicles to enable more accurate drug targeting to the right tissue terminal is well-known. Diverse deformable and fatty, stiff supramolecular vesicular structures have been shown to be capable of delivering medications for the targeted administration of various bioactives. In recent years, a number of researches have been carried out with the aim

of enhancing targeting and lowering dose frequency. To accomplish these aims, these might be produced in semi-liquid or liquid drug delivery systems with a matching lipid composition. The novel vesicular systems actively showed their therapeutic potential to genetic levels on a topical basis. The qualities stated might be acquired using supramolecular chemistry principles, an enzymosome, and a novel vesicular system.

Conflicts of Interest

No conflict of interest is declared.

ACKNOWLEDGEMENT

The author acknowledges the college management, principal, teachers, non-teaching staffs, and colleagues for their kind support.

Funding Information

No agency provided funds.

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