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ENZYMOSOMES – A NANOCARRIER FOR ENZYME DELIVERY: FROM DESIGN TO THERAPEUTIC APPLICATIONS

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ABSTRACT

This study reports the design, characterization, and evaluation of a new generation of SOD-Enzymozomes, where superoxide dismutase (SOD) is covalently attached to the distal terminus of polyethylene glycol (PEG) chemically bound to liposome head groups. The PEG layer shields the exposed enzyme at the liposomal surface from the medium while allowing access to its substrate. The conjugated SOD was prepared through a thiolation reaction and subsequently attached to liposomes containing lipid anchors. In-vitro and in-vivo studies were conducted to compare the newly developed PEG-enzymosomes with surface-exposed SOD (SOD-enzymosomes) and PEG-liposomes with encapsulated SOD in the internal aqueous space (SOD-liposomes). The results demonstrated improved therapeutic activity and stability of the PEG- enzymosomes, highlighting their potential for targeted delivery of enzymes in various biomedical applications, including enzyme replacement therapy, cancer treatment, and organophosphorus antidotal treatment. The development of these novel SOD- enzymosomes offers a promising approach for the treatment of various diseases, where site-specific delivery of enzymes is crucial.

KEYWORDS: Enzymosomes, nanocarrier, enzyme delivery, evaluation, therapeutic applications.

INTRODUCTION

The increasing recognition of oxidative stress as a major factor in the development of numerous chronic diseases, including neurodegenerative disorders, cardiovascular conditions, and cancer, has highlighted the need for more effective antioxidant therapies. Oxidative stress is a key factor in the development of many chronic disease, Superoxide Dismutase (SOD) is an important enzyme that helps protect the body by neutralizing harmful superoxide radicals. However, using SOD as a treatment has its challenges, such as poor stability and fast degradation in the body, which limits its effectiveness. To address this, researchers are developing a new delivery system called "Enzymosomes." These are tiny lipid-based carriers designed to protect and deliver enzymes, like SOD, to where they are most needed in the body. Encapsulating SOD in enzymosomes not only protects the enzyme but also enhances its delivery to tissues experiencing high oxidative stress, increasing its therapeutic impact. The aim of this research is to create and improve novel SOD-enzymosomes that can overcome the limitations of traditional SOD therapies. However, the therapeutic application of SOD is limited by its poor stability, rapid degradation, and inefficient delivery to target tissues. The project will involve developing the formulation of these enzymosomes, studying their properties, and testing their effectiveness in lab models. Enzymosomes are lipid-based nanocarriers specifically designed to encapsulate enzymes, protecting them from degradation and enhancing their delivery to specific sites of action. In the case of SOD, encapsulation within enzymosomes offers the potential to improve its pharmacokinetic profile, enhance its stability, and ensure targeted release in tissues affected by oxidative stress. This study aims to develop and optimize novel SOD-enzymosomes to improve the therapeutic efficacy of SOD. The research will focus on formulating these enzymosomes using advanced nanotechnology techniques, characterizing their physicochemical properties, and evaluating their in vitro and in vivo therapeutic potential. By enhancing the stability and delivery of SOD, this approach holds great promise for advancing antioxidant therapies and providing new treatment options for diseases driven by oxidative stress.

Structure of Enzymosomes

Enzymosomes are an innovative, currently emerging system for targeted vesicular drug delivery. Enzymosomes fundamentally utilize enzymes that have a targeted catalytic function for a substrate that are

embedded in cellular structures with a high lipid background.[1] They provide newly designed liposomes in which enzymes are covalently bound to the surface of lipid molecules. Liposomes designed to provide the right microenvironment for enzymes that can be deactivated in them.[2] Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids. A typical property of lipids forming a bilayer is their amphiphilic nature: a polar head group covalently attached to one or two hydrophobic hydrocarbon tails.[3] Their unique chemical composition allows them to encapsulate hydrophilic biomolecules or drugs in an aqueous core and improve penetration through lipophilic membranes. On the other hand, the lipid bilayer can trap lipophilic drugs and thus increase their solubility in aqueous body fluids.[4] Enzymes play a role in catalysis, site-specific pharmacological action, prodrug activation, etc. But reviewing their low lipid membrane penetration, if the enzyme is captured on the surface of liposomes, enzyme degradation and transmutation are minimized, thus promoting their half-life. with targeted action. Enzymosomes are a method of a novel rate-controlled vesicular drug delivery system that guides the active form of the drug to the site of action and rapidly degrades it for easy uptake.[1] Enzymes can be confined to the surface of liposomes by two approaches; by linking functional hydrophobic compartments, such as long-chain fatty acids, to the enzyme or by linking the enzyme and the phospholipids of the liposome layer.[5] Using a modified approach, polymers encapsulate, protect, and deliver hydrophobic and hydrophilic drugs. self-assembling systems capable of delivering more than one drug simultaneously. Thus, mutated vesicles have increased solubility, stability, and therapeutic index for the encapsulated drug molecule. By modulating the shape of the nanoparticles, we are able to increase the uptake of both hydrophilic and hydrophobic drugs into neural cells, which provides promising results towards increasing uptake in other cell lines, with the ultimate goal of efficiently delivering the payload to blood cells. brain barrier. One application of such lipid nanocarriers is the treatment of CNS disorders such as epilepsy, seizures, which act as natural BBB attractants due to their lipophilicity.[6] Surface modification of nanocarriers by adsorption or covalent binding of hydrophilic polymers leads to increased circulation of nanocarriers in the blood, while derivatization of the surface with molecules that recognize cell receptors facilitates the penetration of nanoparticles through the BBB, which play a vital homeostatic role in the blood. brain. Enzymes acting as therapeutic proteins are delivered through polymeric carriers such as liposomes, lipoplexes, among which the attachment of enzymes to the exposed areas of liposomes shows the maximum response. A drug-loaded vesicular delivery system shows precise results at the site of infection or inflammation with the least drug toxicity and side effects.[7] The covalently bound enzyme and liposome will have minimal changes in enzymatic activity, and the vesicle-loaded enzyme will retain its structural integrity and enzymatic activity when tested in-vitro and in-vivo.[1] This prolonged blood circulation with increased half-life of the drug as well as increased residence time in the living organism.[8] Advances in enzyme design are variably applicable in areas such as production of new recombinant proteins, biotechnological products, etc.[1] enzymes with sufficient surface-exposed enzyme load while preserving the structural integrity of liposomal particles and enzyme activity in a new and successful therapeutic approach to inflammatory situations/diseases associated with oxidative stress.[9]

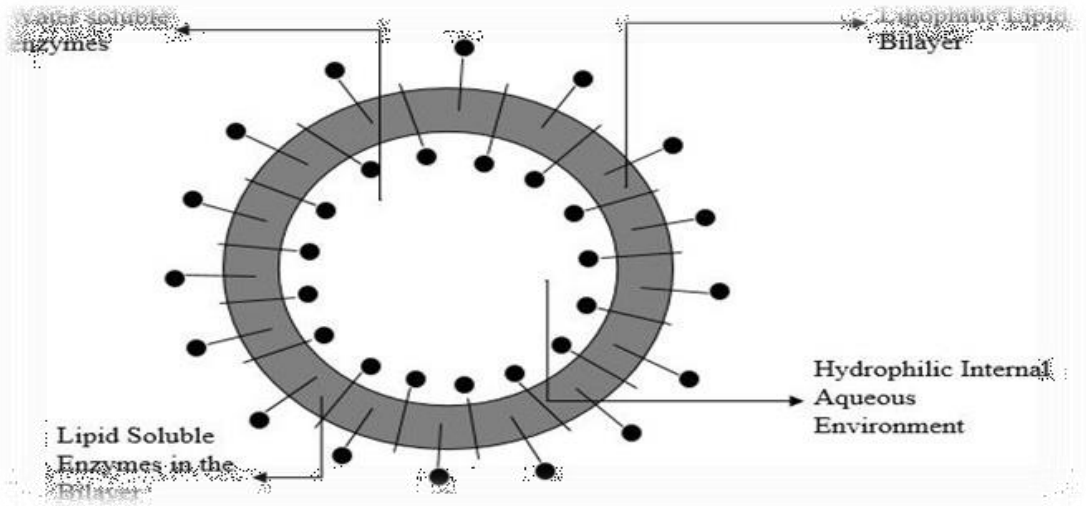

Figure 1: Diagrammatic representation of structure of lipoid bilayer.[10]

Introduction to disease and delivery system

In living organisms, the production of reactive oxygen species (ROS) is balanced by their elimination and/or prevention of formation, which in concert can typically maintain a stable (steady-state) level of ROS. However, this balance can be disturbed and lead to increased levels of ROS called oxidative stress.[11] Oxidative stress caused by an imbalance between reactive oxygen species (ROS)

or reactive nitrogen species (RNS) and biological antioxidant systems can lead to the modification of macromolecules. such as DNA, lipids, and proteins. Because the redox state (oxidation/reduction conditions) of cells is involved in the regulation of various transcription factors/activators (e.g., activator protein 1 (AP-1), nuclear factor-kB (NF-kB), and p53), affecting target gene expression and modulation of cellular signaling pathways, appropriate levels of ROS and RNS are necessary for the normal physiological function of living organisms. However, excessive redox-active species can cause DNA damage, suppress the activity of cellular enzymes, and induce cell death through the activation of kinases and caspase cascades.[12] Superoxide is a normal byproduct of aerobic metabolism and is generated in many reactions, including oxidative phosphorylation, photosynthesis, and the respiratory burst of stimulated neutrophils and macrophages. Cu, Zn superoxide dismutase (SOD) enzymes are found primarily in eukaryotes, but have also been found in bacterial pathogens and symbionts. Major interests in SOD structure and function include Cu(II) and Zn(II) enzyme binding sites and active site geometry, highly stable folding, and extremely fast reaction rates due to long-range electrostatic conduction of the anionic substrate. These essential SOD enzymes are critical components in the physiological response to oxygen toxicity and are being actively investigated as potential therapeutic agents in oxidative stress-related pathological conditions [e.g. reperfusion injury after ischemia, lung and tissue damage, and overall inflammation. The effective mitigation of pulmonary oxygen toxicity recently led to SOD being designated an orphan drug by the Food and Drug Administration for the prevention of bronchopulmonary dysplasia in premature infants, and expansion to other applications is expected. SOD is also involved in the pervasive bioregulatory functions of nitric oxide by preventing the peroxidation of nitric oxide by superoxide. The biological and medical importance of SOD has prompted efforts to develop mutant enzymes with potentially improved clinical efficacy through increased serum stability or half-life. The identification of Cu, Zn SOD enzymes in parasites suggests the design of selective inhibitors that could block parasite SOD without affecting the human enzyme. All of these studies have been hampered by the lack of a structure for human SOD (HSOD), which has been cloned and expressed in yeast. Here we report the determination, refinement, and analysis of natural and engineered thermostable mutant human enzymes at 2.5 Å resolution.[13] Sod gene regulation plays a key role in balancing ROS concentration. The compartmentalization and control of SOD at the level of both expression and activity contributes to the level of SOD and the subsequent localized level of ROS.[12] Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of motoneurons in the motor cortex of the brainstem and spinal cord, leading to paralysis and death, usually within 1–5 years of diagnosis. Approximately 10% of ALS cases are familial, and of these, about 10–20% are caused by mutations in the gene for the ubiquitously expressed human protein Cu/Zn superoxide dismutase (SOD).[14] An increased prevalence of oxidative stress (SOX), inflammation and cardiovascular disease (CVD) has been reported in end-stage renal disease (ESRD), but their association with kynurenine (KYN) pathway activation remains unknown. ESRD patients showed significant increases in Cu/Zn SOD, total peroxide and has CRP levels between controls and all patient groups. KYN(kynurenine) and 3-HKYN(3- hydroxykynurenine) were positively associated with inflammation and SOX markers in uremic subjects. Logistic regression analysis showed that age, gender, presence of DM (all $p < 0.001$), elevated has CRP (C-reactive protein) ($p < 0.01$) and 3-HKYN levels ($p < 0.05$) were independently associated with the presence of CVD (cardiovascular disease) in this population.[15]

However, the therapeutic success of SOD was hindered by rapid clearance from the blood by glomerular filtration and subsequently low accumulation in pathological areas. Thus, the therapeutic potential of systemically administered SOD can be better utilized if its plasma half-life is extended, which could also lead to increased enzymatic activity at the target site. Several strategies have been used to prolong the circulation time and improve the therapeutic effect of SOD.[16]

PEG–SOD has been the enzyme most studied in PEGylation. It can be said to represent the preferred model for evaluating PEG activation chemistry, analytical procedures suitable for conjugate characterization, effect of PEG size on conjugate clearance and elimination of immunogenicity and antigenicity, and effect of route of administration. at the blood vessel level, PEG-SOD has been shown to provide greater resistance to oxidative stress, improve endothelial relaxation, and inhibit lipid oxidation. In the heart, PEG-SOD has been shown to be at least as effective as native SOD in the treatment of reperfusion arrhythmias and myocardial ischemia. In the lung, PEG-SOD appears to be able to reduce oxygen toxicity and lung damage induced by E. coli. it is surprising that no approved application in human therapy has been established and approved.[17]

SOD is a powerful radical scavenger capable of interrupting inflammatory cascades by tissue deprivation of free oxygen radicals. It has been hypothesized that reducing or interrupting the inflammatory process early in Peyronie's disease may stop or even reverse the typical symptoms associated with the disease. Due to the withdrawal of bovine SOD from the market due to severe allergic reactions, these early results have not been confirmed in controlled studies. Recently, human recombinant Cu/Zn-SOD has been produced in a liposomal formulation (lrhSOD) that can be administered as a topical gel and adequate tissue penetration of the active ingredient has been demonstrated. Patients with

painful Peyronie's disease were randomly assigned to treatment with lrhSOD or placebo for 4 weeks. All patients received a 20 ml syringe containing either verum or placebo and were instructed to apply the gel twice daily to the plaque sites (1.4 mg SOD/day).[18]

In previous work, we have shown that delivery of SOD coupled with carrier systems minimizes tissue damage in oxidative stress-related disease models.[16]

Superoxide dismutase (SOD) has been chemically modified by covalently linking fatty acid chains to the available q-amino groups of the enzyme. This acylation method gave rise to a distinct enzymatic entity (Ac-SOD) as evidenced by different physicochemical properties such as octanol/water partition coefficient and isoelectric point (pl) compared to SOD. Ac-SOD was incorporated into conventional and long- circulating liposomes (LCL) and characterized in terms of incorporation efficiency, protein-to-lipid ratio (Prot/Lip), retention of enzymatic activity, and zeta potential. Comparative characterization of Ac-SOD and SOD liposomal formulations showed that the two enzyme forms differ substantially in terms of their intraliposomal location: SOD tends to be localized in internal aqueous spaces, whereas Ac-SOD is expected to be localized in lipid bilayers liposomes, partially buried in the outer surface and exposed to the external medium. The properties of these enzymes may affect the therapeutic effect, since enzyme release from extravasated vesicles is no longer a necessary requirement to achieve dismutation activity at the target site of inflammation.[19]

In this work, we focused on the development and optimization of distal surface- exposed SOD enzymes (SOD-enzymosomes) with a long blood circulation time enabling their accumulation in inflamed target sites and rapid imaging of enzymatic activity.[16] Our goal and concept is to create enzymes that covalently bind the therapeutic enzyme either directly to the outer surface of the phospholipid bilayer, or bind the enzyme to the end of polyethylene glycol polymer chains located on the surface of lipid vesicles. In both cases, we optimized the enzyme covalent binding process to minimize changes in enzyme activity. According to in vitro and in vivo experiments with superoxide dismutase enzymes, appropriate enzyme loading, preservation of vesicle structural integrity, and preservation of enzyme activity were achieved. The goal was to develop and optimize SOD enzymes with a long circulation time in the blood so that they could accumulate in inflamed target sites while maintaining the enzymatic activity in its intact form.[20] Enhancement of carrier- mediated delivery of therapeutic enzymes through attachment of the enzyme to the outer surface of liposomes is actually an unusual strategy, as only a few publications report the preparation of carriers with hydrophobized enzymes that are partially exposed on the surface. of liposomes, while many publications report the attachment of antibodies to the surface of liposomes for active targeting. a new “label” of SOD- enzymosomes, by covalently attaching SOD to the distal end of polyethylene glycol (PEG) chemically bound to the head groups of liposome particles. The presence of PEG is expected to protect the exposed enzyme on the liposomal surface from the medium, while not blocking the enzyme’s access to its substrate. After proper design and characterization, in vivo fate and therapeutic activity studies were performed, comparing newly developed PEG-enzymes with surface-exposed SOD (SOD- enzymosomes) with PEG-liposomes with SOD encapsulated in an internal aqueous space (SOD-liposomes).[16] This conjugated SOD is prepared by Conjugation of SOD to liposomes containing lipid anchors MPB-PE or maleimide-PEG-PE was performed according to, with some modifications. Briefly, the thioacetylating agent, SATA, in dimethylformamide was mixed with the enzyme in a buffer solution. The thioacetylated enzyme, SOD-ATA, was deacetylated with hydroxylamine after separation from unreacted SATA on a PD-10 column. Thiolated SOD-AT enzyme was added to liposomes containing reactive MPB or maleimide groups. Enzymosomes were separated by ultracentrifugation and suspended in buffer.[20] SOD-ATA was deacetylated in the presence of hydroxylamine and the thiolated enzyme, SOD-AT, was added to liposomes containing maleimide-PEG-PE. Briefly, deacetylation was performed by adding 0.1 mL of hydroxylamine HCl (0.5 M hydroxylamine, 0.5 M citrate, 25 mM EDTA, pH 6.0) for each mg of protein to be deacetylated. This protein solution was then diluted to 12.5 µM with buffer (13.7 mM NaCl/10 mM citrate buffer with 1.0 mM EDTA, pH 6.0). For the coupling reaction, 0.4 mg of SOD-AT was added to 1.0 ml of freshly prepared liposomes (10 mM lipid) at a maleimide: protein molar ratio ranging from 0:1 to 10:1 and allowed to react overnight at room temperature under gentle but constant rotary shaking. After this reaction, SOD enzymes were The final pellet was dispersed in 2 mL of buffer (13.7 mM NaCl/10 mM citrate buffer with 1.0 mM EDTA, pH 6.0) and stored at 4°C when needed.[16]

Components of Enzymosomes

1, 2 – distearoyl - sn – glycero -3- phosphoethanolamine-N-[maleimide (polyethylene glycol)-2000] (ammonium salt), (maleimide-PEG-PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (PEG-PE) . Egg phosphatidylcholine (PC). 111In-8-hydroxyquinoline (111In-oxine). Mycobacterium butyricum (killed and dried) and the Incomplete Freund Adjuvant. Bovine Cu, Zn- superoxide dismutase (SOD) was from Sigma-Aldrich. N-succinimidyl S-acethyl thioacetate (SATA).[17]

Advantages of Enzymozomes[1,24,25]

- Protects sensitive tissues from dangerous pharmaceutical effects. Encapsulation and stability have been improved.
- Improvement of pharmacokinetics (prolongation of half-life, reduction of elimination).

- It combines with site-specific ligands for active drug targeting.
- Both the therapeutic index and the efficiency have improved.
- The material is biodegradable.
- These systems are a very attractive and efficient technology.
- clinical application of polymer-protein conjugates such as PEGylated enzymes.
- Flexible change of electrical properties. Biocompatible. Non-immunogenic for systemic and non-systemic administered doses.
- The effect of site limitations.

Disadvantages of Enzymozomes[24]

- 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.

METHODS FOR PREPARATION OF ENZYMOZOMES

There are a number of methods that can be used to prepare enzyme-containing lipid vesicles (liposomes), which are lipid dispersions that contain water-soluble enzymes in an entrapped aqueous space. This has been proven by many researches carried out with different enzymes. An overview of these studies is given and some of the main results are summarized. Regarding the vesicle-forming amphiphiles used, most preparations are based on phosphatidylcholine, either natural mixtures obtained from soybeans or egg yolk, or chemically defined compounds such as DPPC (1,2- dipalmitoyl-sn-glycero-3-phosphocholine or POPC (1-palmitoyl-2-oleoyl-sn-glycero- 3-phosphocholine) Charged lipid vesicles containing the enzyme are often prepared by adding some negatively charged amphiphile (typically dicetyl phosphate) or positively charged lipid (usually a stearyl amine membrane). adsorption of the enzyme to an internal or external site of vesicle bilayers. If (i) high encapsulation efficiency of the enzyme is avoided during the entrapment procedure; (iii) relatively monodisperse spherical vesicles of about 100 nm in diameter are required, then the use of the so-called "method dehydration-rehydration" followed by the "extrusion technique" proved superior to other procedures.

With respect to the utilize of enzyme-containing vesicles as sub micrometer-sized nanoreactors, substrates are included to the bulk stage. Upon saturation over the vesicle bilayer(s), the caught chemicals interior the vesicles catalyze the change of the substrate atoms into items. Utilizing physical (e.g. microwave light) or chemical methods(e.g. expansion of micelle-forming amphiphiles at sub lytic concentration), the bilayer porousness can be controlled to a certain degree. A nitty gritty atomic understanding of these (as a rule) sub micrometer-sized bioreactor frameworks is still not there. There are as it were a few approaches towards a more profound understanding and modeling of the catalytic movement of the captured protein atoms upon remotely included substrates. Utilizing micrometer-sized vesicles (so-called 'giant vesicles') as basic models for the lipidic framework of natural cells, chemical particles can be microinjected interior person target vesicles, and the comparing enzymatic response can be checked by fluorescence microscopy utilizing suitable fluorogenic substrate molecules.[26]

i. **Multilamellar vesicles prepared by dry lipid film dispersion (MLV):** Phospholipid vesicles are thermodynamically metastable structures; subsequently, the planning procedure and the lipid and fluid medium compositions utilized, as well as forms such as accumulation and combination, among others, can have a checked impact on vesicle estimate. Liposomal scatterings can be promptly characterized and routinely controlled by QELSS. Most writing values were gotten by the cumulant strategy, which as it were gives the cruel breadth and width of the estimate conveyance, both of which are accepted to be unimodal. Vesicle scatterings, in any case, are more often than not polydisperse and as often as possible multimodal. Their dispersions ought to hence be examined by strategies based on the Laplace transform. The scattering of a dry lipid film in an enzyme-containing watery arrangement leads to the arrangement of a relative polydisperse vesicle suspension with basically or maybe huge, multilamellar vesicles (MLV). It has been contended that these sorts of vesicles, in the nonattendance of any protein molecules—may on normal be composed of up to 10 bilayers. With this strategy, the vesicle-forming amphiphiles are to begin with broken up in a natural dissolvable (regularly chloroform) and the dissolvable is totally evacuated by rotatory dissipation and tall vacuum drying in a circular foot carafe. In this way, a lean, dry lipid film shapes to which a fluid arrangement containing the chemical to be captured is included. Overwhelming shaking with the help of a Vortex mixer over Tm of the lipids leads to a scattering of the lipid multilayers in the fluid arrangement, which comes about in the arrangement of a heterogenous populace of vesicles. A Vortex blender more often than not works with up to 3000 rpm. most of these vesicles are multilamellar, water-soluble chemicals can be localized not as it were in the central center, but too in the watery inter-lamellar spaces, coming about hence in moderately tall embodiment efficiency.[27]

In numerous cases the MLV-formation is a to begin with step in the planning of more characterized, sub

micrometer measured vesicles.[26] Some arrangements have treated the enzyme-containing MLV arrangements briefly with a mellow shower sonication in arrange to dispose of exceptionally huge vesicles show, e.g. in the case of superoxide dismutase.[28]

ii. Vesicles prepared by repetitive freezing and thawing a MLV suspension (MLV-FAT)

Phospholipid vesicles were measured by the spin-label method in conjunction with the stopped-flow ESR technique. The internal volume was defined as the volume from which the spin broadening agent, tris-(oxalate)-chromate ion, but not the spin label TEMPONE, was excluded. The internal volume of sonicated phospholipid vesicles was approximately 0.4 µl/mg lipid. Freeze-thaw treatment increased this value 10-50 fold. However, repeated freezing and thawing did not further increase the value. The permeability of the vesicle phospholipid membranes, measured as the rate of transmembrane diffusion of the spin-scattering agent into the vesicles, was very low even after freezing and thawing. These findings clearly demonstrated that freeze-thaw treatment induced the fusion of hundreds of small vesicles to form giant liposomes whose membrane was impermeable to complex ions.[16] After repeated freezing of the MLV suspension in liquid nitrogen (at –195°C) and thawing at a temperature above Tm, the vesicle suspension may undergo some physicochemical changes that often equilibrate the aqueous interior of the vesicles and the outer bulk aqueous phase, leading to an increased capture yield and may lead to the formation of an increased population of multivesicular vesicles (MVV) and to the elimination of fusion processes of possibly present very small vesicles depending on the lipid used and depending on the salt content. The freeze-thaw procedure can also lead to the fragmentation of MLV into smaller vesicles in the presence of electrolytes, as demonstrated in the case of vesicle suspensions prepared from DOPC.[26]

iii. Unilamellar vesicles obtained by sonicating a MLV dispersion (SUV)

Cation permeability and size of sonicated phospholipid vesicles were found to be relatively resistant to osmotic strain. The density of vesicles of 4% phosphatidic acid and 96% phosphatidylcholine was found to be almost unaffected by the presence of cholesterol up to a molar ratio of 1:1, the mean value being 1.014. A possible influence of the water structure was found in the difference in sign between the dependence of the diffusion coefficient on the concentration of lipids in 0.16 M KCl and 0.16 M NaCl. The SUV size depends mainly on the sonication conditions and the composition of the vesicle membrane (e.g. cholesterol content).[30] This method involves harsh conditions (sonification) that can lead to the inactivation of the enzymes to be captured. In addition, ultrasound treatment is not highly reproducible and generally does not result in complete elimination of MLV. In enzyme capture experiments using rulactin, the reported diameter of SUV obtained (egg PC– cholesterol–dicetyl phosphate, 150:82.5:22.5, mass ratio) was 50-200 nm.[26] Enzyme entrapment rates ranged from 3 to 9% depending on the type of liposomes and liposome retention in curd from 35 to 65%. An electrophoretic study of protein cleavage in cheeses provided correlative data.[18] Other unexpectedly high EE values were reported for superoxide dismutase: 25–40% (DPPC–cholesterol-stearyl amine, 7:1:2).[26]

iv. Vesicles prepared by the dehydration–rehydration method (DRV)

A novel liposome preparation method has been developed that is easy to use, uses mild conditions, and is capable of efficiently entrapping a wide variety of materials. Conditions were set to allow optimal capture levels; The procedure is based on the induction of fusion of preformed vesicles by means of dehydration and controlled rehydration. Preliminary evidence suggests that liposomes are primarily oligo and multilamellar. Scaling up the process for industrial use is expected to be straightforward.[32] This method is a relatively mild procedure and has been used very often in the past for entrapping enzymes, especially for applications in food technology. To achieve high capture yields, enzyme-free SUVs are usually prepared first in distilled water and then mixed with an aqueous solution containing the enzyme to be captured. The use of MLV as the initial vesicle preparation appears to result in lower encapsulation efficiency.[26] Optimal encapsulation efficiency by the DRV method was achieved when the lipid was fully hydrated before lyophilization, as small unilamellar vesicles (SUVs) were by drying in vacuum without freezing or under a stream of nitrogen at slightly elevated temperatures as DRVs are substantially larger than the SUVs originally present and multilamellar, and now contain largely entrapped enzyme molecules, depending on the experimental conditions used, such as the freezing procedure, initial vesicle properties, lipid composition, and aqueous solution used. Reported capture efficiencies were in most cases between about 15 and45%.[26,32]

v. Vesicles prepared by the extrusion method (VET)

The large unilamellar vesicles can be efficiently produced by extrusion of multilamellar vesicles through 100 nm pore size polycarbonate filters. The unilamellarity and capture efficiency of these vesicles can be greatly increased by freezing and thawing the multilamellar vesicles before extrusion.[34] A suspension of MLVs containing the enzyme is repeatedly (usually 10 times) passed through these filters above Tm under moderate pressure, resulting in the mechanical conversion of large vesicles into smaller ones. The entire process is called the "extrusion technique" and the corresponding vesicles are called vesicles prepared by the extrusion technique (VET).[26]

vi. Vesicles prepared by applying the extrusion technique to repetitively frozen and thawed vesicle dispersions (FAT-VET)

The extrusion technique can also be conveniently used in combination with the dehydration-rehydration method. In this case, the DRV suspension is adequately extruded, leading to a relatively monodisperse vesicle preparation and high entrapment yield. Trapping of superoxide dismutase in DRV-VET100 or DRV- VET200 (egg PC–cholesterol–DSPE-PEG2000, 1.85:1 :0.15) resulted in vesicles with a diameter of 110-60 nm (EE 7%) and 200-20 nm (EE 20%).[26]

vii. Vesicles prepared by microfluidization of a DRV suspension (DRV-MFV): The solute-containing DRVs were micro fluidized into smaller vesicles (to a mean diameter of approximately 100 nm, as measured by photon correlation spectroscopy) that retained 10–100% of the originally entrapped solute. Solute retention was found to depend on the number of microfluidization cycles, the medium in which the microfluidization was performed, and whether or not untrapped solute was removed prior to processing. Under suitable conditions, vesicles with a mean diameter of less than 200 nm were formed, retaining about 35–78% of the initially entrapped solute. These would be suitable for in vivo use, where a small vesicle size and an increased ratio of entrapped solute to liposomal lipid are required.[35] The microfluidization procedure can be used not only for DRV but also for MLV. Furthermore, in principle, it would be possible to use another type of high-pressure homogenizers instead of a microfluidizer.[26]

viii. Vesicles prepared by the reverse-phase evaporation method (REV)

The solute-containing DRVs were micro fluidized into smaller vesicles (to a mean diameter of approximately 100 nm, as measured by photon correlation spectroscopy) that retained 10–100% of the originally entrapped solute. Solute retention was found to depend on the number of microfluidization cycles, the medium in which the microfluidization was performed, and whether or not untrapped solute was removed prior to processing. Under suitable conditions, vesicles with a mean diameter of less than 200 nm were formed, retaining about 35–78% of the initially entrapped solute. These would be suitable for in vivo use, where a small vesicle size and an increased ratio of entrapped solute to liposomal lipid are required.[35] The microfluidization procedure can be used not only for DRV but also for MLV. Furthermore, in principle, it would be possible to use another type of high-pressure homogenizers instead of a microfluidizer.[26]

ix. Vesicles prepared by the detergent dialysis method (DDV)

The process of lipid vesicle formation is investigated using the detergent removal technique from mixed micelles. Recent studies of liposome solubilization and reconstitution have contributed to our knowledge of the structure and properties of mixed lipid-detergent systems. First, the mechanisms involved in both lipid self- assembly and the micelle-vesicle transition are summarized.[37] To elucidate critical steps during liposome preparation by removing detergent from mixed micelles, bile salt removal kinetics were measured by monitoring radiolabeled cholate, deoxycholate (DC), or chenodeoxycholate (CDC) in dialysis buffer. Three different phases of the interaction of bile salts with lipids were found. In the first stage, rapid removal of cholate from mixed micelles was estimated. At the end of phase I, liposome formation was complete after 4 h using pure egg lecithin and after 3–5 hours when mixing 30 mol% cholesterol or sphingomyelin with egg lecithin. Phases II and III showed significantly lower release of bile salts from the liposomes and were correlated with desorption from the outer surface of the liposome and flip-flopping from the inner monolayer to the outside. DC and CDC are only slowly removed from mixed micelles. Liposome formation is therefore complete only after ≈20 hours. When cholate and high lipid concentrations are used, the formation of oligolamellar structures is due to the fusion of liposomes containing a large amount of bile salt.[38] Commercially available devices for the preparation of DDV are known as Mini Liposomal Preparation (0.5-1.0 ml) or Liposomal solvent (3-50 ml). The detergent-assisted vesicle preparation method has been shown to be particularly useful not for the encapsulation of water-soluble enzymes, but rather for the reconstitution of membrane-bound enzymes.[26]

x. Vesicles prepared by the ethanol injection method (VEI)

Injection of a small amount of an ethanolic solution of a bilayer-forming amphiphile (usually PC) into an aqueous solution results in rapid vesicle formation when the amphiphiles are exposed to water. Vesicles formed in this way are denoted by the abbreviation VEI, "vesicles prepared by the ethanol injection method." In principle, methanol can be used instead of ethanol, which is not only polar but also poisonous.

Both alcohols are completely miscible with water. If the alcohol is not specifically removed (e.g. by dialysis), the vesicles remain in the preparation. If the aqueous solution contains enzyme molecules, they will be trapped to some extent in the vesicles formed. Depending on the experimental conditions (e.g., lipid concentration, addition rate of alcohol solution, and stirring rate), VEIs are more or less homogeneous in terms of size and lamellarity.[26] the method was applied in the case of superoxide dismutase using DSPC–cholesterol–stearyl amine (14:7:4), resulting in vesicles with a diameter of 250–50 nm.[26]

xi. Vesicles prepared by the pro-liposome method (VPL)

In the so-called "Pro-liposomal Method," a starting

mixture containing vesicle- forming amphiphiles, ethanol and water is converted into vesicles by a simple dilution step. The resulting vesicles are denoted by the abbreviation VPL, which stands for "Vesicles" prepared by the "Pro-liposomal Method." If the enzymes are present in the aqueous phase, they are partially trapped in the thus formed vesicles.[26]

xii. Multilamellar spherulites (MLS)

MLS stands for (concentric) multilamellar spherulites (or just spherulity), prepared by the so-called "spherulite technology." This technology involves the application of controlled shear to the aqueous lamellar phase of amphiphiles followed by dilution of the polyhedral-type structures thus formed, which ultimately leads to the formation of spherical vesicles. MLS typically have diameters between 0.1 and 10-20 nm and have a dense multilamellar structure. If the aqueous phase contains enzyme, it will be partially trapped in the MLS formed. The spherulite technology was applied in the case of alkaline phosphatase using soy PC (Phospholipon 90) aqueous solution of sodium oleate - alkaline phosphatase (40:50:10, w/w) as the starting lamellar phase. The reported encapsulation efficiency was up to 70–95%.[26]

EVALUATION PARAMETERS OF ENZYMOZOMES

i. **Mean Particle Size:** Mean particle size was measured by dynamic light scattering using a Malvern Zeta III. Protein bound to liposomes was determined according to a modification of the method described by Lowry et al. Phospholipids were assessed by the Fiske and Subbarrow colorimetric assay. Free amino groups were assayed by the method described by Bohlen et al. The catalytic activity of SOD was measured according to the method of Misra and Fridovich.[20]

ii. **Gel Filtration Chromatography:** Gel filtration chromatography was found to be inadequate for the fractionation of the PEG-SOD components present in the reaction mixture due to the high mass of the conjugates and the high hydrodynamic volume of the PEG molecules.[17] These surfaces are highly biocompatible because protein adsorption to them is low. Both the amount of protein adsorption and the magnitude of other biochemical phenomena such as platelet adhesion decrease rapidly with increasing molecular weight of PEG. This decrease is most marked at molecular weights up to 1000, after which the biological interactions tend to gradually level off. One of the primary goals of these studies was to quantify the amount of water tightly bound to PEG in solution. This was investigated using a variety of techniques, including NMR spectroscopy, water-PEG phase diagram analysis, and differential thermal analysis. These studies showed that two to three water molecules are bound to each repeating unit of PEG and led to the development of structural models to account for bound water.[21] Modification of proteins by covalent attachment of PEG chains creates a complex product that exhibits heterogeneity in both size and charge of the resulting species. A number of chromatographic and electrophoretic techniques have been developed that have demonstrated the ability to separate and quantify individual species. These methods should be useful in characterizing the reaction of PEG with SOD under different conditions and also serve as a qualitative tool to verify the consistency of the product from batch to batch.[22] The conjugates are usually eluted from the columns together. The potential of gel filtration has usually only been used to separate low mass SOD-PEG or conjugated species from unconjugated products. At best, only mono-, di-, tri-, and tetra-PEGylated SOD can be fractionated by GPC. Satisfactory fractionation results were obtained by cation exchange chromatography, reverse phase high performance liquid chromatography (HPLC), capillary electrophoresis and hydrophobic interaction chromatography.[17] Polyethylene glycol (PEG) and derivatives of polyethylene glycol are analyzed by a modification of Kurfürst's sodium dodecyl sulfate-polyacrylamide stacking gel electrophoresis procedure. Gels using a discontinuous buffer system but not having a separate stacking gel are used without loss of resolution and with less tendency to produce multiple artifacts or distorted bands. Examination of several commercial preparations of PEG and PEG derivatives on such gels shows heterogeneity other than the expected unimodal size distribution. SDS-gel electrophoresis of proteins or other materials in PEG-containing samples can yield gels with zones of PEG contamination. Methods are proposed to reduce such contamination.[23]

APPLICATIONS OF ENZYMOZOMES

There are two main areas of use (or potential application) of lipid vesicles containing enzymes: (i) in the medical or biomedical field, especially for enzyme-substitution therapy or (ii) in the ripening process of cheeses (acceleration of the process and control of flavor development). In (potential) medical applications, vesicles are used as a drug delivery system, with the drug being the entrapped enzyme. Vesicles in most cases carry enzyme molecules with the aim of replacing or supporting endogenous enzymes in the treatment of specific diseases (enzyme replacement therapy). Entrapped enzyme molecules must be released at a specific location in the body where they are needed. In another medical application, lipid vesicles containing enzymes were used to treat myocardial infarction. In this case, the trapped enzyme t- plasminogen activator must be released at the site of the thrombus to catalyze its dissolution.[26]

i. **Immunoliposomes**: It can effectively mediate the targeting of enzymes to be used for site-specific activation of the prodrug (immuno enzymosomes). the generation of sufficient and site-specific antitumor activity refers to enzymes capable of

locally converting relatively non-toxic prodrugs into active cytotoxic agents. These enzymes can be attached to the outer surface of immunoliposomes targeting cancer cells. After binding these immuno enzymosomes to the target cells, the prodrug is administered and the active drug is formed in close proximity to the tumor cell. The use of enzymes to selectively activate a prodrug at the tumor site has previously been described as antibody enzyme prodrug therapy (ADEPT). In this approach, the enzyme is linked to an antibody that binds to an antigen preferentially expressed on tumor cells. Several studies performed with antibody-enzyme conjugates in in vitro and in vivo models have shown that selective conversion of prodrug to active drug at the tumor site can be achieved. the immuno-enzymosomal system is able to completely convert the daunorubicin-glucuronide precursor to its parent compound.[39]

ii. **Organophosphorus (OP) antidotal treatment:** It can be done by encapsulating OP hydrolyzing, OPA anhydrolase (OPAA), in sterically stabilized liposomes. This enzyme carrier system serves as a biodegradable protective environment for the recombinant OP-metabolizing enzyme OPAA, resulting in prolonged concentration of the enzyme in the body. These studies suggest that protection against OP intoxication can be significantly enhanced by adding OPAA encapsulated inside (SL) to pralidoxime and atropine.[40]

iii. **Alkaline uricase enzymes**: It can be used with increased stability and anti- hyperuricemic effects induced by a favorable microenvironment. By using the right type of buffer to create a liquid medium where catalysis reactions take place; trapping of UCU inside the selectively permeable membranes of lipid vesicles; and sequestration of catalase along with UCU inside membranes. Nanoscale alkaline enzymes containing UCU/(UCU and catalase) (ESU/ESUC) in bicine buffer had better thermal, hypothermal, acid-base, and proteolytic stability, in vitro and in vivo kinetic properties, and uric acid-reducing effects. A favorable microenvironment helped to create enzymes with excellent properties. This was the first time that two therapeutic enzymes were simultaneously captured in one enzymosomes with the right type of buffer to achieve added treatment efficacy. The development of ESU/ESUC in bicine buffer provides a valuable tactic in hypouricemic therapy and enzymosomal application.[41]

iv. **Biotin**: It is a vitamin that is essential for a number of biological functions, including cell growth, in living cells. When biotin was added to the molecule, a biotin tag was previously used to enhance the affinity purification process using an immobilized biotin-binding protein. The avidin-biotin interaction is used in ELISA, immunohistochemistry (IHC), cell surface labeling, and fluorescence-activated cell sorting (FACS), among others.[24]

v. **The enzyme β-glucuronidase (GUS)**: It is capable of activating anthracycline- glucuronide precursors, can bind to the surface of immunoliposomes directed against human ovarian carcinoma cells (OVCAR-3). This study more focused on the design of an immuno-enzymosomes formulation with Higher enzyme targeting capability. Purification of the commercially available β-glucuronidase (GUS) enzyme resulted in a 2-fold increase in the enzyme specific activity and a 4-fold increase in the enzyme activity of the immuno-enzymosomes. Consequently, cell-associated enzyme activity was correspondingly increased after incubation with human ovarian carcinoma (OVCAR-3) cells. The optimized immuno-enzymosomes were shown to bind to target cells in a specific manner. Above a GUS/Fab′ molar ratio of 0.5, a deterioration in the binding ability of immuno-enzymes to the target cell was observed. This was probably due to the steric hindrance effect mediated by the presence of a large number of bulky GUS molecules on the liposome surface. However, increasing the density of GUS on the surface of immuno-enzymosomes to levels well in excess of a GUS/Fab′ molar ratio of 0.5 resulted in a markedly improved targeting ability of the enzyme.[42]

vi. **The application of catalase and superoxide dismutase Enzymozomes:** In everyday practice encounters a number of pitfalls connected mainly with their loss of activity or even degradation. Both enzymes, catalase, and superoxide dismutase, practically do not penetrate biological membranes, which limits or even disables their protective activity against ROS. At the same time, it prolongs their half-life and also increases their activity and stability.[43]

vii. **Enzymosomes as Nanoparticle drug delivery system:** are a kind of lipid nanoparticle drug delivery system consisting mainly of phospholipids structured in bilayer form, which can contain any chemical substance independent of solubility, electric charge, or molecular weight, thus improving 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 others. As the cell membrane has been used as a target for therapeutic intervention, ether and alkyl phospholipids provide a current collection of drugs that do not interact with DNA. Clinical research has shown them to be effective in the treatment of metastases, breast cancer, anti-inflammatory and other disorders.[24]

viii. **Lipid-based vesicles as Dietary and Nutrition Supplements:** have a huge impact on dietary supplements, nutrition, pharmaceuticals, and

cosmetic applications. They offer much needed flexibility in design with ease of manufacture, filling, noticeable retention, and targeted gradual release.[44] Phytotherapy, a science-based medical practice, is thus newly positioned as the most interesting source of new pharmaceutical approaches, resuming its ancient role in solving medical problems, where herbal extracts were for centuries the primary source of natural compounds used in medicine with potential therapeutic effects. activities such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, antimycobacterial and others. Therefore, nanocarriers for the effective delivery of plant bioactives show a viable way to improve the delivery rate of herbs, which ultimately leads to the peak.[45]

MARKETED FORMULATIONS OF ENZYMOZOMES

i. **GliSODin:** Intense physical activity can have some detrimental effects on the health and sports performance of athletes. One of the main reasons for these effects appears to be oxidative stress. Therefore, this study was conducted to determine whether supplementation with an enzymatic antioxidant containing plant superoxide dismutase, GliSODin, can reduce some of the negative effects of oxidative stress associated with exhaustive exercise. According to the results of this study, GliSODin can protect athletes from muscle damage and reduce inflammation caused by intense physical activity and have some positive effect on the sports performance of elite rowers. supplementation with the antioxidant GliSODin 500 mg daily in a population of elite rowers during a 6-week mesocycle period of basic training had a significant effect on parameters of muscle damage, inflammation and sports performance, therefore more studies with a larger number of participants are needed to confirm this positive effect of GliSODin supplementation.[46]

ii. **SOD B Dimpless:** A randomized, double-blind, placebo-controlled clinical trial was conducted on 41 women with cellulite aged 31 to 50 years. 21 of them were orally supplemented with SOD B Dimples®, a natural dry melon juice highly concentrated in superoxide dismutase (SOD) at a dose of 40 mg daily (480 IU SOD), for 56 consecutive days. Cellulite was measured visually by scoring fat nodules on the abdomen and thighs according to a linear scale. Oral SOD B Dimpless® supplementation significantly reduced thigh cellulite compared to placebo after 28 days. This reduction increases after 56 days of supplementation. The main mechanism of action is induction of expression of endogenous antioxidant enzymes, leading to inhibition of fibrosis and stimulation of lipolysis. Further research is needed to document the mode of action of SOD B Dimpless against cellulite.[47]

iii. **MitoQ:** Mitochondria are metabolically active organelles that produce significant reactive oxygen species associated with aging and degenerative diseases. In recent years, mitochondria-targeted antioxidants have received particular attention to reduce the concentration of reactive oxygen species and help mitigate the accumulation of oxidative damage and associated aging. MitoQ is a mitochondria-targeted antioxidant that has been reported to promote healthy aging. The purpose of this systematic review is to detection of the effects of MitoQ on oxidative outcomes related to the aging process. One such mitochondria-targeted antioxidant is MitoQ, which consists of a quinone moiety linked to a 10-carbon triphenyl phosphonium (TPP) moiety. alkyl chain.[48]

iv. **Onivyde:** Security and viability of Onivyde (irinotecan liposome infusion) for the treatment of metastatic pancreatic cancer taking after gemcitabine-based treatment. Patients with progressed and metastatic pancreatic cancer headstrong to gemcitabine- based treatment have a dreary forecast and constrained helpful options. Recently, the FDA approved nano-liposomal irinotecan combined with fluorouracil/folinic acid for such patients based on the results of the NAPOLI-1 study, which showed that this regimen significantly prolonged progression-free survival compared with fluorouracil/folinic acid (3.1 vs. 1.5 months) and overall survival (6.2 vs. 4.1 months). Nano-liposomal irinotecan in combination with 5-FU/folinic acid represents an important step forward in improving second-line treatment options for patients with metastatic progression of pancreatic cancer.[49] It encapsulates the chemotherapy drug irinotecan to improve its delivery and reduce side effects.

v. **Vyxeos, CPX-351:** The pivotal study showed a similar result in all age subgroups (60–69 years and 70–75 years), although it excluded patients younger than 60 years. Most patients receiving standard treatment must be eligible for intensive chemotherapy, and therefore the performance status of patients and their suitability for treatment depends on the clinical judgment and decision of the physician. For patients younger than 60 years with high-risk AML who are to receive standard 7+3, Vyxeos appears to be an optimized formulation that is likely to be superior to 7+3 given the results in patients older than 60 years since the pivotal study. There appeared to be no differences in disease biology between the adult subgroups in treatment-related AML or AML with myelodysplasia-related changes. The prognosis is very poor and these AML subtypes represent an unmet medical need regardless

of age. An exposure-response analysis performed in adult patients included several patients under 60 years of age and showed that the suggested dose is the most appropriate.[50]

vi. **DepoCyt (Cytarabine Liposome Injection**: Cytarabine liposomal injection (DepoCyt), a sterile suspension of the antimetabolite cytarabine, encapsulated in lipid- based multivesicular particles, was developed to improve the treatment of neoplastic meningitis (NM) through sustained release of cytarabine. Administration of intrathecally encapsulated cytarabine prolongs sustained tumor exposure to cytotoxic concentrations of cytarabine (>0.02 µg/mL) with slow, continuous release of cytarabine from DepoFoam particles so that drug exposure is prolonged over time, resulting in lower peak cytarabine levels and longer duration of exposure compared to standard cytarabine (Ara-C).[51]

vii. **Kaneka QH (Ubiquinol):** The safety and bioavailability of ubiquinol (reduced form of coenzyme Q10), a naturally occurring fat-soluble nutrient, was first evaluated in single-blind, placebo-controlled studies in healthy subjects after a single oral dose of 150 or 300 mg and after oral administration of 90, 150 or 300 mg for 4 weeks. No clinically significant changes in the results of standard laboratory tests, physical examination, vital signs, or ECG induced by ubiquinol were observed in any dose group. Cmax and AUC0 is for 48 hours derived from mean plasma concentration-time curves of ubiquinol increased non-linearly with dose from 1.88 to 3.19 g/ml and from 74.61 to 91.76 g h/ml after a single dose. Trough concentrations nearly stabilized at 2.61 g/mL for 90 mg, 3.66 g/ml for 150 mg, and 6.53 g/mL for 300 mg on day 14 and increased non-linearly with dose in the 4-week study. In conclusion, after one or more doses of ubiquinol in healthy volunteers, significant absorption of ubiquinol from the gastrointestinal tract was observed, and in standard laboratory safety tests, even in the assessment of adverse effects at doses up to 300 mg for up to 2 weeks after discontinuation of treatment.[52]

viii. **ASP-Enzymosomes with Saccharomyces cerevisiae Asparaginase II Expressed in Pichia pastoris: Formulation Design and in-vitro Studies of a Potential Antileukemic Drug:** A new transport system (ASP-enzymosomes) was successfully developed for the therapeutic enzyme asparaginase (ASP) by covalently binding the enzyme to functionalized polymer chains of polyethylene glycol (PEG) on the outer surface of liposomes. After incubation at 4 °C, the formulation remained fully stable for 4 days and retained 82% enzymatic activity for 37 days, indicating a stability compatible with its use as a biopharmaceutical. ASP-enzymosomes demonstrated in vitro antiproliferative activity against K562 and Jurkat cell lines, which was equivalent to that of ASP. The antiproliferative reaction of ASP chemicals against Jurkat cells proposes comparability to that of free commercial E. coli asparaginase (Aginasa).Ongoing studies on the production and use of ASP enzymes could potentially lead to a valuable biopharmaceutical alternative for the treatment of acute lymphoblastic leukemia.[53]

Table 1: Marketed Formulations of Enzymozomes.

Marketed Formulation	Enzyme	Uses	Reference
GliSODin	Superoxide Dismutase	Reduce some of the negative effects of oxidative stress associated with exhaustive exercise. It can protect athletes from muscle damage and reduce inflammation caused by intense physical activity.	[46]
SOD B Dimpless	Superoxide Dismutase (SOD)	Induction of expression of endogenous antioxidant enzymes, leading to inhibition of fibrosis and stimulation of lipolysis and help to reduce cellulite.	[47]
MitoQ	Mitochondria-targeted antioxidant	It can reduce age-related vascular endothelial dysfunction, which can help prevent cardiovascular disease and also reduced aortic stiffness. It can protect cells from oxidative damage in animal models and human patients with Alzheimer's disease.	[48,54,55]
Onivyde	Nano-liposomal irinotecan in combination with 5-FU/folinic acid	Onivyde given by irinotecan liposome infusion for the treatment of metastatic pancreatic cancer taking after	[49]

		gemcitabine-based treatment.	
Vyxeos, CPX-351	Liposomes formation of daunorubicin and cytaracine	Used in intensive chemotherapy that treats certain types of acute myeloid leukemia.	[50]
DepoCyt (Cytarabine Liposome Injection)	A sterile suspension of the antimetabolite cytarabine, encapsulated in Lipid-based multivesicular particles.	It was developed to improve the treatment of neoplastic meningitis through sustained release of cytarabine.	[51]
Kaneka QH (Ubiquinol)	Coenzyme Q10 (Ubiquinone)	Used as dietary supplement and in various cardiac disorders. It provides defense against oxidative damage to the body's cells, including lipid, proteins, and DNA.	[52]

CONCLUSION

The development of novel SOD-enzymosomes represents a groundbreaking advancement in enhancing the therapeutic potential of superoxide dismutase (SOD). These tailored nanocarriers significantly improve enzyme stability, bioavailability, and targeted delivery, overcoming the limitations of traditional SOD therapies. By efficiently protecting SOD from degradation and ensuring its controlled release at the site of oxidative stress, SOD-enzymosomes offer superior therapeutic efficacy. This approach demonstrates a substantial reduction in oxidative damage, inflammation, and disease progression across a range of conditions, including neurodegenerative diseases, cardiovascular disorders, and cancer. The enhanced therapeutic activity of SOD-enzymosomes marks a pivotal step forward in oxidative stress-related therapies, with strong potential for clinical applications. Further studies and trials will be critical to fully harness the clinical effectiveness and safety of this novel therapeutic platform.

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