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REVIEW ARTICLE

A REVIEW ON: LIPOSOMES

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ABSTRACT:

Liposomes are spherical vesicles that contain one or more phospholipids bilayers. Today they are highly useful replicas, reagents, and tools in various scientific fields, including mathematics, theoretical physics, biophysics, chemistry, colloid science, biochemistry, and biology. Numerous clinical studies have demonstrated the superiority of liposomal formulations over conventional delivery systems. Liposomes are the hallmark of advanced technology. Liposome research has progressed from conventional vesicles to second generation liposomes,' which produce long-circulating liposomes by varying the lipid composition, size, and charge of the vesicles. Surface-modified liposomes have also been created by combining several molecules, including glycolipids and sialic acids. The antineoplastic agents doxorubicin, daunorubicin and cytarabine are in advanced stages of human clinical trials. One or more of these should prove to be medically useful and commercially viable products in the years to come. This white paper summarizes only the scalable approach and focuses on advantages/limitations related to industrial applicability and regulatory requirements for liposomal formulations based on FDA and EMEA documents.

Keywords: *Liposomes, Drug delivery system.*

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INTRODUCTION:

Liposomes were discovered in 1960s. The discovery of liposomes, or lipid vesicles, resulted from the self-formation of confined lipid bilayers upon hydration. Phospholipid vesicles, liposomes, are spherical lipid bilayers, or lipid molecules within lipid bilayers, that can entrap water-soluble solutes within aqueous domains. They are inherently biodegradable, biocompatible, and non-immunogenic, making them ideal drug delivery systems in therapy. Liposomes are small spherical vesicles that can be made from cholesterol, nontoxic surfactants, sphingolipids, glycolipids, long-chain fatty acids, and even membrane proteins3. Liposomes are drug carriers that can carry small drug molecules, proteins, nucleotides, and even plasmids. Liposomes can be designed and engineered to have different sizes, compositions, charges, and lamellarities. To date, liposomal formulations of antitumor are commercialized4. Liposomes' clinical potential as vehicles for replacement therapy in genetic deficiencies of lysosomal enzymes was first demonstrated in the 1970s5,6. In the 1970s and 1980s, significant progress was made in the field of liposome stability, resulting in longer circulation times

of liposomes after intravenous administration and improved liposome biodistribution. Doxorubicin, an important antineoplastic drug, was formulated as a liposome in the 1980s to improve its therapeutic index. Liposome research is of increasing importance in biological, pharmaceutical, and medical research. This is because liposomes appear to be the most effective carriers for introducing drugs into cells and selectively introducing their contents to specific cells or anatomical sites. Several types of liposomal formulations have been investigated. Such as those with carbohydrate determinants (lectins), monoclonal antibodies, glycoproteins and antigens for targeting liposomes to selected tissues or anatomical sites [8,9].

ADVANTAGES

1. Allows selective passive targeting of tumor tissue (liposomal doxorubicin).
2. Improved efficacy and therapeutic index.
3. Improved stability through encapsulation.
4. Reduced toxicity of encapsulated active ingredients.
5. Position avoidance effect.
6. Pharmacokinetic effects have been improved (increased circulatory life).
7. The ability to combine with site-specific ligands in order to achieve active targeting.

DISADVANTAGES

1. Short half-life.
2. Low solubility.
3. Encapsulated drug/molecule leakage and fusion.
4. High production costs.
5. Fewer stables.
6. Phospholipids can undergo reactions such as oxidation and hydrolysis.

CLASSIFICATION OF LIPOSOMES:

Liposomes can have unilamellar or bilayer membranes. Liposome circulation half-life is determined by vesicle size, and both the size and number of bilayers influence the amount of drug encapsulation within liposomes. [10].

Liposomes are classified into two types based on their size and number of bilayers:

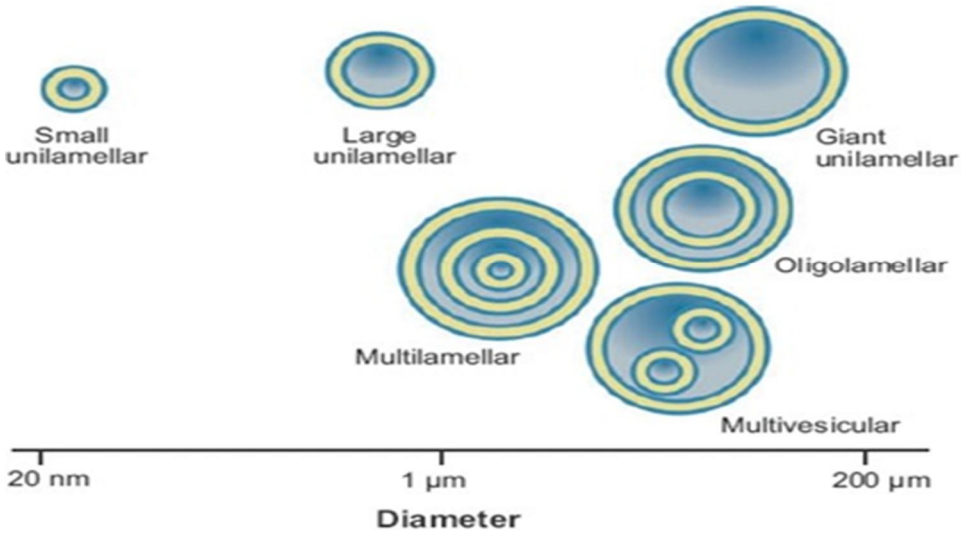
A. Multilamellar vesicles (MLV).
B. Unilamellar vesicles.

Unilamellar vesicles are further classified into the two types listed below:

a. Large unilamellar vesicles (LUV).
b. Small unilamellar vesicles (SUVs).

A single phospholipid bilayer sphere surrounds an aqueous solution in unilamellar liposomes. The vesicles in multilamellar liposomes have an onion structure [11].

Fig.1. The commonly used liposome classification scheme applied is depicted schematically.



PREPARATION OF LIPOSOMES:

A. General method of preparation
B. Specific methods of preparation

A. GENERAL METHOD OF PREPARATION:

Lipids dissolve in organic solvents. The solvent evaporates, leaving a small lipid film on the walls of the container. An aqueous solution of drug is added. In the first method, the mixture is agitated to generate multilamellar vesicles and sonicated to obtain SUVs. In the second method, he obtains the LUV by sonicating the mixture and evaporating the solvent. After extrusion, an SUV is formed. Drugs can be incorporated in aqueous solutions or buffers if they are water soluble, or in organic solvents if they are hydrophobic. Free drug and liposomes can be separated by gel chromatography.

B. SPECIFIC METHODS:

These are classified as 3 types based on the modes of dispersion. They are.

1. Physical Dispersion methods
2. Solvent Dispersion methods
3. Detergent Solubilization methods

1. Physical dispersion methods:

With these methods, the amount of aqueous solution trapped in the lipid membrane is approximately 5–10%, which is a very small percentage of the total amount used in the preparation. Therefore, a large amount of water-soluble drug is wasted during manufacturing. However, fat-soluble drugs can be encapsulated at a high rate. During these procedures, MLVs are formed and require further processing to generate unilamellar vesicles.

a) Hand Shaken Method: This is the most basic and widely used method. Dissolve the lipid mixture and charged components in a mixture of chloroform and methanol (2:1 ratio) and place this mixture in a 250 mL round bottom flask. Attach the flask to a rotary evaporator connected to a vacuum pump and rotate at 60 rpm. Organic solvents evaporate at about 30 degrees. A dry residue forms and continues to rotate 15 minutes after appearance of dry residue. Remove the evaporator from the vacuum pump

and introduce nitrogen. The flask is then removed from the evaporator and fixed in a freeze dryer to remove residual solvent. The flask is then flushed again with nitrogen and 5 ml of phosphate buffer is added. Place the flask back into the evaporator at approx.60 rpm. Eventually, a milky suspension is formed. The suspension is left for 2 hours to complete the swelling process and obtain MLVs.

b) Non-Shaking Method: This is like the shaking method, except that care is taken during the swelling process: spread a mixture of lipids in chloroform and methanol on the flat bottom of an Erlenmeyer flask. The solution is allowed to evaporate at room temperature by flushing the flask with nitrogen without disturbing the solution. After drying, the flask is blown with water-saturated nitrogen until the dry film is clear. After rehydration, bulk fluid is added to swell the lipids. Tilt the flask to one side, introduce sucrose in distilled water down the side of the flask, and slowly return the flask to the upright position. Gently drain the solution into the bottom lipid layer of the flask. Purge the flask with nitrogen, seal it, and at 37 degrees he inflates it for 2 hours. The vesicles are then mixed to obtain a milky suspension. The suspension is centrifuged at 1200 rpm for 10 minutes. Remove the MLV layer floating on the surface. LUVs are made from the remaining liquid.

c) Freeze Drying: Another method of dispersing the lipids into the final dispersed form prior to adding the aqueous medium is to freeze-dry the lipids. A commonly used solvent is t-butanol.

All the above methods produce MLVs. They are too big or too heterogeneous. For resizing, the manufactured MLVs are further processed using the following methods.

TREATMENT OF LIPIDS HYDROGENATED BY PHYSICAL MEANS:

i. **Micro-emulsification of liposomes:** A device called a microfluidizer is used to create tiny vesicles from a concentrated lipid suspension. As a suspension of large MLVs, lipids can be introduced into the liquefier. This device pumps liquid through a 5-micron screen at very high pressure. A long microchannel is then forced to allow two fluid streams to collide at right angles at very high velocities. The collected liquid can be recycled via pumps and interaction chambers until spherical vesicles are obtained.

ii. **Sonication:** This procedure reduces the size of the vesicles and energizes the lipid suspension. This can be achieved by sonicating the MLV. There are two methods of sonication: A) using a bath sonicator B) using a probe sonicator. Probe sonicators are used for suspensions that require small volumes and high energy. (eg high concentrations of lipids or viscous aqueous phases) Bath sonicators are used for large volumes of dilute lipids. A drawback of probe sonicators is contamination of the preparation with metal from the probe tip. Small unilamellar vesicles are formed by this procedure and purified by ultracentrifugation.

iii. **Membrane Extrusion Liposome:** This method reduces the size by passing it through a membrane filter with a predetermined pore size. Membrane filters are classified into two types. A winding path type and a nucleation trail type. The former is used for sterile filtration. This random path occurs between intersecting fibers. The density of the fibres in the matrix determines the average diameter of these fibres. Liposomes larger than the channel diameter are hit when trying to cross such membranes. The nucleating web type consists of thin continuous sheets of polycarbonate. They consist of straight-sided pore holes of precise diameter drilled left and right, thus offering less resistance to the passage of liposomes. This method is applicable to both LUVs and MLVs.

iv. **Freeze and Thaw Sonication:** It's a way to rip and flip an SUV while balancing the inner and outer solutes. This process improves capture volume and capture efficiency. This process results in the formation of vesicles that contain interlamellar vesicles. This method can

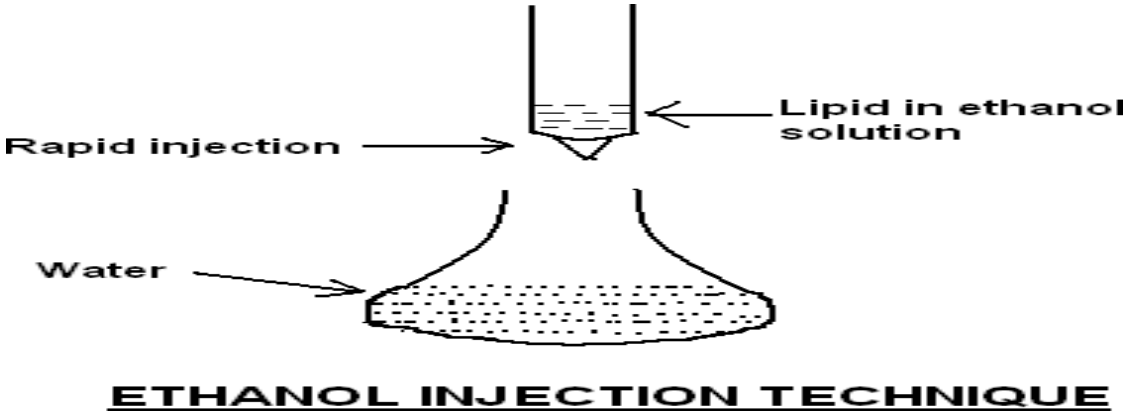
increase trap volume by up to 30%

2. Solvent dispersion methods:

In these methods, the lipids are first dissolved in an organic solution and then contacted with an aqueous phase containing the substance to be encapsulated in the liposomes. The phospholipids self-align to form a monolayer at the interface between the organic and aqueous phases. This is a critical step in the formation of liposome bilayers.

a) Ethanol injection method: This is the easy way. In this procedure, an ethanolic lipid solution is directly injected into excess saline or other aqueous medium using a fine needle. Diluting the ethanol with water distributes the phospholipid molecules evenly throughout the medium. This method yields a high percentage of SUVs (approximately 25 nm in diameter).

Figure 2: Ethanol injection Technique:



b) Ether injection: This method is like the above. At the vaporization temperature of the organic solvent, the immiscible organic solution is slowly injected into the aqueous phase via a thin needle. In this method the lipids are carefully processed, and the risk of oxidative degradation is very low. The drawback is that the procedure is time consuming, and the introduction of the lipid solution requires careful control.

3. Detergent solubilization methods:

Surfactants that bind the phospholipid molecules bring the phospholipids into direct contact with the aqueous phase during this process. The structure formed by this association is called a micelle. The concentration of detergent in water at which micelles begin to form is known as the CMC. Under the CMC, surfactant molecules are in free solution. Detergent molecules are soluble in water at higher concentrations than CMC, resulting in the formation of large amounts of micelles. As the concentration of added surfactant increases, more surfactant is incorporated into the bilayer until a point is reached at which a transformation from the lamellar morphology to the spherical micellar morphology occurs. The micelle size decreases as the surfactant concentration increases.

PURIFICATION OF LIPOSOMES:

In general, liposomes are purified using gel filtration chromatography, dialysis, and centrifugation. Sephadex-50 is the most used chromatographic separation solvent. SUVs in normal saline can be separated using the centrifugation method by centrifuging at 200000 g for 10-20 hours. MLVs are separated by centrifuging them for less than an hour at 100000 g. [15,16]

EVALUATION OF LIPOSOMES:

The formulation and processing of liposomes for specific purposes are characterized to ensure

predictable performance. Physical, chemical, and biological parameters are the three broad categories of characterization parameters for evaluation purposes. [16-20]

a. Physical characterization assesses a variety of parameters.
b. Chemical characterization studies include those that determine the purity and potency of various lipophillic constituents.
c. Biological characterization parameters can help determine the safety and suitability of a formulation for therapeutic use. Some of parameters are:

1. **Vesicle shape and lamellarity:** Freeze-fracture electron microscopy and P-31 Nuclear Magnetic Resosance Analysis are used to determine the lamellarity of vesicles, or the number of bilayers present in liposomes.

2. **Size and distribution of vesicles**: Various techniques for determining size and size distribution are described in the literature. These include Light Microscopy, Fluorescent Microscopy, Electron Microscopy (specially Transmission Electron Microscopy), Laser light scaterring Photon correlation Spectroscopy, Field Flow fractionation, Gel permeation and Gel Exclusion. Electron microscopy is the most precise method of determining liposome size because it allows one to view each individual liposome and obtain precise information about the profile of the liposome population across the entire range of sizes. Unfortunately, it requires equipments that may not always be immediately to hand. In contrast, laser light scattering method is very simple and rapid to perform but having disadvantage of measuring an average property of bulk of liposomes. All these methods require costly equipments. If only approximate idea of size range is required then gel exclusion chromatography techniques are recommended, since only expense incurred is that of buffers and gel material. Another more recently developed microscopic technique known as atomic force microscopy has been utilized to study liposome morphology, size, and stability. Most of methods used in analysis can be grouped into various categories namely microscopic, diffraction, scattering, and hydrodynamic techniques.

a) Microscopic Techniques:

i. **Optical Microscopy**: The microscopic method, which employs a bright-field, phase contrast, and fluorescent microscope, is useful in determining the size of large vesicles.

ii. **Negative Stain TEM**: Microscopic examination of electrons Negative-stain TEM and Scanning Electron Microscopy are the most used techniques for determining liposome shape and size. The latter method is less popular. Bright areas are visualised against a dark background using negative stain electron microscopy (hence termed as negative stain). The negative stains used in TEM analysis are ammonium molybdate, phosphotungstic acid (PTA), and uranyl acetate. PTA and ammonium molybdate are both anionic, whereas uranyl acetate is cationic.

iii. **Cryo-Transmission Electron Microscopy Techniques (cryo-TEM):** This technique has been used to elucidate the surface morphology and size of vesicles.

b) Techniques of Diffraction and Scattering

Laser Light Scattering: PCS is the study of the time dependence of intensity fluctuations in scattered laser light caused by Brownian motion of particles in solution/suspension As a result, the translational diffusion coefficient (D) can be calculated, and the mean hydrodynamic radius (Rh) of particles can be calculated using the Stoke-Einstein equation. Using this technique, particles as small as 3nm can be measured.

c) Hydrodynamic Techniques: This technique includes Gel Permeation and Ultracentrifuge. A thin

layer chromatography system using agarose beads has been introduced as a convient, fast technique for obtaining a rough estimation of size distribution of liposome preparation. However, it was not reported if this procedure was sensitive to a physical blockage of pores of the agarose gel as is the more conventional column chromatography.

3) **Encapsulation Efficiency and Trapped Volume:** These determine amount and rate of entrapment of water-soluble agents in aqueous compartment of liposomes.

a) **Encapsulation Efficiency:** It is usually expressed as % entrapment/mg lipid and describes the percentage of the aqueous phase and thus the percentage of water-soluble drug that becomes ultimately entrapped during liposome preparation. Encapsulation efficiency is measured using two techniques: minicolumn centrifugation and Protamine aggregation. Minicolumn centrifugation is used on a small scale for liposome purification and separation. The hydrated gel is placed in a 1ml syringe barrel without plunger and plugged with a Whatman GF/B filter pad in the mini column centrifugation method. This barrel is sitting inside a centrifuge tube. This tube is spun at 2000 rpm for 3 minutes to remove excess saline solution from the gel. After centrifugation, the gel column should be dry and detached from the side of the barrel. The eluted saline is then extracted from the collection tube. After dropping 0.2ml of liposome suspension onto the gel bed, the column is spun at 2000 rpm for 3 minutes to expel the void volume containing the liposomes into a centrifuge tube. The elute is then collected and set aside for analysis. For neutral and negatively charged liposomes, the protamine aggregation method can be used.

b) **Trapped volume:** It is a critical parameter that governs vesicle morphology. The trapped or internal volume is the amount of lipids entrapped in aqueous per unit volume. This can range between 0.5 and 30 microlites/micromol. To determine trapped/internal volume, various materials such as spectroscopically inert fluid, radioactive markers are used. Internal volume is best measured directly by replacing the external medium (water) with a spectroscopically inert fluid (deuterium oxide) and then measuring the water signal using NMR. To determine trapped volume, disperse lipid in an aqueous medium containing a non-permeable radioactive solute. Centrifugation is used to remove external radioactivity before determining the proportion of solute trapped, and then residual activity per lipid is calculated.

4) **Transitional Behavior and Phase Response:** Liposomes and lipid bilayers undergo a variety of phase transitions that are being investigated. Understanding phase transitions and the fluidity of phospholipid membranes is important in the manufacture and use of liposomes because the phase behaviour of the liposomal membrane determines protein binding. Freeze fracture electron microscopy was used to assess the phase transition. Differential scanning colorimeter (DSC) analysis verifies them more thoroughly.

5) **Drug Release:** A well-calibrated in vitro diffusion cell can be applied to assess the mechanism of drug release from liposomes. Before conducting costly and time-consuming in vivo studies, the liposome-based formulation can benefit from in vitro assays that predict drug pharmacokinetics and bioavailability. The pharmacokinetic performance of liposomal formulations was predicted using dilution-induced drug release in buffer and plasma, and another assay was used to assess drug bioavailability, which determined intracellular drug release induced by liposome degradation in the presence of mouse-liver lysosome lysate.

APPLICATIONS OF LIPOSOMES:

A. Respiratory Drug Administration System Liposome:

Liposomes are commonly used to treat a variety of respiratory disorders. Liposomal aerosols can be

designed to achieve sustained release, avoid local irritation, have lower toxicity, and be more stable. Composition, size, charge, and drug/lipid ratio should all be considered when preparing liposomes for lung delivery. During nebulization, the liquid or dry form is inhaled. Milling or spray drying is used to create drug powder liposomes 26.

B. Liposome as Vaccine Adjuvant:

Liposome has been established firmly as an immune adjuvant that is potentiating both cells mediated and noncell mediated immunity. Liposomal immuno-adjuvant acts by slow release of encapsulated antigen on intramuscular injection and also by passive accumulation within the regional lymph node. Liposomes can either entrap the antigen in an aqueous cavity or incorporate it into the bilayer, depending on the lipophilicity of the antigen 27. The targeting of liposome does the accumulation of liposome to lymphoid with the help of phosphotidyl serine. The liposomal vaccine can be prepared by inoculating microbes, soluble antigens and cytokinesis of deoxyribonucleic acid with the liposomes 28, 29.

C. Liposomes in Cosmetics:

As they are like cell membrane, they release material into the cells 30.

D. Liposomes in Intracellular Drug Delivery:

Drugs with intracellular receptors are required to cross the plasma membrane to show pharmacological activity. Liposomal delivery of drugs that normally enter the cells by pinocytosis can be very effective because liposomes can contain greater drug concentrations than the extracellular fluid. The endocytosis process by which negatively charged liposomes are predominantly taken up by the cells is more efficient than pinocytosis. Liposomes increase cytosolic delivery of certain drugs which is normally poorly taken up into cells 31.

E. Liposomes in Sustained Release Drug Delivery:

Sustained release systems are required to achieve and then maintain the concentration of drug administered within the therapeutically effective range required for medication; this method of drug administration is frequently required to take several times per day. As a result, the drug level fluctuates, resulting in poor efficiency and undesirable toxicity. To minimise this fluctuation, niosomes and liposomes have been developed [32].

TABLE 01: LIST OF APPROVED (MARKETED) LIPOSOMAL DRUG PRODUCTS

Product	Drug	Company	Indication target
Atragen™	Tretinoin	Aronex Pharmaceuticals Inc.	Acute promyelocytic leukemia
Amphotec	Amphotericin B	Sequus Pharmaceuticals Inc.	Fungal infections leishmaniasis
Abelcet™	Amphotericin B	The Liposome Company.	Serious fungal infections
Avian retrovirus vaccine	Killed avian retrovirus	Vineland lab, USA	Chicken pox
DaunoXome™	Daunorubicin citrate	NeXstar Pharmaceuticals Inc., USA	Kaposi sarcoma in AIDS
DepoDur	Morphine	Pacira Pharmaceuticals Inc	Post-surgical pain reliever
Daunoxome	Daunorubicin citrate	Galen Ltd	Kaposi sarcoma in AIDS
Doxil	Doxorubicin	Sequus Pharmaceuticals Inc.	Kaposi sarcoma in AIDS

Estrasorb	estradiol	Novavax	Menopausal Therapy
Evacet™	Doxorubicin	The liposome company, USA	Metastatic breast cancer
Fungizone	Amphotericin B	Bristol-Myers Squibb, Netherland	Serious fungal infections
Mikasome®	Amikacin	NeXstar Pharmaceuticals Inc.	Bacterial infection
Nyotran™	Nystatin	Aronex Pharmaceuticals Inc.	Systemic fungal infections
Topex Br	Terbutaline sulphate	Ozone Pharmaceuticals Ltd.	Asthma
Ventus	Prostoglandin-E1	The liposome company	Systemic inflammatory disease

CONCLUSION:

The key advantages of liposomes is their ability to target specific cells or tissues in the body. This is achieved by incorporating specific ligands or antibodies on the surface of the liposome that bind to receptors on the target cells. This targeted delivery can greatly increase the efficacy of the drug, while reducing the amount of drug needed and minimizing the potential for side effects.

Liposomes have been the subject of extensive research and have been used in various clinical trials for a variety of indications, including cancer, infectious diseases, and inflammatory disorders. While liposomes have already shown promise as drug delivery systems, researchers continue to investigate new ways to improve their properties, such as increasing their stability and circulation time in the body. It is likely that liposomes will undergo further developments in the future and will continue to be studied as a potential way to improve the efficacy and safety of drugs.

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