

CRYPTOSOMES: A REVOLUTIONARY BREAKTHROUGH IN NOVEL DRUG DELIVERY

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ABSTRACT

The vesicular drug delivery systems are promising approaches to overthrow the problems of drugs having lesser bioavailability and rapid elimination from the body. The four type of lipid based drug delivery systems are: solid-lipid particulate system, emulsion based system, solid lipid tablet and vesicular system. Cryptosomes, a novel emerging vesicular drug delivery system which can overcome the disadvantages associated with conventional drug delivery systems like high stability, increased bioavailability, sustained release, decreased elimination of rapidly metabolizable drugs etc. The word Cryptosome was originated from Greek word "Crypto" means hidden and "Soma" means body. It is formed from the mixture of phospholipids like distearoyl phosphatidyl ethanolamine-polyethylene glycol (DSPE-PEG) with distearoylphosphatidylcholine. These entire information regarding its origin and formation is explained in Dinesh Kumar *et al.* Vesicular systems symbolizes the use of vesicles in the different fields as carrier system or additives. This review disclose various vesicular drug delivery system and point out the advancement of cryptosome in the world of drug delivery.

This review would help researchers involved in the field of vesicular drug delivery.

Keywords: Vesicular system, Liposome, Phospholipids, Poloxamer

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INTRODUCTION

In the present scenario of drug discovery systems, various drug delivery systems are introduced to enhance the therapeutic activity and to reduce the adverse effect of various new drugs, one such drug delivery system is Cryptosomes. It is a type of lipid based drug delivery system or liposome. Over the past three years the study of various liposomes are carried out in hope that they can be used for drug delivery in humans and animals [1, 2]. This system enabled a remarkable growth in drug discovery, development, and use. The lipid based drug delivery systems (LDDS) contain different group of formulations based on varying structural and functional characteristics by varying the composition of lipids and other additives. LDDS has advanced over time from micro to nano-scale by improving the efficacy and therapeutic application of this systems. The LDDS is classified into four types, including the solid lipid particulate dosage form, emulsion based system, solid lipid tablets and vesicular systems. The Cryptosomes is a type of vesicular drug delivery system.

The vesicular system plays the central role in novel drug delivery (NDD), particularly in sorting of diseased cell, diagnostics, gene and genetic materials safe, effective and targeted *in vivo* drug delivery. They act as sustained release system and reduces elimination of rapidly metabolizable drugs. Over the past few decades they were widely used as drug carriers. The term Cryptosomes was derived from the Greek word Crypto means hidden and Soma means body or carrier. This lipid vesicle circulate in blood for long period of time after systemic applications and have decreased phagocyte mononuclear uptake [3, 4]. They have a surface coating formed by the assembly of phosphatidylcholine and polyoxyethylene, which are the derivatives of phosphatidylethanolamine. The Cryptosomes is formed from the mixture of phospholipids like distearoyl-phosphatidylethanolamine-polyethylene glycol (DSPE-PEG) with distearoylphosphatidylcholine [5, 6]. Cryptosomes are also known as Immune-liposomes, as they can evade detection in an immune system. Properties of Cryptosomes may vary according to the way in which the polyethylene glycol (PEG) is linked to the lipid. [7, 8] Cryptosomes are long lived lipid vesicles and their longevity is

explained on the basis of rigidity of phospholipid bilayer, surface hydrophilicity, which are essential to keep this vesicle in the blood circulation [9-11]. Another main factor affecting longevity of Cryptosomes circulation *in vivo* is the suppression of adsorption of macromolecules on to the surface of such vesicle. This adsorption can be prevented by mobile steric hindrances near the lipid surface.

There are different type of vesicular drug delivery systems [12] like enzymosome, virosome, ufasome, cryptosome, emulsosomes, discosomes, aquasomes, genosomes, ethosomes, archaeosomes, hemosomes, vesosome, proteosome, erythroosomes, photsome, cubosome, collidosome, layerosome, erythroosome etc. The complete information regarding the various types of vesicular drug delivery systems and their applications are explained in Biju SS *et al.* and Priyanka Rathore *et al.*

Composition of cryptosome

Cryptosomes are liposomal composition which comprises of poloxamer molecules (polymers) and liposomes embedded with one or more delivery agents. The poloxamer is also termed as pluronics. The generally used poloxamers are Polyethylene Oxide (PEO), Polypropylene Oxide (PPO), PEO-triblocks co-polymers of varying molecular weights (fig. 1). The hydrophobic Polypropyleneoxide groups at the centre is bonded with two hydrophilic polyethylene oxide groups. The polymers of hydrophilic PEO groups on each side of the PPO units can provide steric hindrance and thus protection to the bilayer surface. This nature make them useful as emulsifiers and stabilizers. The liposomes are made up of various lipids, it include either phospholipids (fig. 2) like phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidyl-ethanolamine, phosphatidylinositol, or the other lipids like sphingolipids, glycolipids, fatty acid, and cholesterol at various proportions [31].

The phospholipid based drug delivery system has provided the evidence of increased pharmacokinetic and pharmacodynamics activity of drug compared to conventional one [32].

Table 1: Types of vesicular drug delivery systems

Vesicular systems	Characterization	Application	Reference
Enzymosome	The liposomes that are generated to act as a bio-environment, in which the enzymes are covalently bound to their surface.	It is used in tumour for targeted drug delivery.	[13]
Virosomes	Liposomes with viral glycoprotein embedded to the liposomal bilayer	They are used in ligand-mediated drug delivery system and immunological products. It is used in the influenza vaccine.	[14]
Ufasomes	Liposomes embedded with fatty acid obtained from long chain fatty acid like oleic acid or linolenic acid. They have better penetration through skin layers.	They are used in targeted drug delivery system. It exhibit high stability, enhanced drug entrapment.	[15]
Cryptosomes	Liposomes with surface coat formed by PC and polyoxyethylene which are the derivatives of phosphatidylethanolamine.	They act as efficient ligand-mediated drug delivery system.	
Emulsosomes	A polar core with microscopic assembly of lipids having nanosized particle.	It is used in the parenteral administration of hydrophilic drug moiety.	[16]
Discosomes	Non-ionic surfactant hydrolysed solubilised niosome.	It act as a ligand-mediated drug delivery system. Used as an ophthalmic drug carrier.	[17]
Aquasomes	Nanocrystalline particulate core coated with an oligomeric film.	They act as an efficient targeted drug delivery system. In gene and antigen delivery it is used. They maintain conformation and enhance drug stability.	[18, 19]
Genosomes	They are synthetic macromolecular complexes for gene transfer. Usually, positively charged lipids are used as they exhibit increased biodegradability and stability in blood	They are used in cell specific gene transfer.	[20]
Ethosomes	They are lipid malleable vesicle embedding permeation enhancer formed by phospholipid, ethanol and water.	They are used in targeted drug delivery to the skin.	[21]
Archaeosomes	They contain glycerolipids of archaeobacteria membrane. And having very high potent activity	Enhanced stability on varying conditions of temperature, pressure and pH.	[22]
Hemosomes	They are liposome containing haemoglobin used by immobilizing with phospholipids which are polymerisable.	They are having very high oxygen carrying property.	[23]
Vesosome	They have interdigitated bilayer phase formed by incorporating ethanol to different type of saturated phospholipids.	The multiple compartments of vesosome is highly beneficial for protection of internal contents.	[24]
Proteosome	They have subunits of enzymes with high molecular weight complexed with particular catalytic activity which is specifically due to the difference in the arrangement pattern of enzymes.	They exhibit higher catalytic activity.	[25]
Erythroosomes	They are liposome in which cross-linked human red blood cells or erythrocytes cytoskeletons used as a support	They are used in targeted drug delivery of macromolecular drugs.	[26]
Photosome	Liposome incorporated with photolyase enzyme which deliver the compound by photo-triggered charges in the membrane.	They are used in photodynamic therapy.	[27]
Cubosome	They are bicontinuous cubic phases, composed of two separate continuous phases. And the non-intersecting hydrophilic regions are separated by a lipid layer.	Targeted drug delivery.	[28]
Collidosome	The self-orientation of colloidal particles at the interface of emulsion droplet result in the formation of solid microcapsules. The collidosomes are elastic and hollow in nature, whose permeability and elasticity can be varied.	Targeted drug delivery.	
Layerosome	Multi-layered liposome, with each layer consisting of biocompatible electrolytes to increase the structural stability.	Potential for oral use of administration.	[29]
Erythroosome	It consist of an erythrocyte cytoskeleton with lipid bilayer membrane.	It is used as an entrapment system for macromolecular drugs.	[30]

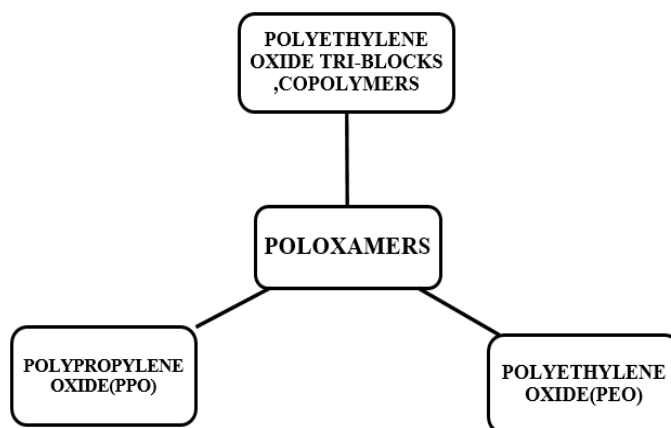


Fig. 1: Types of poloxamers

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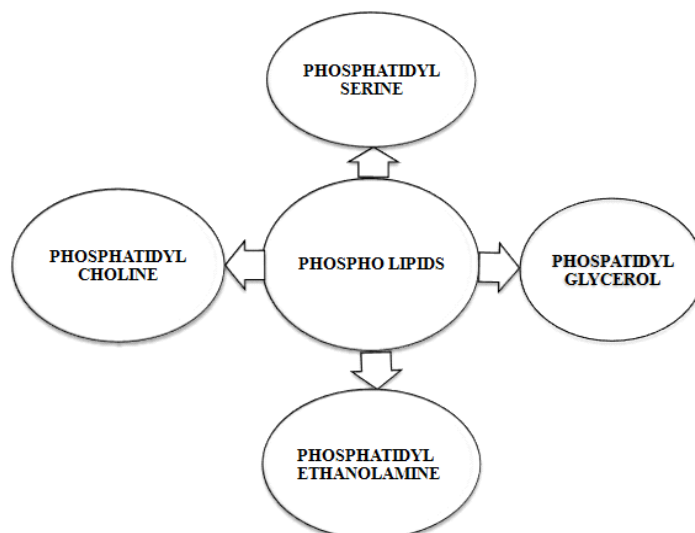


Fig. 2: Types of phospholipid involved are
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Lipid core

It is composed of lipids. They are naturally occurring substances like fat, wax, sterols and the fat-soluble vitamins [like A, D, E and K]. Lipids are widely used in immunology, membrane biology and diagnostic purposes. Either one or more lipids can be used for the synthesis of lipid core. The lipids are generally hydrophobic in nature and are determined by the concentration of fatty acid, melting point, hydrophilic-lipophilic balance (HLB). For sustained release of drugs, lipids with lower HLB and increasing melting point are used. The lipids with higher HLB cause rapid drug release and increased bioavailability [33-36].

Phosphatidylcholine

Lecithin is mainly composed of the phosphatidylcholine. Its solubility in water is very less. Based on temperature and hydration, phospholipid in aqueous medium forms bilayer sheets, micelles, or lamellar structure. This kind of surfactants are classified into amphipathic type. It forms the important constituent of biological membranes like egg yolk or soya bean. Based on the origin from which they are derived they are known to be as egg lecithin and soya lecithin. By the introduction of lecithin the drug entrapment to the vesicle also increases to a great extent [37, 38].

Cholesterol

The cholesterol form an important constituent of a vesicles. The introduction of cholesterol increases the stability of vesicle to a great extent [39, 40]. The composition of cholesterol influence the drug entrapment in vesicle [41]. As the concentration of cholesterol increases drug entrapment ability of vesicle also increases. But if the concentration of cholesterol is very high the entrapment efficiency decreases to a great extent. This is because of the fact that after a particular level cholesterol cause disturbances in the bilayer and thereby reducing the drug entrapment [42].

Negatively charged particles

The phosphatidylinositol is a type of negatively charged phospholipid. The incorporation of negatively charged particle decreases the aggregation of particles and it also reduces the coalescence, flocculation or fusion [43].

Surfactants

Surfactants are selected based on (HLB). It act as indicator of ability of a surfactant to form a vesicle. HLB of range 4-8 are suitable for vesicle formation [44]. The variation in temperature of surfactant influence the entrapment of drug in to the vesicle. The spans which

are having highest phase transition temperature exhibit increased drug entrapment [45, 46]. The high phase transition temperature and less permeability cause leaching of drug from the lipid vesicle [47]. High HLB value of span 40 and 60 cause decrease in surface free energy which results in vesicles of larger size and thereby increasing the area exposed to the dissolution medium [48-51].

Formulation of cryptosome

Cryptosomes are formed from liposomes and poloxamer molecules. Above the critical micellar temperature the poloxamer molecules form micelles, and a fraction of them get introduced into the surface of liposome thus preventing their adhesion to the cells. Poloxamer molecules dissociate below their critical micellar temperature to form monomers, permitting the liposome to adhere to the neighbouring cells and influence the holding of liposome on the adjacent cells. The targeted release of agents involves the introduction of components into the monomers and cooling the target site, which confines the liposomes at or near the targeted site.

The liposomes are prepared by the various method. The lipid vesicles can be prepared by standard techniques like sonication and extrusion. They can also be synthesised from reversed phase evaporation, detergent dialysis and freeze-thawing. After various steps involved in the synthesis of liposome (fig. 3) the poloxamer molecules are incorporated into it to form the Cryptosomes.

The liposomes collected is treated with poloxamer molecules and get embedded in it. The stealthing of liposome is primarily done by using polyethylene glycol and poloxamer or polymer. This results in the formation of Cryptosomes. The complete information regarding the formulation is explained in Mayank Gangwar *et al.* The PEG of molecular weight in the range of 1000 to 5000 exhibit prolonged circulation and decreased uptake by mononuclear phagocytic system (MPS) [52]. The most commonly used phospholipid in the formulation is phosphatidylcholine, it form the elementary unit of plasma membrane, it act as a safeguard of polyphenolics, and it also exhibit both hydrophilic and lipophilic analogs, the phosphatidyl moiety being lipophilic and choline moiety being hydrophilic nature [53].

Methods of formulation

Sonication method

To a round bottom flask varying molar ratios of phosphatidylcholine, cholesterol, lipids were taken and dissolved in chloroform having 3-4 drops of methanol. The proper amount of drug was weighed and added to that. Then evaporated the organic layer until dry in the presence of rotary evaporator in a reduced

pressure condition. This result in the formation of a lipid film on sides of round bottom container [54, 55].

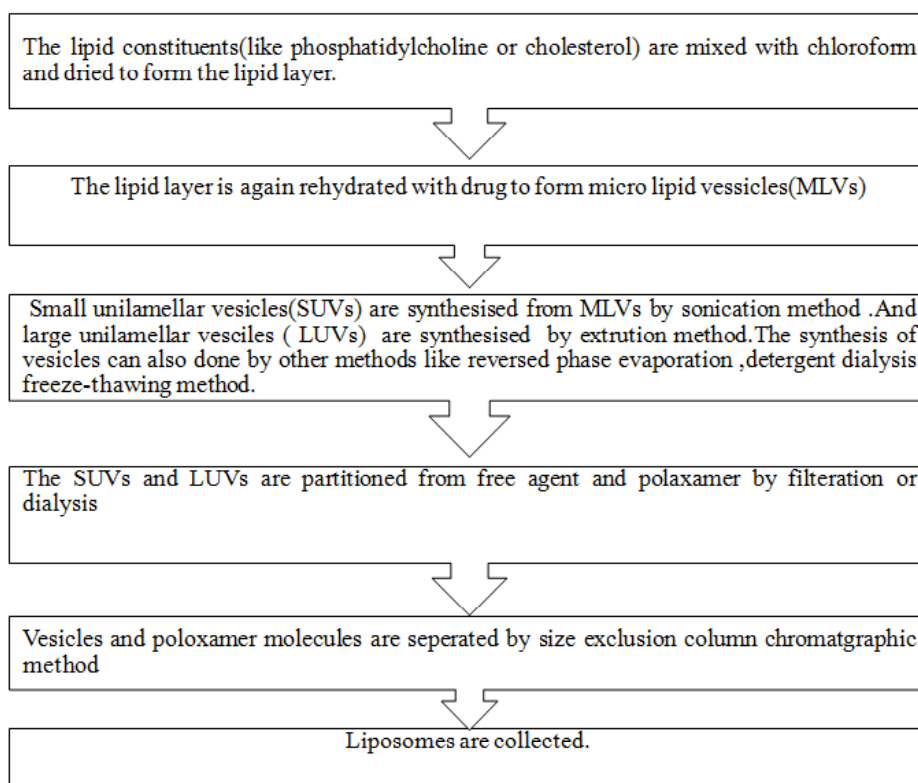


Fig. 3: The steps involved in the synthesis of liposome as follows
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Detergent removal technique

Micellar mixture is formed by mixing the phospholipid and detergent. Then the detergent is removed from the mixture by adsorption or column chromatographic technique. The phospholipid contents in the micelles increase and lipids come closer to form a vesicle of single bilayer [56].

Reversed phase evaporation

It consists of two steps. By using phospholipid and buffer, a water in oil type of emulsion is prepared. Then under reduced pressure organic layer is separated and removed. Both water and

phospholipid layer is emulsified by sonication or by other mechanical methods. Due to the removal of organic layer in vacuum condition, a gel like matrix is formed, as the phospholipid coated water droplets come closer. A paste of smooth texture is formed on further loss of organic phase under vacuum. This paste forms the suspension of LUVs [57]. The efficiency of incorporation of drug can be achieved up to 60-65% by this method. Therefore it can be used for incorporation of both small and large molecules [58].

The information regarding the structure is from Biju SS *et al.* The structure of cryptosome is given below (fig. 4).

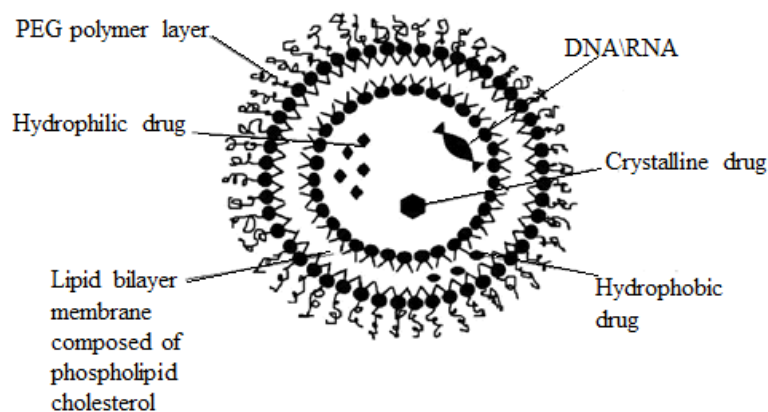


Fig. 4: Structure of cryptosome
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Drug release from cryptosomes

The phospholipid bilayer of Cryptosomes fuse with other bilayer membranes (e. g. cell membrane) and thus releasing the liposomal

contents. This takes place either by endocytosis or adsorption to the cell surface. In endocytosis, the lipid bilayer fuses with the plasma membrane and releasing the contents. But in the case of adsorption to the cell surface, transfer of liposomal contents takes place.

Table 2: Cryptosomes V/S ordinary liposome [59]

Cryptosomes	Ordinary liposome
They are sterically well stabilized	They are not sterically well stabilized as compared to Cryptosomes.
It is used for targeted drug delivery.	It is not efficiently used for targeted drug delivery.
It exhibits Reduced recognition and uptake by macrophages	It exhibit enhanced recognition and uptake by macrophages
Prolonged circulation and half-life.	Reduced circulation and half-life.
It is efficiently used in dose-independent pharmacokinetics.	It may be efficiently used in dose-independent pharmacokinetics.
It exhibit increased uptake <i>in vivo</i> by solid tumors and breast cancer.	It exhibits reduced uptake <i>in vivo</i> by solid tumours and breast cancer.
Decreased tendency to leak the drug during blood circulation.	Increased tendency to leak the drug during blood circulation.

Uses and applications of cryptosome

- Cryptosome can be used as potential carriers of biologically active compounds.
- Polyethylene glycols (PEG)-coated long-circulating sustained release liposomes, exhibit improved efficacy of ciprofloxacin administered for the treatment of Klebsiella-pneumoniae causing pneumonia [60].
- Doxil the liposomal formulation of doxorubicin containing polyethylene-glycols shows increased therapeutic activity, prolonged

circulation time, and accumulation time in murine tumors over free (unencapsulated) doxorubicin (DOX). Liposome longevity in malignant effusions is related to improved drug accumulations [61].

- It can be used for slow release of drug, tumour imaging and therapy [62, 63].
- It is enhanced the drug delivery in solid tumours [64, 65] and breast cancer [66-71].
- Cryptosomes is used as ligand-gated drug delivery system (fig. 5) [72-76].

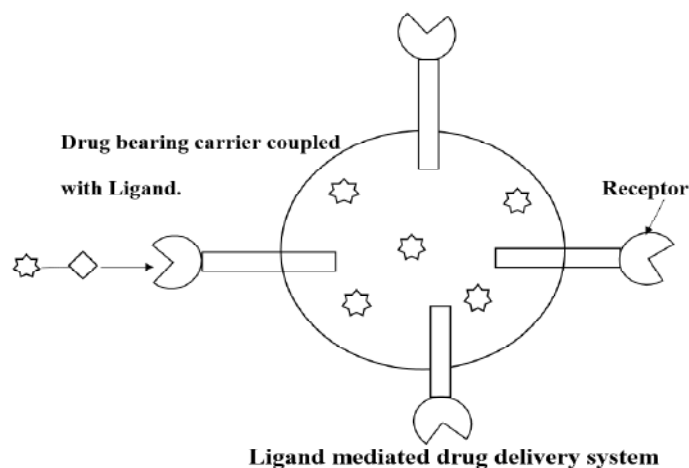


Fig. 5: Ligand mediated drug delivery system
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CONCLUSION

One of the greatest challenge faced by the scientist is to improve the dosage forms for increasing their half-life or duration of action. Cryptosome, due to high stability, prolonged circulation, increased half-life, reduced recognition and uptake by macrophages, are considered as one of the most efficient vesicular drug delivery systems. This is a lipid vesicle with surface coat, circulate in the blood for a long period of time after systemic applications. This system enabled a remarkable growth in drug discovery, development, and use. They have attained a huge engrossment among different novel vesicular drug delivery systems with a phospholipid bilayer and a lipid core. Vesicular systems symbolises the use of vesicles in the different fields as a carrier system or additives. It is used as a potential carrier of biologically active compounds. It is found applicable in the field of tumour imaging and therapy. In future in association with other strategies, Cryptosome like vesicle will play the central role in novel drug delivery in diagnosis and targeted drug delivery.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

Declared none

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