Development, Characterization and Performance Evaluation of Liposomes Containing an Antihypertensive Drug (Valsartan)

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ABSTRACT

The objective of this study was to formulate and evaluate liposomes loaded with Valsartan for the treatment of hypertension. Liposomes were prepared using the rapid film evaporation method, with Soya Lecithin and Cholesterol as lipid components. Drug-polymer compatibility was confirmed through Fourier-transform infrared (FTIR) studies, indicating no significant interactions between the drug and the excipients. The formulations were evaluated for drug content, entrapment efficiency, particle size distribution, zeta potential, and in vitro drug release.

The formulation was assessed for drug content, entrapment efficiency, particle size distribution, zeta potential, and in vitro drug release. The prepared liposomes found to be creamy, white and homogeneous in appearance. Entrapment efficiency ranged from 79% to 95.25%, and zeta potential values varied from -4.42 mV to -74.6 mV, representing good stability. Among the all formulations, F12 was found to most favourable performance with a vitro drug release of 94.79% at the end of 12 hours. Based on these finding, it can be concluded that the formulated Valsartan – loaded liposomes demonstrated efficient drug encapsulation, stability, and sustained release, making them promising candidate for controlled drug delivery in the management of hypertension treatment.

Keywords: Valsartan; soya lecithin; cholesterol; FTIR; Entrapment efficiency; *In vitro* drug release, zeta potential.

1.INTRODUCTION

Targeted Drug Delivery:

In traditional drug delivery systems such as oral ingestion or intravascular injection, the medication is distributed throughout the body through the systemic blood circulation. For most therapeutic agents, only a small portion of the medication reaches the organ to be affected. Targeted drug delivery seeks to concentrate the medication in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. For example, by avoiding the host's defence mechanisms and inhibiting non-specific distribution in the liver and spleen, a system can reach the intended site of action in higher concentrations.
Targeted drug delivery systems are made to increase the accuracy and effectiveness of medication delivery to particular organs or tissues, improving therapeutic outcomes and reducing adverse effect. Conventional techniques such as intravascular injection or oral administration frequency cause the medication to be distributed throughout the body by systemic circulation, which may result in undesired exposure to other tissues and less than ideal drug concentration at the larger site.

Targeted drug delivery often referred to as smart drug delivery is a method aimed to increasing the concentration of a medication in specific areas of the body while reducing its presence elsewhere. The primary goal of such systems is to prolong the drug's residence time, localize its action, and ensure controlled interaction with the target tissue.

Targeted delivery systems can be broadly classified into two categories:

- **Active Targeted Drug Delivery –** This involves the use of ligands or antibodies that recognize and bind to specific receptors on the target cells, thereby enhancing drug accumulation at the desired site.
- **Passive Targeted Drug Delivery –** This exploits the enhanced permeability and retention (EPR) effect commonly observed in tumour tissues and inflamed regions, allowing nanocarriers such as liposomes to accumulate preferentially at these sites due to leaky vasculature and poor lymphatic drainage**.**

In the context, liposomes serve as effective carries for targeted drug delivery due to their biocompatibility, ability to encapsulate both hydrophilic and lipophilic drugs, and potential for surface modification to achieve active targeting. The present study focuses on the formulation and evaluation of Valsartan-loaded liposomes as a targeted delivery system for the treatment of hypertension.

Advantages:

- Reduction in the frequency of the dosages taken by the patient.
- More uniform effect of the drug,
- Reduction of drug side-effects.
- Reduced fluctuation in circulating drug levels.

Disadvantage:

- High cost, which makes productivity more difficult and the reduced ability to adjust the dosages.

Liposomes

Liposomes are spherical vesicles derived from the Greek words “Lipos” (meaning fat) and “soma” (meaning body). They are characterized by a phospholipid bilayer membrane that encloses an aqueous core, enabling the encapsulation of both hydrophilic (water-soluble) and hydrophobic (fat soluble) substances. This structural versatility makes liposomes highly valuable in pharmaceutical applications.

The liposomal membrane is primarily composed of phospholipids-amphiphilic molecules consisting of a hydrophilic (water-repelling) tail. The hydrophilic head interacts with the aqueous environment, while the hydrophobic tail forms the interior of the bilayer, away from water. These lipids can be either natural or synthetic and are generally biocompatible, biodegradable, and non-immunogenic, making them suitable for biomedical use.

Due to their unique bilayer architecture, liposomes are capable of encapsulating a wide range of therapeutic agents. Hydrophilic drugs are confined within the aqueous core, while lipophilic drugs are primarily incorporated into the lipid bilayer. This dual encapsulation capability enhances the stability, bioavailability, and targeted delivery of many drugs, making liposomes an essential platform in advanced drug delivery technologies.[2]

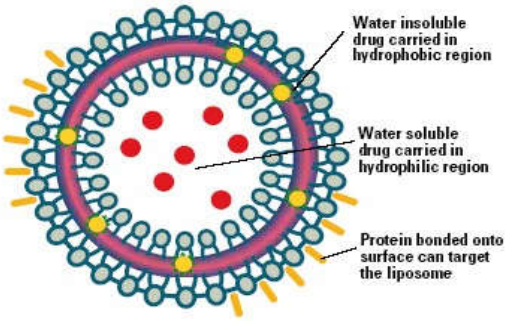


Figure 1.1 A Typical Liposome

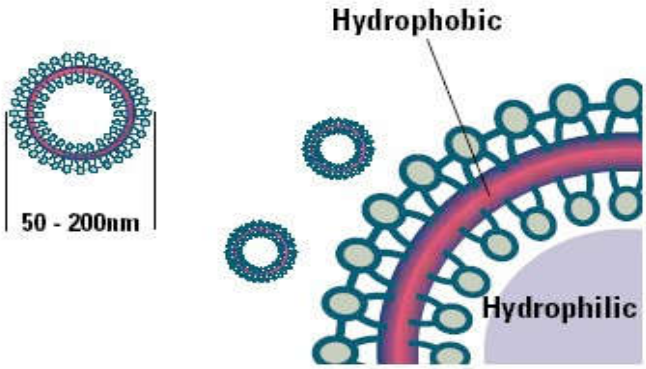


Figure 1.2 Bilayer Membrane

Liposomes are first described by British Haematologist Dr. Alec D. Bangham FRS in 1961 (published 1964). He and his colleague R.W. Horne worked at the Babraham Institute in Cambridge (UK). They were testing the institute’s new electron microscope and decided to examine dry phospholipids by adding a negative stain. When they observed the phospholipid samples under high magnification, they noticed the formation of closed, sphere-shaped bilayer membranes, which they later termed liposomes.

Classification:

Liposomes can be classified based on their composition and mechanism of intracellular delivery into five main types and they are:

1. Conventional Liposomes
2. pH-Sensitive Liposome
3. Cationic Liposomes
4. Immuno-Liposomes
5. Long-Circulating Liposomes

In addition, liposomes are typically Classified by their size and the number of bilayers into the following categories:

1. Small Unilamellar Vesicles (SUVs): 20-100 nm
2. Large Unilamellar Vesicles (LUVs): > 100 nm
3. Giant Unilamellar Vesicles (GUVs): > 1000 nm
4. Oligolamellar Vesicles (OLVs): 100 – 500 nm
5. Multilamellar vesicles (MLV): > 500 nm.[3]

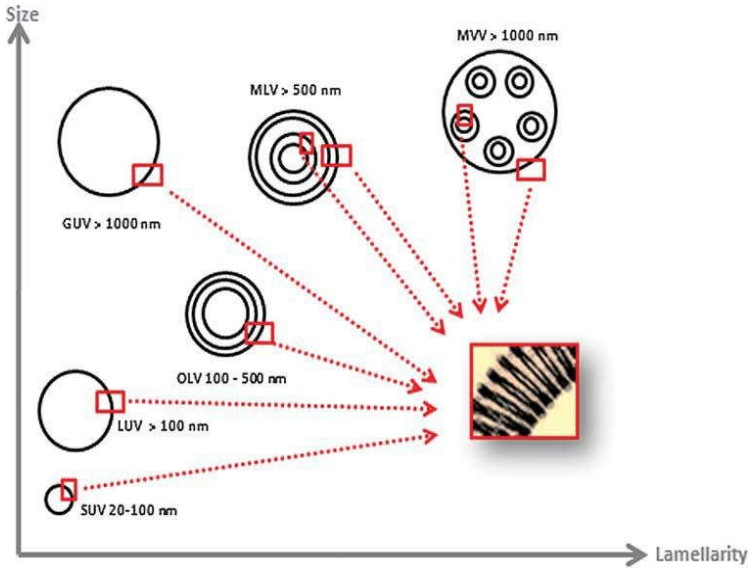


Figure 1.3 Liposomes classification based on size and lamellarity.

2.Mechanism of formation of liposomes:

Liposomes are formed when phospholipids are introduced into an aqueous environment. This Process is driven by the amphipathic nature of phospholipids and their self-assembly behaviour in polar solvents.

1. Amphipathic Nature of Phospholipids

Phospholipids consist of:

- **A hydrophilic (polar) head**, typically composed of a phosphate group bound to a water-soluble molecule.
- **A hydrophobic (non-polar) tail**, made up of two fatty acid chains, each containing **10-24 carbon atoms** and possibly **0-6 double bonds.**

This dual-affinity structure makes phospholipids amphipathic, meaning they have both water-attracting and water-repelling properties.

2. Self-Assembly in Aqueous Environments

When Phospholipids are hydrated:

- The **polar head** align towards the water to maximize interaction with the aqueous environment.
- The **non-polar tails** avoid water and aggregate inward away from the solvent.

This spontaneous arrangement leads to the **self-organization of Phospholipids into bilayer structures**. These bilayers can close into spherical vesicles, forming **liposomes**, to eliminate the exposure of hydrophobic regions to water.

3. Formation of Concentric Bilayers

Phospholipids often from **concentric bilayers** (multiple layers like an onion), particularly when used in drug delivery systems. Among various amphiphiles (e.g., soaps detergents), **polar lipid** are most suitable for liposome formation due to their stable bilayer-forming capacity. However, in aqueous medium these molecules are able to form various phases, some of them are stable and others remain in the metastable state. At high concentrations of these polar lipids, liquid-crystalline phases are formed that upon dilution with an excess of water can be dispersed into relatively stable colloidal particles. The macroscopic structure most often formed includes lamellar, hexagonal or cubic phases dispersed as colloidal nano construct (artificial membrane) referred to as liposomes, hexasomes or cubosomes respectively. The most common natural polar phospholipids are phosphatidylcholine. These are amphipathic molecule in which a glycerol bridge links to a pair of hydrophobic acyl chains with a hydrocarbon chain with a hydrophilic polar head group, phosphocholine. Thus, the amphipathic (amphiphilic) nature of the phospholipid and their analogues render them the ability to form closed concentric bilayers in the presence of water.[4]

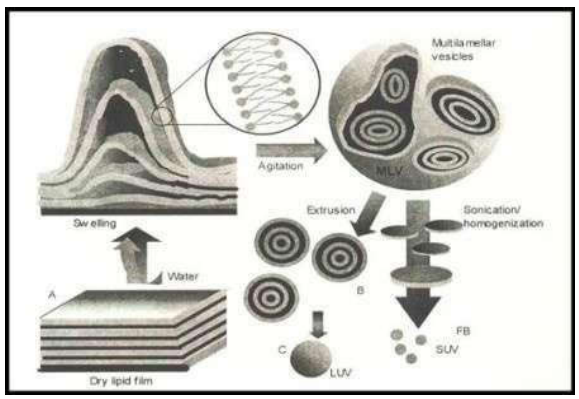


Figure 2.1 Mechanism of liposome formulation

3. Advantages of liposomes:

- Non-ionic. Biodegradable drugs can be stabilised from oxidation.
- Improve protein stabilisation.
- Controlled hydration.
- Provide sustained release.
- Targeted drug delivery or site-specific drug delivery.
- Stabilisation of entrapped drug from a hostile environment.
- Alter the pharmacokinetics and pharmacodynamics of drugs.
- Can be administered through various routes.
- Can incorporate micro and macro molecules.
- Act as a reservoir of drugs.
- The therapeutic index of drugs is increased.
- Site avoidance therapy.
- Can modulate the distribution of the drug.
- Direct interaction of the drug with the cell.
- Biodegradable and flexible.

4. Disadvantages of liposomes:

- Less stability.
- Low solubility.
- Short half-life.
- Phospholipid undergoes oxidation, hydrolysis, Leakage, and fusion.
- High production cost.
- Allergic reactions may occur to liposomal constituents.
- The problem to targeting various tissues due to their large size.

5. Structural components

a) Phospholipids: Glycerol-containing phospholipids are the most commonly used component of liposome formulation and represent greater than 50% of the weight of lipid in biological membranes. These are derived from phosphatidic acid. The backbone of the molecule is the glycerol moiety. At the C3 position OH group is esterified to phosphoric acid. OH, at C1 & C2 are esterified with a long chain. Fatty acid gives rise to the lipidic nature. One of the remaining OH groups of phosphoric acid may be further esterified to a wide range of organic alcohols, including glycerol, choline, ethanolamine, serine, and inositol. Hence, the parent compound of the series is the phosphoric ester of glycerol.

b) Sphingolipids: Backbone is sphingosine or a related base. These are important constituents of plant and animal cells. A head group that can vary from simple alcohols, such as choline, to very complex carbohydrates. Most common Sphingolipids – Sphingomyelin. Glycosphingolipids. Gangliosides – found in grey matter, used as a minor component for liposome production. This molecule contains complex saccharides with one or more Sialic acid residues in their polar head group & thus has one or more negative charges at neutral pH. These are included in liposomes to provide a layer of surface-charged groups.

c) Sterols: Cholesterol & its derivatives are often included in liposomes used for reducing the fluidity or micro viscosity of the bilayer, reducing the permeability of the membrane to water-soluble molecules, and stabilising the membrane in the presence of biological fluids such as plasma. (This effect is used in the formulation of, e.g., liposomes).

6. LIPOSOMES PREPARATION PROCEDURES:

Preparation Method

Classical Technique

There are four classical methods of liposome manufacture.

The difference between the various methods is the way in which lipids are drying down from organic solvents and then redispersed in aqueous media.

6.1 Hydration of a Thin Lipid Film: *Bangham Method.*

This is the original method, which was initially used for liposome production. A mixture of phospholipids and cholesterol was dispersed in the organic solvent. Then, the organic solvent was removed utilising evaporation (using a Rotary Evaporator at reduced pressure). Finally, the dry lipidic film deposited on the flask wall was hydrated by adding an aqueous buffer solution under agitation at a temperature above the lipid transition temperature

6.2 Reverse-Phase Evaporation (REV) Technique:

A lipidic film is prepared by evaporating organic solvent under reduced pressure. The system is removed with nitrogen, and the lipids are re-dissolved in a second organic stage, which is usually formed by diethyl ether and/or isopropyl ether. Large unilamellar and oligolamellar vesicles are formed when an aqueous buffer is introduced into this mixture. The organic solvent is subsequently removed, and the system is maintained under continuous nitrogen.

6.3 Solvent (Ether or Ethanol) Injection Technique:

The solvent injection methods involve the dissolution of the lipid into an organic phase (ethanol or ether), followed by the injection of the lipid solution into aqueous media, forming liposomes. The Ethanol Injection method was first described in 1973. The main relevance of the ethanol injection method resides in the observation that a narrow distribution of small liposomes (under 100 nm) can be obtained by simply injecting an ethanolic lipid solution in water, in one step, without extrusion or sonication. The ether injection method differs from the ethanol injection method since the ether is immiscible with the aqueous phase, which is also heated so that the solvent is removed from the liposomal product. The method involves an injection of ether-lipid solutions into warmed aqueous phases above the boiling point of the ether. The ether vaporises upon contacting the aqueous phase, and the dispersed lipid forms primarily unilamellar liposomes.

6.4Treatment of human immunodeficiency virus (HIV) infections:

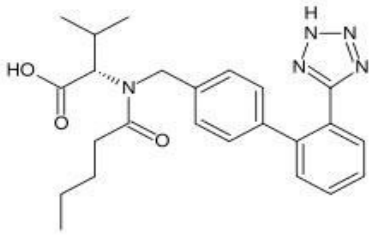
Several antiretroviral nucleotide analogues have been developed for the treatment of patients suffering from the acquired immunodeficiency syndrome (AIDS). These include antisense oligonucleotide, which is a new antiviral agent that has shown potential therapeutic application against HIV-1.

7. Method of preparation of Liposomes of Valsartan

7.1 Valsartan

Valsartan is an angiotensin II receptor antagonist (more commonly called an "ARB", or angiotensin receptor blocker), with particularly high affinity for the type I (AT_1) angiotensin receptor. By blocking the action of angiotensin, Valsartan dilates blood vessels and reduces blood pressure.

Chemical structure of Valsartan



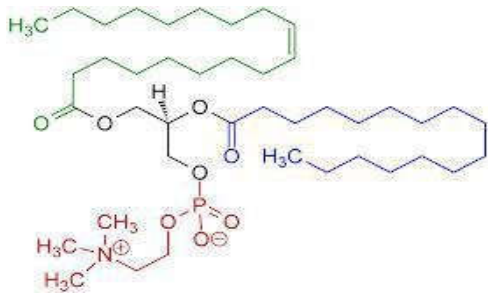
Medical uses:

Valsartan is used to treat high blood pressure, congestive heart failure, and to reduce death for people with left ventricular dysfunction after having had a heart attack. There is contradictory evidence about treating people with heart failure with a combination of an angiotensin receptor blocker like Valsartan and an angiotensin converting enzyme inhibitor, with two major clinical trials (CHARM-additive and Val Heft) showing a reduction in death, and two others (VALIANT and ONTARGET) showing no benefits, and more adverse including heart attacks.

7.2 Soya Lecithin:

The term "lecithin" as used today refers to the material obtained by degumming crude vegetable oils and drying the hydrated gums the specific phospholipids formerly called lecithin is now referred to as phosphatidylcholine. Lecithin contains choline, which is an important precursor for many biochemical functions. Choline helps provide structure and fluidity in cell membranes, improves transfer of nerve impulses to the brain and circulatory system, and aids in lipid and cholesterol transport and metabolism.

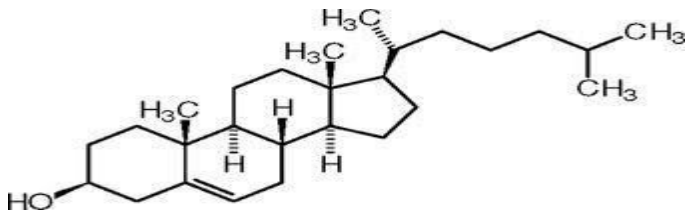
Structural formula:



7.3 Cholesterol:

Cholesterol is used in cosmetics and topical pharmaceutical preparations at absorptions of 0.3–5.0% w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity. Cholesterol also has a biological role. It is the major sterol of the higher animals, and it is found in all body tissues, especially in the brain and spinal cord. It is also the main constituent of gallstones.

Structural Formula:



8. Applications of liposomes:

a) Protection against enzyme degradation of drugs:

Liposomes are used to protect the entrapped drug against enzymatic degradation whilst in circulation. The basis is that the lipids used in their formulation are not susceptible to enzymatic degradation; the entrapped drug is thus protected while the lipid vesicles are in circulation in the extracellular fluid.

b) Drug targeting:

The approach for drug targeting via liposomes involves the use of ligands (e.g., antibodies, sugar residues, apoproteins, or hormones), which are tagged on the lipid vesicles. The ligand recognises specific receptor sites and, thus, causes the lipid vesicles to concentrate at such target sites.

c) Topical drug delivery:

Liposomes increase the permeability of skin for various entrapped drugs and at the same time diminish the side effects of these drugs because lower doses are now required.

d) Treatment of human immunodeficiency virus (HIV) infections:

Several antiretroviral nucleotide analogues have been developed for the treatment of patients suffering from the acquired immunodeficiency syndrome (AIDS). These include antisense oligonucleotide, which is a new antiviral agent that has shown potential therapeutic application against HIV-1.

a) Enhanced antimicrobial efficacy/ safety:

Antimicrobial agents have been encapsulated in liposomes for two reasons. First, they protect the entrapped drug against enzymatic degradation. Secondly, the lipid nature of the vesicles promotes enhanced cellular uptake of the antibiotics into the microorganisms, thus reducing the effective dose and the incidence of toxicity as exemplified by the liposomal formulation of amphotericin B.

9. Conclusion

The purpose of the study was to develop and evaluate liposome containing Valsartan using various suitable polymers. The novelty of the work resides in the formulation of liposomes of Valsartan, using polymers i.e. soya lecithin, cholesterol. Hence, an attempt was made to formulate and evaluate liposome for the treatment of hypertension.

Using the Thin film method, Valsartan has been successfully incorporated into liposome formulations, which can be potentially useful for the delivery of this drug. Results of the present work have shown that the content of cholesterol and lecithin affects the encapsulation efficiency and drug release rate from liposomes. A maximum encapsulation efficiency of 95.25 % and drug release of 94.79 % after 12 hr have been attained. Liposome formulation with a higher concentration of soya lecithin had more encapsulation efficiency compared with a higher concentration of cholesterol.

To design and evaluate liposomes of Valsartan that would release the drug over a prolonged period, thus avoiding first-pass metabolism, and to improve its systemic availability. The IR spectra analysis suggested that there is no interaction between the drug and formulation additive, the drug exists in its original form and is available for biological action.

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