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:[1]

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]

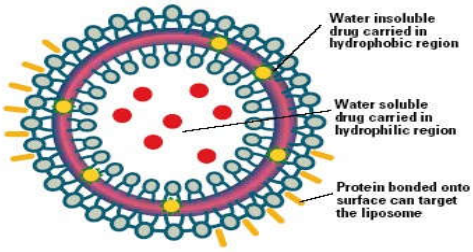


Figure 1.1 A Typical Liposome

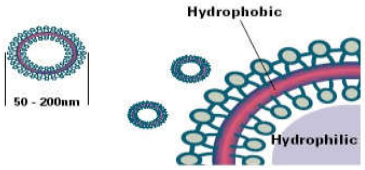


Figure 1.2 Bilayer Membrane

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.

2.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 Liposomes
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]

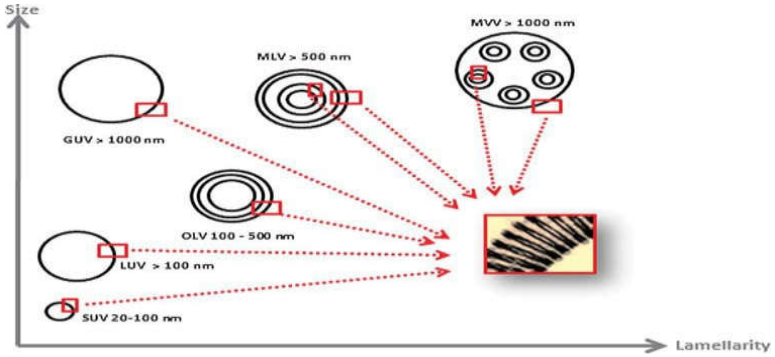


Figure 1.3 Liposomes classification based on size and lamellarity.

Advantage of liposome:

- Non-ionic.
- Can carry both water and lipid soluble drugs.
- Biodegradable drugs can be stabilized from oxidation.
- Improve protein stabilization.
- Controlled hydration.

Disadvantages:

- Less stability.
- Low solubility.
- Short half-life.
- Phospholipid undergoes oxidation, hydrolysis Leakage and fusion.
- High production cost.

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]

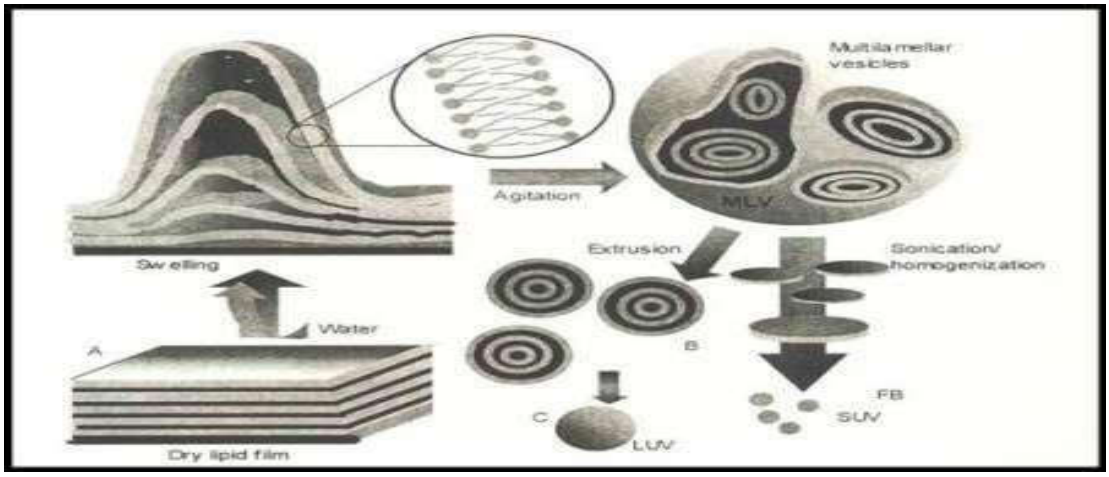


Figure 1.4 Mechanism of liposome formulation

Structural components[5, 6]

a) Phospholipids: Glycerol containing phospholipids are most common used component of liposome formulation and represent greater than 50% of weight of lipid in biological membranes. These are derived from phosphatidic acid. The back bone of the molecule is glycerol moiety. At C3 position OH group is esterified to phosphoric acid. OH, at C1 & C2 are esterified with long chain. Fatty acid giving rise to the lipidic nature. One of the remaining OH group of phosphoric acid may be further esterified to a wide range of organic alcohols including glycerol, choline, ethanolamine, serine and inositol. Thus, 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. Glycosphingo lipids. Gangliosides – found on 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 have one or more negative charge at neutral pH. These are included in liposomes to provide a layer of surface charged group.

c) Sterols: Cholesterol & its derivatives are often included in liposomes for decreasing the fluidity or microviscocity of the bilayer reducing the permeability of the membrane to water soluble molecules stabilizing the membrane in the presence of biological fluids such as plasma. (This effect used in formulation of i.e. liposomes).

d) Polymeric materials: Synthetic phospholipids with diacetylenes group in the hydrocarbon chain polymerizes when exposed to U.V, leading to formation of polymerized liposomes having significantly higher permeability barriers to entrapped aqueous drugs. e.g. for other Polymer sable lipids are – lipids containing conjugated diene, methacrylate etc.

Other Substances:

- Varieties of other lipids of surfactants are used to form liposomes.

- Many single chain surfactants can form liposomes on mixing with cholesterol.
- Non-ionic lipids.

3. REVIEW OF LITERATURE

Nagasamy Venkatesh Dhandapani *et al* 2013;4(2):187-193., had given information about the method of production and extensive therapeutic potential of liposomes as carriers for targeted and controlled delivery.[7]

Eskandar Moghimipour *et al* 2012;2(1):93-98., worked on to formulate and characterize liposomal vesicles loaded with celecoxib. Liposomes were prepared by thin film method using soya lecithin and cholesterol. The release of drug was determined using a dialysis membrane method. Liposomes were characterized by Differential Scanning Calorimetry (DSC), Transmission Electron Microscopy (TEM) and their particle size was also determined. The results of characterization of the vesicles indicated the potential application of celecoxib loaded liposome as carrier system.[8]

Supaporn Sriwongsitanont *et al* 2011;(4):1-6., studied the influence of the freeze-thawing process on the size and lamellarity of multilamellar PEG-lipid liposomes prepared from a mixture of egg yolk phosphatidylcholine (EggPC) and Distearoyl Phosphatidylethanolamine polyethylene glycol (DSPE-PEG) 2000 was investigated. Trapped volume measurement, quasielliptical light scattering (QELS) and freeze-fracture electron microscopy were used to estimate the morphology and lamellarity of liposomes. During the freeze-thawing process, the lamellarity of multilamellar vesicles (MLVs) depended strongly on both PEG-lipid concentration and the number of freeze-thaw cycles. The decrease in the number of lamellae was a function of the number of freeze-thaw cycles. The increase in trapped volume coincided with the decrease in the number of lamellae observed in electron micrographs. After comparing the results obtained from EggPC/DSPE-PEG2000 MLV and from pure EggPC MLV, it was concluded that during the freeze-thawing the liposomes with DSPE-PEG2000 achieved a unilamellar structure more readily than the pure EggPC liposomes. [9]

Soleiman Mohammadi-Samani *et al* 2009;5(4):199-204., in his study loaded Cyproterone acetate (CA) to liposome by solvent evaporation and thin film formation technique. The effects of some formulation variables such as temperature of organic solvent evaporation, rotary evaporator speed, volume of organic solvent, volume of balloon and temperature of hydrating buffer has been evaluated. The data showed that bigger balloon with higher surface area has better capacity for lipoid film formation; also, the best temperature for solvent evaporation and film hydration was 40 °C. According to the data with higher drug/lipid ratio, it is possible to load higher amount of drug into liposome but optimum loading could be obtained at phosphatidylcholine (PC): cholesterol/drug ratio of 1:2:0.5. The results showed that liposomal formulation has better penetration potential than conventional CA formulation.[10]

N Manjunatha et al 2009;1(1):25-31., designed and development acyclovir liposomes by reverse phase evaporation method using various ratios of phosphatidyl choline with cholesterol and Cephalin (phosphatidyl ethanolamine) with cholesterol. Based on evaluation of entrapment efficiency, the best formulations were subjected to physicochemical studies i.e., photo microscopy, in vitro drug release and stability studies. The liposomes stored at 40°c were found to be stable for duration of two months compared to other storage conditions.[11]

S.Rathod *et al.*, formulated et al 2010;72(2):155–160., prolonged release drug delivery system of pilocarpine nitrate by optimizing thin layer film hydration method. Egg phosphatidylcholine and cholesterol were used to make multilamellar vesicles. Effects of charges over the vesicles were studied by incorporating dicetylphosphate and Sterylamine. Various factors, which may affect the size, shape, encapsulation efficiency and release rate, were studied. Biological response in terms of reduction in intraocular pressure was observed on rabbit eyes. Pilocarpine nitrate liposomes were lyophilized and stability studies were conducted.[12]

Mohammad Riaz *et al* 1996;19(1):65-77., had given information about various methods of liposome preparation (including the large-scale manufacture) has been reviewed. The advantages and disadvantages of the methods have been described in terms of size distribution and encapsulation efficiency.[13]

Judith H. Waknine *et al* 2013;8(8): e72722., formulated a novel formulation of liposome-encapsulated water-soluble glucocorticoid prodrugs, and in particular b-methasone hemi - succinate (BMS), for treatment of experimental cerebral malaria (ECM), using the murine P. berghei ANKA model. BMS is a novel derivative of the potent steroid b-methasone, and was specially synthesized to enable remote loading into nano-sterically stabilized liposomes (nSSL), to form nSSL-BMS. The novel nano-drug, composed of nSSL remote loaded with BMS, dramatically improves drug efficacy and abolishes the high toxicity seen upon administration of free BMS. nSSL-BMS reduces ECM rates in a dose-dependent manner and creates a survival time-window, enabling administration of an anti- plasmodial drug, such as artemisone. Administration of artemisone after treatment with the nSSL-BMS results in complete cure.[14]

AV jithan *et al* 2010;3(2)., had developed a diclofenac liposomal gel intended for better anti-inflammatory activity compared to a normal gel after topical administration. Liposomal gel formulations were then prepared by incorporation of SUVs into Carbopol 934 gel previously prepared. The formulation Were the characterised for in-vitro drug release, ex-vivo permeation studies, drug-lipid compatibility and rheology behaviour. Diclofenac liposomal gel formulation prepared showed more sustained and prolonged anti-inflammatory effect compare with diclofenac gel.[15]

Vladimir P. Torchilin *et al* 2005; 4:160., had mentioned that Liposomes-microscopic phospholipid bubbles with a bilayer membrane structure have received a lot of attention during the past 30 years as pharmaceutical carriers of great potential. More recently, many new developments have been seen in the area of liposomal drugs from clinically approved products to new experimental applications, with gene delivery and cancer therapy still being the principal areas of interest. For further successful development of this field, promising trends must be identified and exploited, albeit with a clear understanding of the limitations of these approaches.[16]

Marcela Achim *et al* 2009;57(6)., developed doxorubicin (DOX)-loaded thermos sensitive liposomes (TSL). Liposomes were prepared by lipid film hydration and extrusion method and

characterized with respect to DOX loading, entrapment efficiency, size, morphology and in vitro DOX release. DOX entrapment efficiency was low, especially for TSL.[17]

Francis szoka *et al* 1978;75(9):4194-98., in his study mentioned that large unilamellar and oligolamellar vesicles are formed when an aqueous buffer is introduced into a mixture of phospholipid and organic solvent and the organic solvent is subsequently removed by evaporation under reduced pressure.[18]

Kant Shashi *et al* 2012;3(7)., in this review article, discussed about liposomes these are one amongst the various drug delivery system use to target the drug to particular tissue because of structure similarity b/w lipid bilayer and cell membrane.[19]

Kataria Sohil *et al* 2011;2(5):1534-1538., study involves the significant step in the development of long circulating liposome came with inclusion of the synthetic polymer PEG (poly ethylene glycol) in liposome composition. The presence of PEG on the surface of the liposomal carrier has been shown to extend blood-circulation time while reducing Mononuclear phagocyte system uptake (stealth liposome). this technology has resulted in a large number of liposome formulation encapsulated active molecules with target efficiency and activity.[20]

Bizhan Malaekeh - Nikoue *et al* 2011;02(06):1-4., prepared and characterized liposomes containing clindamycin (Lip-CL), liposomes containing tretinoin (Lip-TRT) and liposomes loaded with both tretinoin and clindamycin (Lip-CL-TRT). Lip-TRT were prepared by solvent evaporation method whereas Lip-CL and Lip-CL-TRT were prepared by dehydration method. The morphologic, mean size and drug encapsulation efficiency were evaluated. Also, the amount of drug which was passed through or retained inside the skin was determined by Franz cell diffusion method and compared with the TRT cream. The particles of the liposomes were obtained in submicron size. The encapsulation efficiency (EE) of TRT and CL were high in the liposomal formulations. The retention of TRT and CL inside the skin from the Lip-CL-TRT were obtained more than 80%. Generally, the results of the present study showed that it is possible to select liposomes as drug carrier for both CL and TRT.[21]

K L Senthilkumar *et al* 2012;1(1):81-86., prepared 6 formulations of liposomal carrier for Norboletone for the treatment of arthritis that is capable of delivering the drug to the specific target site by topical route by using different ratios of phospholipid and cholesterol with a desired amount of drug by thin film hydration technique and to find out the drug release from the liposome's of different ratios, mechanism kinetics of drug release pattern and also to find out the size distribution of liposome's of different ratios and also to increase the bioavailability and efficacy of the drug.[22]

P. Divakar *et* al 2013;4(2):479-485., prepared liposomal formulations containing Metformin hydrochloride. Liposomal formulations were evaluated for drug entrapment, surface characterization, *invitro* drug release studies, release kinetics and its mechanisms. Drug excipient compatibility was determined by using U.V spectroscopy, AT-IR spectral studies. Liposomal suspensions were prepared using film hydration technique using varying concentrations of phosphatidylcholine and cholesterol and optimize the ideal combination for required drug release. The prepared liposomes were rigid, intact and fulfilled all official requirements. The results of *in vitro* drug release studies showed that release from liposomal formulation was slow and sustained for >12 hrs period. The formulations followed first order kinetics and release mechanism was non-fickian diffusion from all the formulations. Thus, the liposomal suspensions of Metformin hydrochloride were successfully developed.[23]

Eskandar Moghimipour *et al* 2013;4(1):101-107., formulate and evaluate liposomal vesicles loaded with triamcinolone acetonide. liposomes containing triamcinolone acetonide were

prepared using thin film method, Carbomer 940 was used as gel base and four different gel formulations including hydro alcoholic gel, MLV liposomal gel, SUV liposomal gel and blank MLV gel containing free drug were prepared. The release profile of triamcinolone acetonide was determined using dialysis membrane method. Liposomes were also characterized by optical microscope and their particle size was also determined. Formulation containing lecithin: drug: cholesterol (100: 10: 10) having about 90.05 percent encapsulation was selected as the best formulation and the results of release showed SUV liposomal gel has the most regular and the least interaction between the drug and polymer. Results of particle size determination showed 50% of MLV and SUV liposomes had diameter below 33.80 µm and 22.09 µm, respectively. The results of characterization of the vesicles indicated the potential application of triamcinolone acetonide loaded liposome as carrier system.[24]

Rishu Kakkar *et al* worked on Non-ionic surfactant vesicles of valsartan, an angiotensin II inhibitor, were prepared by coacervation phase separation method. The prepared systems were characterised for encapsulation efficiency, shape, size and in vitro drug release. Stability study was carried out to investigate the leaching of drug from the proniosomal system during storage. The results showed that valsartan in all the formulations was successfully entrapped and a substantial change in release rate and an alteration in the encapsulation efficiency of valsartan from proniosomes were observed upon varying the type of surfactant and cholesterol content. The encapsulation efficiency of proniosomes prepared with Span 60 was superior to that prepared with Span 40. A preparation with 9:2:9 ratio of Span 60, cholesterol and lecithin gave maximum encapsulation efficiency (71.50%) and release results (Q24h=75%) as compared to other compositions. Proniosomal formulations showed fairly high retention of valsartan inside the vesicles at refrigerated temperature (4-8oC) up to 1 month.[25]

4. METHODOLOGY

Melting point determination of valsartan:

Melting point of Valsartan was determined by using Thale's tube method by taking a small amount of drug in a capillary tube closed at one end and placed in Thale's tube containing liquid petroleum and temperature at which drug melts was recorded. This was performed in triplicates and average value was reported.

Solubility of Valsartan:

Solubility of Valsartan was performed in various solvents like ethanol, methanol. Accurately weighed one gm of drug was transferred in a clean and dry test tube followed by addition of the solvents individually and shaken vigorously and the solubility of drug was checked visually.

Infrared spectral studies:

Method: In this technique, approximately 1 mg of the Valsartan was allowed to mix with about 100 mg of KBr (which is transparent to IR) in the ratio of 1:100 and then thoroughly mixed in a mortar. The mixture was pressed in a pellet die manually and placed it in a Fourier transform infrared (FTIR) spectrophotometer (Shimadzu corporation 8400S, Japan).

Preparation of standard graph of Valsartan:

Procedure:

Preparation of Standard solution (Ethanol):

1st Stock: 100 mg of Valsartan was accurately weighed into 100 ml volumetric flask and dissolved in small quantity of Ethanol, finally the volume was made up to 100 ml with Ethanol (1000 µg/ml).

2nd Stock: 1 ml of the above solution was pipette into another 100 ml volumetric flask and the volume was made up to 100 ml with Ethanol (10 µg/ml). From standard solution of 2nd stock i.e., 2ml, 4ml, 6ml, 8ml and 10ml were pipette into 10 ml volumetric flasks. The volume was made up with Ethanol. The spectrum of this solution was run in 200-400 nm range in UV-Visible spectrophotometer. The λ_{max} of Valsartan was found to be 250 nm. The absorbance of each concentration was measured at 250 nm using Ethanol as blank. This was performed in triplicates and average value was reported.

Preparation of Standard solution (Methanol):

1st Stock: 100 mg of Valsartan was accurately weighed into 100 ml volumetric flask and dissolved in small quantity of methanol; finally the volume was made up to 100 ml with methanol (1000 µg/ml).

2nd Stock: 1ml of the above solution was pipette into another 100 ml volumetric flask and the volume was made up to 100 ml with methanol (10µg/ml).

From the standard solution of 2nd stock i.e., 2ml, 4ml, 6ml, 8ml and 10ml were pipette into 10 ml volumetric flasks. The volume was made up with methanol in order to get 2 µg/ml,4 µg/ml, 6 µg/ml, 8 µg/ml and 10 µg/ml respectively. The spectrum of this solution was run in

200-400 nm range in UV-Visible spectrophotometer. The λ_{max} of Valsartan was found to be 250 nm. The absorbance of each concentration was measured at 250 nm using) methanol as blank. This was performed in triplicates and average value was reported.

Preparation of Standard solution (phosphate buffer pH-7.4):

1st Stock: 100 mg of Valsartan was accurately weighed into 100 ml volumetric flask and dissolved in small quantity of phosphate buffer 7.4, finally the volume was made up to 100 ml phosphate buffer 7.4 with (1000 µg/ml).

2nd Stock: 1ml of the above solution was pipette into another 100 ml volumetric flask and the volume was made up to 100 ml phosphate buffer 7.4 with (10µg/ml).

From the standard solution of 2nd stock i.e., 2ml, 4ml, 6ml, 8ml and 10ml were pipette into 10 ml volumetric flasks. The volume was made up with phosphate buffer 7.4 in order to get 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml and 10 µg/ml respectively. The spectrum of this solution was run in 200-400 nm range in UV-Visible spectrophotometer. The λ_{max} of Valsartan was found to be 250 nm. The absorbance of each concentration was measured at 250 nm using phosphate buffer 7.4 as blank. This was performed in triplicates and average value was reported.

4.4. Drug-Polymer compatibility:

A. FTIR spectrophotometer:

The compatibility of drug and polymer was analysed using FTIR spectrophotometer. In this technique, 1mg of the sample and 100mg of potassium bromide (KBr) (1:100 ratio) was finely ground using mortar and pestle. A small number of mixtures was placed for 2 minutes under a hydraulic press compressed at 7 Kg/cm^2 to form a transparent pellet. The pellet was

kept in the sample holder and scanned from 4000 cm^{-1} to 400 cm^{-1} in Shimadzu FT-IR spectrophotometer. Samples were prepared for drug (Valsartan), polymer (Cholesterol, Lecithin soya) and physical mixture of drug and polymers. The spectra obtained were compared and interpreted for the functional group peaks.

B. Differential Scanning Calorimetry (DSC):

DSC analysis of the samples was carried out by using Mettler Toledo DSC 822e. Samples were heated at a heating rate of 10^0 C/min over the temperature range of 30.0-220.0^0C.

4.5. Method of preparation of Liposomes of Valsartan:

Method:

Thin film hydration technique

1. A thin film was prepared from the mixture of vesicles forming ingredients that is phospholipids and Cholesterol by dissolving in volatile organic solvent (chloroform: methanol 3:2). Organic solvent was then evaporated at 40°C using rotary evaporator. Final traces of solvent were removed under vacuum.

2. Then prepared thin film was hydrated with buffer (pH 7.4) by rotation at 100 rpm for 30min. at the 40°C temperature. The resulting vesicles were swollen for 2 hr at room temperature.[7]

Formulation of Valsartan liposome

Code	Polymer ratio	Solvent ratio	rpm	Temp
F1	1:0.25	3:2	100	40°C
F2	1:0.50	3:2	100	40°C
F3	1:0.75	3:2	100	40°C
F4	1:1	3:2	100	40°C
F5	1:1.25	3:2	100	40°C
F6	1:1.50	3:2	100	40°C
F7	1:1.75	3:2	100	40°C
F8	1:2	3:2	100	40°C

F9	0.25:1	3:2	100	40°C
F10	0.50:1	3:2	100	40°C
F11	0.75:1	3:2	100	40°C
F12	1.25:1	3:2	100	40°C
F13	1.50:1	3:2	100	40°C
F14	1.75:1	3:2	100	40°C
F15	2:1	3:2	100	40°C

Evaluation of Liposomes:

4.6. 1. Drug content determination:

The amount of drug contained in the liposomes was determined by dissolving 1ml of the formulation in 9 ml of chloroform: methanol (2:1) and the volume was made up to 100 ml with methanol. The mixture was analysed by a UV-Visible spectrophotometer at 250 nm against methanol as a blank.[7]

4.6. 2. Photo microscopy:

All batches of liposomes prepared were observed under light microscope.[10]

4.6. 3. Zeta potential and particle size distribution:

The zeta potential measurement was carried out using a NanoZS (Malvern UK) employing a 532 nm laser at a back scattering angle of 173^0.

4.6. 4. Entrapment Efficiency (%EE):

Percentage entrapment efficiency was conducted by the centrifuge method. The liposome dispersion obtained was centrifuged (REMI LJ 01, Mumbai, India) at 15000 rpm for 40 min. The clear fraction (supernatant) was used for the determination of free drug. The drug concentration in the resulting solution was assayed by a UV spectrophotometer (Shimadzu-1800, Japan) at 250 nm.

The percentage of drug encapsulation was calculated by the following equation:

Entrapment efficiency (%) = [(Ct − Cf) / Ct] × 100

Where Ct is the concentration of total drug and Cf is the concentration of unentrapped drug.[10]

5. *In Vitro* Permeation Study:

The egg membrane was used for the in vitro permeation experiment using locally fabricated Franz diffusion cell. The membrane was clamped between the donor and the receptor chamber of diffusion cell with an effective diffusion area of 2.5 cm2. The receptor chamber was filled with freshly prepared PBS (pH 7.4). The diffusion cell was maintained at 37^0C and the solution

of the receptor chamber was stirred continuously by using magnetic stirrer (Remi equipment, Mumbai). All the formulation (1ml from each) was gently placed in the donor chamber. At 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hr intervals 2ml of the solution in receptor compartment was removed and replaced immediately with equal volume of fresh buffer analysed using UV spectrophotometer and the data was recorded.[7]

4.6. Storage-Physical Stability Evaluation of liposomes:

Stability of a drug has been defined as the ability of particular formulation in specific container to remain within its physical, chemical, therapeutic and toxicological specification.

Factor affecting stability

Extrinsic: Temperature, light, gases, moisture

Intrinsic: pH, complexation, microbial growth

Boundary: Container composition, porosity, dosage form interaction.

Stability testing is an integral part of formulation development. It generates information on which to base proposals for shelf lives of drug substance and products. And their recommended storage conditions. Stability data also are a part of the dossier submission to regulatory agencies for licensing approvals.

Salient features of ICH guidelines:

- The stability test should be conducted using the containers and closures proposed for storage and distribution.
- The stability plan must include different types of containers and closures such as those used for marketing, physician and promotional samples and bulk storage.

However, for bulk containers testing in prototype container that simulates the actual packaging is allowed in ICH and FDA guidelines.

- A sampling frequency of every 3 months during the first year, every 6 months during the second year and then annually for drug substances and products stored for real time testing.
- At least two containers are required to be sampled during the stability study.
- To predict the shelf life of the dosage form for clinical zone III and IV, the predictive factor is 3.3 at 30°C (6 months at 40°C corresponds to 20 months at 30°C).

Procedure: Liposomes were evaluated for drug retentive potential at 25 ± 2^0C for a period of 90 days. The elastic liposome suspensions were kept in sealed ampoules glass containers (10 ml capacity). Sample were withdrawn periodically and analysed for the physical appearance and drug content.[28]

5. RESULTS

Preformulation test of Valsartan:

5.1.1 Determination of Solubility:

Valsartan was soluble in ethanol, methanol, partially soluble in phosphate buffer pH-7.4.

5.1.2 Determination of Melting point:

Table 5.1 Data of melting point of drug

Compound name	Melting Point	
	Observed*	Standard
Valsartan	105±1^0 C	116-117^0 C

(n=3)

5.1.3 IR Spectroscopy:

The IR spectra of pure drug was carried out and the graph is shown in Figure 5.1 And peaks are shown in Table 5.2

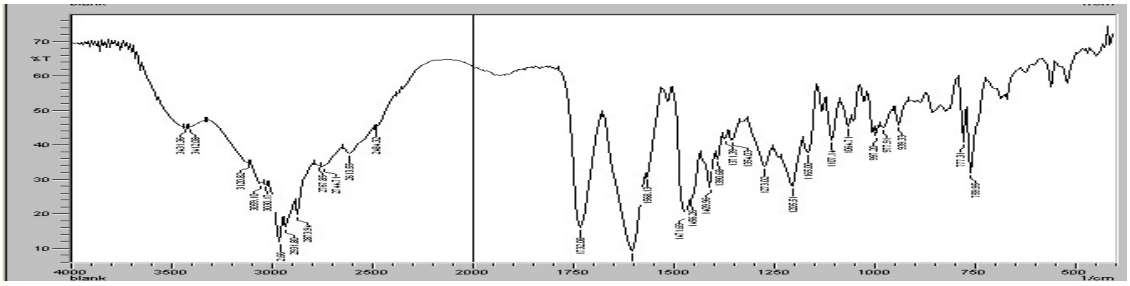


Figure 5.1 IR spectra of Valsartan

Table 5.2 IR spectral data of valsartan

Compound name	Functional group	Standard range	Observed peak
Valsartan	COOH stretch	1618 cm^{-1}	1602 cm^{-1}
	N-O stretch	1345-1385cm^{-1}	1354 cm^{-1}
	O-H stretch	3450 cm^{-1}	3412 cm^{-1}
	N-H stretch	3350 cm^{-1}	3120 cm^{-1}

5.1.4 Differential Scanning Calorimetery (DSC):

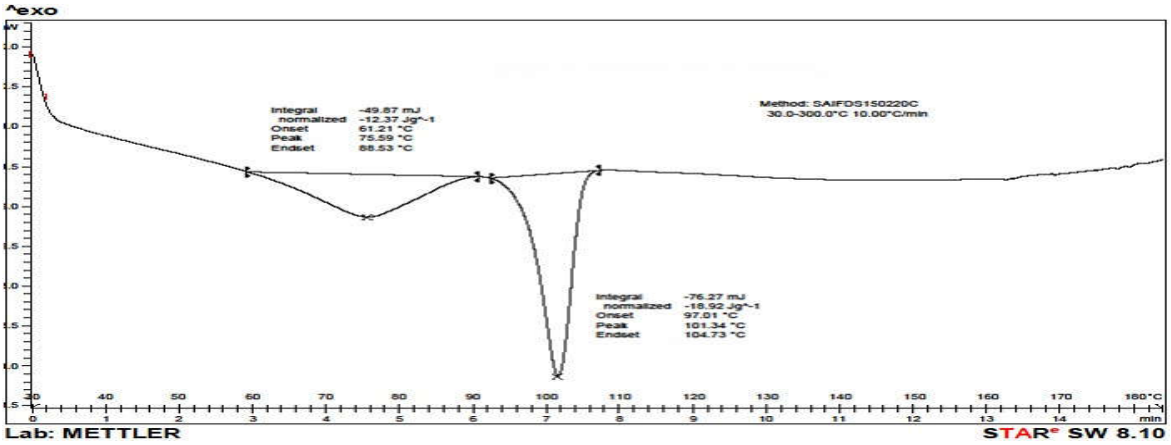


Figure 5.2 DSC for pure drug

Calibration curve of valsartan:

a) Calibration curve of Valsartan in Ethanol

Table 5.3 Calibration curve data of Valsartan in Ethanol

Concentration (mcg/ml)	**Absorbance (Mean ±SD) ***
0	0
2	0.07 ± 0.036
4	0.138 ± 0.025
6	0.186 ± 0.024
8	0.229 ± 0.0026
10	0.302 ± 0.019

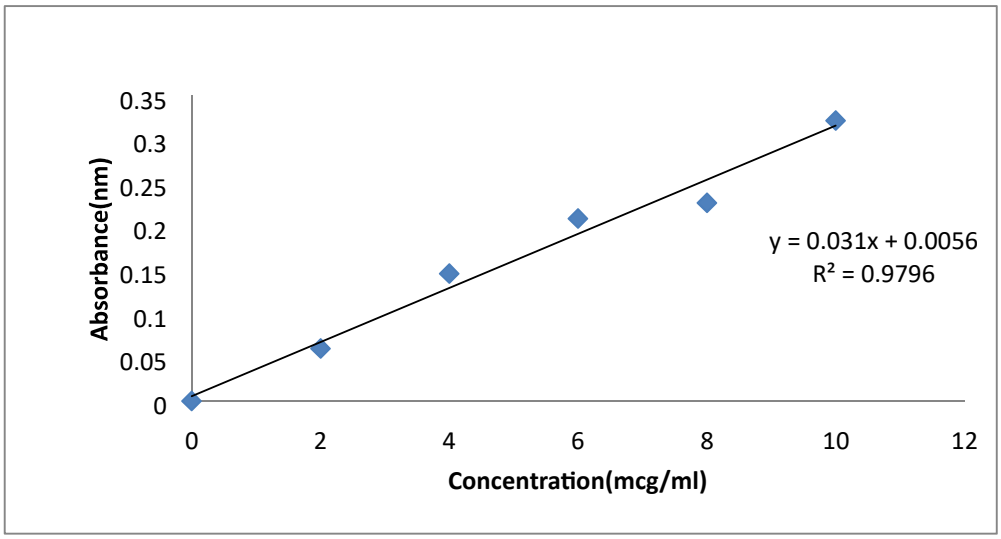


Figure.5.3 Standard graph of Valsartan in Ethanol

b. Calibration curve of Valsartan in Methanol:

Table.5.4 Calibration curve data of Valsartan in Methanol

Concentration (mcg/ml)	Absorbance (Mean ±SD)*
0	0
2	0.093 ± 0.034
4	0.138 ± 0.0122
6	0.192 ± 0.0137
8	0.27 ± 0.0112
10	0.333 ± 0.0168

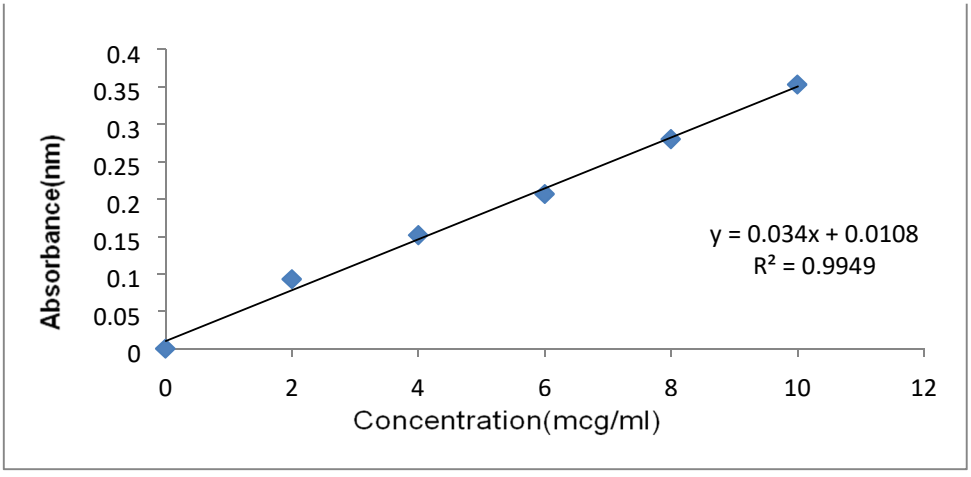


Figure 5.5 Calibration curve of valsartan in Methanol

c. Calibration curve of Valsartan in buffer pH 7.4:

Concentration (mcg/ml)	Absorbance (Mean ±SD) *
0	0
2	0.051±0.0015
4	0.141± 0.0015
6	0.212± 0.0020
8	0.284±0.0047
10	0.356±0.003

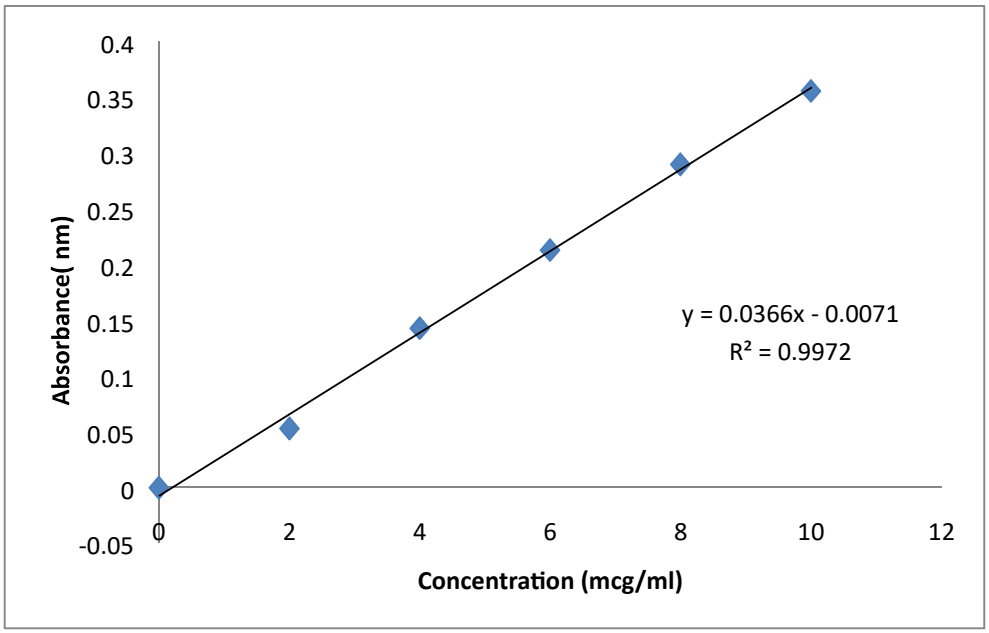


Figure 5.6 Calibration curve data of Valsartan in buffer pH 7.4

Evaluation studies:

5.5.1 Drug content study:

The drug content of the formulation was recorded and shown in Table 5.6 below

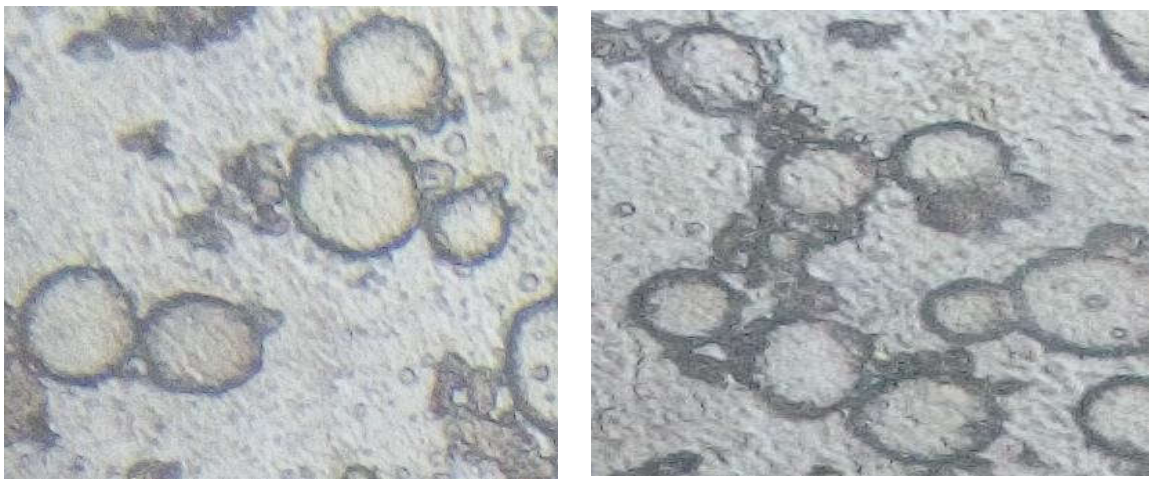
Table 5.6 Data of drug content

Formulations code	Mean % ± SD
F1	45%±0.07
F2	73.89%±0.06
F3	54.79%±0.10
F4	77.26%±0.12
F5	82.89%±0.18
F6	67.08%±0.11
F7	50.88%±0.10
F8	39.09%±0.03
F9	53.43%±0.08
F10	83.89%±0.020
F11	46.97%±0.011
F12	48.22%±0.05
F13	64.62%±0.14
F14	59.91%±0.17
F15	46.97%±0.02

The drug content in the formulation varied from 39.09 -83.89 % which indicates that the drug is stable in each of the formulations.

5.5.2 Photo microscopy:

The photograph of selected formulations of liposomes is shown in the photograph.



F-10 **F-11**

Figure 5.7 Microscopic image of selected formulations

5.5.3 Zeta potential:

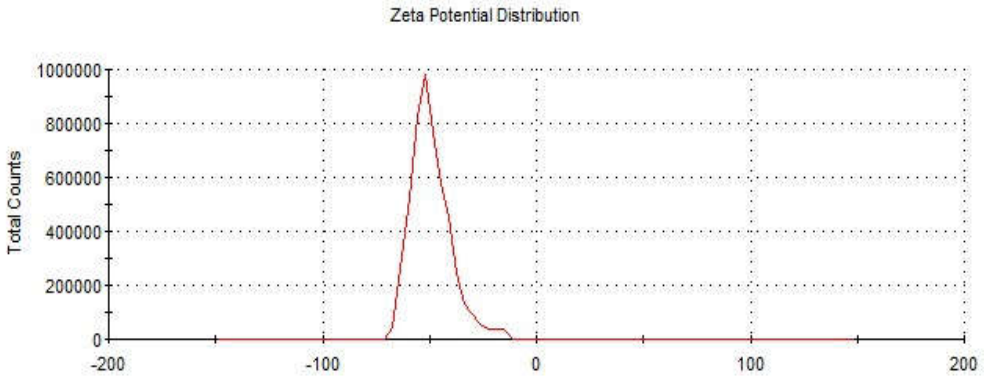


Figure 5.8 Zeta potential distribution for F-10

The zeta potential was found in the range of - 4.42 mv to - 74.6 mv, which indicates that formulation is stable.

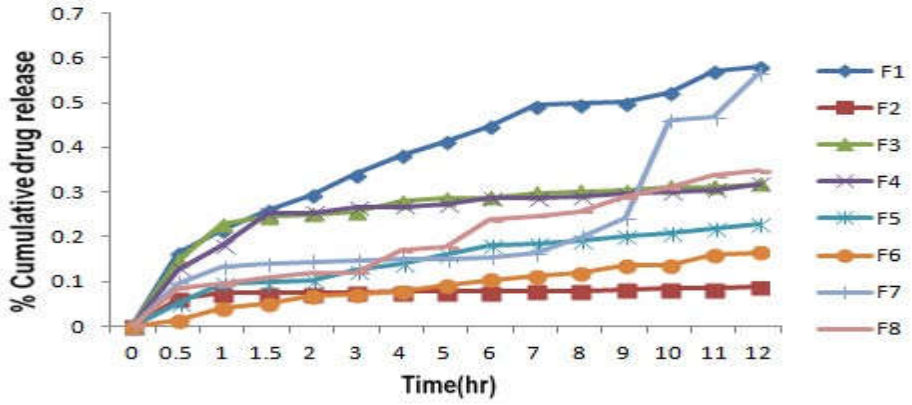
5.5.4 *In vitro* drug release study of liposomal suspension:

Time (hr)	Cumulative % drug release							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
0.5	16.50±0.051	6.27±0.062	14.78±0.138	12.75±0.148	5.08±0.121	1.37±0.058	9.62±0.140	8.54±0.120
1	21.50±0.323	7.33±0.018	22.68±0.113	18±0.123	9.62±0.148	4±0.140	13.20±0.018	9.37±0.128
1.5	26.06±0.166	7.33±0.113	24.40±0.148	25.25±0.146	9.76±0.136	5±0.112	13.89±0.218	10.93±0.123
2	29.50±0.112	7.42±0.123	24.97±0.168	25.38±0.153	10.31±0.112	6.75±0.181	14.44±0.123	11.87±0.134
3	33.99±0.234	7.47±0.146	25.55±0.088	26.63±0.143	12.51±0.431	7.25±0.171	14.57±0.109	12.08±0.153
4	38.18±0.167	7.7±0.089	27.81±0.138	26.75±0.112	14.02±0.089	7.75±0.163	14.85±0.112	16.97±0.138
5	41.25±0.013	7.74±0.018	28.53±0.128	27.13±0.142	15.95±0.078	9.25±0.120	14.85±0.133	17.70±0.166
6	44.26±0.138	7.74±0.112	28.76±0.123	28.50±0.124	18.01±0.140	10.37±0.143	15.40±0.128	23.75±0.143
7	49.37±0.128	7.79±0.138	29.79±0.143	28.63±0.120	18.65±0.183	11.25±0.124	16.22±0.141	24.6±0.113
8	49.74±0.148	7.83±0.218	30.13±0.188	29±0.163	19.11±0.168	11.87±0.163	19.94±0.123	25.62±0.149
9	50.06±0.183	8.29±0.112	30.36±0.153	29.75±0.013	20.21±0.109	13.62±0.120	24.20±0.112	28.85±0.129

10	**52.19±0.068**	**8.38±0.140**	**31.05±0.058**	**30±0.138**	**20.90±0.238**	**13.75±0.113**	**46.07±0.166**	**31.04±0.079**
11	**57±0.079**	**8.43±0.018**	**31.16±0.166**	**30.50±0.166**	**21.73±0.143**	**16±0.141**	**46.62±0.171**	**33.75±0.143**
12	**57.94±0.189**	**8.8±0.123**	**31.73±0.171**	**31.88±0.121**	**22.83±0.167**	**16.50±0.158**	**56.52±0.133**	**34.68±0.128**

The release study was performed for the formulations and study data were collected. The release

Table no. 5.8 Data of *In vitro* release



6 .Stability study:

The stability study of the formulation was recorded and is shown in Table 5.8 below

Table 6.1 Stability study data

No. of days	**% drug content**
Initial	83.89
30 days	83.89
60 days	81.23
90 days	80.82

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

evaluate liposomes of Valsartan that would release the drug over a prolonged period of time thus avoiding first pass metabolism and to improve its systemic availability Liposome formulation could be conveniently prepared by rapid film evaporation method using soya-lecithin, cholesterol at different concentrations. The drug content of all liposome formulations were uniform with low SD and CV values and the results were reproducible. The zeta potential of the liposome formulation was determined by using NanoZS (Malvern UK). The zeta potential was found to be in range of to -4.42 mv to -74.6 mv. This indicates that the formulation is stable. The In-vitro diffusion rate was studied by Franz diffusion cell. The drug release from vesicles was dependent on concentration of cholesterol and lecithin in liposome formulation.

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