DESIGN AND DEVELOPMENT OF PROLIPOSOMAL DRY POWDER INHALATION FOR PULMONARY DELIVERY OF ANTIHYPERTENSIVE DRUG

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY,

Chennai-600 032

In partial fulfilment for the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

BRANCH-I→PHARMACEUTICS

SUBMITTED BY

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October 2021

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ISO 9001 : 2015
Reg. No : RQ91/7949

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DECLARATION

The thesis entitled "**DESIGN AND DEVELOPMENT OF PROLIPOSOMAL DRY POWDER INHALATION FOR PULMONARY DELIVERY OF ANTIHYPERTENSIVE DRUG**" was carried out by me in the Department of Pharmaceutics, C.L. Baid Metha College of Pharmacy, Chennai-600097 during the academic year 2019-2021. The work embodied in this thesis is original and is not submitted in part or full for any other degree of this or any other university.

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ACKNOWLEDGEMENT

I take this privilege and pleasure to acknowledge the contributions of many individuals who have been inspirational and supportive throughout my work undertaken and endowed me with most precious knowledge to see success in my attempt.

It gives me immense pleasure to express my deep sense of gratitude to my esteemed guide **Dr. Priyanka Sinha, M. Pharm, Ph.D.,** Associate Professor, Department of Pharmaceutics, C.L Baid Metha College of Pharmacy for his unflagging interest, constant source of inspiration and guidance throughout the course of the study.

I would be failing in my duties if I did not record my sincere thanks to respected **Dr. Grace Ratnam, M. Pharm, Ph.D.,** Professor and Head, Department of Pharmaceutics, C.L Baid Metha College of Pharmacy for her benevolent help in the completion of the study.

I am very much thankful to **Dr.Kumaravel Rajan, M.Pharm Ph.D.,** and **Dr. U. Ubaidullah, M.Pharm Ph.D.,** Associate professor and Department of Pharmaceutics for their valuable suggestions and guidance throughout my course.

I also thank the lab assistants **Mrs.Thenmozhi** and **Mr.Rajaram** for their assistance and help rendered in the completion of this project.

I extend my sincere thanks to Chief Librarian **Mrs. M. Rajalakshmi**, C.L. Baid Metha College of Pharmacy in helping me to utilize the library facilities for refrences.

We are thankful to **Mr. Ganesh Bahadur**, Chief security officier, C.L. Baid Metha College of Pharmacy, Chennai for providing us the uninterrupted service at college campus during the work period.

I thankful all non-teaching staff members of our college for their help extended during my project work.

I am extending my thanks to my Industrial guide **K. Yuvaraja M.Pharm, Senior Manager, Formulation R&D, Softgel Healthcare Private Ltd, Chennai** for giving me the opportunity to proceed my research work in formulation research and development laboratory and providing invaluable guidance throughout this project. His dynamism, vision, sincerity and motivation have deeply inspired me. He has taught me the methodology to carry out the research and to present the research works as clearly as possible. It was a great privilege and honor to work and study under his guidance.

It is an honor to pay my respect and heartfelt thanks to all the Staffs of Formulation R&D - **Mr. Siraj Rahmath, Mr. M. Anand Kumar, Mr. A. Anand, Mr. B. Prakash, Miss R. Priya Varshini, Miss K. Ammu, D. Jagan** and **Mr. Navaneetha Krishnan**, Manager, Analytical R&D, Softgel Healthcare Private Ltd, Chennai and heartfelt thanks to all the Staff of Analytical R&D-**Mr. Karupaaiha** and **Mr. RajeshKanna**, Assistant Manager, Microbilogy Department, Softgel Healthcare Private Ltd, Chennai and heartfelt thanks to all the Staff **Mr. Krishnan** who stood by my side in providing me with necessary knowledge and giving me access to use the facilities required to complete my dissertation work.

The completion of this dissertation is not only fulfillment of my dreams but also the dreams of my parents **Mr. A. Pradeep** and **Mrs. P. Kalaiselvi** and also my heartly thanks to my brother Mr. **P. Deepakiran** and my lovely sister **P. Sneha** who have taken a lot of pain for me in completion of higher studies successfully, with their full-hearted co-operation, love and moral support.

I express my special heartfelt thanks to my seniors for their support and suggestions throughout the work.

I have no words to express my pleasure in thanking my dear friends A. Charumathy, K. Maheshwari, M. Krithika who were behind me in providing invaluable assistance for completing my project.

My acknowledgement was incomplete without a heartfelt thanks to all those peoples who directly or indirectly helped and contributed to this dissertation in their own way.

Thanks To All

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INTRODUCTION

Liposomes, defined as microscopic spherical-shaped vesicles, consist of an internal aqueous compartment entrapped by one or multiple concentric lipidic bilayers. Liposome's membrane is composed of natural and/or synthetic lipids which are relatively biocompatible, biodegradable and non-immunogenic material. Because of their unique bilayer-structure properties, liposomes are used as carriers for both lipophilic and water-soluble molecules. Hydrophilic substances are encapsulated in the interior aqueous compartments. Lipophilic drugs are mainly entrapped within lipid bilayers [1].

Liposomes have attractive biological properties, including the biocompatibility and biodegradability. They show promise as active vectors due to their capacity to enhance the encapsulant performance by increasing drug solubility, and stability; delivering encapsulated drugs to specific target sites, and providing sustained drug release. Their sub-cellular size allows relatively higher intracellular uptake than other particulate systems; improving in vivo drug bioavailability. Other advantages of liposomes include high encapsulation efficiency inspite of drug solubility, low toxicity due to phospholipids content, drug protection against degradation factors like pH and light and the reduction of tissue irritation.

However, aqueous dispersion forms of liposomes suffer from serious stability problems associated with the aggregation, fusion, and phospholipid hydrolysis that could limit their shelf-lives. Although freeze drying is most frequently used today as a possible tool in improving the stability of liposomes, it still exhibits some difficulties in terms of residual water content and chemical stability problems caused by the lyoprotectants used.

This technique itself consumes a larger amount of energy in the production process with considerably high cost due to the lyophilization step as compared to other techniques. For large-scale production of liposomes, not only the desired product properties should be focused on, but efficiency and feasibility of the production process should be recognized as well.

Dry liposomal formulations in the form of proliposomes appear to be the promising candidate with respect to its simplicity and practicality as compared to freeze-dried products. Proliposomes are definitely dry free-flowing particles composed of the drug, phospholipid, and water-soluble porous powder, which upon addition of water are hydrated to form a liposomal dispersion [2].

Proliposomes could be fabricated into various dosage forms including tablets/capsules, transdermal delivery systems, and those for vaginal administration.

Although various methods for producing proliposomes have been developed including fluidized bed method, film-deposition on carriers' method, spray drying method, and crystal-film method, there are still some limitations with respect to both stable formulations in gastrointestinal tract and manufacturing processes on an industrial scale.

Pro-liposome is one of the most widely used and cost-effective methods. As they are available in dry powder form, it's easy to distribute, transfer, measure and store, making it a diverse system. Liposomes can either be formed in vivo by the influence of biological fluids in the body or in vitro using a suitable hydrating fluid before the administration. Solubility and bioavailability problems of many drugs can be overcome by developing pro-liposomal formulations [3].

Method of Preparation Pro-liposomes (PLs) is prepared by many methods such as:

a) Film-deposition on carrier method.
b) Spray drying method.
c) Fluidized-bed method.
d) Supercritical anti-solvent method.
e) Slurry method.

a) Film deposition on carrier method:

Film deposition on carrier method is used for the composition of Pro-liposomes. In this procedure, the coat of drug and phospholipids is discharged on a previous, water-soluble carrier substance. An evaporative solution containing a solution of drug and phospholipids is injected drop by drop by a feed tube onto a core of carrier substance which is carried in a vessel of a rotary flash evaporator under vacuum. At any stated moment, the matrices over wetting are circumvented and the following aliquot of the organic mixture is feeding solely when a free-flowing powder matrix is procured [4].

Selected carriers should exhibit great surface area and permeability in order to regulate the quantity of carrier which is needed to assist the lipids. This also permits great surfactant to carrier mass proportion for the pro-liposomes production. As they are water-soluble, they enable fast production of liposomal dispersion on hydration, and by properly managing the size of the previous powder, a comparatively limited variety of reconstituted liposomes can be

acquired. Mostly used carriers are maltodextrin, sorbitol, microcrystalline cellulose, magnesium aluminum silicates, mannitol, etc. The stride of solvent inclusion and evaporation is sluggish.

To circumvent this issue, alter the procedure by dispersing the carrier substance in an organic mixture of drug and phospholipids in the vessel of the rotary evaporator and then directing it to vacuum evaporation. By doing so, a highly consistent and well-organized lipid distribution is achieved and a steady and less time taking procedure is gained in contrast to the actual procedure [5].

b) Spray drying method:

The distinctive attribute of this process is reclined in its propensity to include particle composition and drying together in a consistent stride, permitting more desirable production of particles. This method can be used for any of the aqueous or non-aqueous systems for particles production. Predominantly, this process is utilized when invariable sized and shaped particles are needed and can be simply scaled up. Its price is effective and acceptable for the massive preparation of PLs.

This spray drying procedure consists of four phases: the atomization of the product into a spray nozzle, spray-air association, drying of the spray droplets, and collection of the solid product. Firstly, the preparation of liquid dispersions carrying pure lipid or lipids and carriers in the organic mixture is done and then it is poured into the dry cell. By utilizing a spray nozzle, dispersions are atomized into drying cells and desiccated in a simultaneous airflow which is then gathered in a tank.

Prime factors which affect this method are high temperatures, shearing stresses, and absorption episodes and these can result in the thermal and mechanical degradation of active molecules. It can be upgraded by making the working variables better. Examples of working variables are drying air temperatures and liquid spraying rate. For shielding the unification of active molecules, stabilizing adjuvants e.g., disaccharides, cyclic oligosaccharides, and polyols can be utilized and by augmenting the surface area of lipids, the effectiveness of hydration can be intensifying [6].

c) Supercritical anti-solvent method:

In the Supercritical anti-solvent method for the production of PLs, we use Supercritical Carbon dioxide (SCCO2) which actually is carbon dioxide's fluid state when it is held at some level above its critical temp and pressure.

Because of three main factors which includes

• Lower residual solvents,

• Simple steps,

• Mild operation temperatures

We use anti-solvent technology for the preparation of PLs. An apparatus consisting of three parts (e.g. a sample delivery unit, a precipitation unit, and a separation unit) is basically used in those simple steps. Two pumps, one for the delivery of CO2 which is supplied through CO2 cylinder (72 cm^3) after being cooled down by refrigerator and a high-pressure pump is used introduce it to the buffer tank (-7°C) for preheating thus The conditions of temperature and pressure of the reaction vessel or CO2 cylinder should be 45°C and 10 MPa and one for the drug solutions which is introduced via HPLC pump combines up to make the sample delivery unit .

The solvent which is completely miscible with CO2 should be used for dissolving the drugs. For both preparations, phospholipids, cholesterol, and drugs were dissolved in organic solvents followed by sonication until a clear and homogeneous solution was obtained. For the entrance of CO2 into the vessel through nozzle valves, A and B will be opened. CO2 is sprayed through the outer tubule whereas the solution is sprayed through the inner tubule of the nozzle. The second part of the apparatus consists of a heated air bath vessel, and the last part comprises a wet gas meter and a separator. SCCO2 is separated from organic solvent in the last part's separator because of its low pressure and on the other hand, a wet gas meter is used to measure the CO2 [7].

After reaching the preset value of temp and pressure, valve A is open for the entrance of CO2 right after that, valve B allows drug solution to enter the nozzle. The solution is mixed with SCCO2 and diffused into each other rapidly like it is sprayed through the coaxial nozzle. Thus, the solute will dissolve in an organic solvent to reach supersaturation in a very short period of time about 30 minutes, and this is all because the solubility of the solute in the organic solvent

decreases gently, thus the PLs are precipitated in the vessel. After the complete utilization of solution, A and B valves are closed and valve C is opened to depressurize the vessel at the opening temp in the end we collect these samples at the bottom of a vessel on the filter. The pressure, temp, and flow rate of the drug solution need to be optimized to obtain the high drug loading PLs.

d) Fluidized bed method:

On the large-scale production of PLs whose principle relies on particle coating technology, in which carrier material can vary from crystalline powder to nonpareil beads. While using nonpareil beads as carrier material, first for getting smooth surface pareil beads are coated with seal coating which can help further in the coating of phospholipids and which also ensure thin uniform coating formation of phospholipids around the core and small-sized liposomes upon hydration. Carrier materials are then sprayed with the solution of organic solvent and solution of drugs through the nozzle, and by applying vacuum at the same time to the fluid bed organic solvent is removed. The trace amount of residual solvent is removed by the finished lipid-coated powder/beads when dried under vacuum overnight [8].

- Number of various ways of coating
- Cost-effective method

d) Slurry method:

Proliposome powders were prepared via slurry method [9]. Combinations of different phospholipids i.e., SPC, HSPC or DMPC and different carbohydrate carriers i.e. LMH, MCC or Starch were employed in proliposome formulations. SPC and HSPC are from natural source, whereas DMPC is from synthetic source. The lipid phase prepared, consisted of phospholipid and cholesterol in 1:1 M ratio. A drug was incorporated at a 2 mol% concentration based on lipid phase. Carbohydrate carrier was transferred to a round bottom flask (RBF) (100 ml). A lipid phase, comprised of phospholipids and cholesterol (83.33 mg) with 2 mol% of drug was dissolved in 20 ml of absolute ethanol; this solution was then poured over the carbohydrate carrier forming a slurry. Subsequently, the RBF was subjected to rotary evaporation in a water bath previously adjusted to 45°C. A negative pressure was created by a vacuum pump and the evaporation of organic solvent was continued with a rotation speed of 270 RPM for 1 h. Following release of negative pressure, dry proliposome (i.e., powder form) were collected in a dry air-tight glass bottle (100 ml) and stored at −18°C for subsequent studies.

- **Classification of liposomes:**

The liposome size can vary from very small (0.025 μm) to large (2 .5 μm) vesicles. Moreover, liposomes may have one or bilayer membranes. The vesicle size is an acute parameter in determining the circulation half-life of liposomes, and both size and number of bilayers affect the amount of drug encapsulation in the liposomes.

On the basis of their size and number of bilayers, liposomes can also be classified into one of two categories:

(1) multilamellar vesicles (MLV) and (2) unilamellar vesicles.

a. Unilamellar vesicles can also be classified into two categories:

(1) large unilamellar vesicles (LUV) and (2) small unilamellar vesicles (SUV)

In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. In multilamellar liposomes, vesicles have an onion structure. Classically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water.

The use of liposomal aerosols intended for inhalation has received increasing interest not only for the treatment of lung diseases such as asthma, chronic obstructive pulmonary disease, cystic fibrosis or tuberculosis, but also shows several advantages for systemic delivery [10].

This non-invasive route of delivery exhibits several advantages:

(i) the lung has a large surface area (~100 m^2) available for absorption;
(ii) in the distal lung only a thin alveolar epithelial lining separates the airspace from a vast capillary network;
(iii) the enzymatic activity in the lungs is believed to be much lower than in the GIT/liver;
(iv) a faster onset of action in comparison to oral administration can be achieved.

a. Pulmonary drug delivery:

In pulmonary drug delivery, there are three main barriers against the deposition of the aerosol in the deep lung (i.e., respiratory bronchioles and the alveolar region), and these are:

(a) the anatomic barrier – the tracheobronchial tree structure of the pulmonary system is the main protection mechanism against the deposition of deleterious particles and pollutants;

(b) the pathological barrier – the disease status may affect the viscoelastic properties of the mucous covering the respiratory tract epithelium, hence affecting the clearance of deposited material and absorption profile, and

(c) the immunological barrier – alveolar macrophages are involved in the defense mechanism and hence particles depositing in the alveolar region might be engulfed and transported to the upper respiratory tract where the mucociliary escalator can eradicate the particles because there is always competition between clearance and absorption. The above barriers are discussed further in this review; however, it is important to bear in mind that for inhaled drug particles to be regarded 'therapeutically useful' they should be in 'fine particle fraction' (FPF; i.e., capable of reaching the bronchioles and alveoli).

For this to happen, the aerodynamic size of inhaled particles should be small er than 5 or 6 μm, with particles smaller than 2 μm being the most suitable for deposition in the alveolar region.

Hence, the size of inhaled particles is the prime es sential factor to consider for overcoming the anatomical barrier of the lung. Accordingly, liposome formulations should be aerosolised into particles that have a high FPF, and these liposomes should encapsulate a therapeutically feasible concentration of the drug which can then exhibit prolonged release from the liposome vesicles to the de sired target within the lung [11].

b. Safety of Liposome Formulations for Pulmonary Drug Delivery

Liposomes for pulmonary delivery have attracted a marked interest owing to the ability of liposome vesicles to entrap therapeutic molecules and, following inhalation, localize the drug effect in the pulmonary system for a prolonged duration. This had been reported to enhance the therapeutic benefit of the drug and reduce the potential of systemic adverse effects.

Liposomes are prepared using phospholipids with or without cholesterol; these components are highly similar to pulmonary surfactants in mammals.

Many studies have established the high biocompatibility and biodegradability of liposomes as drug carriers in inhaled formulations. Historically, liposomes in this field were suggested as surfactant replacement therapy in patients with respiratory distress syndrome. Recently, lung surfactants based on mixtures of phospholipids have been commercialized (e.g., Survanta ®) for prophylaxis against respiratory distress syndrome in neonates.

Many studies have also shown that drugs entrapped in liposomes are safe for pulmonary delivery since liposomes can control the mode of drug release, hence reducing the drug amount available to exert adverse effects. The safety of drugs in liposome formulations given via inhalation is not confined to anticancer agents. Genes, antimicrobial agents, and antidiabetic drugs are also safe when administered in liposome formulations.

Steroids are commonly and widely used as anti-inflammatory agents in prophylaxis against asthma. Investigations have demonstrated that inhaled liposome-entrapped beclometasone was well tolerated when given in therapeutic doses to humans [12].

c. Devices Used for the Pulmonary Delivery of Liposomes

There are four types of inhalation device:

- pressurized metered-dose inhalers (pMDIs),
- dry powder inhalers (DPIs), soft mist inhalers (SMIs) and
- medical nebulizers.

All these devices have been investigated for the delivery of liposomes.

1. Pressurized Metered-Dose Inhalers

pMDIs are robust canisters enclosing a drug dissolved or dispersed in liquefied propellants. Actuation of the device with coordinated inspiration results in the release of a precise dose.

The propellant rapidly evaporates owing to its high vapour pressure, leaving an accurate dose of the aerosolised drug particles to be inhaled by the patient. pMDI devices have traditionally been used in the treatment of asthma since the 1950s, but serious concerns have been raised about using them both clinically, because of the limited dose reaching the deep lung, and environmentally, because propellants like chlorofluorocarbons (CFCs) have been reported to be depleting the ozone layer.

An approach to delivering liposomes using pMDIs was reported by dissolving the phospholipid in CFC propellant in which drugs like salbutamol and cosolvents like ethanol are included. Actuation of the device in front of an impinger resulted in the deposition of a drug and lipid mixture and subsequent hydration and formation of liposomes within the impinger.

The ozone-depleting effect of CFCs necessitated the introduction of the safe alternative propellant family, namely hydrofluoroalkanes, in which phospholipids have very limited solubility. Thus, pMDI formulations were made by dispersing phospholipids in PEG-

phospholipids followed by the delivery of the subsequent in situ formation of liposomes in the aqueous environment of the impinger.

Issues of complicated formulation, poor FPF of the aerosolised dose and stability are all major limitations in the development of liposomal formulations for delivery via pMDIs. The inclusion of cosolvents in phospholipid formulations may compromise deposition in FPF [13].

2. Dry Powder Inhalers

DPIs are breath actuated, thus the problem of co-ordi nated inspiration with actuation, as in the case of pMDIs, is avoided. The delivery of liposomes using DPIs has been investigated using a range of drying technologies such as spray drying, freeze drying, spray freeze drying or air jet micronisation. For example, the spray drying of drugs in liposome formulations has been shown to be appropriate for manufacturing particles with a small aerodynamic size (i.e. high FPF), and it was presumed that the rehydration of liposomes may take place following the deposition of the powder in the aqueous environment of the lung.

An approach to gene therapy was introduced by spray drying a lactose solution incorporating lipid-polycation-pDNA, resulting in an enhanced transfection compared to formulation prior to spray drying.

More recently, proliposomes have been studied for delivery via DPIs. In this context, proliposomes are powdered phospholipid formulations that can generate liposomes when they come into contact with an aqueous environment.

Inhalable proliposome formulations have been made by spray drying an ethanolic solution of phospholipid.

The FPF was reported to reach up to 35% of the formulation and it is presumed that hydration of the powdered lipid particles would happen in the aqueous milieu of the lung following inhalation of the proliposome powder [14].

3. Soft Mist Inhalers

SMIs are hand-held propellant-free metered dose inhalation devices that generate slow-moving aqueous aerosols for deep-lung deposition.

An example is the AERx ® (Aradigm Corp., Novo Nordisk, Hayward, Calif., USA), an SMI that is able to deliver liposome-DNA complexes in respirable aerosols [15].

Large doses are needed for the treatment of many diseases in the lung (e.g. cancers, infectious diseases, etc.); however, all the aforementioned devices (i.e. pMDIs, DPIs and SMIs) can deliver only small amounts of aerosol, and thus are more appropriate for treating diseases that require small doses of the therapeutic agent (e.g. asthma).

4. Medical Nebulisers

Compared to other inhalation devices, nebulizers can generate large volumes of 'respirable' aerosol, with no need to perform drying procedures, as in the case of DPIs, or involve propellants, as in the case of pMDIs.

Nebulizers are the most commonly used inhalation devices for the delivery of liposomes.

There are three types of nebulizers: air jet, ultrasonic and vibrating mesh. Using many types of formulations, the air jet type is the best-established nebulizer for the delivery of liposomes.

Whilst the ultrasonic nebulizer has generally been shown to be the least suitable for delivering liposomes, the vibrating mesh nebulizer demonstrated excellent suitability of delivering vesicles in FPF, including large liposomes and liposome aggregates (median size around 50 μm) [16].

This suggests that the mesh nebulizer was capable of breaking the aggregates into discrete vesicles suitable for aerosolization and subsequent inhalation.

HYPERTENSION:

Systemic arterial hypertension (hereafter referred to as hypertension) is characterized by persistently high blood pressure (BP) in the systemic arteries. BP is commonly expressed as the ratio of the systolic BP (that is, the pressure that the blood exerts on the arterial walls when the heart contracts) and the diastolic BP (the pressure when the heart relaxes). The BP thresholds that define hypertension depend on the measurement method (Table 1). Several aetiologies can underlie hypertension. The majority (90–95%) of patients have a highly heterogeneous 'essential' or primary hypertension with a multifactorial gene-environment aetiology. A positive family history is a frequent occurrence in patients with hypertension, with the heritability (a measure of how much of the variation in a trait is due to variation in genetic factors) estimated between 35% and 50% in the majority of studies [17].

Signs suggestive of secondary hypertension
● Features of Cushing syndrome ● Neurofibromatosis (pheochromocytoma)

● Enlarged kidneys (polycystic kidney) ● Abdominal murmurs (renovascular hypertension) ● Precordial murmurs (aortic coarctation, aortic disease)
Signs of target organ damage
● Brain: motor or sensory deficit ● Retina: hypertensive retinopathy ● Heart: atrial fibrillation, arrhythmias, pulmonary congestion and peripheral oedema ● Peripheral arteries: absent, reduced or asymmetrical pulses and ischaemic skin lesions ● Carotid arteries: murmurs
Evidence of obesity
● BMI (body weight/height2) > 30 ● Waist circumference* >102 cm in man and >88 cm in women

Table: 1; Factor causes hypertension

Genome-wide association studies (GWAS) have identified ~120 loci that are associated with BP regulation and together explain 3.5% of the trait variance.

These findings are becoming increasingly important as we search for new pathways and new biomarkers to develop more modern 'omics'-driven diagnostic and therapeutic modalities for hypertension in the era of precision medicine.

Hypertension is the most common preventable risk factor for cardiovascular disease (CVD; including coronary heart disease, heart failure, stroke, myocardial infarction, atrial fibrillation and peripheral artery disease), chronic kidney disease (CKD) and cognitive impairment, and is the leading single contributor to all-cause death and disability worldwide [18].

The relationship between BP and the increased risk of CVD is graded and continuous, starting as low as 115/75 mmHg, well within what is considered to be the normotensive range. Successful prevention and treatment of hypertension are key in reducing disease burden and promoting longevity in the world's population. In treating hypertension, it is important to consider a person's predicted atherosclerotic CVD (ASCVD) risk more than the level of BP alone, as persons with high CVD risk derive the greatest benefit from BP lowering treatment [19].

EPIDEMIOLOGY

In pre-industrial societies, BP levels had narrow distributions with mean values that changed little with age and averaged around 115/75 mmHg, a value that probably represents the normal (or ideal) BP for humans. However, in most contemporary societies, systolic BP levels rise steadily and continuously with age in both men and women. This ubiquitous finding could be explained because age is a proxy for the probability and duration of exposure to the numerous

environmental factors that increase BP gradually over time, such as excessive sodium consumption, insufficient intake of dietary potassium, overweight and obesity, alcohol intake and physical inactivity.

Other factors, such as genetic predisposition or adverse intrauterine environment (such as gestational hypertension or pre-eclampsia), have small but definite associations with high BP levels in adulthood.

Even modest rises in mean population BP lead to large increases in the absolute number of people with hypertension [20].

Disease burden

Globally, 3.5 billion adults now have non-optimal systolic BP levels (that is, >110–115 mmHg) and 874 million adults have systolic BP ≥140 mmHg. Thus, approximately one in four adults has hypertension.

Between 1990 and 2015 there was a 43% increase in the total global number of healthy life years lost to non-optimal BP, driven by population increase, population aging and a 10% increase in the age-standardized prevalence of hypertension.

The Global Burden of Disease study has shown that non-optimal BP continues to be the biggest single risk factor contributing to the global burden of disease and to global all-cause mortality, leading to 9.4 million deaths and 212 million lost healthy life years (8.5% of the global total) each year [21].

CVD risk

Prospective observational studies have repeatedly demonstrated a strong, continuous positive relationship between BP and CVD, with no evidence of a threshold for risk throughout the usual range of BP observed in clinical practice.

The relationship between BP and CVD applies to both systolic BP and diastolic BP, but is somewhat more robust for systolic BP in adults.

It is noted in both sexes, at all ages throughout adulthood and for all major manifestations of CVD, including stroke (ischaemic and haemorroagic), coronary artery disease, heart failure, peripheral vascular disease and end stage renal disease.

The relationship is independent of other CVD risk factors, and level of BP has proven to be a major component of CVD risk in all prediction models.

Approximately two-thirds of all adults who have hypertension or receive treatment with BP lowering medication at 30 years of age have a ~40 % higher risk of experiencing a CVD event than their age-matched and sex-matched counterparts with a lower level of BP.

In addition, CVD events in individuals with hypertension tend to manifest about five years earlier than in individuals with a lower level of BP.

In individuals of 40–69 years of age, a 20 mmHg rise of systolic BP or a 10 mmHg rise of diastolic BP regardless of baseline values is associated with more than a doubling of the risk for stroke or ischaemic heart disease mortality, whereas a systolic BP reduction of 5 mmHg can decrease stroke mortality by 14% and CVD mortality by 9%. At older ages (≥80 years), the corresponding relative risk is slightly lower, but the absolute risk is far greater than earlier in life. For example, a 20 mm Hg difference in systolic BP between 120 and 140 mmHg is associated with an annual difference in absolute risk that is nearly ten times larger at ages 80–89 years than that at ages 50–59 years [22].

MECHANISMS/PATHOPHYSIOLOGY

BP regulation

BP is determined by several parameters of the cardiovascular system, including blood volume and cardiac output (the amount of blood pumped by the heart per minute) as well as the balance of arterial tone that is affected by both intravascular volume and neurohumoral systems (discussed in the following sections). The maintenance of physiological BP levels involves a complex interplay of various elements of an integrated neurohumoral system that includes the renin-angiotensin-aldosterone system (RAAS), the role of natriuretic peptides and the endothelium, the sympathetic nervous system (SNS) and the immune system. Malfunction or disruption of factors involved in BP control in any of these systems can directly or indirectly lead to increases in mean BP, BP variability or both, over time resulting in target organ damage (for example, left ventricular hypertrophy and CKD) and CVD outcomes.

The pathophysiological mechanisms responsible for hypertension are complex and act on a genetic background. Primary hypertension involves multiple types of genes; some allelic variants of several genes are associated with an increased risk of developing primary hypertension and are linked in almost all cases to a positive family history (Box 1) This genetic

predisposition, along with a host of environmental factors, such as high Na+ intake, poor sleep quality or sleep apnoea, excess alcohol intake and high mental stress, contribute to the development of hypertension.

Finally, the probability of developing hypertension increases with aging, owing to progressive stiffening of the arterial vasculature caused by, among other factors, slowly developing changes in vascular collagen and increases in atherosclerosis.

Immunological factors can also play a major part, especially on the background of infectious or rheumatological diseases such as rheumatoid arthritis. The mosaic theory of hypertension describes its multifaceted pathophysiology.

Sodium homeostasis regulation Sodium (Na+) is a crucial regulator of blood volume: high serum Na+ concentration promotes fluid (water) retention, thereby increasing blood volume and BP. When dietary Na + increases in normotensive individuals, compensatory haemodynamic changes occur to maintain constant BP. These changes include reduction in renal and peripheral vascular resistance and increased production of nitric oxide (a vasodilator) from the endothelium. However, if the effect of nitric oxide is impaired or absent, an increase in BP occurs. Endothelial dysfunction is a risk factor for the development of salt sensitivity and subsequent hypertension. Salt sensitivity is defined as a marked elevation in BP following a Na+ load of ≥5 g and is characterized by an elevation of systolic BP of at least 10 mmHg within a few hours of ingestion. Salt sensitive individuals have underlying endothelial dysfunction due to genetic or environmental influences. In response to a high salt load these individuals generally manifest overproduction of transforming growth factor β (TGF-β), which increases the risk of fibrosis, and oxidative stress, and have limited bioavailable nitric oxide. Chronic high salt ingestion can result in endothelial dysfunction, even in salt-resistant individuals, and also affects the gut microbiota, with resultant changes that contribute to increased salt sensitivity and the development of hypertension.

High salt intake also appears to drive autoimmunity by inducing T helper 17 (TH17) cells. High salt intake in mice has been shown to deplete Lactobacillus murinus in the gut microbiota. Treatment of mice with L. murinus prevented salt-induced exacerbation of salt-sensitive hypertension by modulating TH17 cells. In line with these findings, a moderate high-salt challenge in a pilot study in humans reduced intestinal survival of Lactobacillus spp., increased the activity of TH17 cells and increased BP. Thus, the gut microbiota appears to contribute to salt sensitivity of BP and the pathogenesis of hypertension [23].

Renin-Angiotensin-Aldosterone System

The RAAS has wide-ranging effects on BP regulation, mediating Na+ retention, pressure natriuresis (that is, the mechanism whereby increases in renal perfusion pressure (the gradient between renal arterial and venous blood pressure) lead to decreased Na+ reabsorption and increased Na+ excretion), salt sensitivity, vasoconstriction, endothelial dysfunction and vascular injury, and plays an important part in the pathogenesis of hypertension.

The RAAS is present at the cellular level in many organs, but its most crucial role is to help regulate pressure-volume homeostasis in the kidney, where it maintains perfusion in volume depleted states (that is, when there is a reduction in extracellular fluid volume as a result of sodium and fluid loss) and is suppressed in volume expanded (fluid overload) conditions. Renin and its precursor pro-renin are synthesized and stored in the juxtaglomerular cells of the kidney and are released in response to various stimuli (Figure 3). The main function of renin is to cleave angiotensinogen to form angiotensin I. Angiotensin-converting enzyme (ACE) cleaves angiotensin I to form angiotensin II, which is at the center of the pathogenetic role of the RAAS in hypertension.

Angiotensin II enhances Na+ reabsorption in the proximal tubule by increasing the activity of the sodium-hydrogen exchanger (NHE3), sodium-bicarbonate exchanger and sodium potassium ATPase, and by inducing aldosterone synthesis and release from the adrenal glomerulosa.

Angiotensin II is also associated with endothelial dysfunction and has profibrotic and pro-inflammatory effects, mediated in large part by increased oxidative stress, resulting in renal, cardiac and vascular injury. Angiotensin II is tightly linked to target organ damage in hypertension via these mechanisms [24].

Angiotensin-converting enzyme 2 (ACE2) has emerged as an important modulator in the pathophysiology of hypertension, CVD and renal disease, owing to its role in metabolizing angiotensin II into angiotensin-(1–7). Ang-(1–7) induces systemic and regional vasodilation, diuresis and natriuresis, and exerts antiproliferative and antigrowth effects on vascular smooth muscle cells, cardiac myocytes and fibroblasts as well as glomerular and proximal tubular cells. Ang-(1–7) also has cardiorenal protective effects that are mediated by the proto-oncogene Mas receptor through signaling pathways that include mitogen activated protein kinases (MAPK), PI3K-AKT, NADPH oxidase, TGF-β1, the EGF receptor, and NF-κB activity.

Aldosterone plays a crucial part in hypertension: by binding to the mineralocorticoid receptor, it induces non-genomic effects (that is, without directly modifying gene expression) that include activation of the amiloride-sensitive sodium channel, commonly known as the epithelial sodium channel (ENaC) and result in the stimulation of renal Na+ reabsorption in the cortical collecting duct.

Aldosterone also has many non-epithelial effects that contribute to endothelial dysfunction, vasoconstriction and hypertension.

These include vascular smooth muscle cell proliferation, vascular extracellular matrix deposition, vascular remodeling, fibrosis, and increased oxidative stress [25].

The Endothelium

Endothelial dysfunction plays a seminal part in the pathogenesis of hypertension. Normotensive offspring of parents with hypertension often have impaired endothelium dependent vasodilation, which implies a genetic component in the development of endothelial dysfunction.

Endothelial dysfunction in the setting of chronic hypertension is related to a combination of direct pressure-induced injury and increased oxidative stress. Several enzyme systems, including NADPH oxidase, xanthine oxidase and cyclooxygenase, as well as decreased activity of superoxide dismutase generate reactive oxygen species.

Excess superoxide anions bind to NO, decreasing NO bioavailability and generating the pro inflammatory oxidant, peroxynitrite. Decreased NO bioavailability is the central factor that links oxidative stress to endothelial dysfunction and hypertension.

Salt-sensitive individuals may be very sensitive to the hemodynamic stress of increased blood volume, leading to overproduction of TGF-beta, oxidative stress, and limiting bioavailable NO.

Angiotensin II, along with other factors, including cyclic vascular stretch as a result of BP changes, endothelin-1 (ET-1), uric acid, systemic inflammation, norepinephrine, free fatty acids, and tobacco smoking, enhances NADPH oxidase activity and plays a central part in the generation of oxidative stress in hypertension [26].

Sympathetic Nervous System

The importance of the SNS in the pathogenesis of hypertension has been defined in a variety of experimental models. Models of obesity-related hypertension demonstrate that increased

renal sympathetic nerve activity and its attendant increase in renal sodium reabsorption are key factors in the maintenance of sustained hypertension.

In another animal model, rats that received daily infusions of phenylephrine for 8 weeks developed hypertension during the infusions; their BP normalized under a low salt diet after discontinuation of phenylephrine, but once re-challenged with a high salt diet, the animals became hypertensive again.

The degree of BP elevation on the high salt diet was directly related to the degree of renal tubulo-interstitial fibrosis and decrease in glomerular filtration rate, suggesting that catecholamine-induced hypertension causes renal interstitial injury and a salt-sensitive phenotype that persists even after sympathetic overactivity is no longer present. In addition, enhanced SNS activity results in alpha-1 adrenergic receptor mediated endothelial dysfunction, vasoconstriction, vascular smooth muscle proliferation and increased arterial stiffness, which contribute to the development and maintenance of hypertension.

Finally, there is evidence that sympathetic overactivity enhances salt-sensitivity owing to a reduction in activity of the WNK lysine deficient protein kinase 4 (WNK4) gene, which encodes a serine/threonine kinase that inhibits the thiazide-sensitive-Na-Cl co-transporter, resulting in increased distal tubular Na+ retention. These mechanisms have been reviewed recently [27].

Inflammation and the immune system

Inflammation makes an important contribution to the genesis of hypertension and related target organ damage. Inflammation is associated with increased vascular permeability and release of potent mediators, such as reactive oxygen species, NO, cytokines and metalloproteinases. Cytokines mediate the formation of neo-intima (a new or thickened layer of arterial intima), thereby decreasing the lumen diameter of resistance vessels (small arteries and arterioles highly innervated by autonomic nerves and the primary vessels involved in the regulation of BP), and promoting vascular fibrosis, leading to increased vascular resistance and stiffness. Cytokines also affect renal tubular function by increasing local synthesis of angiotensinogen and angiotensin II, as well as promoting sodium and volume retention in hypertension.

Matrix metalloproteinases stimulate the degradation of the extracellular matrix, allowing infiltration of immune cells through the vessel wall into the interstitium of the affected organs,

promoting apoptosis and enhancing collagen synthesis and matrix deposition, leading to target organ damage [28].

DIAGNOSIS, SCREENING AND PREVENTION

Diagnosis and screening

Essential or primary hypertension is usually asymptomatic; thus, in clinical practice all adults should have their BP measured at regular office visits. Hypertension is most commonly diagnosed based on repeated BP measurements in a clinical office setting. Accurate measurement and recording of BP are essential to categorize the level of BP, ascertain BP-related CVD risk and guide management. Since 2010, methods to measure out of-office BP have been increasingly introduced to guide diagnosis and treatment of hypertension.

These include home BP monitoring (HBPM) and ambulatory BP monitoring (ABPM). HBPM refers to the measurement of BP at regular intervals by an individual at their home or elsewhere outside the clinic setting. ABPM consists of measuring and recording the BP at regular intervals (usually every 20–30 minutes), typically for the 24- hour period and while individuals go about their daily activities. The ability to measure out of-office BP has enabled the identification of distinct BP phenotypes, including white coat or isolated clinic hypertension and masked or isolated ambulatory hypertension.

White coat hypertension is characterised by elevated office BP but normal ABPM or HBPM readings. By contrast, masked hypertension is characterised by normal office readings but elevated out –of-office readings (ABPM and HBPM) [29].

Prevention

The association between BP and risk of CVD highlights the importance of treating hypertension, especially when severe. Further, it also underscores the importance of strategies to reduce BP-related CVD risk in those who have a higher-than-normal level of BP (average systolic BP 120–129 mmHg) but below the hypertension threshold. Reducing BP in adults with a high normal BP (referred to as elevated BP in the 2017 US guidelines) provides the potential to directly reduce CVD risk and to prevent or at least slow the age-related tendency for individuals to develop hypertension. In most countries there is a strong tendency for BP, especially systolic BP, and the prevalence of hypertension to increase progressively from childhood until late in life [30].

However, studies in isolated societies that have limited contact with the outside world indicate that high BP is not an inevitable consequence of aging and that the rise in BP associated with local migration by members of isolated societies is related to changes in diet, decreased physical activity and consumption of alcohol. These reports underscore the logic of efforts to prevent high BP in settings where an age-related increase in BP is common.

Antihypertensive Pharmacotherapy

Antihypertensive pharmacotherapy has evolved over several decades driven by development of various antihypertensive medication classes and large-scale outcomes trials proving their benefits on CVD morbidity and mortality.

Clinicians are now faced with a plethora of antihypertensive medications of different drug classes and a variety of fixed dose combinations. Typically, antihypertensive pharmacotherapy begins with first-line antihypertensive medications either in monotherapy or in combination.

Combination therapy may be preferable in patients with higher levels of pretreatment BP. First-line antihypertensive medications include ACE inhibitors, angiotensin II receptor blockers (also known as sartans), dihydropyridine calcium channel blockers, and thiazide diuretics.

Beta-blockers are also indicated in patients with heart failure and reduced left ventricular ejection fraction or post myocardial infarction, and some guidelines recommend beta blockers as first line antihypertensive medications.

The choice should be based on individual efficacy and tolerability. Ethnicity affects the response to antihypertensive medications, and it has been suggested that calcium channel blockers and diuretics may be the first choice in blacks.

Further, in specific clinical situations, for example hypertension in pregnant women, other medications such as alpha-methyldopa (an agonist of alpha adrenoreceptors in the central nervous system that inhibits the sympathetic nervous system) or labetalol (a beta adrenoreceptor blocker) are preferable, whereas some first line antihypertensives, for example ACE inhibitors and angiotensin II receptor blockers, are contraindicated because of increased risk for renal teratogenicity. Divided dosing of antihypertensive drugs tends to decrease adherence and should be avoided when possible [31].

BP cannot be controlled with monotherapy in many patients, particularly those with severe hypertension. When combining antihypertensive medications, it is important to consider

whether the drugs have additive effects on BP or adverse effects, and whether the patient has comorbidities that mandate particular drug choices.

ACE inhibitors or angiotensin II receptor blockers, thiazide diuretics and dihydropyridine calcium channel blockers are additive in lowering BP and can be combined as double or triple combination therapies. By contrast, combining ACE inhibitors and angiotensin II receptor blockers adds little BP lowering while increasing the risk for renal dysfunction and hyperkalemia (high blood potassium levels, which can lead to cardiac arrhythmias). Similarly, combining RAAS inhibitors with beta-adrenoreceptor blockers adds little BP reduction, but this combination is indicated in patients following acute myocardial infarction or heart failure with reduced left ventricular ejection fraction for reasons beyond BP reduction.

LITERATURE REVIEW

1. **Laouini, C. Jaafar-Maalej et al., (2012)** Liposomes, spherical-shaped nanovesicles, were discovered in the 60ies by Bangham. Since that, they were extensively studied as potential drug carrier. Due to their composition variability and structural properties, liposomes are extremely versatile leading to a large number of applications including pharmaceutical, cosmetics and food industrial fields. This bibliographic paper offers a general review on the background and development of liposomes with a focus on preparation methods including classic (thin film hydration, reverse-phase evaporation, ethanol injection…) and novel scalable techniques. Furthermore, liposome characterization techniques including mean size, zetapotential, lamellarity, encapsulation efficiency, in vitro drug release, vesicles stability and lipid analysis synthesized from different published works are reported. The current deepening and widening of liposome interest in many scientific disciplines and their application in pharmaceutics, cosmetics and food industries as promising novel breakthroughs and products were also handled. Finally, an opinion on the usefulness of liposomes in various applications ranging from unsubstantiated optimism to undeserved pessimism is given. The obtained information allows establishing criteria for selecting liposomes as a drug carrier according to its advantages and limitations.
2. **Song KH, et al., (2002)** Salmon calcitonin (sCT)-containing proliposomes were prepared by penetrating a methanol-chloroformic solution of sCT and phosphatidylcholine (PC) into microporous sorbitol particles, followed by vacuum evaporation of the solvent. As a result, sCT proliposomes with free-flowing flowability were obtained. On contact with water, the proliposomes were rapidly converted into a liposomal dispersion, in which a certain amount of sCT was entrapped by the liposomes. The apparent permeability of sCT across Caco-2 cell monolayers was increased as the result of incorporating sCT into the proliposomes, suggesting that the pharmacokinetics of sCT would be modified through the administration of proliposomes. This is the first study that reports the successful loading of sCT, a protein drug, in proliposomes. The development of various dosage forms of sCT, especially solid dosage forms, appears be feasible using proliposomes.
3. **Hongtao Xu, et al., (2009)** Free-flowing proliposomes which contained vinpocetine were prepared successfully to increase the oral bioavailability of vinpocetine. In this study the proliposomes were prepared by a novel method which was reported for the first time and the formulation was optimized using the centre composite design (CCD). The optimized formulation was Soybean phosphatidylcholine: 860 mg; cholesterol: 95 mg and sorbitol: 8000 mg. After the proliposomes were contacted with water, the suspension of vinpocetine

liposomes formed automatically and the entrapment efficiency was approximately 86.3% with an average particle size of about 300 nm. The physicochemical properties of the proliposomes including SEM, TEM, XRD and FTIR were also detected. HPLC system was applied to study the concentration of vinpocetine in the plasma of the New Zealand rabbits after oral administration of vinpocetine proliposomes and vinpocetine suspension. The pharmacokinetic parameters were calculated by the software program DAS2.0. The concentration–time curves of vinpocetine suspension and vinpocetine proliposomes were much more different. There were two absorption peaks on the concentration–time curves of the vinpocetine proliposomes. The pharmacokinetic parameters of vinpocetine and vinpocetine proliposomes in New Zealand rabbits were T_{max} 1 h and 3 h (there was also an absorption peak at 1 h); C_{max} 163.82 ± 12.28 ng/ml and 166.43 ± 21.04 ng/ml; $AUC_{0-\infty}$ 1479.70 ± 68.51 ng/ml h and 420.70 ± 35.86 ng/ml h, respectively. The bioavailability of vinpocetine in proliposomes was more than 3.5 times higher than the vinpocetine suspension. The optimized vinpocetine proliposomes did improve the oral bioavailability of vinpocetine in New Zealand rabbits and offer a new approach to enhance the gastrointestinal absorption of poorly water-soluble drugs.

4. **Abdelbary Elhissi, et al., (2005)** In this study, particulate-based proliposomes were investigated as a formulation which would form, an isotonic liposome dispersion in situ when nebulised within a medical nebuliser. Transmission electron microscopy and particle size analysis indicated that liposomes were generated from soya phosphatidylcholine /cholesterol/sucrose proliposomes in air-jet (Pari LC Plus), ultrasonic (Liberty) and vibrating-mesh (Omron NE- U22) nebulisers, without the need for a prolonged hydration step. With jet and vibrating-mesh nebulisers, liposomes were efficiently delivered to the second (lower) stage of a two-stage impinger, indicating such liposomes would be predicted to be delivered to the peripheral airways. Phos- pholipid output from these nebulisers was less than the total mass output, suggesting some accumulation of liposomes in the nebuliser reservoir. The ultrasonic nebuliser delivered less than 6% of available phospholipid to the impinger, demonstrating that this device was inappropriate for delivering a therapeutic proliposome formulation for pulmonary administration. These results show that proliposomes offer a means of producing stable formulations which can readily generate isotonic liposome formulations in situ for delivery to the airways using either an air-jet or vibrating-mesh nebuliser.
5. **Lancet, et al., (2014)** We aimed to investigate whether the benefits of blood pressure-lowering drugs are proportional to baseline cardiovascular risk, to establish whether

absolute risk could be used to inform treatment decisions for blood pressure-lowering therapy, as is recommended for lipid-lowering therapy. Methods: This meta-analysis included individual participant data from trials that randomly assigned patients to either blood pressure-lowering drugs or placebo, or to more intensive or less intensive blood pressure-lowering regimens. The primary outcome was total major cardiovascular events, consisting of stroke, heart attack, heart failure, or cardiovascular death. Participants were separated into four categories of baseline 5-year major cardiovascular risk using a risk prediction equation developed from the placebo groups of the included trials (<11%, 11-15%, 15-21%, >21%). Findings: 11 trials and 26 randomised groups met the inclusion criteria, and included 67,475 individuals, of whom 51,917 had available data for the calculation of the risk equations. 4167 (8%) had a cardiovascular event during a median of 4·0 years (IQR 3·4-4·4) of follow-up. The mean estimated baseline levels of 5-year cardiovascular risk for each of the four risk groups were 6·0% (SD 2·0), 12·1% (1·5), 17·7% (1·7), and 26·8% (5·4). In each consecutive higher risk group, blood pressure-lowering treatment reduced the risk of cardiovascular events relatively by 18% (95% CI 7-27), 15% (4-25), 13% (2-22), and 15% (5-24), respectively (p=0·30 for trend). However, in absolute terms, treating 1000 patients in each group with blood pressure-lowering treatment for 5 years would prevent 14 (95% CI 8-21), 20 (8-31), 24 (8-40), and 38 (16-61) cardiovascular events, respectively (p=0·04 for trend).

6. **Suzanne Oparil, et al., (2014)** Systemic arterial hypertension is the most important modifiable risk factor for all-cause morbidity and mortality worldwide and is associated with increased risk of cardiovascular disease (CVD). Fewer than half of those with hypertension are aware of their condition, and many others are aware but not treated or inadequately treated, although successful treatment of hypertension reduces the global burden of disease and mortality. The aetiology of hypertension involves the complex interplay of environmental and pathophysiological factors that affect multiple systems, as well as genetic predisposition. Evaluation of patients with hypertension includes accurate standardized blood pressure (BP) measurement, assessing patients' predicted risk of atherosclerotic CVD, evidence of target organ damage, detection of secondary causes of hypertension and presence of comorbidities, including CVD and kidney disease. Lifestyle changes, including dietary modifications and increased physical activity, are effective in lowering BP and preventing hypertension and its CVD sequelae. Pharmacological therapy is very effective in lowering BP and preventing CVD outcomes in most patients; first line antihypertensive medications include angiotensin-converting enzyme (ACE) inhibitors,

angiotensin II receptor blockers, dihydropyridine calcium channel blockers and thiazide diuretics.

7. **Eoin O'Brien, et al., (2013)** Ambulatory blood pressure monitoring (ABPM) is being used increasingly in both clinical practice and hypertension research. Although there are many guidelines that emphasize the indications for ABPM, there is no comprehensive guideline dealing with all aspects of the technique. It was agreed at a consensus meeting on ABPM in Milan in 2011 that the 34 attendees should prepare a comprehensive position paper on the scientific evidence for ABPM. This position paper considers the historical background, the advantages and limitations of ABPM, the threshold levels for practice, and the cost-effectiveness of the technique. It examines the need for selecting an appropriate device, the accuracy of devices, the additional information and indices that ABPM devices may provide, and the software requirements. At a practical level, the paper details the requirements for using ABPM in clinical practice, editing considerations, the number of measurements required, and the circumstances, such as obesity and arrhythmias, when particular care needs to be taken when using ABPM. The clinical indications for ABPM, among which white-coat phenomena, masked hypertension, and nocturnal hypertension appear to be prominent, are outlined in detail along with special considerations that apply in certain clinical circumstances, such as childhood, the elderly and pregnancy, and in cardiovascular illness, examples being stroke and chronic renal disease, and the place of home measurement of blood pressure in relation to ABPM is appraised. The role of ABPM in research circumstances, such as pharmacological trials and in the prediction of outcome in epidemiological studies is examined and finally, the implementation of ABPM in practice is considered in relation to the issue of reimbursement in different countries, the provision of the technique by primary care practices, hospital clinics and pharmacies, and the growing role of registries of ABPM in many countries.
8. **M Iskedjian, et al., (2002)** The purpose of this study was to compare the rates of adherence with QD, twice-daily (BID), and MDD antihypertensive drug regimens. Methods: MEDLINE, Embase, and International Pharmaceutical Abstracts databases were searched to identify comparative trials of patient adherence to antihypertensive medication in solid, oral formulations. Data were combined using a random-effects meta-analytic model. Results: Eight studies involving a total of 11,485 observations were included (1830 for QD dosing, 4405 for BID dosing, 4147 for dosing >2 times daily [>BID], and 9655 for MDD), in which the primary objective was to assess adherence. The average adherence rate for QD dosing (91.4%, SD = 2.2%) was significantly higher ($Z = 4.46$, $P < 0.001$) than for MDD

(83.2%, SD = 3.5%). This rate was also significantly higher (Z = 2.22, P = 0.026) than for BID dosing (92.7% [SD = 2.3%] vs 87.1% [SD = 2.9%]). The difference in adherence rates between BID dosing (90.8%, SD = 4.7%) and >BID dosing (86.3%, SD = 6.7%) was not significant (Z = 1.82, P = 0.069).

9. **Nazareth E. Ceschan, et al., (2022),** Hypertension is a chronic pathology where blood pressure levels are continuously high, causing cardiac, renal, cerebral, and vascular damage leading to early morbi-mortality. This illness is the main risk factor for cardiovascular diseases and the main cause of atrial fibrillation. Atenolol (AT) is a β-1 blocker drug useful for antihypertension and antiarrhythmic treatments. However, this drug possesses low oral bioavailability associated to its low permeability and extensive hepatic first-pass metabolism. To solve the conventional AT-administration problems, oral controlled-release and transdermal delivery have been reported. In this work, an alternative AT inhalatory system administered by nebulization is presented. This system is based on an ionic complex between acidic groups of alginic acid and cationic groups of AT (AA-AT), which was obtained by spray-drying. Pharmaceutical and biopharmaceutical properties for AA-AT inhalatory administration using a jet nebulizer were investigated. The aerodynamic performance (assayed at different cup-nebulizer loadings) of the nebulized system demonstrated that around 40% of the formulation would deposit in the respiratory membrane, with mass median aerodynamic diameters of 3.4–3.6 μm. The AT carried in the AA-AT system was released adequately by ionic exchange in saline solution by permeation through a cellulose membrane. The presence of AA as polyelectrolyte conferred mucoadhesive properties to the ionic complex. Even at high relative AA-AT concentrations, no cytotoxic effect was detected in A-549 cell line. Finally, the preliminary pharmacokinetic assay in the in vivo model confirmed that AT was absorbed from the lung to the systemic circulation, with a greater plasmatic AUC compared to the pure drug (around 50% higher). Then, the system and the nebulization administration demonstrated potential for drug cardiac targeting.
10. **W. Kirch., et al, (1981),** Atenolol is a hydrophilic betareceptor blocking drug, which is predominantly eliminated via the kidneys, only about 5% of the atenolol is metabolised by the liver. After oral administration atenolol is incompletely absorbed from the intestine, so about 50 % of the beta blocker are finally biovailable. In plasma only 3% of atenolol are protein-bound. There exists a linear relationship between the atenolol plasma levels and the degree of beta blocking effect measured by inhibition of the exerciseinduced tachycardia. No correlation was found between plasma levels of atenolol and blood pressure lowering

activity of the drug. After oral administration elimination half-life of atenolol is calculated from 6 to 9 h by different authors. In patients with impaired renal function elimination half-life of atenolol gradually increases to values of 36 h in uraemic patients (glomerular filtration rate (GFR) < 10 ml/min). Between GFR and atenolol plasma clearance as well as renal clearance a close significant correlation is described. Prolongation of elimination half-life requires a dosage adjustment of atenolol in patients with renal failure. A marked interaction of atenolol is found when calcium or aluminium hydroxide are concurrently administered with the beta blocker whereas cimetidine does not influence atenolol kinetics.

11. **Byung-nak ahn., et al, (1995),** Proliposomes were prepared by the penetration of a methanol-chloroform solution of propranolol hydrochloride (PH) and lecithin into microporous sorbitol, with subsequent vacuum drying. They were characterized for surface morphology and flowability, and following the conversion to liposomes upon hydration, size distribution, drug content and in vitro drug release of the reconstituted liposomes were examined. The porous structure of sorbitol was maintained in the proliposomes, affording the proliposomes good flowability at lecithin-to-sorbitol ratios (w/w) of not more than 0.2. Multilamellar liposomes were reconstituted spontaneously from the proliposomes upon hydration. The mean diameter of the resultant liposomes was highly dependent on the PH-to-lecithin ratio, but less dependent on the lecithin-to-sorbitol ratio and sorbitol particle size (105-710pm). Entrapment efficiency of PH in liposomes showed a maximum 10% at PH-to-lecithin ratio < 0.5 and a lecithin-to-sorbitol ratio > 0.1. Sustained release of propranolol from the proliposomes was achieved when the lecithin-to-PH ratio was > 2, and the lecithin-to-sorbitol ratio was in the range examined (0.06742). In conclusion, granular proliposomes of PH with good flowability and sustained release characteristics could be prepared by controlling the drug/lecithin/sorbitol ratio and sorbitol particle size. PH proliposomes can be potential candidates for the sustained drug delivery of propranolol when applied directly onto the mucosal membranes.
12. **Mohamed Rahamathulla., et al, (2020),** Simvastatin a cholesterol-lowering agent used to treat hypercholesterolemia, coronary heart disease, and dyslipidemia. However, simvastatin (SV) has shown low oral bioavailability in GIT. The main purpose of the work was to develop proliposomal formulations to increase the oral bioavailability of SV. Film deposition on the carrier method has been used to prepare the proliposomes. The proliposomes were assessed for morphology, particulate size, entrapment efficacy, drug-polymer compatibility, in vitro and in vivo studies. FTIR and DSC results revealed no drug-polymer interaction. SEM and XRD analysis conform; proliposomes are spherical,

amorphous in nature, so that it enhances the solubility of SV between 15.01 ± 0.026 and 57.80 ± 0.015 µg/mL in pH 7.4 phosphate buffer. The optimised formulation (PL6) shows drug release up to 12 h (99.78 ± 0.067%). The pharmacokinetics of pure SV and SV proliposomes (SVP) in rats were Tmax 2 ± 0.5 and 4 ± 0.7 h, Cmax 10.4 ± 2.921 and 21.18 ± 12.321 µg/mL, AUC0-∞ 67.124 ± 0.23 and 179.75 ± 1.541 µg/mL h, respectively. Optimised SVP shows a significant improvement in the rate and absorption of SV. The optimised formulation showed enhanced oral bioavailability of SV in Albino Wister rats and offers a new technique to improve the poor water-soluble drug absorption in the gastrointestinal system.

13. **Krishna Mohan Chinnala., et al, (2017),** Acyclovir is a synthetic acyclic purine nucleoside analog that is currently used for the prevention and treatment of Herpes Simplex Virus (HSV) and Varicella-Zoster Virus (VZV) infection that occurs at epidermis. The oral bioavailability of acyclovir is low, variable and species dependent. The aim of the study is to prepare 10 formulations of liposomal carrier for Acyclovir for the treatment of viral infections that is capable of delivering the drug to the specific target site by topical route by using different ratios of phospholipids and cholesterol with a desired amount of drug by thin film hydration technique and rotary flash evaporation technique and to find out the drug release from the liposome's of different ratios, 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.
14. **Karthik Y. Janga., et al, (2012),** The present systematic study focused to investigate the combined advantage of proliposomes and surface charge for improved oral delivery of zaleplon. The zaleplon loaded proliposomes were prepared using hydrogenated soyphosphatidylcholine (HSPC) and cholesterol (CHOL) in varying ratios, and the optimized formulation was tailored with dicetyl phosphate and stearylamine to obtain negative and positive charged vesicles, respectively. The formulations were characterized for micromeritics, size, zeta potential, and entrapment efficiency. Further, in vitro release and dissolution study carried out provide an insight on the stability and enhanced dissolution of zaleplon from proliposome formulations. The solid state characterization (SEM, DSC, and PXRD) studies unravel the transformation of zaleplon to amorphous or molecular state from the native crystalline form. To depict the conclusions, in situ single-pass perfusion and bioavailability studies were carried out in rats. The significant increase in effective permeability coefficient (Peff) and rate and extent of absorption from cationic vesicles indicate the importance of surface charge for effective uptake across the

gastrointestinal tract. Overall, a two- to fivefold enhancement in bioavailability in comparison with control confers the potential of proliposomes as suitable carriers for improved oral delivery of zaleplon.

15. **Junting Jia., et al, (2011),** This study was designed to evaluate the in vitro release, pharmacokinetics (PK), pharmacodynamics (PD) and PK–PD relationships of atenolol sustained-release pellets (AT-SRPs), compared with those of atenolol immediate-release pellets (AT-IRPs). Blood sampling for AT plasma concentration was performed in normal rats and blood pressure-lowering effects were recorded continuously in hypertensive rats (HRs) before and at 1, 4, 8, 12, 16 and 24 h after drug administration. The parameters were calculated using DAS1.0 program and WinNonlin software. The release profile of SRPs was steadier and more sustained than that of IRPs. The mean Cmax and area under concentration–time curve from 0 to 24 h after administration (AUC0–24 h) of SRPs were significantly lower than that of IRPs ($p < 0.05$), while area under concentration–time curve from 0 to infinity (AUC0–∞) was almost equivalent between the two formulations. The mean half life time (t1/2) of AT-SRPs was almost 2 times longer compared to that of AT-IRPs. The SRPs approximately achieved half of peak drug effect (Emax) of IRPs, while there were no significant differences in the area under effect–time curve from 0 to 24 h after administration (AUEC0–24 h) and the area under effect–time curve from 0 to infinity (AUEC0−∞). The value of the rate constant of equilibration between plasma and the effect-site (ke0) for SRPs was about 4 times higher than IRPs. The effect–concentration–time course for AT-SRPs was represented by the clockwise hysteresis loop, while the counter-clockwise hysteresis loop well showed that for AT-IRPs. The more favorable characteristics of SRPs would make it more appropriate as a potential dosage form for the treatment of hypertension.
16. **Iftikhar Khan., et al, (2017),** Proliposome powders were prepared via a slurry method using sorbitol or D-mannitol as carbohydrate carriers in 1:10 or 1:15 w/w lipid phase to carrier ratios. Soya phosphatidylcholine (SPC) and cholesterol were employed as a lipid phase and Beclometasone dipropionate (BDP) was incorporated as a model drug. Direct compaction using a Minipress was applied on the lipid-enriched powder in order to manufacture proliposome tablets. Sorbitol-based proliposome tablets in a 1:15 w/w ratio were found to be the best formulation as it exhibited excellent powder flowability with an angle of repose of 25.62 ± 1.08°, and when compacted the resultant tablets had low friability (0.20 ± 0.03%), appropriate hardness (crushing strength) (120.67 ± 12.04 N), short disintegration time (5.85 ± 0.66 min), and appropriate weight uniformity. Moreover,

upon hydration into liposomes, the entrapment efficiency for sorbitol formulations in both 1:10 and 1:15 lipid to carrier ratios were significantly higher (53.82 ± 6.42% and 57.43 ± 9.12%) than D-mannitol formulations (39.90 ± 4.30% and 35.22 ± 6.50%), respectively. Extended stability testing was conducted for 18 months, at three different temperature conditions (Fridge Temperature (FT; 6°C), Room Temperature (RT; 22°C) and High Temperature (HT; 40°C)) for sorbitol-based proliposome tablets (1:15 w/w ratio). Volume median diameter (VMD) and zeta potential significantly changed from 5.90 ± 0.70 µm to 14.79 ± 0.79 µm and from -3.08 ± 0.26 mV to -11.97 ± 0.26 mV respectively at month 18, when samples were stored under HT conditions. Moreover, the entrapment efficiency of BDP decreased from 57.43 ± 9.12% to 17.93 ± 5.37% following 18 months storage under HT conditions. Overall, in this study for the first time, proliposome tablets were manufactured and thoroughly characterized, and sorbitol showed to be a promising carrier.

17. **Pradeep Kumar Y., et al, (2012),** The aim of the present study was to develop proliposomal formulations to enhance the oral bioavailability of agomelatin by improving solubility, dissolution and/or intestinal permeability. Proliposomal powder formulations were prepared using different ratios of agomelatin, phospholipon 90H and cholesterol by solvent evaporation method. The effect of phospholipid composition and drug:lipid ratio on in vitro performance of proliposomes was studied. Proliposomes were characterized for their particle size distribution, zeta potential, micromeritics, and entrapment efficiency. Further, the formulated proliposomes were subjected to in vitro drug release performance in both simulated gastric and intestinal fluid demonstrate improved dissolution characteristics compared to pure drug. Multimedia dissolution profiles were carried out to demonstrate enhanced dissolution characteristics compared to pure drug. Proliposomes provided enhanced agomelatin dissolution due to incorporation into the phospholipid bilayers and change in the physical state from crystalline to amorphous. These proliposomal formulations of agomelatin could provide improved oral bioavailability due to enhanced solubility, permeability and enhanced absorption.
18. **Nicholas I. Payn., et al, (1985),** Photographic evidence of the process of proliposc ie hydration is provided together with comprehensive particle size analysis of both hydrated dimyristoyl phosphatidylcholine: dimyristoyl phosphatidyl glycerol: ergosterol: amphotericin B proliposomes and egg 1ecithin:ergostero1:amphotericin B proliposomes using photon correlation spectroscopy and Coulter Counter analysis. Proliposome particle size and the temperature during hydration have been shown to have little effect on subsequent liposome size. A short tc*rl stability study of proliposomes indicated that only

minor changes in the size distribution profile of the hydrated product are apparent after storage at 20°C for 9 months. Furthermore, no drop in amphotericin B potency was noticed over a 6- month period.

19. **Suvakanta Dash., et al, (2010),** In this paper we review the mathematical models used to determine the kinetics of drug release from drug delivery systems. The quantitative analysis of the values obtained in dissolution/release rates is easier when mathematical formulae are used to describe the process. The mathematical modeling can ultimately help to optimize the design of a therapeutic device to yield information on the efficacy of various release models.
20. **Gamal Shazly., et al, (2007),** The aim of these studies was to compare dialysis and dispersion methods for determining in vitro release of propranolol, metoprolol, pindolol, and atenolol from multilamellar liposomes. Multilamellar vesicles (MLV) were prepared using hydrogenated soy-lecithin phospholipon 90H (Ph 90H) as the primary lipid. The same volume of pH 7.4 phosphate buffered saline was used as a receptor medium for both methods. Samples were withdrawn, and drug concentration was determined using HPLC. All drug-containing liposomes exhibited an initial burst release followed by a slower rate of release. The rate and extent of drug release from MLV was dependent on the physicochemical properties of the drug. For all drugs investigated, the rate of release was higher for the dispersion method as compared with the dialysis method.

AIM AND OBJECTIVE

Atenolol, a beta-2 receptor blocking agent, is widely used for treatment of hypertension and coronary artery disease. Atenolol (4-[2-hydroxy-3-isopropylaminopropoxy-] phenyl-acetamide) is a hydrophilic β-adrenoceptor blocking drug. It is classified as class III drug in the biopharmaceutics classification system (BCS). This means that, orally administered atenolol possesses high solubility but low permeability. Hydrophilic drugs have difficulty in crossing cellular membranes and tend to be poorly absorbed from the gut. After oral administration, atenolol is incompletely absorbed from the intestine, so about 50 % of the beta blocker are finally bioavailable. The oral recommended dose for AT tablets is between 25 and 100 mg, twice daily. Conventional oral administration usually results in erratic drug concentrations in plasma because of Atenolol hydrophilicity, leading to reduction in the pharmacological effect or development of undesirable side effects. Ischemic colitis, nausea and diarrhoea have been attributed to frequent oral administration and patients can also suffer central nervous system side effect.

These disadvantages make it an appropriate candidate for proliposomes. Pro-liposomes are dry, free-flowing granular products that on hydration or on contact with biological fluids in the body, form liposomal dispersion. They are composed of water-soluble porous powder and phospholipid. Solubility and bioavailability problems of many drugs can be overcome by developing pro-liposomal formulations. Liposomal suspension may have limited shelf life and to overcome the stability issue associated with liposome, a novel formulation “pro-liposome” method is developed that can produce liposomes quickly when there is a need and without excessive manipulation.

Inhalation is one of the most common non-invasive medication delivery methods, and it is especially useful for cardiac targeting. This is because, during absorption, drugs are predominantly first transported to the heart via the pulmonary vein. Considering this, inhaling Atenolol may be a viable alternative for reaching heart tissue directly while reducing systemic exposure. The pulmonary epithelium is relatively extensive and widely vascularized. Furthermore, there is little presence of efflux transporters, which favors drug absorption. Via this route, the onset of action can be relatively fast, metabolic enzymes levels are lower compared to hepatic ones, drugs even with different physicochemical properties can be absorbed and frequent administration of atenolol is reduced. Hence the novel formulation

powder liposome holds immense versatility to be explored in drug delivery system, bypassing the limitations of former liposomal system.

PLAN OF WORK

❖ LITERATURE REVIEW

❖ PREFORMULATION STUDY

a) Morphological study
   - ➢ Colour, odour, taste, and appearance of Atenolol is observed.

b) Solubility

c) Calibration curve

d) Drug excipient compatibility study
   - FTIR spectroscopy

❖ **FORMULATION AND DEVELOPMENT**

- Optimization of Atenolol proliposomes by Box Behnken design
- Preparation of Proliposomes

❖ **EVALUATION OF PROLIPOSOMES**

- Microscopic observation of hydrated proliposomes
- Particle size analysis
- SEM (Scanning electron microscope)
- Drug entrapment efficiency
- Stability study
- In-vitro drug diffusion study
- In-vitro drug release kinetics
- In-vivo pharmacodynamic study
- In-vivo pharmacokinetic study

DRUG PROFILE

ATENOLOL

Atenolol is used with or without other medications to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. This medication is also used to treat chest pain (angina) and to improve survival after a heart attack.

Atenolol belongs to a class of drugs known as beta-blockers. It works by blocking the action of certain natural chemicals in your body, such as epinephrine, on the heart and blood vessels. This effect lowers the heart rate, blood pressure, and strain on the heart. β− 1 blocker drugs are one of the most used treatments of hypertension. Also, this drug family is widely prescribed for treating irregular heart rhythm as antiarrhythmic treatment.

Atenolol may also be used to treat irregular heartbeat, heart failure, alcohol withdrawal symptoms, and to prevent migraine headaches.

CVS: It is important to distinguish these effects in normal subjects from those in subjects with cardiovascular disease (CVD) such as hypertension or myocardial ischemia. The negative ionotropic and chronotropic effects are modest when the simulation of b-receptors is low. However, in presence of activated sympathetic nervous system as during exercise or stress; atenolol attenuates the expected rise in heart rate (HR) and myocardial contractility. The exercise-induced increase in cardiac output (CO) is less affected because of an increase in stroke volume. It decreases the effects of catecholamine on determinants of myocardial oxygen consumption (heart rate; contractility and systolic pressure), hence improving the relationship between cardiac oxygen supply and demand; exercise tolerance is improved in patients with angina.

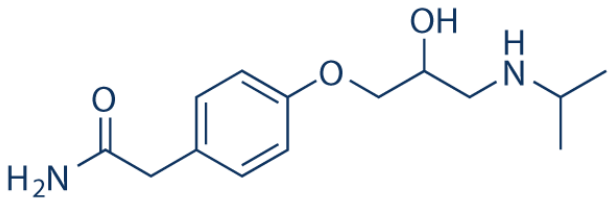
The duration of action is dose-related and also bears a linear relationship to the logarithm of plasma atenolol concentration. Besides reducing HR, cardiac index, and blood pressure; effects on total peripheral resistance have been documented; though less uniformly. Acute intravenous administration is usually followed by an increase in total peripheral resistance of 20-30%.

Studies during chronic oral administration of atenolol have found either no change in vascular resistance7-8 or an increase of about 5%.

IUPAC NAME	benzene acetamide, 4- [21 hydroxy 31 [(1- methyl ethyl) amino] propoxy]
MOLECULAR FORMULA	$C_{14}H_{22}N_2O_3$
MOLECULAR WEIGHT	266.34
MELTING POINT (°C)	158-160
WATER SOLUBILITY	26.5 mg/ml at 37◦C
LogP	0.16
PROTEIN BINDING	6-16

Table: 2; Physicochemical properties of Atenolol

MOLECULAR STRUCTURE:



MECHANISM OF ACTION:

Atenolol is a cardioselective beta-blocker, called such because it selectively binds to the β1-adrenergic receptor as an antagonist up to a reported 26 fold more than β2 receptors.15 Selective activity at the β1 receptor produces cardioselectivity due to the higher population of this receptor in cardiac tissue. Some binding to β2 and possibly β3 receptors can still occur at therapeutic dosages but the effects mediated by antagonizing these are significantly reduced from those of non-selective agents. β1 and β2 receptors are Gs coupled therefore antagonism of their activation reduces activity of adenylyl cyclase and its downstream signaling via cyclic adenosine monophosphate and protein kinase A (PKA).

In cardiomyocytes PKA is thought to mediate activation of L-type calcium channels and ryanodine receptors through their phosphorylation. L-type calcium channels can then provide

an initial rise in intracellular calcium and trigger the ryanodine receptors to release calcium stored in the sarcoplasmic reticulum (SR) and increased contractility. PKA also plays a role in the cessation of contraction by phosphorylating phospholamban which in turn increases the affinity of SR Ca2+ ATPase to increase reuptake of calcium into the SR. It also phosphorylates troponin I to reduce affinity of the protein for calcium. Both of these events lead to a reduction in contraction which, when coupled with the initial increase in contraction, allows for faster cycling and consequently higher heart rate with increased contractility. L-type calcium channels are also a major contributor to cardiac depolarization and their activation can increase frequency of action potentials and possibly the incidence of ectopic potentials.

Similar inhibitory events occur in the bronchial smooth muscle to mediate relaxation including phosphorylation of myosin light-chain kinase, reducing its affinity for calcium. PKA also inhibits the excitatory Gq coupled pathway by phosphorylating the inositol trisphosphate receptor and phospholipase C resulting in inhibition of intracellular calcium release. Antagonism of this activity by beta-blocker agents like atenolol can thus cause increased bronchoconstriction.

PHARMACOKINETICS

Absorption

Approximately 50% of an oral dose is absorbed from the gastrointestinal tract, with the remainder being excreted unchanged in the feces. Label Administering atenolol with food can decrease the AUC by about 20%. While atenolol can cross the blood-brain barrier, it does so slowly and to a small extent.

Half-life

6-7 hrs.

Volume of distribution

Total Vd of 63.8-112.5 L. Atenolol distributes into a central volume of 12.8-17.5 L along with two peripheral compartments with a combined volume of 51-95 L. Distribution takes about 3 hrs for the central compartment, 4 hrs for the shallower peripheral compartment, and 5-6 hrs for the deeper peripheral compartment.

Protein binding

6-16% bound in plasma. Label Atenolol binds to two sites on human serum albumin.

Metabolism

Minimal metabolism in the liver. Label The sole non-conjugated metabolite is the product of a hydroxylation reaction at the carbon between the amide and benzene groups. The only other metabolite to be confirmed is a glucuronide conjugate. These metabolites make up 5-8% and 2% of the renally excreted dose with 87-90% appearing as unchanged drug. The hydroxylated metabolite is exerting 1/10th the beta-blocking activity of atenolol.

Route of elimination

85% is eliminated by the kidneys following IV administration with 10% appearing in the feces

Clearance

Total clearance is estimated at 97.3-176.3 mL/min with a renal clearance of 95-168 mL/min.

Side effects of atenolol include:

- Tiredness
- Low blood pressure (hypotension)
- Slow heart rate
- Cold extremities
- Dizziness upon standing
- Depression
- Nausea
- Dreaming
- Drowsiness
- Diarrhea
- Fatigue
- Leg pain
- Lethargy

- Lightheadedness
- Spinning sensation (vertigo)
- Shortness of breath
- 2°/3° atrioventricular (AV) block

Other side effects of atenolol include:

- Severe congestive heart failure (CHF)
- Sick sinus syndrome
- Catatonia
- Disorientation
- Mood swings
- Hallucinations
- Headache
- Impaired performance on neuropsychiatric tests
- Psychoses
- Short-term memory impairment
- Purple-colored spots on the skin
- Rashes
- Nausea
- Low platelet count (thrombocytopenia)
- Visual disturbances
- Dry eyes
- Raynaud phenomenon

Warnings

Ischemic heart disease may be exacerbated after abrupt withdrawal.

Hypersensitivity to catecholamines has been observed during withdrawal.

Exacerbation of angina and, in some cases, myocardial infarction (MI) may occur after abrupt discontinuance.

When long-term beta-blocker therapy (particularly with ischemic heart disease) is discontinued, dosage should be gradually reduced over 1-2 weeks with careful monitoring.

If angina worsens markedly or acute coronary insufficiency develops, beta-blocker administration should be promptly reinitiated, at least temporarily (in addition to other measures appropriate for unstable angina).

Patients should be warned against interruption or discontinuance of beta-blocker therapy without physician advice.

Because coronary artery disease (CAD) is common and may be unrecognized, beta-blocker therapy must be discontinued slowly, even in patients treated only for hypertension.

This medication contains atenolol. Do not take Tenormin if you are allergic to atenolol or any ingredients contained in this drug.

Keep out of reach of children. In case of overdose, get medical help or contact a Poison Control Center immediately.

Cautions

• Use with caution in anesthesia or surgery (myocardial depression), bronchospastic disease, cerebrovascular insufficiency, diabetes mellitus, hyperthyroidism or thyrotoxicosis, liver disease, renal impairment, peripheral vascular disease, compromised left ventricular function, advanced age, heart failure.

• May mask the effects of hyperthyroidism.

• Risk of hypoglycemia and bradycardia in neonates born to mothers who receive the drug at parturition or while breastfeeding, especially in premature infants and those with renal impairment.

• Use with caution in patients taking calcium-channel blockers or cardiac glycosides or using inhaled anesthetics.

• Avoid abrupt withdrawal; sudden discontinuance can exacerbate angina and lead to MI.

• Increased risk of stroke after surgery.

• In patients receiving clonidine, atenolol should be discontinued several days before the withdrawal of clonidine.

• May cause or exacerbate CNS depression (use with caution in patients with psychiatric illness).

• Use in pheochromocytoma (alpha blockade required before use of beta-blocker).

• Consider preexisting conditions such as sick sinus syndrome before initiating therapy.

• May potentiate hypoglycemia and may mask its signs and symptoms in patients with diabetes mellitus; use caution.

• Monitor for worsening of heart failure symptoms in patients with compensated heart failure.

• Use caution in patients with myasthenia gravis; may precipitate or aggravate symptoms of arterial insufficiency in patients with Raynaud's disease and peripheral vascular disease; use caution and monitor for progression of arterial obstruction.

• Avoid beta-blockers without alpha1-adrenergic receptor blocking activity in patients with Prinzmetal variant angina; unopposed alpha1-adrenergic receptors mediate coronary vasoconstriction and can worsen anginal symptoms.

• Exacerbation or induction of psoriasis reported with beta-blocker use; cause and effect not established.

Pregnancy and Lactation

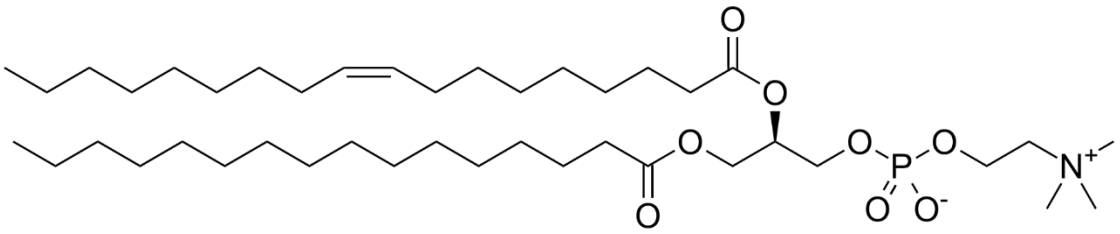
• Use atenolol during pregnancy only in LIFE-THREATENING emergencies when no safer drug is available. There is positive evidence of human fetal risk.

• Atenolol enters breast milk; neonates born to mothers who are receiving atenolol at parturition or breastfeeding may be at risk for hypoglycemia and bradycardia; use with caution if breastfeeding.

EXCIPIENT PROFILE

a) Soya phosphatidylcholine:

Soya Phosphatidylcholines (PC) are a class of phospholipids that incorporate choline as a headgroup. They are a major component of biological membranes and can be easily obtained from a variety of readily available sources, such as egg yolk or soybeans, from which they are mechanically or chemically extracted using hexane. They are also a member of the lecithin group of yellow-brownish fatty substances occurring in animal and plant tissues.



Molecular Formula	$C_{42}H_{80}NO_8P$
Chemical Name	(Z)-3-(oleoyloxy)-2-(palmitoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate.
Molecular Weight	758.1
Appearance	Yellow to light brown semi-solid or wax solid.
Solubility	Soluble in DMSO, not in water

Table:3; Physicochemical properties of Soya phosphatidylcholine

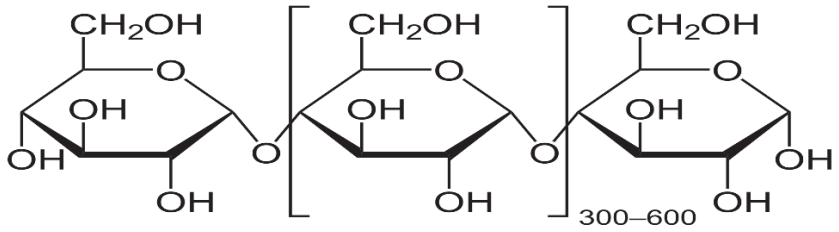
Uses:

Used as an emulsifier, as a wetting agent, for viscosity reduction, as release agents, and for crystallization control.

b) STARCH:

Starch is a complex carbohydrate. Starch or amylum is a polymeric carbohydrate consisting of numerous glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants for energy storage. Worldwide, it is the most common carbohydrate in human

diets and is contained in large amounts in staple foods like wheat, potatoes, maize (corn), rice, and cassava (manioc).



Chemical formula	$C_{12}H_{22}O_{11}$
Molecular Weight	342.297
pH	It has a pH level of 7
Molar mass	105 g·mol−1
Appearance	White powder
Solubility in water	insoluble
Density	1.8±0.1 g/cm3
Boiling Point	667.9±55.0 °C at 760 mmHg
Melting Point	256-258 °C (dec.) (lit.)

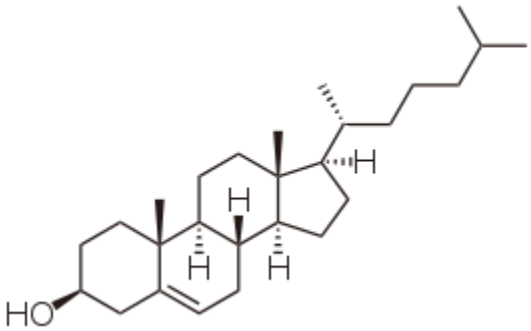
Table: 4; Physicochemical properties of Starch

Uses:

Starches are used in the pharmaceutical industry for a wide variety of reasons, such as an excipient, a tablet and capsule diluent, a tablet and capsule disintegrant, a glidant, or a binder.

C) CHOLESTEROL:

Cholesterol is any of a class of certain organic molecules. Cholesterol is a sterol (or modified steroid), a type of lipid. Cholesterol is biosynthesized by all animal cells and is an essential structural component of animal cell membranes. When chemically isolated, it is a yellowish crystalline solid.



Chemical formula	$C_{27}H_{46}O$
Molar mass	386.65 g/mol
Appearance	white crystalline powder[2]
Density	1.052 g/cm^3
Melting point	148 to 150 °C (298 to 302 °F; 421 to 423 K) [2]
Boiling point	360 °C (680 °F; 633 K) (decomposes)
Solubility in water	0.095 mg/L (30 °C)[1]
Solubility	Soluble in acetone, benzene, chloroform, ethanol, ether, hexane, isopropyl myristate, methanol
Magnetic susceptibility (χ)	$-284.2 \cdot 10^{-6}$ cm^3/mol

Table: 5; Physicochemical properties of cholesterol

Uses:

Cholesterol has been shown to increase liposome stability through various mechanisms, including increased retention time, modulating phospholipid packing, Tm, and plasma stability.

MATERIALS USED

S.No	Material	Function	Source
1	Atenolol	Active Pharmaceutical Ingredients (API)	Softgel Healthcare Pvt, Ltd, Chennai.
2	Soya phosphatidylcholine	phospholipid	Softgel Healthcare Pvt, Ltd, Chennai.
3	Cholesterol	Stabilizing agent	Softgel Healthcare Pvt, Ltd, Chennai.
4	Starch	Carrier	Softgel Healthcare Pvt, Ltd, Chennai.
5	Ethanol	Solvent	Softgel Healthcare Pvt, Ltd, Chennai.

Table: 6; Materials used in the formulation

METHODOLOGY

A. PREFORMULATION STUDYS:

1. Description: The colour, odour, taste and appearance of Atenolol was observed.

2. Calibration curve:

25mg of atenolol reference standard was accurately weighed and dissolved in 50 ml (0.1N) HCL, and from the above solution, a1ml was diluted to 50ml with (0.1N) HCL to produce 10μg/ml of the atenolol reference standard. A suitable aliquot of this stock solution of atenolol was diluted with methanol to obtain 10-50 μg/ml of atenolol reference standard and their absorbance was determined at 275 nm. So, the standard curve was plotted between absorbance and concentration [32].

3. Compatibility study:

Fourier Transformed Infrared

Fourier transformed infrared (FT-IR) analysis was performed to verify potential interactions among drugs and excipients. The blend of drug and polymers or formulation was dispersed in potassium bromide powder and pellets were made using a KBr press. Powder diffuse reflection on the FT-IR spectrum was taken from the FT-IR spectrophotometer [33].

B. Optimization by design of expert:

Box–Behnken experimental design:

A Box–Behnken optimization design with three variables was applied to find the optimum conditions and to analyze how sensitive the responses were to variations in the settings of the experimental variables. This design is a factorial design with three levels, using middle points instead of corner points and is useful for estimating the coefficients in a second-degree polynomial. A total of 15 experiments were performed including triplicates of the center point. The center points improve the assessment of the response surface curvature and simplify the estimation of the model error. The traditional approach to develop a formulation is to change one variable at a time. It is difficult to develop an optimized formulation, as the method reveals nothing about the interactions among the variables. Hence, a Box–Behnken statistical design with three factors, three levels was selected for the optimization study. The polynomial equation generated by this experimental design (Instat + software) is as follows:

$$Yi = b0 + b1 X1 + b2 X2 + b3 X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X12 + b22X22 + b33X32$$

Where Yi is the dependent variable; b0 is the intercept; b1 to b33 are regression coefficients: and X1, X2 and X3 are the independent variable selected from the preliminary experiments [34].

1. Preparation of Atenolol loaded Proliposomes via the slurry method:

Proliposomes were prepared using the slurry method, using starch as carbohydrate carriers. Soya phosphatidylcholine (SPC) and cholesterol were employed in combination as the lipid phase in a 1:1mol ratio. Atenolol was incorporated into the lipid phase. The lipid phase constituting SPC, cholesterol and Atenolol was dissolved in absolute ethanol. Starch was placed in a glass beaker (100ml) and the ethanolic solution was poured onto the carbohydrate carrier to form a slurry, ensuring uniform distribution of the lipid phase and drug over the carrier particles. The beaker was subjected to a magnetic stirrer in a water bath previously adjusted to 45◦C and the evaporation of the organic solvent was continued with a rotation speed of 270 RPM for 1 h. Dry Proliposomes were collected in a dry airtight glass bottle and stored at -18◦C for subsequent studies [35-37].

Formulation	Drug (mg)	SPC	Cholesterol	Starch	RPM
F1	25	2	2	25	220
F2	25	6	2	25	220
F3	25	2	6	25	220
F4	25	6	6	25	220
F5	25	2	4	25	170
F6	25	6	4	25	170
F7	25	2	4	25	270
F8	25	6	4	25	270
F9	25	4	2	25	170
F10	25	4	6	25	170
F11	25	4	2	25	270
F12	25	4	6	25	270
F13	25	4	4	25	220

Table: 7; The formulation design matrix for proliposomes

CHARACTERIZATION OF PROLIPOSOMES

A. MICROSCOPIC OBSERVATION OF HYDRATED PROLIPOSOMES

Granules of Proliposomes are hydrated using distilled water, on hydration Proliposomes are converted into liposomes. Using light microscopy microscopic analysis was performed. The lipids were viewed at 10X, 400X, and 100X [38].

B. PARTICLE SIZE ANALYSIS

Particle size and size distribution measurements of the liposomal dispersions were performed using photon correlation spectroscopy (PCS) otherwise known as Dynamic light scattering (DLS). A polydispersion index is an index used to determine the distribution of particles of nanoparticles and it is a dimensional number varying from 0 to 1 for monodispersed particles. Higher value implies a size distribution of less homogenous for nanoparticles [39].

C. SCANNING ELECTRON MICROSCOPY

The particle size of proliposomes was determined by using a scanning electron microscope.

The optimized batch of proliposomes was viewed under a microscope to study their size. The size of proliposomal vesicles was measured at different locations on a slide by taking a small drop of proliposomal dispersion on it and the average size of proliposomal vesicles was determined [40].

D. DRUG ENTRAPMENT EFFICIENCY

The proliposomal dispersion was centrifuged at 10,000 g for 20 min to separate unentrapped drug and washed with phosphate-buffered saline (pH 7.4). The clear supernatant was analyzed by UV-spectrophotometer at 275nm [41].

$$\% \text{ Encapsulation efficiency} = \text{Total drug–free drug} / \text{Total drug} \times 100$$

E. STABILITY STUDY:

The physical appearance of the proliposomes was measured. The formulations were stored at $4^{o}C$, room temperature ($24^{o}C$), and physiologic temperature ($37^{o}C$). At specific time intervals of one month, the samples were taken and their physical appearance was examined. The encapsulation efficiency of the formulation was measured as described above [42].

F. IN-VITRO DRUG RELEASE:

In vitro skin permeation studies were performed by using Franz Diffusion Cells operated at 37◦C were used. Receptor and donor compartments were limited by goat pulmonary mucosal membrane. The receptor compartment was completed with degasified phosphate buffer and was kept under constant magnetic stirring.

Necessary amounts of the formulated proliposomes were dispersed in phosphate buffer to achieve an Atenolol concentration of 5mg/ml. A volume of 5 ml of these dispersions was placed in the donor compartment. Sample of 2ml was withdrawn from the receptor compartment at 5, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min and replaced with fresh medium. Atenolol content in the sample was assayed by UV spectrophotometry at 275nm [43].

G. RELEASE KINETICS:

1. Zero-order kinetics: Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$Q0 - Qt = K0t$$

Rearrangement of equation (3) yields:

$$Qt = Q0 + K0t$$

where Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero-order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as the cumulative amount of drug released versus time

2. First-order kinetics: This model has also been used to describe the absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first-order kinetics can be expressed by the equation:

$$\log C = \log C0 - Kt / 2.303 \quad (6)$$

where C0 is the initial concentration of the drug, k is the first-order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of -K/2.303.

3. Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media and the equation was

$$Q_t = KH - t1/2$$

Q_t was the amount of drug release in time t, KH was the Higuchi dissolution constant.

4. Korsmeyer and Peppa's model: to study this model the release rat are fitted to the following equation.

$$M_t/M_\alpha = K.tn$$

Where was the fraction of drug release, K was the release constant, t was the release time and n was the diffusional exponent for the drug release that was dependent on the shape of the matrix dosage form. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

Diffusion component (n)	**Overall solute diffusion mechanism**	**Rate as a function of time**
0.45	Fickian diffusion	$t^{-0.5}$
0.45<n<0.89	Anomalous diffusion (Non-Fickian)	t^{n-1}
0.89	Case – II transport	Zero-order release
n >0.89	Super case -II transport	t^{n-1}

Table:8; values of korsmeyer peppas model

5. Hixson Crowell erosion equation

$$Q_o^{1/3} - Q_t^{1/3} = K_{HC}\, t$$

Where Q_t amount of drug released at time t, Q_o initial amount of drug and K_{HC} rate constant for Hixson Crowell equation. To study the release kinetics, data obtained from in vitro drug release studies were plotted as the cube root of drug percentage remaining in matrix versus time [44].

H. IN-VIVO PHARMACODYNAMIC STUDY

The study protocol was approved by CPCSEA/ institutional Animal Ethical Committee (IAEC) Approval Number: **11/321/PO/Re/S/01/CPCSEA**

A pharmacodynamic study was performed to evaluate the in vivo performances of developed formulation using an animal model (rat) at C.L. Baid Metha College of Pharmacy. Albino rat weighing 180-200gm (6–12-week-old) was chosen for the study. The animals are kept at room temperature (25 ± 2°C) with 60-70% humidity and a 12-hour light/darkness cycle in the Animal Holding Unit. Guidelines of the institutional animal ethical committee were followed for in vivo experiments.

A blood pressure measuring instrument (Vmed Vet-Dop2) with a non-invasive tail-cuff was used [45]. After the rat's initial BPs were recorded, hypertension was induced by injecting medroxyprogesterone acetate (MPA) (20 mg/kg/week subcutaneously) [46]. 2 weeks later, rats with a minimum mean BP of 150mmhg were selected. Rats were divided into four groups, group I serves as a control, group II as positive control, group III received atenolol, and group IV received optimized atenolol proliposomes formulation. BP was measured at a different time interval (1, 2, 3, 4, 5, and 6 hr).

- Atenolol proliposomes were pulmonary administered by using a jet nebulizer. Around 25mg of the atenolol formulation was weighed, dispersed in 4 ml saline solution, and placed into the nebulizer cup. The rat was nebulized using a custom whole-body inhalation chamber for 20min [47]. Single dose of pure drug will be intragastrically administered with benzene bromide dissolved in olive oil and administered by oral gavage.

S.NO	GROUPING	TREATMENT	ANIMALS REQUIRED
1.	GROUP I	Control	4 albino rat
2	GROUP II	Positive control	4 albino rat
3.	GROUP III	Animal treated with pure atenolol (p.o, 25mg/kg)	4albinorat
4.	GROUP IV	Animals treated with optimized formulation (25mg/kg)	4 albino rat

Table: 9; Grouping of animals for pharmacodynamic study

I. IN VIVO PHARMACOKINETIC STUDY

The study protocol was approved by CPCSEA/ institutional Animal Ethical Committee (IAEC) Approval Number: **11/321/PO/Re/S/01/CPCSEA**

I. 1. Objectives:

The objective of the present study is to determine the In vivo Bioavailability and plasma drug concentration of the developed Atenolol proliposomes using albino rat.

I.2. Pharmacokinetic Study:

A pharmacokinetic study will be performed to evaluate the in vivo performances of developed formulation using animal model (rat) at C.L. Baid Metha College of Pharmacy.

I.3. Experimental Animal Model:

Albino rat weighing 180-200gm (6–12-week-old) will be chosen for the study.

I.4. Preparation Of the Animals:

At all time, rats were handled in accordance with CPCSEA guidelines for the care of laboratory animals, and the ethical guidelines for the investigations of experimental pain in conscious animals.

I.5. Housing And Feeding Conditions:

The animals to be kept at room temperature (25 ± 2°C) with 60-70% humidity and 12-hour light / darkness cycle in the Animal Holding Unit.

I.6. Grouping Of the Animals:

The animals will be divided into three groups; each group consists of 4 rats (n=4) as follows:

GROUP I: Control

GROUP II: Receive pure drug by oral route.

GROUP III (Test group): inhalation of atenolol proliposomes dose 25mg/kg. The percentage absorption of optimized formulation was calculated by comparing with that of pure drug.

S.NO	GROUPING	TREATMENT	ANIMALS REQUIRED
1.	GROUP I	Control	4 albino rat
2.	GROUP II	Animal treated with pure atenolol (p.o, 25mg/kg)	4albino rat
3	GROUP III	Animals treated with optimized formulation (25mg/kg)	4 albino rat

Table: 10; Grouping of animals for pharmacokinetics study

I.7. Test Procedure:

- Animals will be divided in three groups of four each.
- Group I is control
- Group II Receive pure drug by oral route.
- Group III inhalation of atenolol proliposomes.
- Single dose of pure drug will be intragastrically administered with benzene bromide dissolved in olive oil and administered by oral gavage.
- Atenolol proliposomes were pulmonary administered by using a jet nebulizer. Around 25mg of the atenolol formulation was weighed, dispersed in 4 ml saline solution, and placed into the nebulizer cup. The rat was nebulized using a custom whole-body inhalation chamber for 20min [47].
- Serial blood samples will be collected from the tail veins of the rat at predetermined times (t): 0 hours to 12 hours and blood plasma was the obtained by centrifugation of the samples, measured the volume and also frozen.
- Drug quantification was performed using an HPLC equipment coupled to a UV-diode array detector (DAD-3000). The stationary phase was a C-18 column (150 × 4.6 mm, 5 µm, Phenomenex, USA). The mobile phase was a mixture of 20 mM phosphate buffer pH 2.45/MeOH (85/15). An isocratic 1.2 mL/min flowrate was used and the detection wavelength was set at 225 nm [48].

I.8. Parameters:

Pharmacokinetic parameters such as AUC, AUMC, and MRT of pure drug and developed formulation were calculated by using non-compartmental analysis [49]. The total area under the plasma concentration time curve ($AUC_{0-\infty}$) was estimated by adding the area from time zero to the last sampling time (AUC_{0-t}) and the area from the last sampling point to infinity ($AUC_{t-\infty}$).

The ($AUC_{0-\infty}$) and moment plasma level time curve (AUMC) were calculation of mean residence time (MRT). The ($AUC_{t-\infty}$) was determined by dividing the last measurable plasma drug concentration with total elimination rate constant (Kel) [50].

$$Kel = -slope \times 2.303$$

$$MRT = AUMC/AUC$$

RESULT AND DISCUSSION

1. **DESCRIPTION:**
   a) **Colour:** white
   b) **Odour:** odourless
   c) **Appearance:** fine powder
   d) **Solubility:** solubility studies were done to determine the selection of suitable solvent to dissolve Atenolol.

2. **CALIBRATION CURVE:**

The curve of atenolol was derived from the concentration and corresponding absorbance. Values of linear regression analysis gave the equation for the line of best fit as y = 0.0784x + 0.0642. linearity was observed in the concentration range between 10-50 µg/ml.

S. No	Concentration	Absorbance
1	10	0.023
2	20	0.085
3	30	0.164
4	40	0.251
5	50	0.332

Table: 11; Calibration curve value

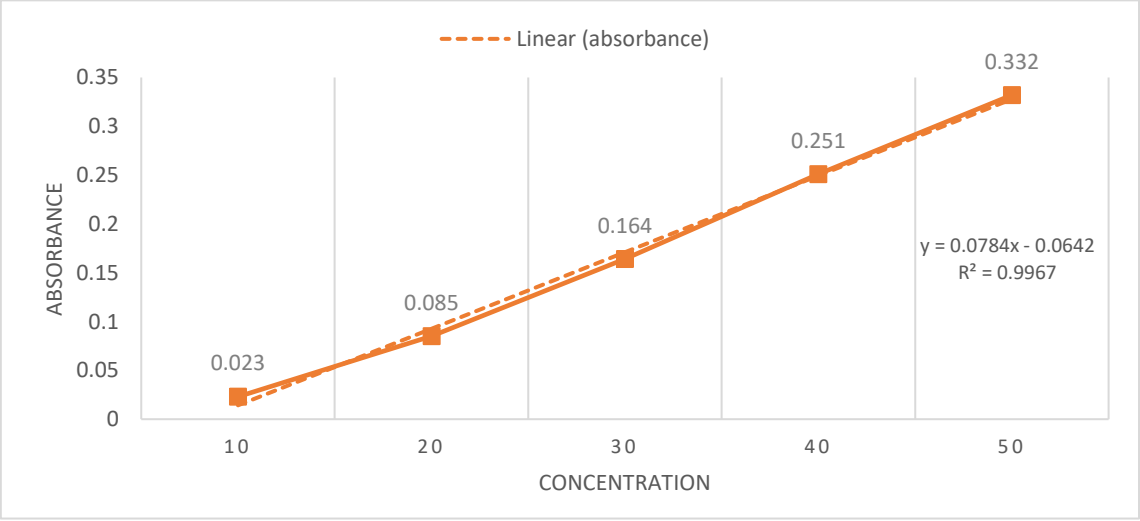


Fig: 1; Calibration curve of Atenolol

3. FOURIER TRANSFORMED INFRARED

The FTIR spectrum shows the interaction between soya phosphatidylcholine, cholesterol, Atenolol, and proliposomes. The spectrum ranges from 400-4000cm^{-1}. The compatibility of the drug in the formulation was confirmed by comparing FT-IR spectra of pure drug with FTIR of its formulation.

a. FTIR OF SOYA PHOSPHOTIDYLCHOLINE (SPC)

Peak values (cm^{-1})	Characteristic functional group
1699.9	C=O
1422.3	C-O-H
1363.9	CH-CH_3
1230.9	C-O
1093.05	C-C
665.6	CH_2

Table: 12; FT-IR peak value and corresponding functional group of SPC

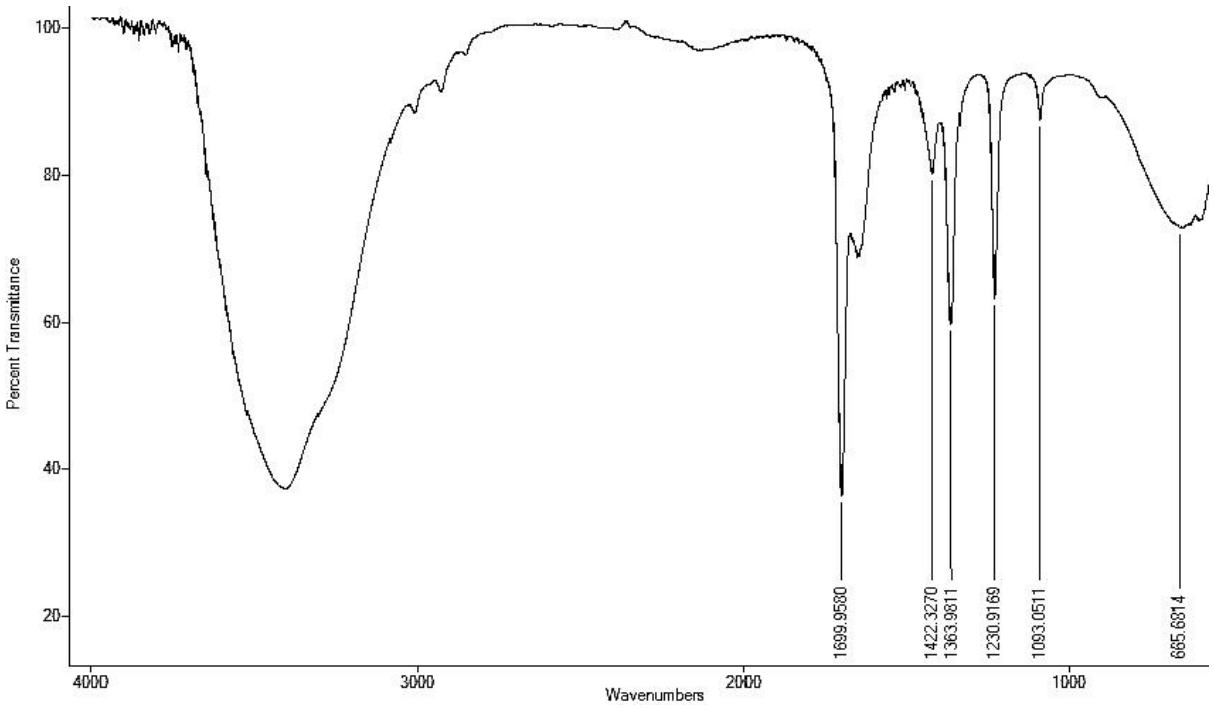


FIG: 2; FTIR spectrum of SPC

Fig 2 presents the result obtained from FT-IR spectroscopy analysis of SPC. The spectrum of SPC shows characteristic peaks of CH_2 stretching bond at 665.6cm^{-1}. The stretching band peak of 1699.9 cm^{-1} was due to stretching between C=O bond of carbonyl group. The spectrum of peak value 1093.05 cm^{-1} was due to the C-C stretching. The stretching band peak value 1422.3 cm^{-1} was due to C-O-H stretching. The spectrum band peak of 1363.9 cm^{-1} was due to the CH-CH_3.

b. FTIR OF CHOLESTEROL

Peak value (cm^{-1})	Characteristic functional group
3432.4	O-H
2933.6	CH2
1466.1	C-H
1245.9	C-H

Table: 13; FT-IR peak value and corresponding functional group of cholesterol

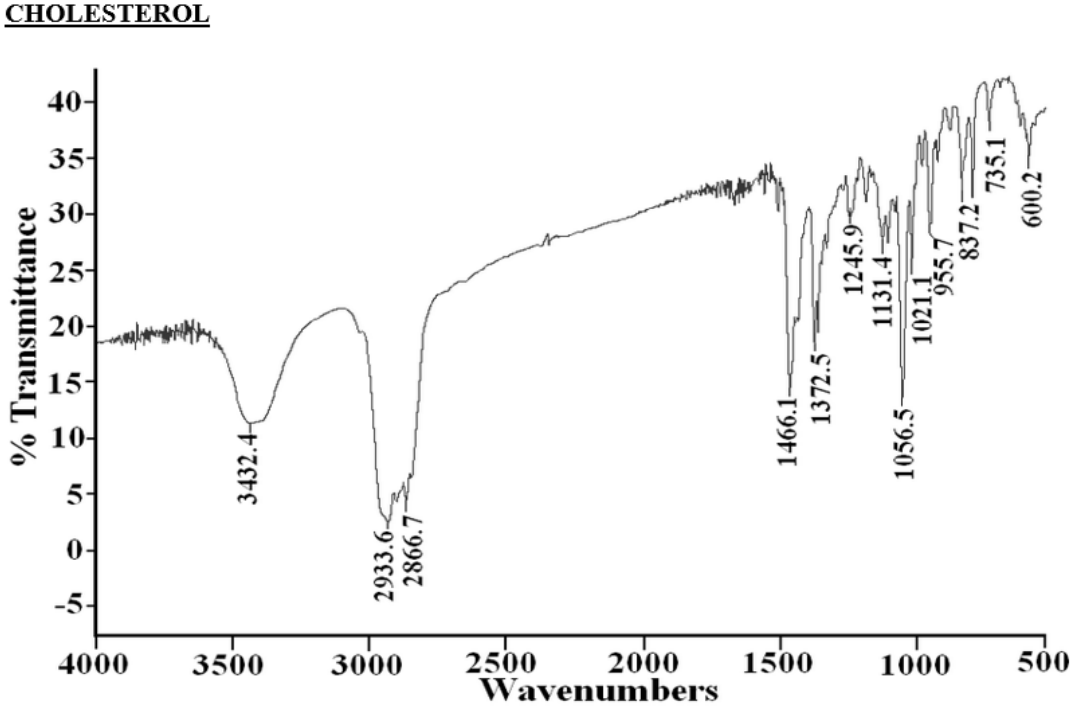


Fig: 3; FTIR spectrum of cholesterol

Figure 3 present the result obtained from FTIR spectroscopy analysis of cholesterol. The spectrum band peak of 3432.4 cm^{-1} was due to O-H. The spectrum band peak of 2933.6 cm^{-1} was due to CH_2. the spectrum band peak of 1466.1 cm^{-1} was due to C-H.

c. FTIR OF STARCH

Peak value (cm^{-1})	Characteristic functional group
3271	O-H
2928	C-H
1642	C=C
1455	C-H

Table: 14; FT-IR peak value and corresponding functional group of starch

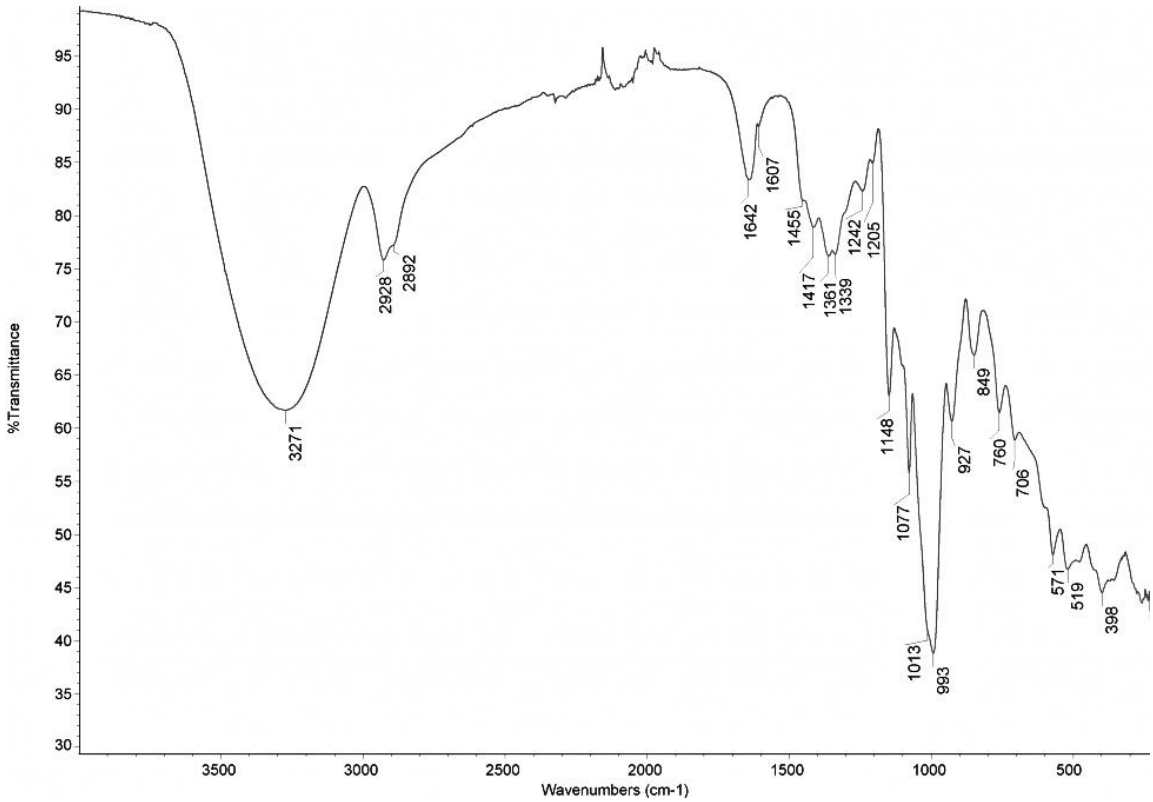


Fig: 4; FTIR spectrum of Starch

Figure 4 present the result obtained from FTIR spectroscopy analysis of Starch. The spectrum band peak of 3271 cm^{-1} was due to O-H. The spectrum band peak of 2928cm^{-1} was due to C-H. The spectrum band peak of 1642 cm^{-1} was due to C=C.

d. FTIR OF ATENOLOL

Peak value (cm^{-1})	Characteristic functional group
3351.09	N-H
2964.62	C-H
1634.48	C=O
1583.56	C=O
1412.79	C-C
1379.69	C-H
1337.62	C-H
1178.72	C-H
1091.38	C-N
942.17	O-H
885.24	N-H
814.50	C-H
702.89	=C-H
671.70	N-H

Table: 15; FT-IR peak value and corresponding functional group of Atenolol

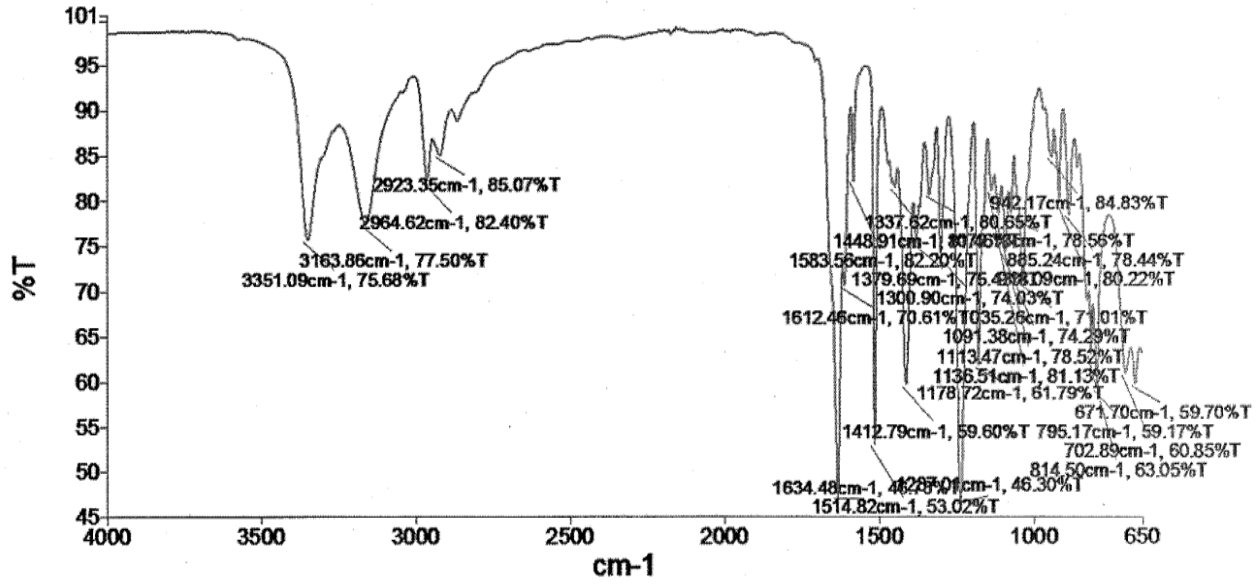


Fig: 5; FTIR spectrum of Atenolol

e. FTIR OF FORMULATION

Peak value (cm^{-1})	Characteristic functional group
3340.1	N-H
2942	C-H
1647.88	C=O
1565.92	C-C
1417.42	C-C
1379	C-H
1332.57	C-H
1238	C-H
1141.65	C-H
1092.48	C-N
919	O-H
852.4	C-H
653.75	N-H

Table: 16; FT-IR peak value and corresponding functional group of Atenolol proliposomes.

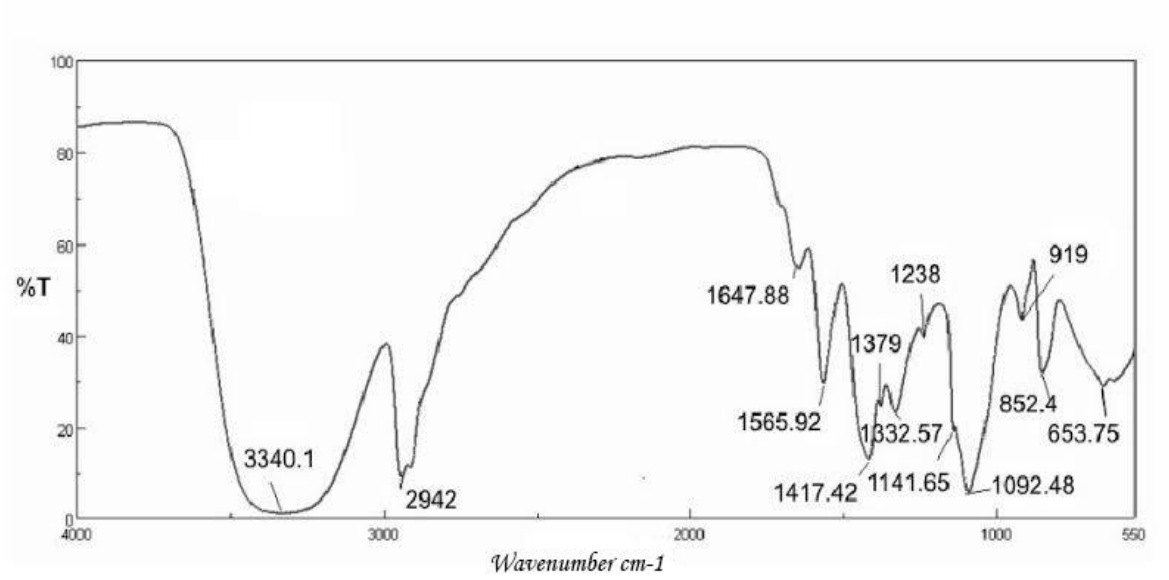


Fig: 6; FTIR spectrum of Atenolol proliposomes

Fig 6 presents the result obtained from FTIR spectroscopy analysis of atenolol proliposome. The spectrum of atenolol shows characteristic peaks of N-H stretching bond at 653.75 cm^{-1} and 3340.1 cm^{-1}. The stretching band peak of 1647.88 cm^{-1} was due to stretching between C=O bond of carbonyl group. The spectrum of peak value 919 cm^{-1} was due to stretching between O-H bond. The spectrum of peak value 1332.57 cm^{-1} was due to N-O bond. The stretching of 1238 and 1141.65 cm^{-1} was due to C-H bond. The stretching of peak value 1092.48 cm^{-1} was due to C-N of amines group. All the peaks corresponding to the respective bonds are shown in the table.

4. OPTIMIZATION – EXPERIMENTAL DESIGN:

Box-Behnken statistical design with 3 factors, 3 levels and 15 runs were employed for the optimization study using design expert software version13. The ratio of soye phosphatidyl choline (SPC), cholesterol and stirring speed (rpm) were main factors that impacted the particle size of the Proliposomes and percentage drug permeated from the atenolol prolipoesomes formulation.

To determine the impact of SPC, cholesterol and stirring speed (rpm) were selected as independent variables and high, medium and low level were set on the basis of the result of initial trials. Based on the design, 13 proliposomes formulations were prepared and characterized for particles size(R1) and Cumulative drug permeated (R2) by the design expert

software the optimized formulation was selected. And according to the DOE final formulation was prepared.

Independent Variables	Level		
	Low (-1)	Medium (0)	High (1)
SPC	2	4	6
Cholesterol	2	4	6
Stirring speed (rpm)	170	220	270

Table: 17; Variables and their levels used in formulation

Based on each independent variable, the design expert software automatically generated the optimized formula. It was observed that the best-fitted model for all the three dependent variables was the quadratic model with a coefficient of correlation (R2) nearly equal to 1. The quadratic mathematical model for three independent factors was provided below equation:

$$Yi = b0+b1X1+b2X2+b3X3+b12X1X2+b13X1X3+b23X2X3+b11X12+b22X22 +b33X32$$

Where Yi is the dependent variable; b0 is the intercept; b1 to b33 are regression coefficients: and X1, X2, and X3 are the independent variables selected from the preliminary experiments.

Std	**Run**	**Factor 1 A: SPC (g)**	**Factor 2 B: cholesterol (g)**	**Factor 3 C: stirring speed (rpm)**	**Response 1 Particle size (nm)**	**Response 2 % Drug permeated**
12	1	4	6	270	2014	51.4
2	2	6	2	220	1554	62.8
11	3	4	2	270	2764	56.5
3	4	2	6	220	1658	48
10	5	4	6	170	2984	54.2
8	6	6	4	270	1982	72.31
9	7	4	2	170	3630	49.1
1	8	2	2	220	1761	55
15	9	4	4	220	683	93.78

4	10	6	6	220	1313	78.2
7	11	2	4	270	2764	42.14
5	12	2	4	170	5650	45.8
13	13	4	4	220	683	93.78
6	14	6	4	170	3201	65.25
14	15	4	4	220	683	93.78

Table: 18; Three level three-factorial Box-Behnken Design

Response surface methodology: From the three-dimensional surface plot the interaction of the different parameters on the particle size of the proliposomes and percentage drug permeated are shown.

A. Particle Size: The three-dimensional (3D) response plots show the impact of the three independent variables on the particle size of the proliposomes.

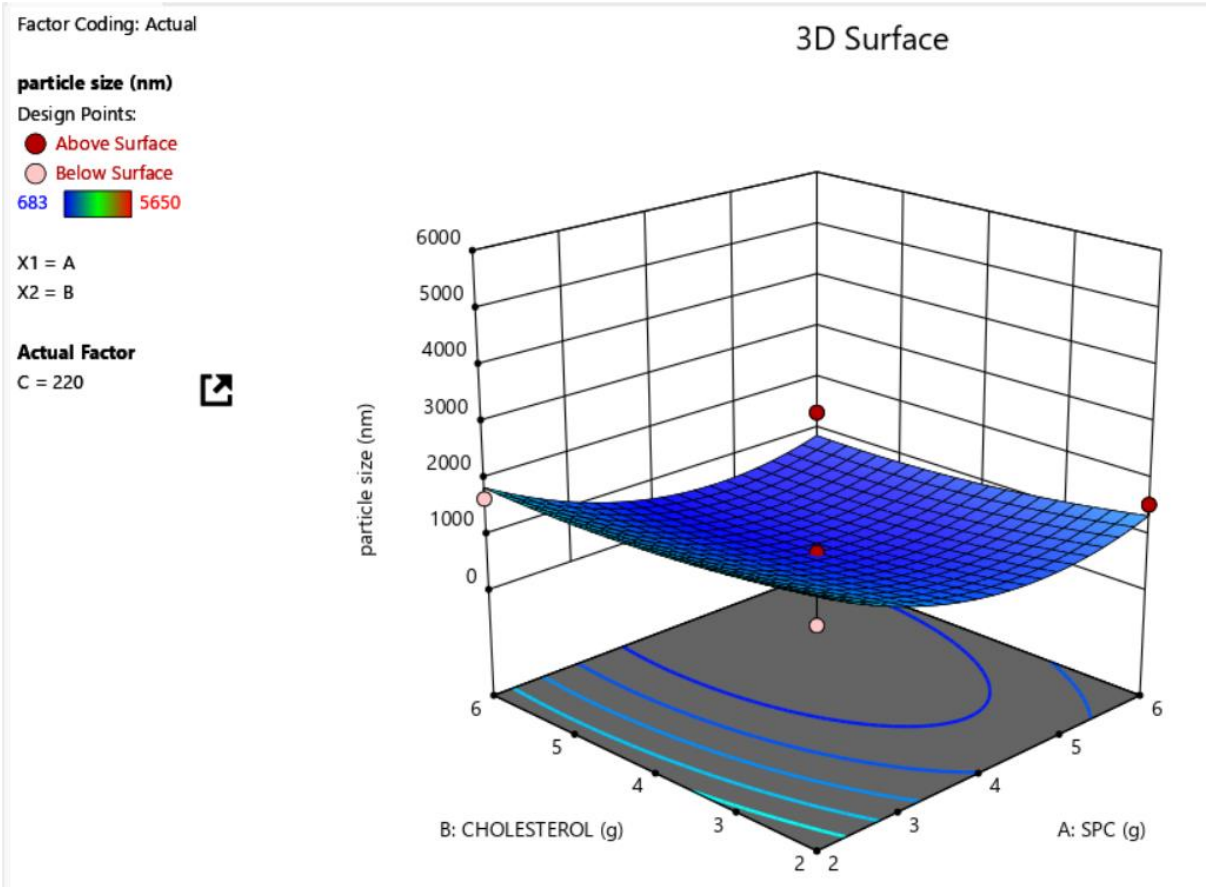


FIG: 7; 3D Surface Showing Impact On particle size

B. Drug permeability: The three-dimensional (3D) response plots show the impact of the three independent variables on the drug permeability of the proliposomes.

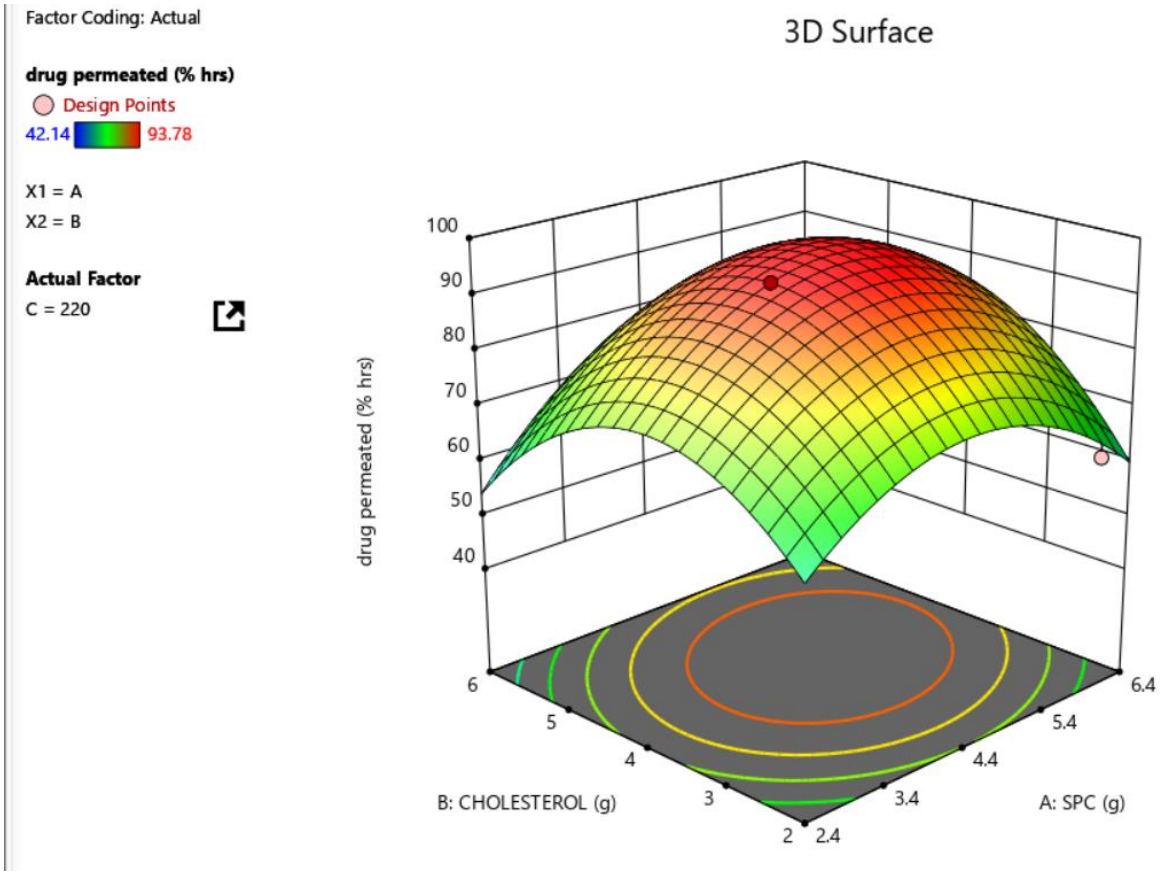


FIG: 8; 3D Surface Showing Impact on percent Drug permeated

Particle size:

Figure 9 shows that the particle size of proliposomes decreases with increase in the stirring speed. When the stirring speed decreases, the particle size of proliposomes gets increased. This is due to the stirring speed. The plotted model depicted a linear decrease in particle size when the stirring speed was increased.

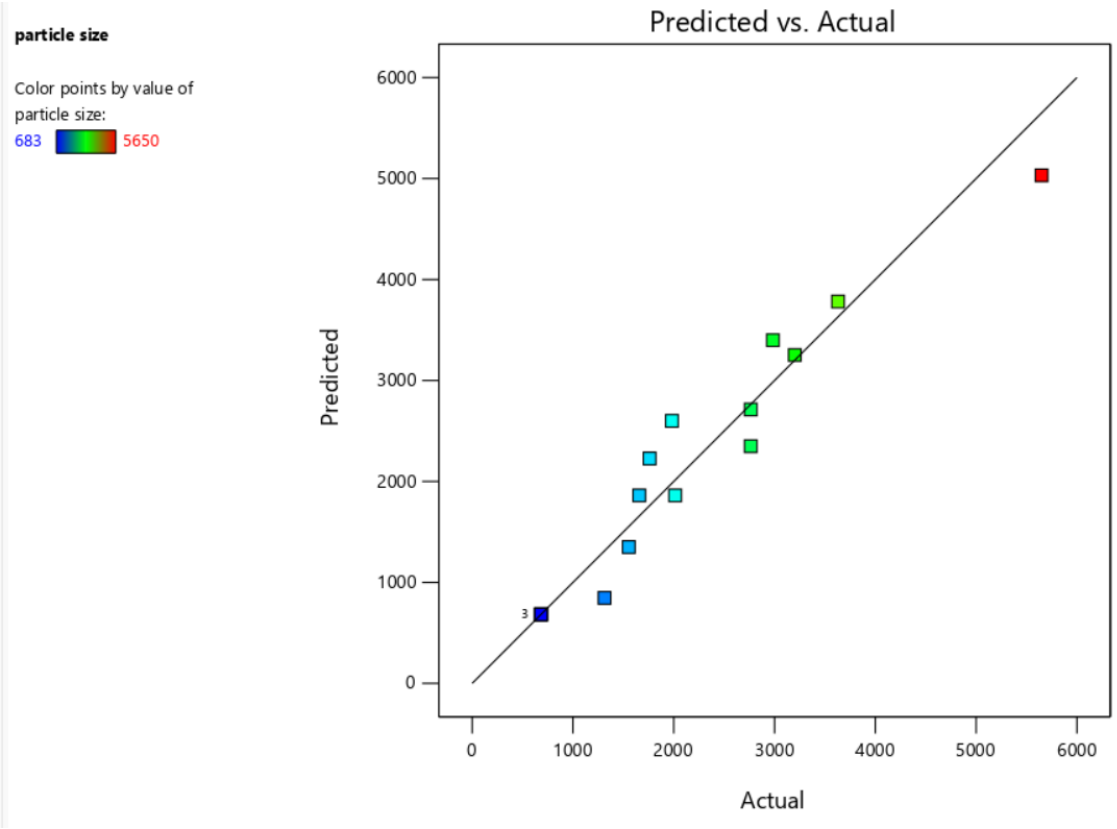


FIG: 9; Showing Linear decrease in particle size When stirring speed Increased

Percent Drug Permeated:

Figure 10 shows that the percent drug permeated is increased toward the increase in the ratio of SPC and cholesterol. However, the permeation of the drug decreased as the ratio of the SPC and cholesterol decrease. The plotted model depicted a linear drug permeability when the ratio of the SPC and cholesterol increases.

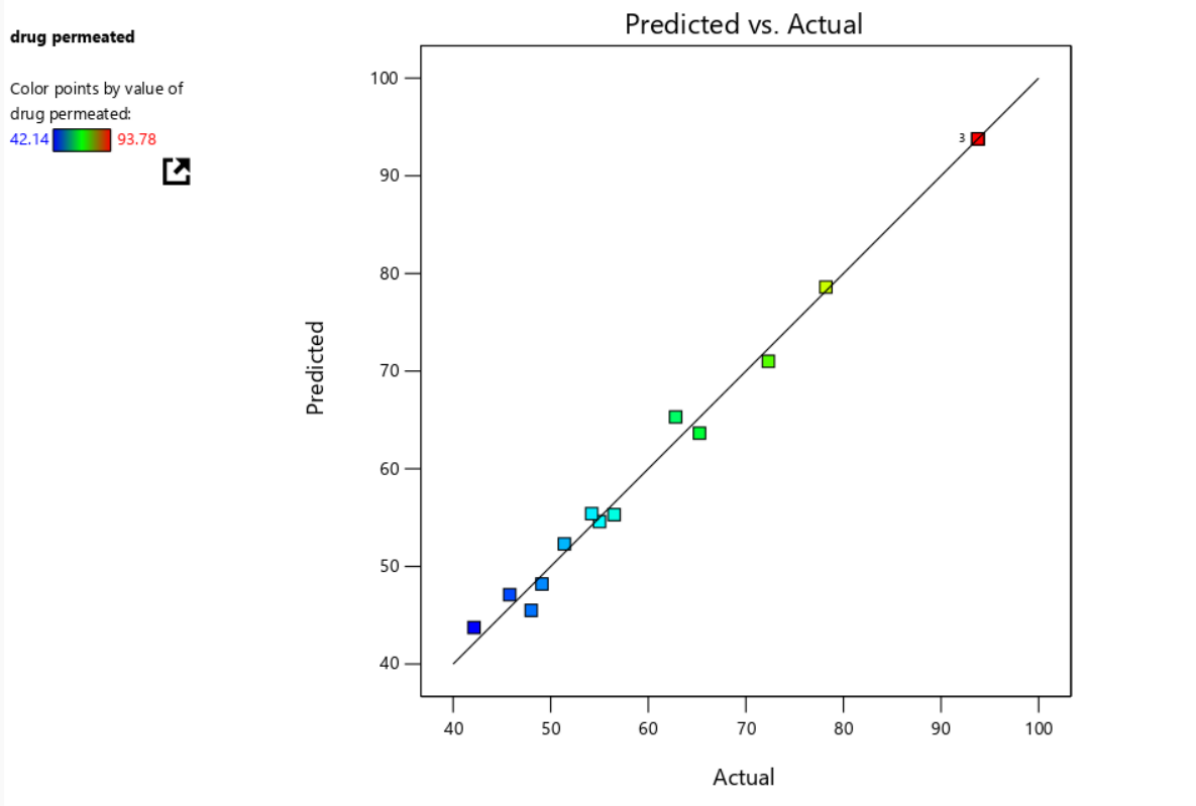


FIG: 10; Showing Linear increase in drug permeability when ratio of SPC and cholesterol increases

Perturbation plots show the impact of each independent variables on particle size.

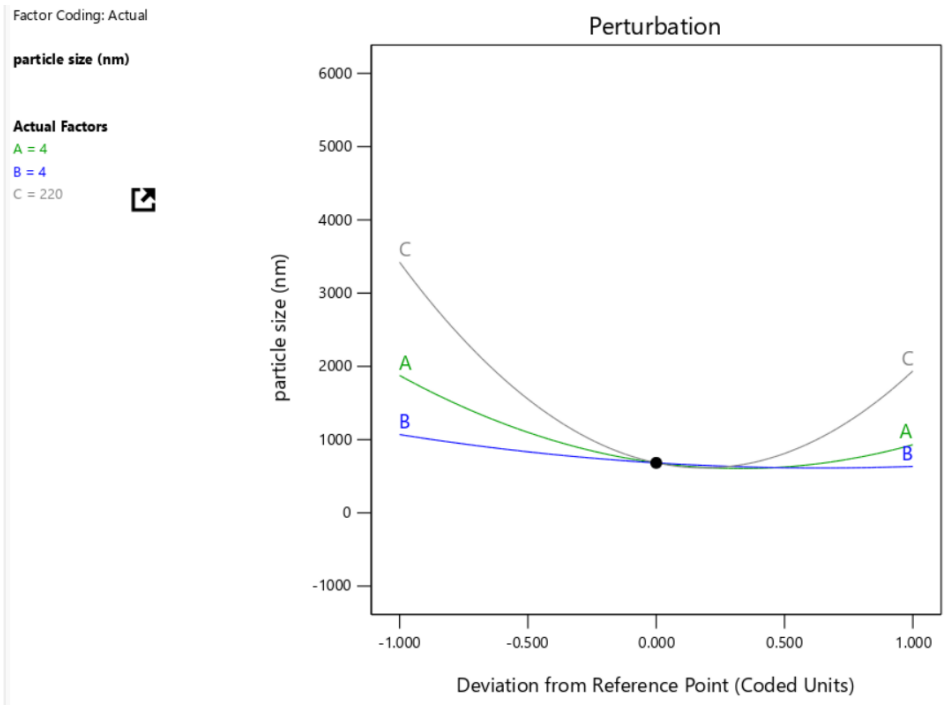


FIG: 11; Perturbation Plots on Particle size (nm)

Perturbation plots show the impact of each independent variables on drug permeated.

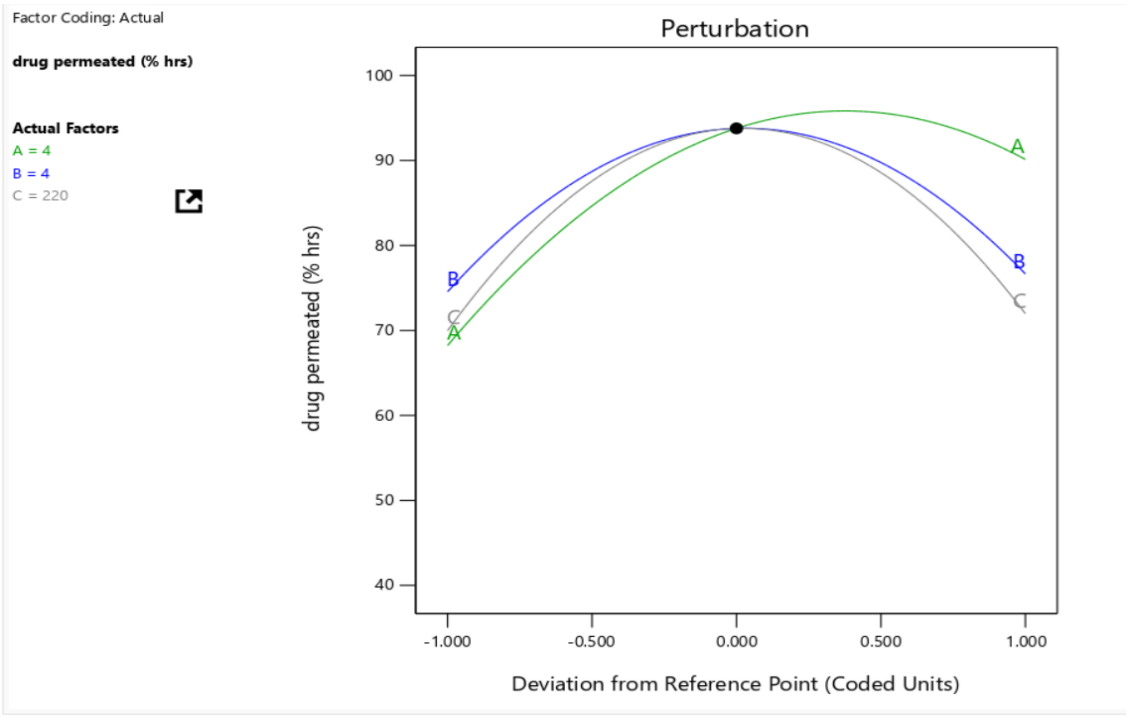


FIG: 12; Perturbation Plot on Drug Permeated (% hr)

ANOVA:

The responses were obtained and adjusted and predicted R^2 was determined based on the Box Behnken Design and ANOVA was calculated. The P values, precision, % CV, adjusted and predicted R^2 are given in table 19.

RESPONSE	Adjusted R^2	Predicted R^2	Model P value	Adequate precision	%CV
Particle size (nm)	0.8110	-0.0802	0.0055	9.1906	26.08
% Drug permeated	0.9843	0.9102	<0.0001	26.93	3.55

Table: 19; Table of ANOVA

After studying the effect of the independent variables on the responses, the levels of these variables that give the optimum response were determined. It is evident that ratio of SPC, cholesterol and stirring speed affects the drug permeability and particle size of proliposomes. Hence, medium level was selected as optimum for the ratio of SPC, cholesterol and stirring speed. The optimum formulation is one that gives a high value of drug permeability and smaller particle size of proliposomes. Using a computer optimization process, for SPC and cholesterol the level of 4 and for stirring speed level of 220 rpm which gives the theoretical values of 93.76% and 683 nm were selected. A decrease in the levels leads to a decrease in the percentage drug permeated and increase in particle size and increase in the level leads to low drug permeability and high particle size. Hence, a medium level was selected as optimum.

5. MICROSCOPIC OBSERVATION OF HYDRATED PROLIPOSOMES

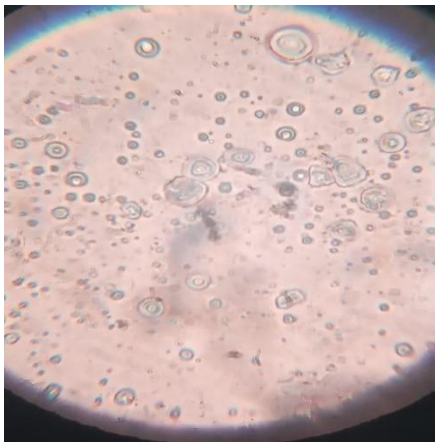


FIG: 13 (a)

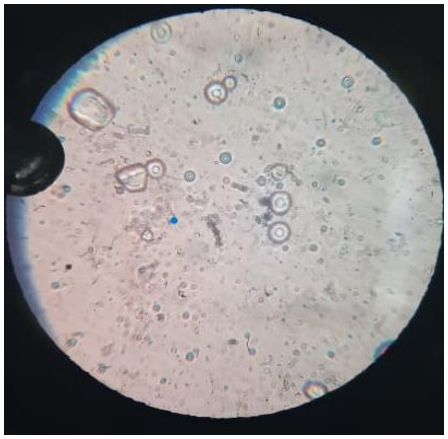


FIG: 13(b)

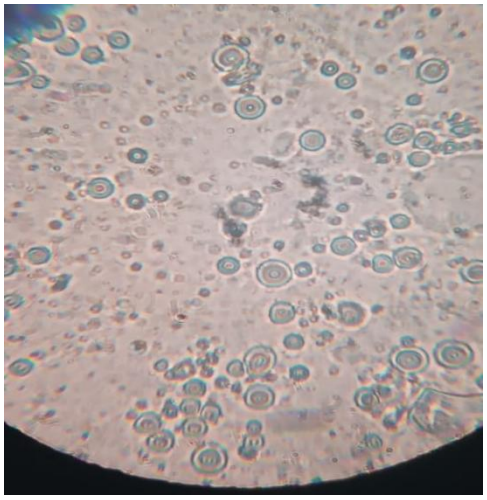


FIG: 13 (c)

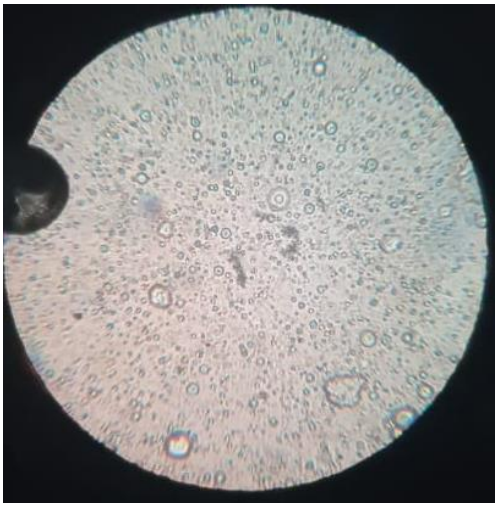


FIG: 13 (d)

Light photomicrography of hydrated optimized proliposome formulation. When the proliposomes get hydrated they are converted into the liposomes. From the image we can visualize the formation of small bilayer vesicle on hydration of proliposomes.

6. PARTICLE SIZE ANALYSIS

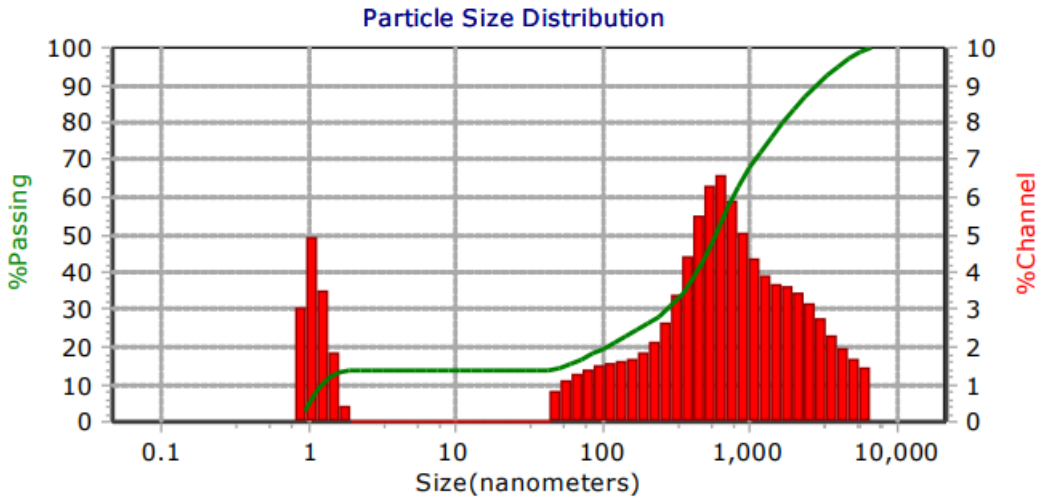


Fig: 14 (a)

Summary	
Data	Value
MI(nm):	1021
MN(nm):	1.030
MA(nm):	7.84
CS:	764.9
SD:	970.0
PDI:	0.903
Mz:	880.7
σι:	1.076
Ski:	0.593
Kg:	1.424

Percentiles	
%Tile	Size(nm)
10.00	1.240
20.00	107.3
30.00	273.5
40.00	427.0
50.00	571.0
60.00	749.0
70.00	1052
80.00	1652
90.00	2758
95.00	3900

Fig: 14(b)

Peaks Summary		
Dia(nm)	Vol%	Width
683	89.4	2010
1.15	10.6	0.38

Fig: 14 (c)

Particle sizes of the Optimized atenolol proliposomes was measured and to be 683nm. The PDI value of atenolol proliposomes was found to be 0.903. the poly dispersity index less than <1 indicates good uniform distribution of the particles. The result was shown in fig 14 (c)

7. SCANNING ELECTRON MICROSCOPY

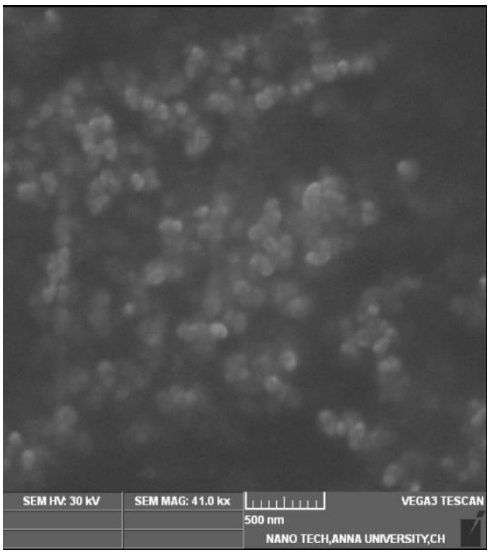


Fig: 15 (a)

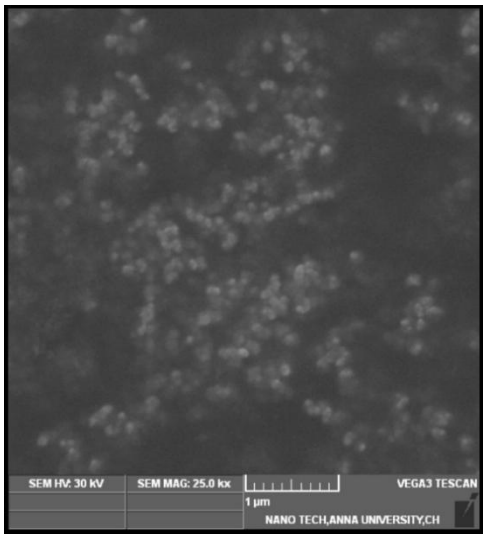


Fig: 15(b)

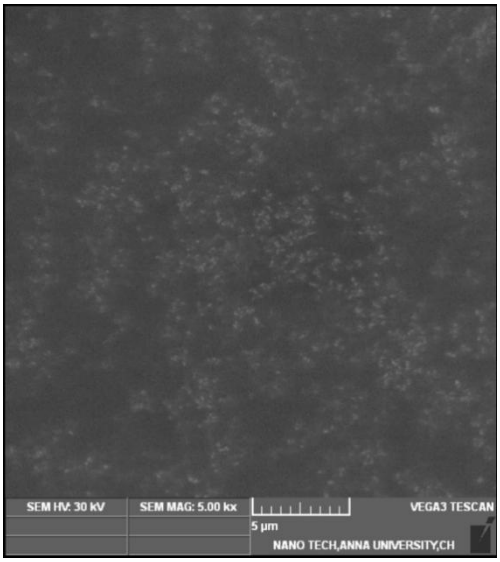


Fig: 15 (c)

Figure 15 (a), 15(b) and 15(c) shows the Scanning electron microscopic visualization of optimized atenolol proliposomes.

8. DRUG ENTRAPMENT EFFICIENCY

FORMULATION	ENTRAPMENT EFFICIENCY (%)
F1	68.26±0.95
F2	74.92±1.02
F3	64.79±1.15
F4	84.27±0.52
F5	66.3±0.27
F6	71.96±0.62
F7	64.6±1.25
F8	83.02±0.16
F9	69.82±1.24
F10	72.43±1.36
F11	63.47±0.29
F12	76.51±1.35
F13	91.04±2.16

Table: 20; Entrapment Efficiency

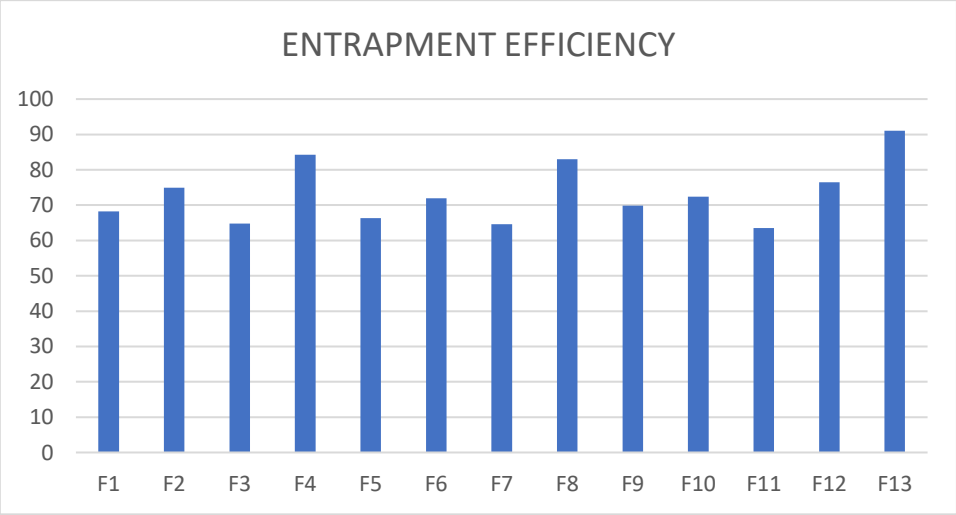


Fig: 16; Entrapment efficiency

The entrapment efficiency of atenolol proliposomes was calculated as percent total drug entrapped. The entrapment efficiency of proliposomes formulation was depends on the ratio of SPC, cholesterol and stirring speed. When the stirring speed and ratio of the SPC and cholesterol decreases the entrapment efficiency get affected. At the moderate level of SPC, cholesterol and stirring speed (rpm) they have formed the optimized formulation (F13) with entrapment efficiency of 91.04%. The entrapment efficiency of atenolol proliposomes was found and results were shown in table 20.

9. STABILITY STUDY:

FORMULATION	TEST CONDITION	TEMPERATURE	%EE	PHYSICAL APPEARANCE
OPTIMIZED FORMULATION	INITIAL	4°C	91.04±2.16	Yellowish fine powder
	1 MONTH	4°C	90.02±0.65	Yellowish fine powder
		24°C	88.26±2.61	
		37°C	85.82±3.15	Yellowish clumps

Table: 21; Stability study

The physical appearance and entrapment efficiency of proliposomes formulations stored at 4°C, 24°C, and 37°C for 1 months was evaluated. At the end of 1 month, the proliposomes stored at 4°C and 24°C were stable. But for the formulations stored at 37°C, a change in appearance was observed. Entrapment efficiency of the proliposomes formulations stored at 4°C decreased by 1.12%, whereas those stored at 24°C decreased by 3.15% and formulation stored at 37°C decreased by 5.73%. The result of entrapment efficiency was shown in table 21.

10. IN-VITRO DIFFUSION:

The drug permeation data for various atenolol proliposomes formulations were given below in table 22.

Fig: 17; Franze diffusion apparatus

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.85	1.02	0.02	12.86	0.01	2.67	0.006	9.26	0.21	0.16	1.04	1.22	17.39
2	3.01	4.3	1.2	19.72	0.89	6.2	0.4	13.5	1.02	0.98	3.44	4.2	25.27
3	7.41	9.6	5.1	27.54	4.51	11.8	2.05	22.5	3.65	2.92	8.09	10.2	33.24
4	11.6	16.2	9.6	36.71	8.4	18.54	7.08	32.11	5.88	6.21	13.4	16.4	35.78
5	17.29	21.8	17.18	43.25	15.47	20.5	11.2	38.7	18.08	19.8	19.6	20.5	41.32
6	26.6	27.33	21.88	49.62	21.01	26.25	17.5	47.51	23.9	24.91	29.07	28.12	48.78
7	33.48	38.1	29.14	56.84	28.1	32.48	24.12	51.42	30.18	31.09	37.21	34.28	62.02
8	36.21	42.62	32.48	59.98	30.65	36.64	29.46	56.28	32.58	34.92	39.28	46.78	71.52

9	39.71	49.5	36.22	60.85	34.24	48.65	31.02	59.4	36.44	37.4	40.8	42.6	76.62
10	43.56	52.37	41.05	63.48	36.28	52.68	36.54	62.84	39.62	40.29	43.85	44.69	80.15
11	46.72	54.2	43.58	68.21	40.5	56.15	38.4	64.27	41.68	44.81	48.2	46.25	83.91
12	55	62.8	48	78.2	45.8	65.25	42.14	72.31	49.1	54.2	56.5	51.4	93.78

Table: 22; Drug release in permeation study

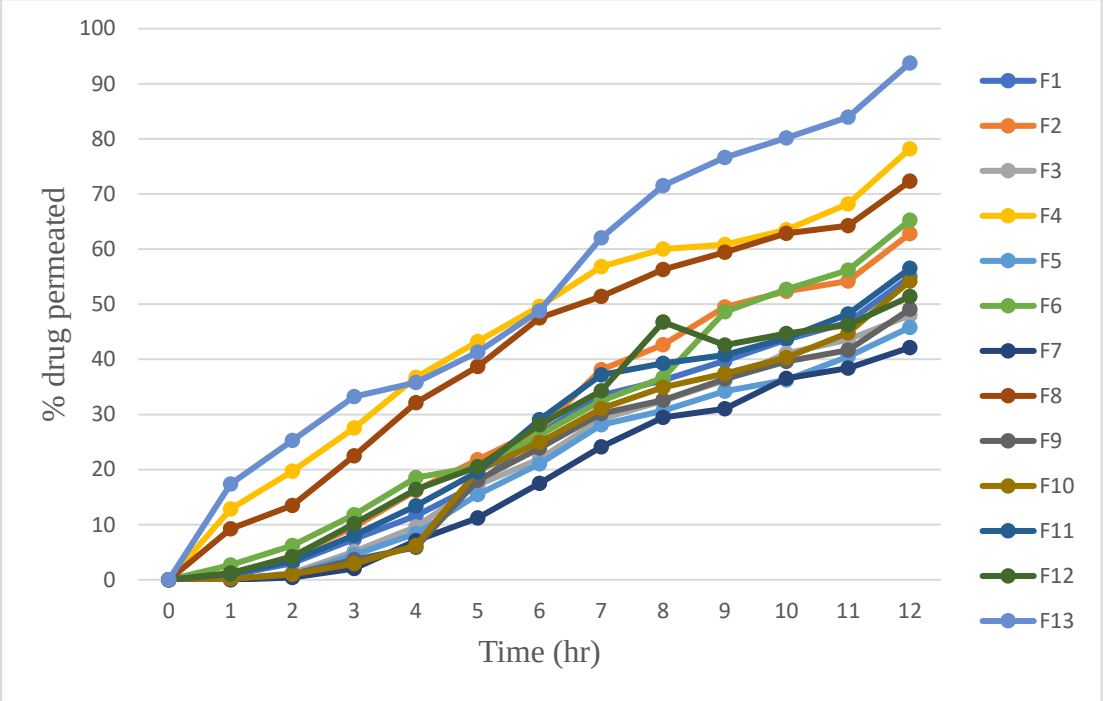


Fig:18; Invitro drug permeability plot

Fig 18 shows the drug permeability profile of Atenolol Proliposomes formulations.

For formulations F2, F5, F7, F9 the cumulative percentage of drug diffusion was found in the range of 48, 45.8, 42.14, 49.1%.

For formulations F1, F10, F11, F12 the cumulative percentage of drug diffusion was found in the range of 55, 54.2, 56.5, 51.4%.

For formulation F2, F6 the cumulative percentage drug diffusion was found as 78.2, 72.31%.

For formulation, F13 was 93.78% this shows that ratio of SPC (4g), cholesterol (4g) and decrease in the particle size of the proliposomes increase the drug release.

11. IN-VITRO DRUG RELEASE KINETICS:

OPTIMIZED FORMULATION	Zero-order kinetics		First-order kinetics		Higuchi		Korsmeyer-Peppas	
	K	r^2	K	r^2	K	r^2	K	N
	11.29	0.9396	-0.139	0.067	51.72	0.9919	1.98	0.6299

Table: 23; Drug release kinetics values of r^2 for optimized formulation

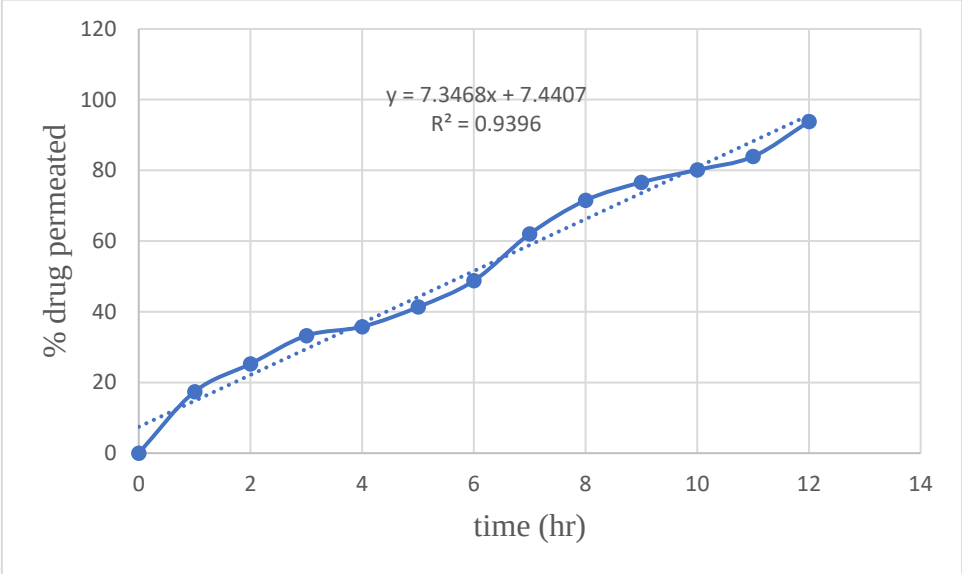


Fig: 19(a); Zero order kinetic plot

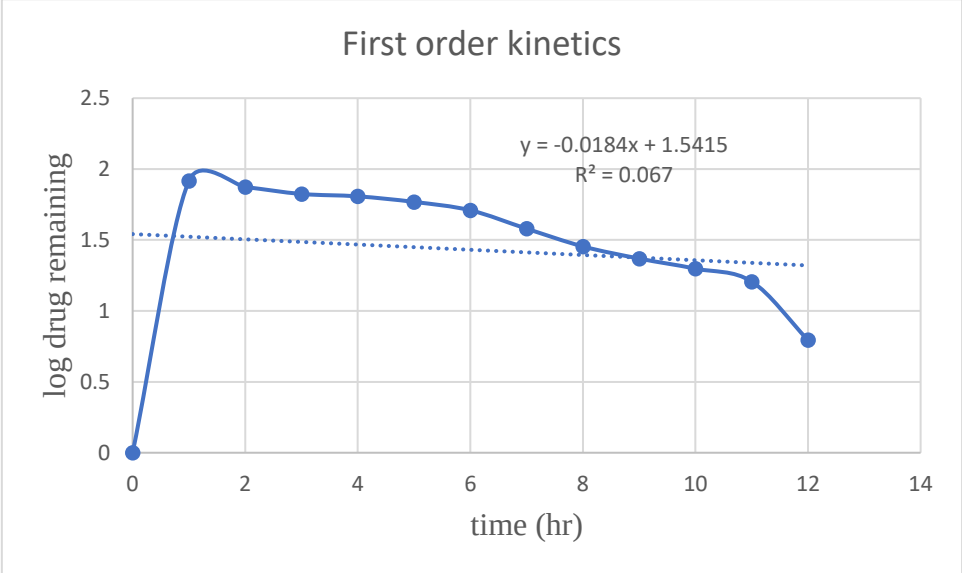


Fig: 19(b); First order kinetic plot

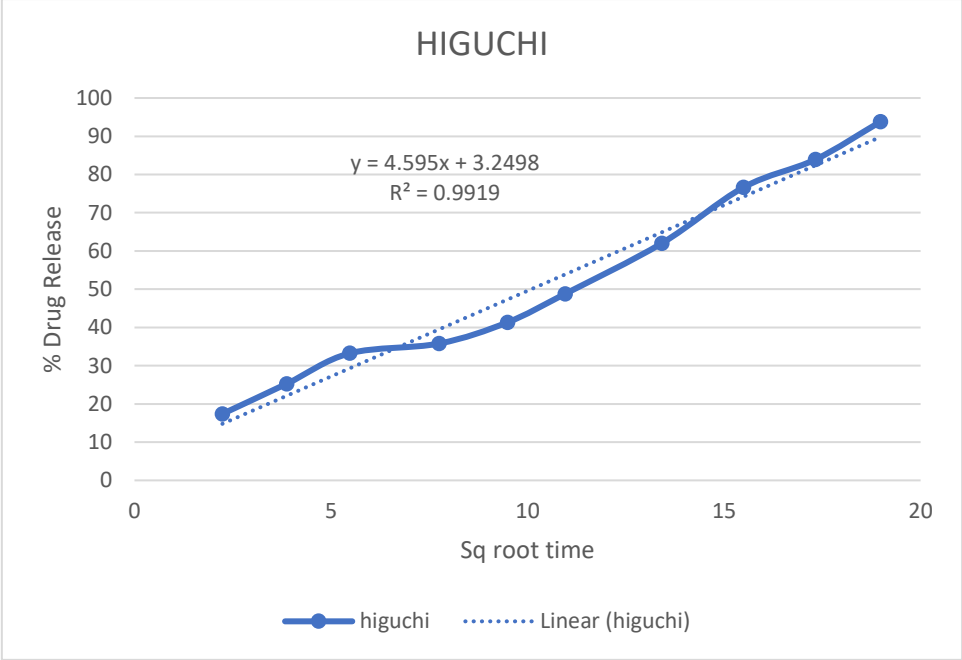


Fig: 19 (c); Higuchi plot

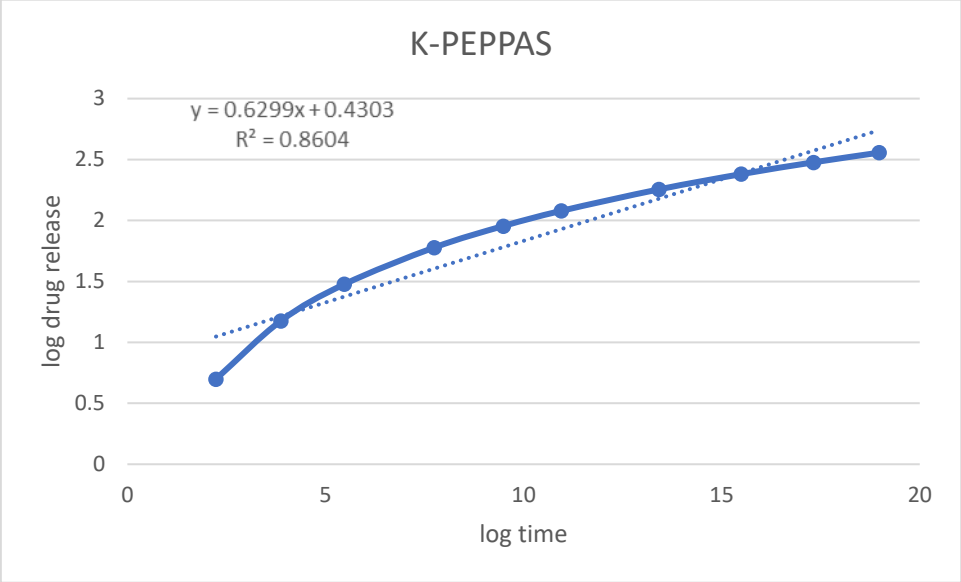


Fig: 19 (d); Korsmeyer-peppas plot

The drug permeation from Atenolol proliposomes of optimal formulation (F13) followed a diffusion mechanism, according to the correlation coefficient (R^2) value.

To study the drug release kinetics, data obtained from In-Vitro drug release studies are plotted in various kinetic models. The curve fitting results of the rate of permeation of the designed formulations gave an idea of the mechanism of drug release. Based on the "n" value of 0.6299 the drug release was found to follow non-Fickian diffusion.

Also, the drug release mechanism was best explained by Higuchi, as the plots showed the highest linearity (r2 = 0.9919), as the drug release was best fitted in Higuchi.

12. IN VIVO PHARMACODYNAMIC STUDY:

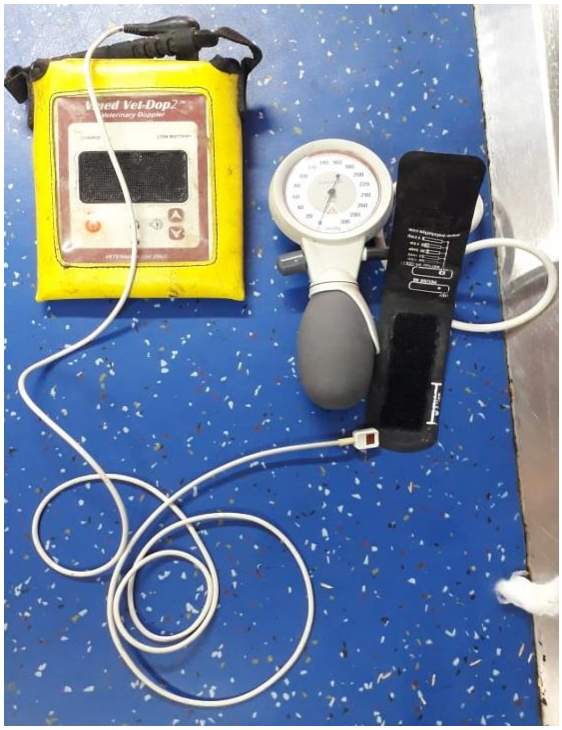


Fig: 20 (a) Vmed Vet-Dop2

Fig: 20 (b); Tail cuffing of rat

Antihypertension effect of proliposomal in comparison to oral route

Group	Treatment	Mean BP (mm/Hg)						
		Initial	1hr	2hr	3hr	6hr	10hr	12hr
I	Control	122±1.5	128±2.4	125±2.1	123±1.6	126±3.4	124±2.8	121±4.1
II	Positive control	168±3.6	162±3.8	164±4.2	162±2.6	167±4.2	166±3.0	163±2.3
III	Standard (Oral Route)	167±3.1	133±6.2	128±7.9	124±1.3	143±5.7	157±3.8	163±3.6
IV	Atenolol Proliposomes	165±3.8	124±2.1	107±2.5	94±1.9	87±3.0	83±3.6	96±4.6

Table: 24; Pharmacodynamic study report

The results in the table 24 indicate that the administration of medroxyprogesterone acetate (MPA) produced significant hypertension in rats. The oral administration of atenolol controlled the hypertension initially, with the maximum effect observed at 3 hours, but after 3 hours the BP started rising gradually until it was the same as the initial value at 12 hours. In contrast, the administration of optimized atenolol proliposome through nebulizer results in gradual decrease of BP, they decreased the BP significantly at the first hour and the effect continued for 12 hrs. Because medications are mostly delivered to the heart via the pulmonary vein during absorption, this is the case. In light of this, inhaling Atenolol could be a viable alternative for reaching heart tissue directly and reducing systemic exposure. The pulmonary epithelium is a large and vascularized structure. Furthermore, efflux transporters are not abundant, which favours medication absorption. This method allows for a faster beginning of effect, lower levels of metabolic enzymes compared to hepatic enzymes, and the absorption of medications with various physicochemical features, as well as less frequent administration of atenolol.

13. INVIVO PHARMACOKINETIC STUDIES:

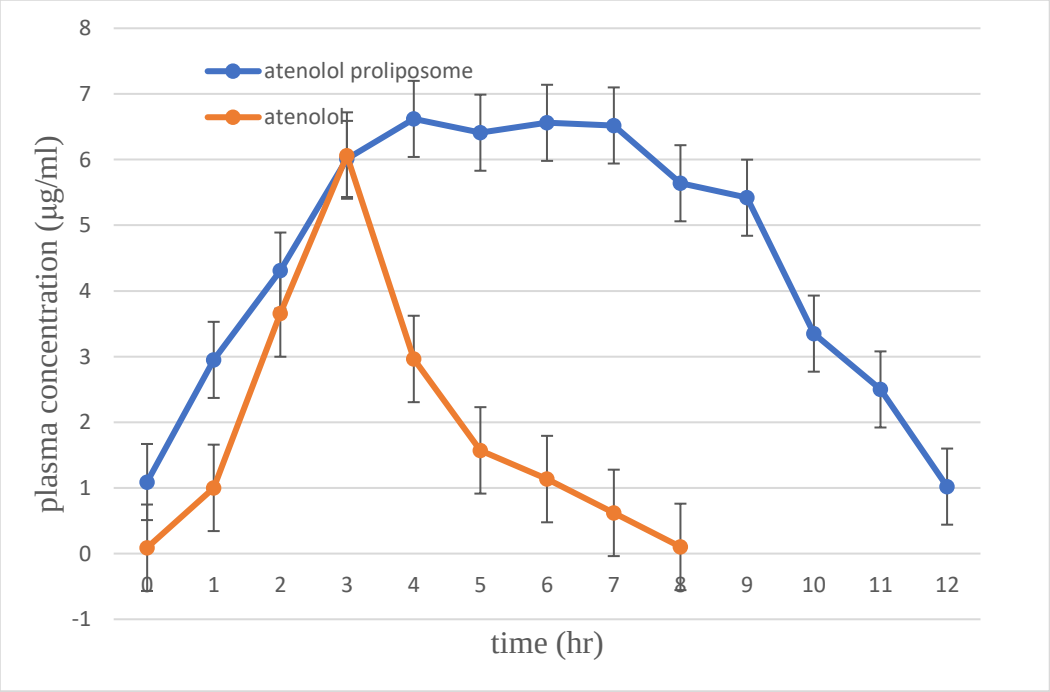


Fig: 21; In-vivo pharmacokinetic plot

Time (hr)	Plasma Concentration (µg/ml) of pure Atenolol	Plasma Concentration (µg/ml) of atenolol proliposomes
0	0.089	1.09
1	3.001	2.95
2	5.656	4.31
3	3.062	6.01
4	1.964	6.62
5	1.572	6.41
6	1.135	6.56
7	0.62	6.52
8	0.102	5.64
9		5.42
10		3.35
11		2.5
12		1.02

Table:25; In-vivo pharmacokinetic plasma drug concentration

Pharmacokinetic parameters	Pure Atenolol	Atenolol Proliposomes
AUC $_{o-\infty}$	13.86 µg/hr/ml	58.89 µg/hr/ml
AUMC$_{o-\infty}$	51.98 µg/hr^2/ml	379.54 µg/hr^2/ml
MRT (hr)	3.75hr	6.4hr

Table:26; Pharmacokinetic parameters

The mean plasma concentration-time curve of Atenolol and Atenolol proliposomes were shown in figure 21. The pharmacokinetic parameters such as AUC $_{o-\infty}$, AUMC$_{o-\infty}$ and MRT are listed in table 25. After oral administration of atenolol, AUC $_{o-\infty}$ was found to be 13.86 µg/hr/ml, AUMC$_{o-\infty}$ was 51.98 µg/hr^2/ml and MRT was found to be 3.75hr for the pure drug. where as in atenolol proliposome formulation, when they are administered using nebulizer, they have shown AUC $_{o-\infty}$ value of 58.89µg/hr/ml, AUMC$_{o-\infty}$ of 379.54 µg/hr^2/ml and MRT value 6.4hr. The above data shows the optimized proliposome formulation attained the Cmax at 3 hr as the case with pure drug. The plasma dug concentration was maintained till 12 hrs, hence the objective of the present study confirms the justification of delivering the AT in proliposomal form through nebulization. Hence the dosing frequency can be minimized and bioavailability of the drug is increased as compared to oral administration.

CONCLUSION

The objective of the present study was to **Design and Development of Proliposomal Dry Powder Inhalation for Pulmonary Delivery of Antihypertensive Drug**. The oral recommended dose for AT tablet is between 25 and 100 mg, twice daily. Conventional oral administration usually results in erratic drug concentrations in plasma because of Atenolol lower permeability, leading to dose dumping and reduction in the pharmacological effect or development of undesirable side effects. Hence, considering these disadvantages inhalatory administration of atenolol is considered one of the main alternative non-invasive drug delivery route and it is particularly interesting for cardiac targeting. This is because during absorption, drugs are predominantly first transported to the heart via the pulmonary vein.

Pulmonary epithelium is relatively extensive and widely vascularised. In addition, there is little presence of efflux transporters, which favours drug absorption. So preparing a targeted drug delivery system in terms of atenolol proliposomes could be an interesting alternative option to directly reach the cardiac tissue and decrease systemic exposure. Via this route, onset of action can be relatively fast, metabolic enzymes levels are lower compared to hepatic ones. Hence the drug, even with different physicochemical properties can be absorbed. In addition, pulmonary administration route exerts may advantages as alveolar membrane is extremely thin and highly permeable so the pulmonary absorption of different APIs also possible.

Hence Atenolol proliposomes were prepared and optimized using Box-Behnken Design is justifiable. Atenolol proliposomes were prepared by using various concentration ratio of SPC (2, 4 & 6g) and Cholesterol (2, 4 & 6g) and stirring speed (170, 220 & 270 rpm) with response of particle size and percent drug permeated.

Based on the characterization of drug permeability and particle size of the atenolol proliposomes, the optimized formulation was selected. The data collected from the invitro drug permeability, were then analysed using RSM to determine the effect of each parameter and the effects of various parameters involved were then interpreted. The best composite of SPC and cholesterol was selected from the various ratios. The ratio of 4 g of SPC, 4g of cholesterol and stirring speed 220 rpm (F13) was selected.

The in-vitro permeability of optimized formulation shows 93.78% for 12 hours. the drug release kinetics, data obtained from In-Vitro drug release studies are plotted in various kinetic models. The curve fitting results of the rate of permeation of the designed formulations gave an idea of the mechanism of drug release. Based on the “n” value of 0.6299 the drug release

was found to follow non-Fickian diffusion, which indicated both diffusion and swelling mechanism. Also, the drug release mechanism was best explained by Higuchi, as the plots showed the highest linearity (r2 = 0.9919), as the drug release was best fitted in Higuchi.

The optimized formulation showed particle size of 683nm. The PDI value of atenolol proliposomes was found to be 0.903. The poly dispersity index less than <1 indicates good uniform distribution of particles.

The results obtained from the in vivo pharmacodynamic study shows that administration of optimized atenolol proliposome results in gradual decrease of BP, they decreased the BP significantly at the first hour and the effect continued for 12 hrs.

The in-vivo pharmacokinetics study states that oral administration of pure Atenolol shows 3.75hrs MRT, whereas in the case of optimized formulation it shows 6.4hrs. The plasma dug concentration was maintained till 12 hrs, when given as proliposomes, hence the objective of the present study confirms the justification of delivering AT in proliposomal form through nebulization. Hence the dosing frequency can be reduced and bioavailability of the drug is increased as compared to oral administration.

From the above result, it can be concluded that, atenolol proliposomes have promising drug delivery attributes for antihypertension.

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