FORMULATION AND EVALUATION OF CURCUMIN -PIPERINE COLOADED LIPOSOMES FOR ENHANCED ANTI- BREAST CANCER ACTIVITY

A THESIS IS SUBMITTED TOASSAM SCIENCE AND TECHNOLOGY UNIVERSITY, ASSAM

IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF MASTER OF PHARMACY IN PHARMACEUTICS

Under the supervision of

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DECLARATION BY THE STUDENT

I hereby declare that the matter embodied in the dissertation entitled **"FORMULATION AND EVALUATION OF CURCUMIN-PIPERINE COLOADED LIPOSOMES FOR ENHANCED ANTI-BREAST CANCER ACTIVITY"** is a bonafide and genuine research work carried out by me under the supervision of Dr. BhanuPratapSahu, Associate Professor, GirijanandaChowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowapara, Azara, Guwahati-17. The work embodied in this thesis is original and has not been submitted the basis for the award of any degree, diploma or fellowship in any other university or institution.

Signature of the student **Date:**

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Date:

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Abstract

The present study was aimed to prepare PiperineColoadedcurcumin liposomes for enhancement of anti-breast cancer activity. Piperine, a major constituent of black pepper, they showed that it inhibits both the drug transporter P-glycoprotein and the major drug-metabolizing enzyme CYP3A4. Because both proteins are expressed in enterocytes and hepatocytes and contribute to a major extent to first-pass elimination of many drugs, our data indicate that dietary piperine could affect plasma concentrations of P-glycoprotein and CYP3A4 substrates in humans. Curcumin is a potential anti-breast cancer and its efficacy can be increased by delivery of curcumin-piperine liposomes.

Curcumin-Piperine liposomes are prepared by thin film hydration method using cholesterol, soya lecithin as lipids and tween 80, tween 20 or poloxamer 188 as surfactant and distilled water was used as hydrophilic phase. The particle size obtained in the formulation was between (160-760) d. nm without using drug. The particle size obtained in the curcumin-piperine liposomal formulation was between (13-380) d. nm. The all liposome formulations were evaluated for mean particle size, polydispersity index.

1.Introduction

1.1 Breast cancer: Breast cancer is a common malingnancy cancer mostly found in female person. It is the second leading reason of death in USA. This cancer is the main reason for death of woman aged between 45-55 years. The breast cancer is found almost 1 in 8 woman. It needs different types of treatment most of the time such as radiotherapy, complete tissue removal, chemotherapy and hormone therapy. It is a type of tissue cancer. It mainly effects the inner layer of milk lobules or glands.The risk factor which is also known as primay risk factors are economic status,age, iodine deficiency in diet and high hormone level.

1.2 Liposomes:Liposomes are spherical shaped vesicle that are produced from phospholipid, cholesterol, surfactant, fatty acids etc.

Liposomes were first discovered by British Hemotologist Dr. Alec D Bangham in 1961 at Babraham Institute in Cambridge, England.ThenBangham and R.W. Horme were testing new microscope in a new institute by adding gram negative stain to dry phospholipids. Then they found automatically formed " Bag Like" arrangement which was named as " multi lamellar smecticmesophase" or "Banghasome" by A.D. Bangham. Later it was named as liposome.

Liposomes are nanoparticles that encapsulate the drug in them and they are used as vehicle to transfer the drug from one place to the target site. Liposomes has both lipothlic and

hydrophilic area. Lipophilic drugs are encapsulated in the lipophilic area of the liposome and are transferred.On the other hand, water soluble drugs are encapsulated in the hydrophilic area of the liposomes and are transferred. They are composed of bi-layer (two layer) membrane of phospholipids and cholesterol. The phospholipid is phosphatidyl choline and the lipid is phosphatidyl ethanolamine. In liposomes the external layer is lipid and the internal layer is aqueous. The external layer i.e., lipid layer has more reactivity than aqueous layer. So, we can attach tags to the exterior lipid layer. Example, ifwe attach antibody to the external lipid layer which is specified for tumor cell (antigen), the liposomes directly go to the tumor cell and release the drugs and show its action.

Methods of preparation of liposomes:

For the preparation of liposomes, it needs four basic stages. These are-

a) The lipids are drying down from organic solvents

b) The lipids are dispersing in aqueous medium

c) The resultant liposomes are purified

d) The final product are analysed

Passive loading and active loading technique are used for the preparation of liposomes.

I) Passive loading technique has three different methods which are-

i) Solvent dispersion method

a)Ethanol injection method

b)Ether injection method

c)Reverse Phase evaporation

ii) Mechanical dispersion method

a) French pressure cell extrusion
b) Sonication
c) Freeze-thawed liposome
d) Non-hand shaking or freeze drying
e) Microemulsification
f) Lipid film hydration by hand shaking
g) Membrane extrusion
h) Dried reconstituted vesicles

iii) Detergent removal method

a) Dialysis
b) Gel-permeation chromatography

II) Active loading technique

I) Passive loading technique:

i) Solvent dispersion method:

a) **Ethanol injection method**:

In this method, a lipid solution of ethanol is quickly injected to a large amount of buffer. After that, formation of MLVs occur immediately. The major disadvantage of the method is that heterogenous size distribution of particles may be obtained (30-110 nm). Another disadvantage of this method is that dilute liposomes is obtained and the removal of all ethanol is very difficult.

b) **Ether injection method**:

In this method, lipids are dissolved in ether-methanol or diethyl ether mixture and make the solution. Then this solution is injected slowly to an aqueous solution containing the drug, which is to be encapsulated at temperature of 55-65C under reduced pressure. Then the ether is removed under vaccum for the formulation of liposomes. The main disadvantage of this method is that degradation may occur due to exsposure of organic solvents and drugs at high temperature. Also,the size of liposomes obtained is heterogenous i.e., 70-190nm.

c) Reverse phase evaporation:

In this method, the mixture of the lipids is taken in a round bottom flask. The organic solvents are removed by using a rotary evaporator under reduced pressure. Then the system is removed with nitrogen and lipids are again dissolved in the organic phase. In this organic phase, there will be formation of reverse phase vesicles. The solvents which are usually used are isopropyl ether and diethyl ether. Then the drug to be encapsulated is dissolved in aqueous phase. So, the aqueous phase is added after the lipids are re-dispersed in this phase. Then this system is placed under continuous nitrogen and the two phase system is sonicated until the mixture becomes clear one phase dispersion. Then the mixture is kept on the rotary evaporator and after sometime organic solvents are removed until a gel is formed followed by removal of non-encapsulated material. The resulting liposomes are called as reverse phase evaporation vesicles.

ii) Mechanical Dispersion method

a) French Pressure cell extrusion:

In this method, extrusion of MLV is done through a small orifice at 20,000 Psi at 4C. This method has several advantage or disadvantage. This method is very simple, quick, reproducible and it involves gentle handling of unstable materials. The prepared liposomes is slightly larger than the SUVs prepared by sonication method. The disadvantage of the method is that the high temperature is difficult to obtained and working volume is comparatively small.

b) Sonication method:

This method is used for the preparation of MLV and SUV.Here, MLVs are sonicated by using probe sonicator or bath type sonicator under passive atmosphere. The main disadvantage of this method is that it has very low internal volume/encapsulation efficiency, exclusion of large molecules, phospholipids degradation, metal contamination from probe tip and presence of MLV along with SUV.

c) Freeze thaw method:

This method involves the SUVs are freezed and after that they are slow thawing. This sonication process converted aggregated materials to LUV. During the process of thawing & freezing, the fusion of SUV leads to the formation of ULV. This fusion type of method is suppressed by increasing phospholipid concentration and increasing ionic strength of the medium. 20-30% of encapsulation efficiencies were obtained by using this method.

d) Freeze drying:

In this method, lipids and solvents are well mixed and the mixture is spread over the conical flask. Then evaporate the solution at room temperature by the nitrogen for drying. Then pass some water saturated nitrogen through conical flask until dried film opacity disappears. Then add water fluid and 10-20 ml of 0.2 M sucrose solution for swelling. After that it is stand for 2 hr at room temperature.Then the suspension is centrifuged at room temperature for 10 min and then the remaining fluid are added to the iso-molar glucose solution. After that LUV will formed.

e) **Micro-emulsification:**

In this method, lipid composition is microemulsified using high shearing stress which is generated from high pressure homogenizer. The speed of rotation of the homogenizer is adjusted from 20-200 and get microemulsion for biological application. This method is used for preparing small lipid vesicles in commercial quantities.

f) Thin film hydration method: In this method, the lipids and the other ingredients are dissolved in organic solvents. Then the mixture is kept under magnetic stirrer by maintaining the rpm 500 for 1 hour. After that transfer the solution into the rotatory flask of the evaporator and run the apparatus by maintaining the temperature 40-45°C for 1 hour time. After the evaporation of organic solvents, remove the flask where the formation of thin film occurs. So, the thin film was re-suspended by aqueous phase which was heated in water bath for 1 hour by maintaining the temperature at 45°C. Then the solution is transferred into the conical flask and kept into rotatory shaker for 1 hour at 80 rpm. Then the solution is transferred into the 50 ml beaker and kept it in the homogenizer by maintain the rpm 500 for 1 hour.Then the solution was suspended into the distilled water taken (1000µl and 800 µl) and sample solution was taken by using 20-200µl). Then the solution was kept into the magnetic shaker for 10 mins.Then the solution was taken into the glass cubate and checked for the size of the particle by using the instrument zeta sizer and required results as obtained.

g)Membrane extrusion:

In this method, a polymer filter is used which has a web like construction. In this, heterogenous size liposome suspension is passed through a polymer filter. The polymer filter provide a tortuous-path capillary pore, interconnected network and a membrane thickness of at least of about 100 microns. The liposomes formed which have a narrow size distribution and selected average size less than 0.4 microns.

h)Dried reconstituted vesicle: In this method, the previously hydrated form of liposomes are again hydrated to an aqueous fluid. The aqueous fluid has an active ingredient.The rehydrated liposomes are then dehydrated.

iii) Detergent removal method:

a)Dialysis: In dialysis method, at critical micelle concentration (CMC), the detergents have been used to solubilize lipid. When the separation of detergent occurs, the micelles become increasingly advantageous in phospholipid and they lastly combine to form LUVs. To eliminate the detergents, dialysis method is used. For the removal of detergents, a commercial device is obtainable which is called Lipo-Prep (Diachema AG, Switzerland)

b)Gel-permeation chromatography:

In this method, special chromatography is used to deplet the size of the detergent. For gel filtration two types of media can be used which are Sephadex G-1 and Sephadex G-50.The

beads are packed in a column and the liposomes do not penetrate into the pores of the beads. Through interbead spaces, the liposomes are percolate at slow flow rates. The liposomes are separated from the detergent monomer is very good. A large amount of amphiphilic lipids are adsorbed by the swollen polysaccharide beads so pre treatment is necessary. The pre-treatment can be done by pre-saturation of the gel filtration column by lipids using suspension of empty liposomes.

II) Passive loading technique

Pro-liposome lyophilization: pro-liposomes are dry, free flowing granular particles composed of phospholipids coatings on water soluble powder particles. Oxidation and other chemical reactions degraded our natural products. Free drying is a standard process which is used for the formulation of many pharmaceutical products. Some of the manufactured pharmaceutical products requires free drying from organic co-solvents system. Free drying is a process of removal of water in the frozen state at very low pressure. Freeze drying process is normally used for drying products which are thermo labile and by using heat drying they would be destroyed.

1.3 Targeted delivery:

- The EPR effect in nanomedicine development:

EPR-based tumor targeting requires macromolecular drugs to have longer half-life time in order to provide a sufficient effective pharmacodynamics level. The usual way to obtain macromolecular drugs is to “mask” conventional small macromolecular drugs by modifying their surface with certain water-soluble polymers with a well-solveted and flexible main chain, such as polyethylene glycol (PEG), styrene maleic acid (SMA), N- (2-hydroxypropyl) methacrylamide (HPMA), and so on. Regarding the tumor targeting strategy, two kinds of targeting are always there, i.e., “ passive targeting” and “ active targeting”. Passive targeting is so far EPR-based tumor targeting. To date, many macromolecular drugs were developed based on EPR effect, some of which are used in clinic and more are in preclinical stages, including liposomes, polymers, micelles, and nanoparticles. EPR effect is a phenomenon due to the unique anatomical and pathophysiological characteristics of solid tumor. Namely, in contrast to normal tissues and organs, most solid tumors show a higher vascular density (hypervasculature), i.e., angiogenesis that is one of the most important features of tumors to sustain their rapid growth. Electron microscopy of a vascular cast of tumor blood vessels that was obtained by using polymer resin showed distinct differences between tumor vessels and normal blood vasculature. Tumor vascular angiogenesis (vascular bed) could be observed even when tumor nodules were smaller than 0.2 mm. Moreover, irregular or inconsistent blood flow is also commonly observed in tumors. Long-circulating pharamaceuticalnanocarriers, such as liposomes, micelles, or polymeric nanoparticles, are capable of accumulatingin various pathological areas with affected vasculature via the EPR effect, and have been repeatedly

used for drug delivery into tumors via passive accumulation. Long-circulating liposomes and other nanocarriers demonstrate dose independent, non-saturable, long-linear kinetics, and increased bioavailability.

- P-gp inhibitor efflux transporter:"

Inhibition of efflux pumps is a emerging approach in cancer therapy and drug delivery. Since it has been discovered that polymeric pharamaceuticals excipients such as Tweens or Pluronics can inhibit efflix pumps, various other polymers have been investigated regarding their potential efflux pump inhibitory activity. Among them are polysaccharides, polyethylene glycols and derivatives, amphiphilic block copolymers, dendrimers and thiolated polymers. In the current review article, natural and synthetic polymers that are capable of inhibiting efflux pumps as well as their application in cancer therapy and drug delivery. Basically, an inhibition of efflux pumps can be applied to (a) improve the transport of efflux pump substrate anticancer agents into MDR cells and (b) drug delivery. The over expression of efflux pumps such as P-gp in tumours consequently leads to low concentrations ofanticancer agents inside MDR cellsduring cancer therapy, so that therapeutic effects are minimized or do not occur at all. To circumvent MDR in cancer cells, researchers either focus on the development of novel therapeutic non-substrate agents or formulations that allow the drug to bypass efflux pump transport, or on auxiliary agents that can inhibit efflux pumps.

1.4 **Nanoformulation in Targeted Delivery**

- Nanocarriers Based Anticancer Drugs:

Anticancer therapies mostly depend on the ability of the bioactivates to reach their designated cellular and subcellular target sites, while minimizing accumulation and side effects at non-specific sites. The development of nanotechnology based drug delivery systems that are able to modify the biodistribution, tissue uptake and pharmacokinetics of therapeutic agents is considered of great importance in biomedical research and treatment therapy. Controlled releases from nanocarriers can significantly enhance the therapeutic effect of a drug. Nanotechnology has the potential to revolutionize in cancer diagnosis and therapy. Targeted nanomedicines either marketed or under development, are designed for the treatment of various types of cancer. Nanocarriers are able to reduce cytotoxic effect of the active anticancer drugs by increasing cancer cell targeting in comparison to conventional formulations. The newly developed nano devices such as quantum dots, liposomes, nanotubes, nanoparticles, micelles, gold nanoparticles, carbon nanotubes and solid lipid nanoparticles are the most promising applications for various cancer treatments.

- Anti-cancer Effects of Curcumin;

Cancer is a hyper-proliferative disorder where a normal cell loses its cellular homeostasis and begins to constitutively activate a plethora of genes that are involved in cell cycle, invasion, survival, metastasis, and angiogenesis. Curcumin is also a potent anti-inflammatory compound.Based on its distinct chemical properties, curcumin interacts with numerous extracellular and intracellular molecules that are actively involved in cancer initiation and progression, thereby inhibiting cancer progression. Increasing evidence

suggests that deregulated inflammatory pathways play a pivotal role in a multitude of chronic diseases, including cancer. The mechanism by which chronic inflammation drives cancer initiation and progression is via increased production of pro-inflammatory drives cancer initiation and progression is via increased production of pro-inflammatory mediators, such as cytokines, chemokines, reactive oxygen species (ROS), overexpression of oncogenes, cyclooxygenase (COX-2), matrix metalloproteinase (MMPs), intracellular signaling pathway mediators, transcription factors such as nuclear factor kB (NF-kB), signal transducer and activator transcription 3 (STAT3), protein kinase B (AKT), and activator protein 1(AP1) that drive tumor cell proliferation, transformation, invasion, metastasis, angiogenesis, chemoresistance, and radioresistance. Numerous studies have also reported the inhibitory effects of curcumin on almost all types of tumor cells, such as cancers of the reproductive, digestive, lymphatic and immune, uninary,pulmonary, nervous,skeletal systems, and the skin. The inhibitory concentrations of curcumin have been found to range from 1μM to 100μM in these studies.

Drug Profile:

Curcumin:

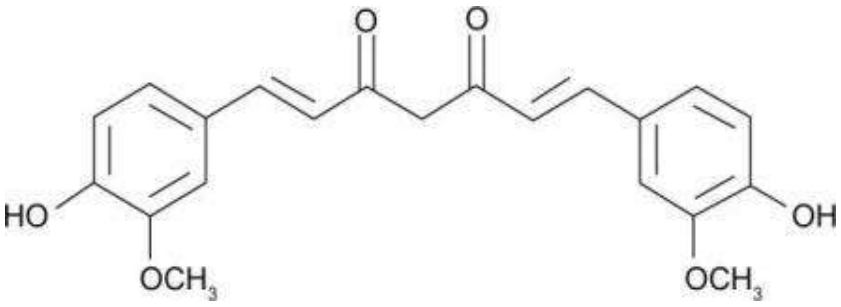


Fig 1: Structure of curcumin

Molecular formula: $C_{21}H_{20}O_6$

Molecular weight: 368.385 $g \cdot mol^{-1}$

Curcumin (diferuloylmethane) is polyphenolic compound derived from spices turmeric, a product of the plant curcuma longa . The pigments responsible for yellow color of curcumin are phenolic in nature and are known by the name curcuminoids, which occur naturally in curcumin.

Curcumin blocks the formation of reactive-oxygen species, possesses anti-inflammatory properties as a result of inhibition of cyclooxygenases (COX) and other enzymes involved in inflammation; and disrupts cell signal transduction by various mechanisms including

inhibition of protein kinase C. These effects may play a role in the agent's observed antineoplastic properties, which include inhibition of tumor cell proliferation and suppression of chemically inducedcarcinogenesis and tumor growth in animal model of cancer.

- **Anticancer activity of curcumin**

Curcumin (diferuloylmethane), a naturally occurring phytochemical responsible for the yellow color of the commonly used spice turmeric (Curcuma longa Linn), is receiving attention from cancer investigators because of the chemopreventive properties against human malignancies. The anti-carcinogenic properties of curcumin in animals have been demonstrated by its inhibition of tumor initiation induced both by various carcinogens and by phorbol esters. Recent report also demonstrated the anti-tumor property of curcumin in cancer of colon, forestomach, breast etc. Besides its anti- carcinogenic effects, curcumin has been reported to have a wide range of pharmacological properties including anti-inflammatory, anti-toxic and anti-oxidative. The pharmacological safety of curcumin is demonstrated by its consumption for centuries at upto 100 mg/day by people in certain countries.

Piperine:

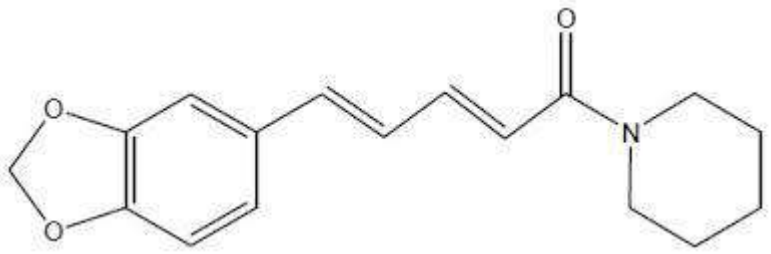


Fig 2: Structure of piperine

Molecular formula: $C_{17}H_{19}NO_3$

Molecular weight: 285.343 $g \cdot mol^{-1}$

Natural products from plants are important sources of new drugs. The genus Piper (Piperaceae), which contains approximately 2000 plant species distributed mainly in tropical areas, is a potential source of drugs based on the use of some Piper species in traditional medicine. For example, nearly 30 out of 60 indigenous Chinese piper species are used medically. Recent, ethnobotanical and medicinal chemistry research focussed on Piper plants leads to the discovery of cytotoxic amides from Piper boehmeriifolium Wall; an anticancer medicine used in India. In China, Piper Plants are also used in some formulae to treat cancers. The genus Piper (Piperaceae) contains approximately 2000 species, of which 10 species have been used in traditional medicines treat cancer or cancer like symptoms. Studies have shown that compounds from piper plants possess cytotoxic activity. Amide alkaloids account for the major active principles. Among them, piplartine (Piper

longumine) shows the most promise, being toxic to dozens of cancer cell lines and having excellent in vivo activity. It is worthwhile to conduct further anticancer studies both in vitro and in vivo on piper plants and their active principles.

- **Piperine as anticancer agent**

Piperine is an alkaloid present in black pepper (Piper nigrum), one of the most widely used spices, in long pepper (Piper longum), and other Piper species fruits belonging to the family of Piperaceae. Piperine is responsible for the black pepper distinct biting quality.Piperine could inhibit both the drug transporter P-glycoprotein and the major drug metabolizing enzyme CYP3A4. So it has been used as a bioavailability enhancer with various structurally and therapeutically diverse drugs.

Excipients:

- **Poloxamer:**

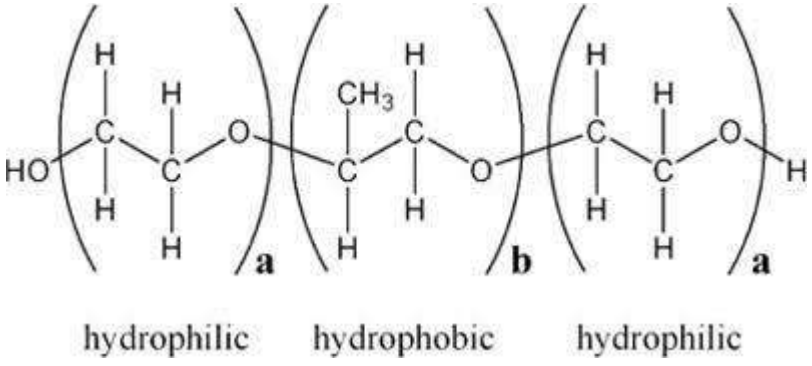


Fig 3: Structure of poloxamer

Poloxamer 188 (P188) is a nonionic triblock linear copolymer that exhibits rheologic, anti-thrombotic, anti-inflammatory, and cytoprotective activities in various tissue injury models . Composed of two hydrophilic side-chains attached to a hydrophobic center core , its average molecular weight is 8400 Daltons.

Molecular formula: $C_8H_{18}O_3$

Application:

Because of their amphiphilic structures, the polymers have surfactant properties that make them useful in industrial applications. Among other things, they can be used to increase the water solubility of hydrophobic, oily substances or otherwise increase the miscibility of two substances with different hydrophobicities. For this reason, these polymers are commonly used in industrial applications, cosmetics, and pharmaceuticals. They have also been evaluated for various drug delivery applications and were shown to sensitize drug-resistant cancers to chemotherapy.

In bioprocess applications, poloxamers are used in cell culture media for their cell cushioning effects because their addition leads to less stressful shear conditions for cells in reactors.

Effect on multi drug resistant cancer cells

Poloxamers have been shown to preferentially target cancer cells, due to differences in the membrane of these cells when compared to noncancer cells. Poloxamers have also been shown to inhibit MDR proteins and other drug efflux transporters on the surface of cancer cells; the MDR proteins are responsible for the efflux of drugs from the cells and hence increase the susceptibility of cancer cells to chemotherapeutic agents such as doxorubicin.

Another effect of the polymers upon cancer cells is the inhibition of the production of ATP in multi-drug resistant (MDR) cancer cells. The polymers seem to inhibit respiratory proteins I and IV, and the effect on respiration seems to be selective for MDR cancer cells,

which may be explained by the difference in fuel sources between MDR and sensitive cells (fatty acids and glucose respectively).

The poloxamers have also been shown to enhance proto-apoptotic signaling, decrease anti-apoptoic defense in MDR cells, inhibit the glutathione/glutathione S-transferase detoxification system, induce the release of cytochrome C, increase reactive oxygen species in the cytoplasm, and abolish drug sequestering within cytoplasmic vesicles.

- **Cholesterol:**

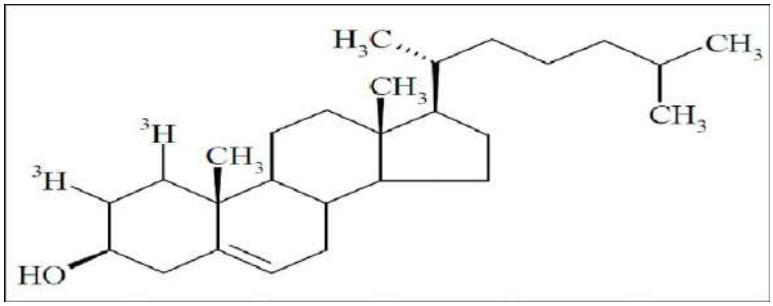


Fig 4:. Structure of cholesterol

Cholesterol is an organic molecule. It 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. It is a yellowish crystalline solid.

It is essential to maintain both membrane structural integrity and fluidity. Cholesterol allows animal cells to function without a cell wall (which in other species protects membrane integrity and cell viability); that allows animal cells to change shape rapidly.

Properties:

Cholesterol esters have a lower solubility in water than cholesterol and are more hydrophobic. They are hydrolysed by the pancreatic enzyme cholesterol esterase to produce cholesterol and free fatty acids. Cholesterol has vital structural roles in membranes and in lipid metabolism in general. It is insoluble in water but slightly soluble in alcohol and somewhat more soluble in ether and chloroform, lipid based formulations are mostly prepared for enhancing solubility and absorption of poorly water soluble drugs. These formulations typically contain long or medium chains triglycerides lipids, long or medium chain mixed mono and di-glycerides, individual or mixed surfactants, and various hydrophilic surfactants.

Molecular Formula: $C_{27}H_{46}O$

Molecular weight: 386.664 g/mol

Lecithin:

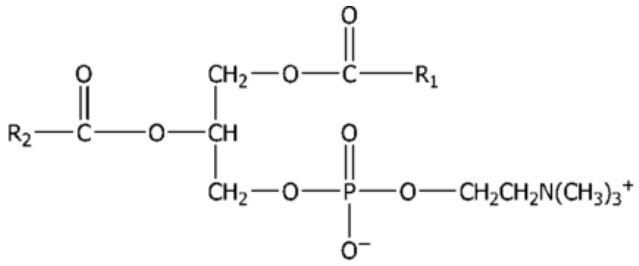


Fig 5: Structure pfPhosphatidylcholine

Lecithin is a fat that is essential in the cells of the body. It can be found in many foods, including soybeans and egg yolks. Lecithin is taken as a medicine and is also used in the manufacturing of medicines. Lecithins are mixtures of glycerophospholipids including phosphatidylcholine, phosphotidylethanolamine, phosphotidylinositol, phosphatidylserine, and phosphatidic acid. Lecithin can easily be extracted chemically using solvents such as hexane,ethanol, acetone, petroleum ether or benzene; or extraction can be done mechanically. It is usually available from sources such as egg yolk,marine sources, soybens, milk, rapessed, cottonseed, and sunflower oil. It has low solubility in water, but is an excellent emulsifier. In aqueous solution, its phospholipids can form either liposomes, bilayer sheets, micelles, or lameller structures, depending on hydration and temperature. This results in a type of surfactant that usually is classified as amphipathic.

Properties:

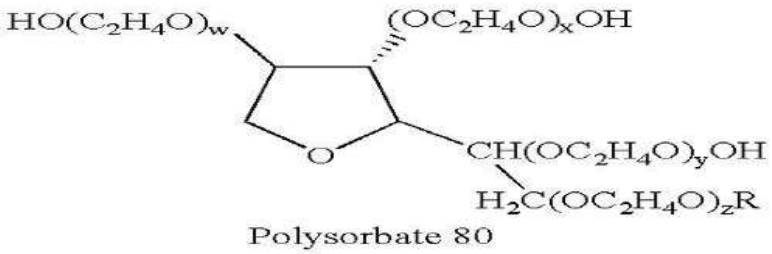
Lecithins have emulsification and lubricant properties, and are a surfactant. They can be completely metabolized by humans, so are well tolerated by humans and nontoxic when ingested; some other emulsifiers can only be excreted via the kidneys

Application: In the pharmaceutical industry, it acts as a wetting agent, stabilizing agent and a choline enrichment carrier, helps in emulsification and encapsulation, and is a good dispersing agent. It can be used in manufacture of intravenous fat infusions and for therapeutic use.

Molecular formula: $C_{35}H_{66}NO_7P$

Molecular weight: 643.9 g/mol

Tween 80:



Polysorbate 80

Fig 6: Structure of tween 80

Tween 80 is a nonionic surfactant and emulsifier often used in foods and cosmetics. This synthetic compound is a viscous, water soluble yellow liquid.

Polysorbate 80 is derived from polyethoxylated sorbitan and oleic acid. The hydrophilic groups in this compound are poly ethers also known as polyoxyethylene groups, which are polymers of ethylene oxide.

Application:

Medical:Polysorbate 80 is an excipient that is used to stabilize aqueous formulations of medications for parenteral administration, and used as an emulsifier in the making of the popular antiarrhythmic amiodarone

Molecular formula: $C_{64}H_{124}O_{26}$

Molecular weight: 1310 g/mol

Tween 20:

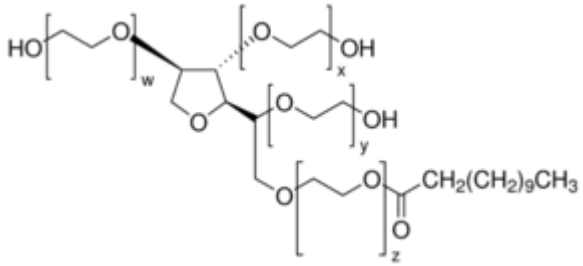


Fig 7: Structure of Tween 20

Tween 20 is a polysorbate -type non-ionic surfactant formed by the ethoxylation of sorbitan before the addition of lauric acid Its stability and relative nontoxicity allows it to be used as a detergent and emulsifier in a number of domestic, scientific, and pharmacological applications. As the name implies the ethoxylation process leaves the molecule with 20 repeat units of polyethylene glycol; in practice these are distributed across 4 different chains, leading to a commercial product containing a range of chemical species

Pharmaceutical Application:

Polysorbate 20 is used as an excipient in pharmaceutical applications to stabilize emulsions and suspensions

Molecular formula: $C_{58}H_{114}O_{26}$

Molecular mass:522.7 g/mol

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2.Literature Review:

2.1 Curcumin-piperine co-loaded formulation:

Shoba G et. al. (1997)demonstrated an experiment on the influence of piperine on the Pharmacokinetics of Curcumin and the results obtained in the study demonstrate that piperine enhances the oral bioavailability of curcumin in both rats and human at does that we devoid of adverse side effects.However, certain differences between rats and human with respect to curcumin were evident.The study shows that in the dosages used, piperine enhances the serum concentrations, extent of absorption and bioavailability of curcumin in both rats and humans with no adverse effects.

Jangra A et. al (2016) presented a study to investigate the protective effects of curcumin alone and in combination with piperine against lipopolysaccharide (LPS) induced neurobehavioral and neurochemical deficits in the mice hippocampus since Curcumin has numerous pharamacological properties; however, it has limited therapeutic usefulness due to its poor oral bioavailability Furthermore, co administration of curcumin with piperine significantly potentiated the neuroprotective effect of curcumin.These results demonstrate

that piperine enhanced the neuroprotective effect of curcumin against LPS-induced neurobehavioral and neurochemical deficits.Studies have evidently proved piperine as a bioavailability enhancer of curcumin in some diseased model. Piperine acts by affecting the pharmacokinetics of other drugs such as curcumin, quercetin, carbamazepine, etc. thereby enhancing the bioavailability through various mechanisms which are still elusive to design curcuminpiperine formulation.

2.2 Piperine inhibits Multi Drug resistance:

Bhardwaj R K et al. (2002) carried out an experiment where Piperine, a Major constituent of Black Pepper, they showed that piperine inhibits both the drug transporter P-ghycoprotein and the major drug-metabolizing enzyme CY3PA4.Because both proteins are expressed in enterocytes and hepatocytes and contribute to a major extent to first-pass elimination of many drugs, our data indicate that dietary piperine could affect plasma concentrations of P-glycoprotein and CYP3A4 substrates in humans.

2.3 Conjugated Curcumin formulation:

Anand P et. al.(2008) suggested an article on synthetic analogues and several other strategies to enhance the biological activity of curcumin. These starategies include adjuvents, nanoparticles, liposomes,micelles,and phospholipid complexes.Theadjuvents were selected on the basis of their ability to prevent the rapid metabolism of curcumin by interfering with the enzyme that catalyse the metabolism of curcumin. All other formulations mentioned are designed primarily to increase absorption of curcumin into tissues. Nanoparticles can provide more penetration to membrane barriers because of their

small size. Besides their size, their potential for modification for targeting specific organs makes them excellent drug carriers. Liposomes, micelles, and phospholipid complexes can reduce the hydrophobicity of curcumin; these carriers also can increase the permeability of membrane barriers by interacting with the membrane components. Recently it was also reported that the water solubility of curcumin could be 12- fold by the use of heat.

2.4 Curcumin as anticancer agent:

Choudhuri T et al. (2002) carried an experiment to determine the mechanisms of curcumin-induced human breast cancer cell apoptosis. Mechanisms that suppress tumorigenesis often involve modulation of signal transduction pathways, leading to alteration in gene expression, arrest of cell cycle progress or apoptosis. Curcumin has been demonstrated to inhibit tumor initiation induced by various carcinogens through p-53 dependent Bax induction.

Shiou H J et al. (2002) carried out a study on where curcumin induces a p53 dependent apoptosis in human basal cell carcinoma cells, they demonstrated that curcumin is able to induce apoptosis in BCC cells, accompanied by an increase in p53, p21CIP1/WAF1, and Gadd45 protein levels.

2.5 Piperine as anticancer agent:

Wang Y H et al.(2014) reviewed carried out an experiment on piperine. Piperine is a major component of black (P. nigrum) and long (P.longum) pepper which exhibited significant inhibitory effects on the growth of at least one tumor cell line in vivo at concentrations less than 15 mg/kg body weight. The compound kills cancer cells by targeting the stress response to reactive oxygen species (ROS).

Siriwiriyajan S et al. (2005) investigated Anticancer and Cancer Prevention Effects of Piperine-Free Piper nigrum extract on N-nitrosomethylurea-Induced Mammary Tumorigenesis in Rats they found that the recrystallized extract of P. nigrum that was piperine free had a potent cytotoxic activity on breast cancer cell lines.

2.6 Targeted drug delivery:

Fumiyoshi Y et al. (2012) reviewed the pharmaceutical consideration for targeted drug delivery where drug delivery system involves technology designed to maximize therapeutic efficacy of drugs by controlling their biodistribution profile. In order to optimize a function of the delivery system, their biodistribution characteristics should be systemically understood. Pharmacokinetic analysis based on the clearance concepts provides quantitative information of biodistribution which can be related to physicochemical properties of the delivery system.. Various delivery systems including macromolecular drug conjugates, chemically or genetically modified proteins and particulate drug carriers have been designed and developed so far in physiological and pharmacokinetic implication of the delivery systems.

Jun L et al. conduct a review on recent advances in targeted nanoparticles drug delivery to melanoma where noval targeted therapeutics (eg. Vemurafenib, debrametinib) having higher initial response rate and clear rate impact on the overall survival, but relapse usually occur within 6-9 months. With the development of nanotechnology the application of nanocarriers are widely expected to change the melanoma therapy for future.In this review, they relate recent advances in the application of multifunctional nanocarriers for targeted delivery to melanoma nanotraotics and combination therapy and nano pharmaceutical associated melanoma clinical trials, followed by challenge and perspective.

2.7 Nanoparticle in Targeted Delivery:

Haree J I et al. (2017) reviewed Anti-cancer nanomedicines in clinical development which can be broadly divided into five main types: liposomes, polymeric conjugates, polymeric nanoparticles, polymeric micelles, and others. They perceived different challenges for the nanomedicine has been investigated by putting some considerations when selecting the delivery system, drug, and target patient population for disease-driven design and developmentof new anticancer nanomedicines.

James D B et al. (2008) studied the active targeting achemes for nanoparticle systems in cancer targets for nanoparticle systems give insight into direction of the field. The major targeting strategies that have been used for the delivery of therapeutics or imaging agents to cancer have been broken into three sections. These sections are angiogenesis-associated

targeting to uncontrolled the cell proliferation markers and tumor cell targeting. The targeting strategies explored for many nanoparticle system suggest the great potential of targeted delivery of revolutionize treatment.

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3.AIM AND OBJECTIVES:

3.1 AIM: Formulation and evaluation of curcumin-piperine co-loaded liposomes for enhanced anti-breast cancer activity.

3.2 Objectives:

The main objectives are:

- To prepare curcumin liposomes to enhance the bioavailability and solubility
- To prepare piperine liposomes to enhance the bioavailability and solubility
- To formulate curcumin-piperine liposome for targeted anti-breast cancer activity.

4. MATERIALS AND METHOD:

4.1 Materials used

- **Collection of the drug curcumin**

Curcumin (cyst 99%) was procured from ottokemi, Mumbai

- **Piperine**

It was gifted by Dr. DamikiLaloo (Associate Professor, GIPS)

- **MATERIALS USED AND THE LIST OF MANUFACTURER**

SL NO.	MATERIAL	SOURCE
1	CHOLESTEROL	SISCO RESEARCH LABORATORIES PVT. LTD ANDHERI E, MUMBAI-400018
2	SOYA LECITHIN	SISCO RESEARCH LABORATORIES PVT. LTD ANDHERI E, MUMBAI-400018
3	POLOXAMER 188	MEREK SPECIALIST PVT. LTD, SHIV NAGAR ESTATE MUMBAI- 40018
4	TWEEN 80	SISCO RESEARCH LABORATORIES PVT. LTD ANDHERI E, MUMBAI-400018
5	TWEEN 20	SISCO RESEARCH LABORATORIES PVT. LTD ANDHERI E, MUMBAI-400018
6	SODIUM HYDROXIDE	MEREK SPECIALIST PVT. LTD, SHIV NAGAR ESTATE MUMBAI- 40018
7	SODIUM DIHYDROGEN PHOSPHATE	MEREK SPECIALIST PVT. LTD, SHIV NAGAR ESTATE MUMBAI- 40018
8	CHLOROFORM	CHANGSHU YANGYUAN CHEMICAL CHINA
9	METHANOL	CHANGSHU YANGYUAN CHEMICAL CHINA
10	ACETONE	CHANGSHU YANGYUAN CHEMICAL CHINA

Table 1: Materials and their list of companies

4.2. Instrument used for the preparation and evaluation of formulation

INSTRUMENT	COMPANY NAME
UV VISIBLE SPECTROPHOTOMETER, MODEL- UV 1800 240 V	SHIMANDJU: JAPAN BRUKER
FT-IR SPECTROPHOTOMETER (MODEL NO.- 10059736)	PERKIN ELMER 2000, USA
ZETASIZER VER 7.01 SERIAL NO. MAL1080846	MALVERN
ROTATORY FALSK EVAPORATOR	

Table 2: Instruments and their company name

4.3 Preformulation studies of Drug and excipients:

- Organoleptic properties
- Solubility studies
- Melting point
- Preparation of standard calibration curve of curcumin in phosphate buffer
- Preparation of standard calibration curve of piperine in phosphate buffer
- FTIR-spectroscopy study
- Different scanning calorimetry study

4.4 Preparation of curcuminpiperine liposomes by thin film hydration method:

Accurately weight and measure drugs and all the measure required excipients.Then dissolve all the excipients into the organic solvents (where organic solvents are taken in the form of 2:1 ratio, organic solvents used chloroform and methanol.Then mixture of the solution is kept under magnetic stirrer by maintaining the rpm 500 for 1 hour.Now transfer the solution into the rotatory flash evaporator and run the apparatus by maintaining the temperature 40-45 for 1 hour time.After evaporation of organic solvents remove the flask and where formation of thin film occurs, so now the thin film was re-suspended in phosphatebuffer saline (pH 7.4).Which was heated in water bath for 1 hour by maintaining the temperature at 45 degree Celsius.Then the solution is transferred into the conical flask and kept into rotatory shaker for 1 hour at 80 rpm.Then the solution is transferred into the 50 ml beaker and kept it in the homogenizer by maintain the rpm 500 for 1 hour.Then the

solution was suspended into the distilled water taken (1000µl and 800 µl) and sample solution was taken by using 20-200µl).Then the solution was kept into the magnetic shaker for 10 mins.Then the solution was taken into the glass cubate and checked for the size of the particle by using the instrument zeta sizer and required results as obtained.

4.5Characterization of the prepared liposomes:

- Particle size analysis
- Polydispersity index (PDI)
- Transmission Electron Microscopy (TEM)
- Drug Entrapment efficiency
- Drug Release
- Zeta potential

Size measurement:

The particle size and polydispersity index (PDI) of the liposomes is measured by using dynamic leser light scattering after suitable dilution (zetasizer, Malvern nano S90). The sample is diluted with distilled water and analysed at 25°C using quartz micro cuvette. Then the instrument is run and the particle size and PDI is measured.

Drug encapsulation efficiency:

Drug encapsulation efficiency of the liposomal formulation is measured by the ultra centrifugation of the formulation at 11000-1500 rpm for 45 min in an ultracentrifuge to separate the loaded drug from free drug. The supernatant was separated and analysed after suitable dilution in solvent by UV-visible spectrophotometer at 425 nm which is indicate the amount of free drug.

The liposome (sediment) was redispersed in same solvent(methanol) and analysed drug content after dilution using UV- visible spectrophotometer which is indicate the amount of drug entrapped.

The drug encapsulation efficiency of liposome is calculated by this equation

$DEE=[(T-C)/T]\times 100$

Where

T= total amount of drug that is detected both in the supernatant & sediment

C= amount of drug selected only in the supernatant

Transmission electron microscopy (TEM):

This is done to study the morphology of the developed liposomes.The liposome solution was diluted 3 times with PBS buffer at room temperature. The sample was carefully dropped into a pure carbon film copper mesh, which gave the droplets a hemispherical liquid surface. After drying, the solution was dyed using phosphotungstic acid solution.The dying agent was blotted around the copper mesh using filter paper. Ultrapure water was

then added dropwise to the copper mesh, followed by blotting dry with filter paper. After air drying, the sample imaged using at transmission electron microscope (TEM).

Drug release: In vitrodrug release was performed using dialysis membrane method. Firstly, membrane was clamped in open glass tube for drug release and consider as a donor compartment.6.8 pH Phosphate buffer solution (PBS) 200ml was used as dissolution medium and taken in receiver compartment. Phosphate buffer pH 6.8 was used as receptor medium.

The entire system was kept at 37°C with continuous magnetic stirring. Samples (1 mL) were withdrawn from the receptor compartment at predetermined time intervals and replaced by

fresh medium. The amount of drug dissolved was determined with UV spectrophotometry using the standard curve equation y = 0.0002x + 0.0011; R2 = 0.9917.

Zeta potential:

The zeta potential of the liposoma formulation is determined by using the clear disposable zeta cell for zeta potential analysis by electrophoretic mobility method (zetasizer Ver. V 2 2 Malvern

5.RESULTS AND DISCUSSION:

5.1 Preformulation study:

- ## Organoleptic Properties:

The organoleptic properties like colour taste odour were observed and the results were compared with official requiorement and found to be acceptable

- **Melting point determination**:

The melting point was determined by using melting point apparatus with the help of capillary tube, the results were shown below

Curcumin- 180° C

Piperine- 127° C

Poloxamer- 59° C

Cholesterol- 149° C

Soya lecithin- 236° C

Tween 80 – 98.9° C

The melting point range was compared with the official requirement and it is found to be acceptable.

- **Determination of pH:** The pH of 1% solution was found to be

Curcumin- 7.4

Poloxamer- 7.3

Piperine- 7.9

Sample	Colour	Odour	Taste
Curcumin	Brightly yellow	Characteristic	Characteristc
Piperine	Light yellow	Characteristc	Characteristc
Cholosterol	White crystalline powder	Odourless	Tasteless
Soya lecithin	Cream light yellow to brown color	Soyabeanodour	Odourless
Tween 80	Amber colour	Faint characteristic odour	Bitter taste
Tween 20	Clear amber yellow	Slightly fatty	Bitter taste
Poloxamer	White crystalline powder	Odourless	Tasteless
Span 80	Brownish yellow viscous liquid	fatty	Bitter taste

Table 3: Evaluation ofColour, odour and taste

FTIR spectrum of the drugs:

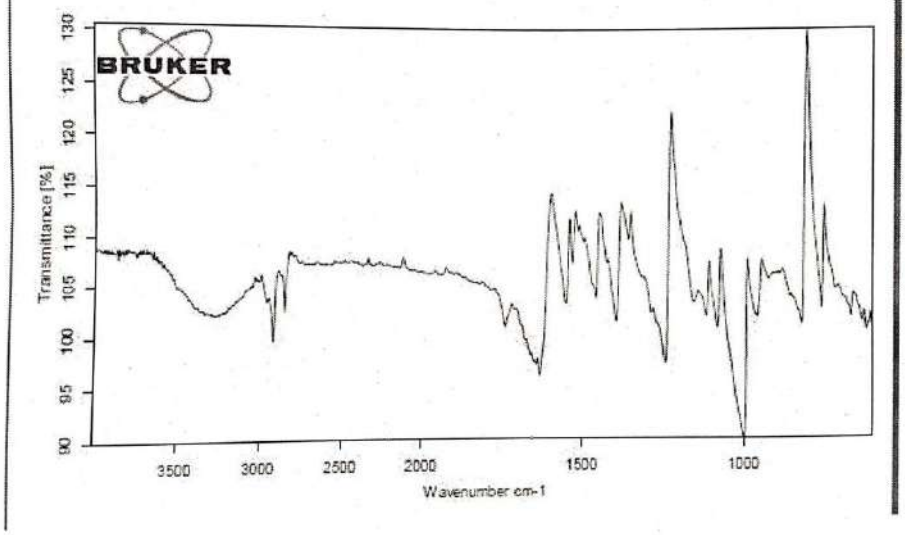


Fig 8: FT-IR spectrum of Curcumin

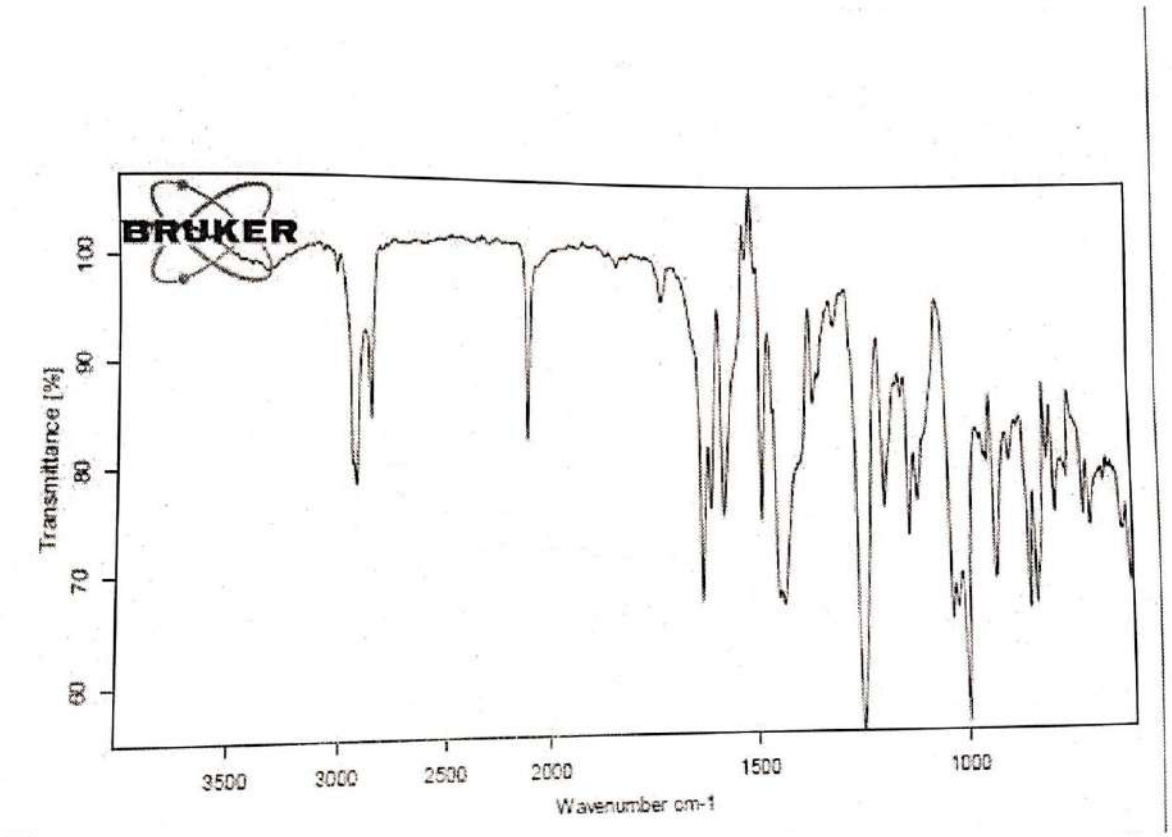


Fig 9: FT-IR spectrum of Piperine

Formulation without using drug:

SLNO.	Surfactant	Cholesterol	Soyalecithin	Chloroform	Methanol	Water
F1	200µl (Tween 20)	50mg	10mg	8ml	4ml	25ml
F2	200µl (Tween 80)	50 mg	10mg	8ml	4ml	25ml

Table 4: Formulation table of liposome (without drug)

SL NO.	Surfactant	Cholesterol	Soyalecithin	Chloroform	Methanol	Buffer
F3	Poloxamer 188 5mg	52mg	30mg	8ml	4ml	10ml
F4	Span 80-70mg	20 mg	90mg	10ml	5ml	10ml
F5	60mg (Span 80)	30mg	60mg	10ml	5ml	10ml
F6	60mg (Span 80)	30mg	60mg	10ml	5ml	15ml
F7	100(1%) Surfactant (Tween 80)	10mg	90mg	10ml	5ml	10ml
F8	200 (2%) Surfactant (Tween 80)	10mg	90mg	10ml	5ml	10ml
F9	100(1%) Surfactant (Tween 80)	10mg	90mg	10ml	5ml	10ml
F10	200 (2%) Surfactant (Tween 80)	10mg	90mg	10ml	5ml	10ml

Table 5: Formulation table of liposome (without drug)

SL NO.	Z-average size (d. nm)	Polydispersity index
F1	161.8	0.355
F2	189.5	0.322
F3	755.7	0.658
F4	396.1	0.585
F5	398.5	0.517
F6	501.7	0.769
F7	238.4	0.523
F8	256.6	0.463
F9	206	0.431
F10	187.8	0.396

Table 6: Particle size analysis table for liposomes (without drug)

SL NO.	Drug 1 (Curcumin)	Drug 2 (Piperine)	Cholesterol	Soyalecithn	Surfactant (Tween 80)	Chloroform	Methanol	Buffer
F1	20mg	20 mg	10mg	90mg	300mg	10ml	5ml	10ml
F2	20mg	20mg	10mg	90mg	300mg	10ml	5ml	10 ml
F3	10mg	10mg	10mg	90mg	300mg	10ml	5ml	10ml
F4	10mg	10mg	10mg	90mg	200mg	10ml	5ml	10ml
F5	10mg	10mg	10mg	90mg	400mg	10ml	5ml	10ml
F6	10mg	10mg	10mg	90mg	100mg	10ml	5ml	10ml

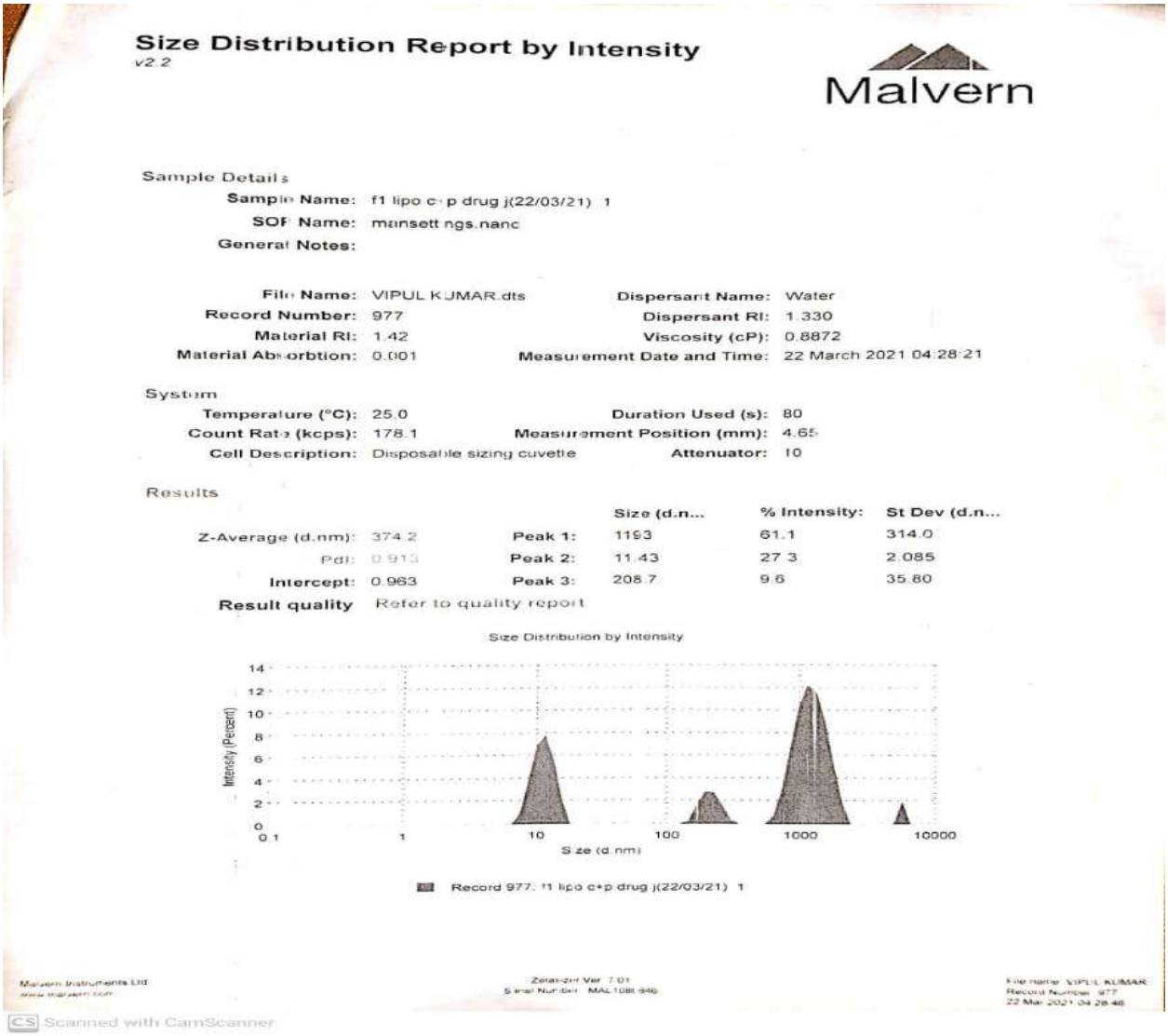
Table 7: Formulation table Curcumin-Piperine liposomes

SL NO.	Zeta- average size	Polydispersity index
F1	374.2	0.913
F2	376.1	0.709
F3	131.6	0.644
F4	48.55	1.000
F5	13.73	0.267
F6	185.3	0.317

Table 8: Particle size analysis table for curcumin-piperine liposomes

Particle size of curcumin-piperinecoloaded liposomes:

From the particle size analysis



Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	f1 lipo c+p drug j(22/03/21) 1		
SOP Name:	mansett ngs.nanc		
General Notes:			
File Name:	VIPUL KUMAR.dts	Dispersant Name:	Water
Record Number:	977	Dispersant RI:	1.330
Material RI:	1.42	Viscosity (cP):	0.8872
Material Absorbtion:	0.001	Measurement Date and Time:	22 March 2021 04:28:21

System

Temperature (°C):	25.0	Duration Used (s):	80
Count Rate (kcps):	178.1	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	10

Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	374.2	Peak 1:	1193	61.1	314.0
PdI:	0.913	Peak 2:	11.43	27.3	2.085
Intercept:	0.963	Peak 3:	208.7	9.6	35.80
Result quality	Refer to quality report			

Size Distribution by Intensity

Intensity (Percent)

Size (d.nm)

Record 977: f1 lipo c+p drug j(22/03/21) 1

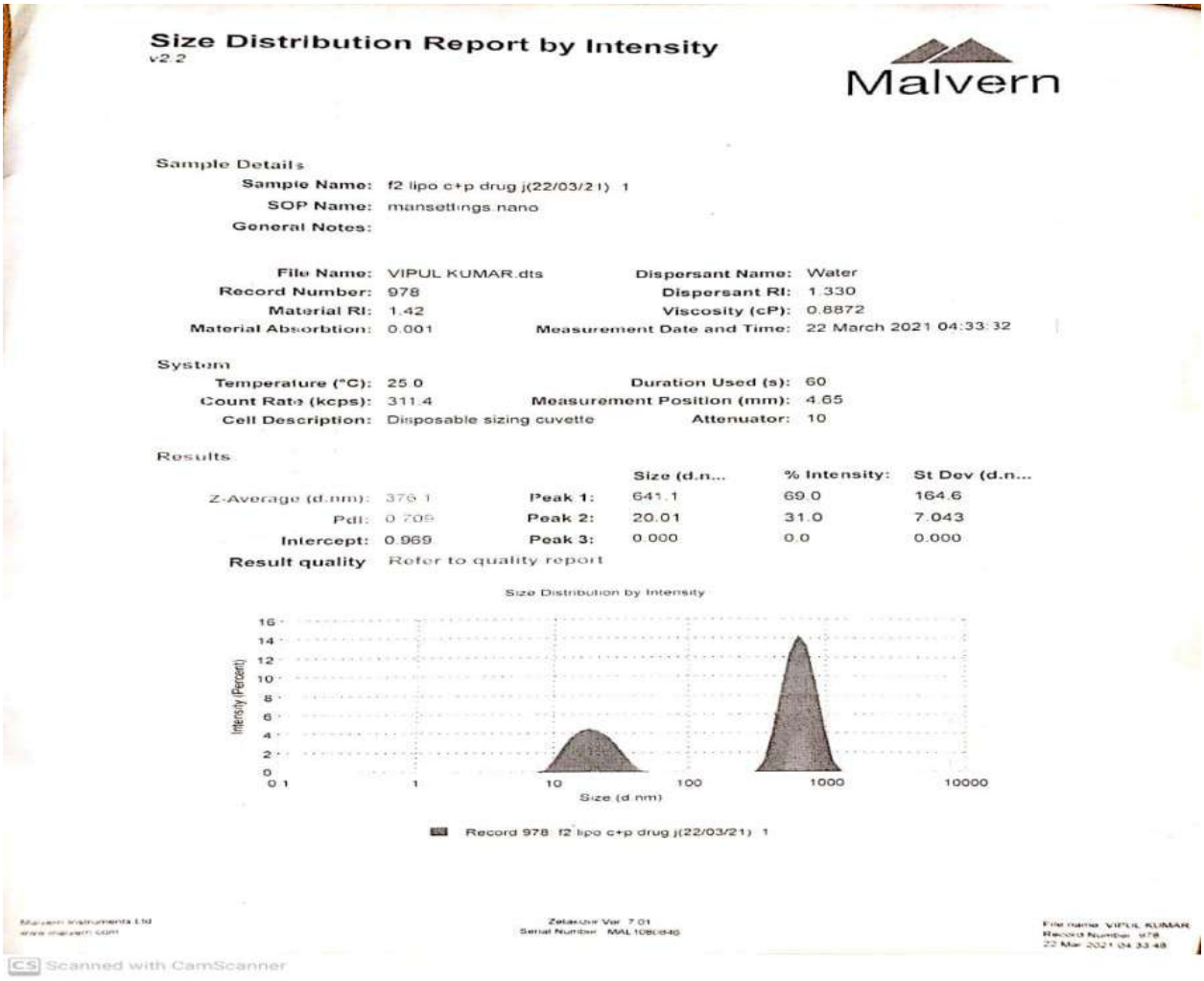
Malvern Instruments Ltd

Zetasizer Ver. 7.01

File name: VIPUL KUMAR
Record Number: 977
22 Mar 2021 04:28:46

Scanned with CamScanner

Fig 10: Particle size analysis of curcuminpiperine liposomes (F1)



Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	f2 lipo c+p drug j(22/03/21) 1		
SOP Name:	mansettings nano		
General Notes:			
File Name:	VIPUL KUMAR.dts	**Dispersant Name:**	Water
Record Number:	978	**Dispersant RI:**	1.330
Material RI:	1.42	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.001	**Measurement Date and Time:**	22 March 2021 04:33:32

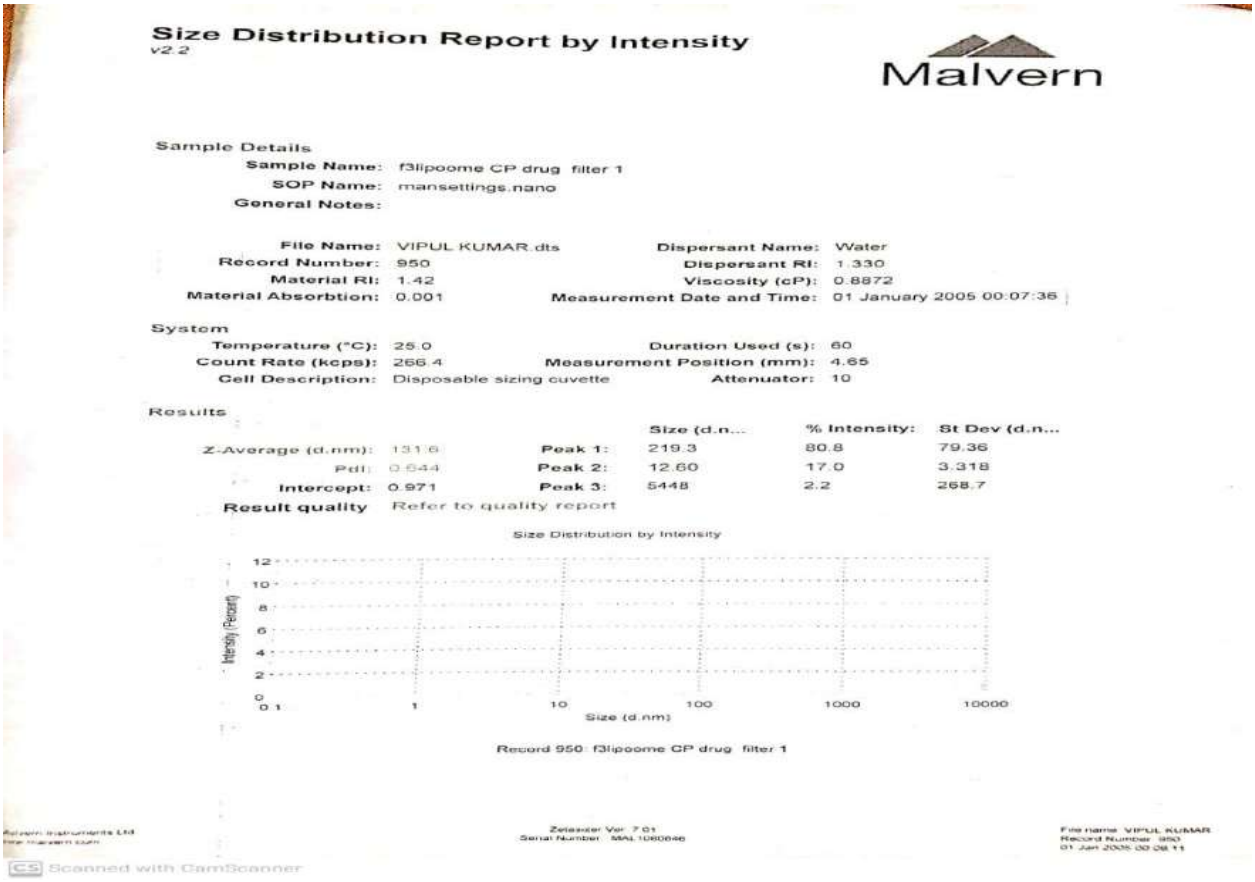
System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	311.4	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	10

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	376.1	**Peak 1:**	641.1	69.0	164.6
PdI:	0.709	**Peak 2:**	20.01	31.0	7.043
Intercept:	0.969	**Peak 3:**	0.000	0.0	0.000
Result quality	Refer to quality report				

Fig 11: Particle size analysis of curcuminpiperine liposomes (F2)



Size Distribution Report by Intensity
v2.2

Malvern

Sample Details
Sample Name: f3lipoome CP drug filter 1
SOP Name: mansettings.nano
General Notes:

File Name: VIPUL KUMAR.dts
Record Number: 950
Material RI: 1.42
Material Absorbtion: 0.001
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: 01 January 2005 00:07:36

System
Temperature (°C): 25.0
Count Rate (kcps): 266.4
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 4.65
Attenuator: 10

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	131.6	Peak 1:	219.3	80.8	79.36
PdI:	0.544	Peak 2:	12.60	17.0	3.318
Intercept:	0.971	Peak 3:	5448	2.2	268.7
Result quality	Refer to quality report				

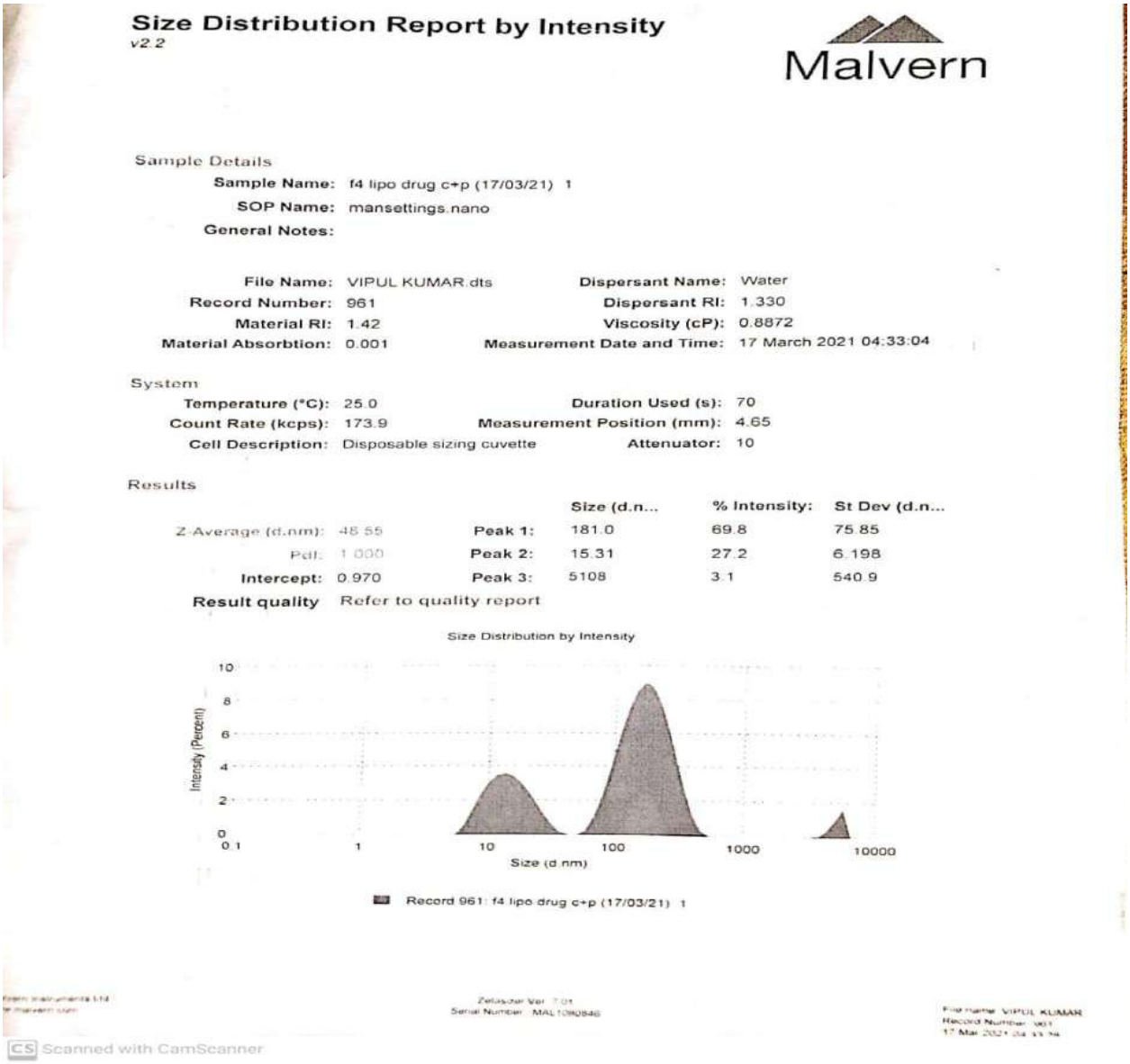
Size Distribution by Intensity

Record 950: f3lipoome CP drug filter 1

Zetasizer Ver. 7.01
Serial Number: MAL1060646

File name: VIPUL KUMAR
Record Number: 950
01 Jan 2005 00:08:11

Fig 12: Particle size analysis of curcuminpiperine liposomes (F3)



Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	f4 lipo drug c+p (17/03/21) 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	VIPUL KUMAR.dts	**Dispersant Name:**	Water
Record Number:	961	**Dispersant RI:**	1.330
Material RI:	1.42	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.001	**Measurement Date and Time:**	17 March 2021 04:33:04

System

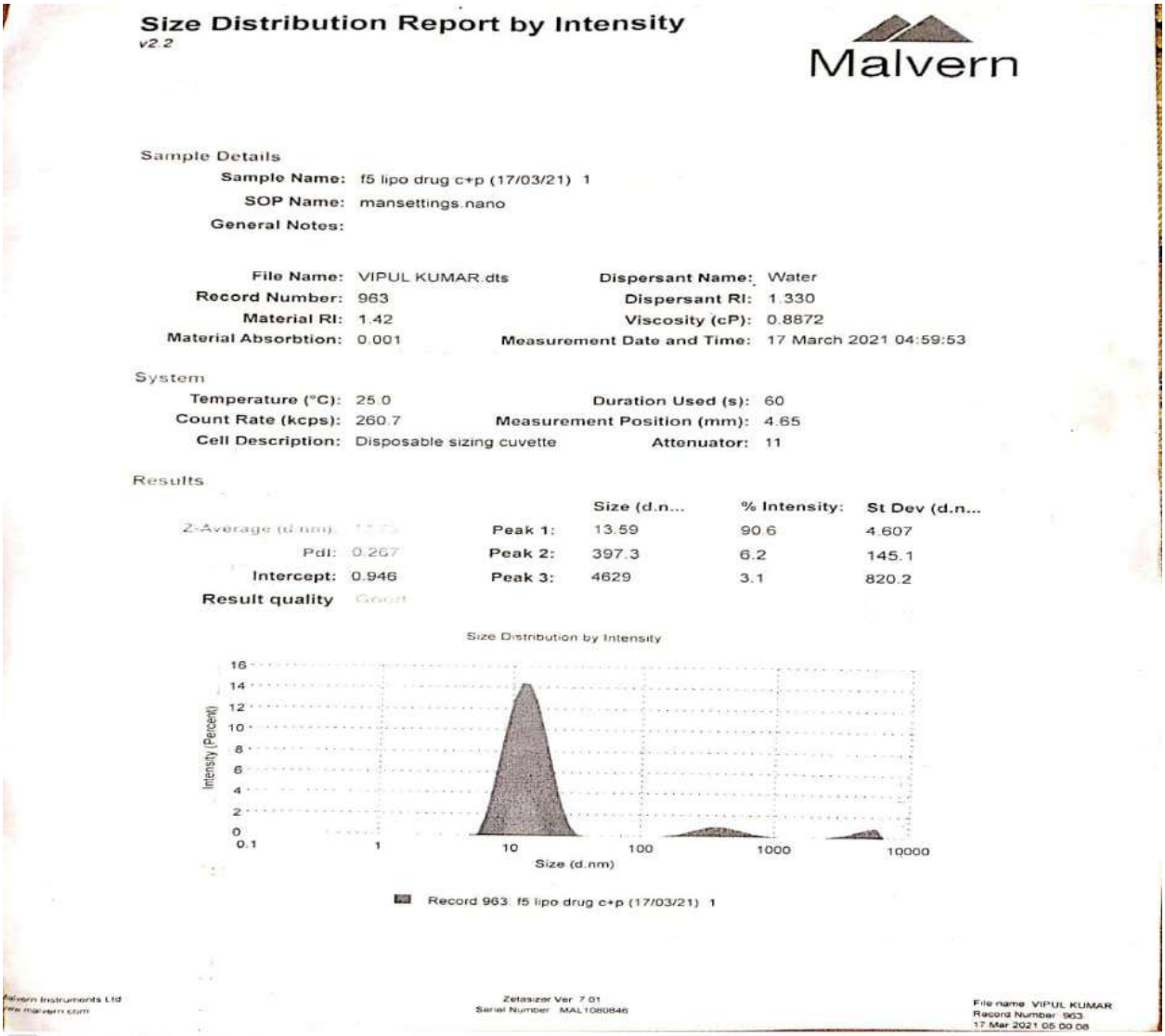
Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	173.9	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	10

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	48.55	**Peak 1:**	181.0	69.8	75.85
PdI:	1.000	**Peak 2:**	15.31	27.2	6.198
Intercept:	0.970	**Peak 3:**	5108	3.1	540.9
Result quality	Refer to quality report				

Size Distribution by Intensity

Fig 13: Particle size analysis of curcuminpiperine liposomes (F4)



Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	f5 lipo drug c+p (17/03/21) 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	VIPUL KUMAR.dts	**Dispersant Name:**	Water
Record Number:	963	**Dispersant RI:**	1.330
Material RI:	1.42	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.001	**Measurement Date and Time:**	17 March 2021 04:59:53

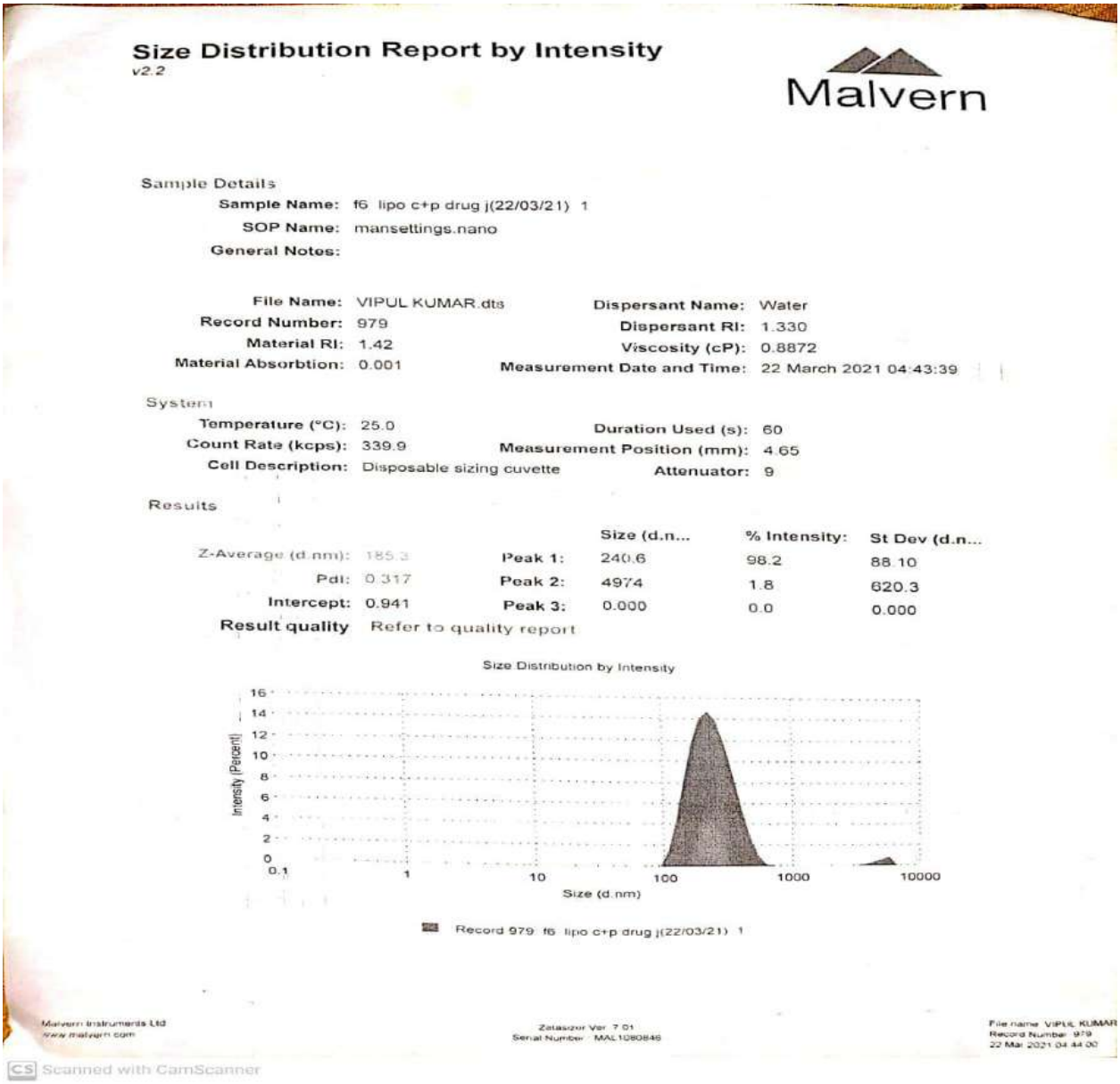
System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	260.7	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	11

Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	Peak 1:	13.59	90.6	4.607
PdI: 0.267	Peak 2:	397.3	6.2	145.1
Intercept: 0.946	Peak 3:	4629	3.1	820.2
Result quality				

Fig 14: Particle size analysis of curcuminpiperine liposomes (F5)



Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	f6 lipo c+p drug j(22/03/21) 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	VIPUL KUMAR.dts	Dispersant Name:	Water
Record Number:	979	Dispersant RI:	1.330
Material RI:	1.42	Viscosity (cP):	0.8872
Material Absorbtion:	0.001	Measurement Date and Time:	22 March 2021 04:43:39

System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	339.9	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	9

Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	185.3	Peak 1:	240.6	98.2	88.10
PdI:	0.317	Peak 2:	4974	1.8	620.3
Intercept:	0.941	Peak 3:	0.000	0.0	0.000

Result quality Refer to quality report

Size Distribution by Intensity

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Zetasizer Ver. 7.01
Serial Number : MAL1080846

File name: VIPUL KUMAR
Record Number: 979
22 Mar 2021 04:44:00

Fig 15: Particle size analysis of curcuminpiperine liposomes (F6)

5.3Reports and results:

The liposomes were prepared by thin film hydration method and 10 formulations were obtained without using drug. The amount of ingredients was changed in different formulation to obtain better size of the formulation. Distilled water and phosphate buffer of pH 7.4 was used as hydrophilic phase in different formulation. Formulation 9 and 10 was obtained under 30 mins of sonication.The size of all formulation was obtained by using Zeta sizer.

The curcumin-piperineliposomes was obtained by thin film hydration method. Total six (6) formulation were obtained by using drug. Before size measuring, formulation 3-6 was passed through membrane filter of 0.45 micron. After that their size was measured using zeta sizer.

Conclusion:

In the present study, an attempt has been made to develop liposomal delivery system of curcumin-piperine for targeted delivery in breast cancer.This study indicates successfully preparation of Curcumin-Piperine liposomes by thin film hydration method using cholesterol, soya lecithin as lipids and tween 80, tween 20 and poloxamer 188 as surfactant and distilled water was used as hydrophilic phase.Preformulation studies were performed for identification of drug i.e. physical appearance, melting point, etc. Phospholipids i.e. soy lecithin, cholesterol used as a carrier for preparation of Curcumin and piperine by thin film hydration method. First liposomes were formulated without using drug and after that drug loaded formulation was prepared.The all liposome formulations were evaluated for mean particle size, polydispersity index. The particle size obtained in the formulation was between (160-760) d. nm without using drug. The particle size obtained in the curcumin-piperine liposomal formulation was between (13-380) d. nm

To increase the efficiency and bioavailability of liposomes, piperine loaded with curcumin liposomes were successfully prepared.