"FORMULATION AND DEVELOPMENT OF LIPOSOME FOR THE TREATMENT OF GASTRIC CANCER"

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in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

BY

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May 2020

CERTIFICATE

This is to certify that the dissertation work entitled "Formulation and development of liposome for the treatment of gastric cancer" submitted by Mr. Dhruv Soni with Regn. No. (18MPH104) in partial fulfillment for the award of Master of Pharmacy in "Department of pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under my/our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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CERTIFICATE OF ORIGINALITY OF WORK

This is to undertake that the dissertation work entitled "Formulation and development of liposome for the treatment of gastric cancer" Submitted by Dhruv Soni (18mph104) in partial fulfillment for the award of Master of Pharmacy in "M.Pharm. Programme" is a bonafide research work carried out by me at the "Name of Department", Institute of Pharmacy, Nirma University under the guidance of "Name of a Guide and Co-guide". I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, this work is original and not reported anywhere as per best of my Knowledge.

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DECLARATION

I hereby declare that the dissertation entitled "Formulation and development of liposome for the treatment of gastric cancer", is based on the original work carried out by me under the guidance of Prof. Tejal Mehta, Professor, and Head, Department of pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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Regards
Dhruv Soni

Contents

ABSTRACT

[1] ABSTRACT

Gastric cancer(GC) one of the common cancer malignancies worldwide. It is on the second rank in death due to cancer worldwide. This high mortality rate is due to late or advance stage diagnosis of cancer; symptoms are begun at an advanced stage of malignancy. Its five-year survival rate is also less and different in all worldwide countries. Treatment of GC is necessary and also there are so many chemotherapies available in market. Problem with this therapy are so many life threatening adverse effect and development of resistance, there are so many project have been started to address such kind of problem. In this project we have also developed liposome of sorafenib for the treatment of gastric cancer. Sorafenib is multi tyrosin kinase inhibitor that is why less chances of development of resistance and also reported for the reverse the drug resistance. We have prepared liposome of sorafenib, as liposome has highest impact on oncology till date, furthermore it is biocompatible, less toxic and easily fabricated. Last but not least by surface fictionalization we can target it to specific site. As the outcome of this project we can get the increased therapeutic efficacy, bioavailability, specific tumor targeting by modifying drug delivery system and the drug loading percentage will also increase with stability, furthermore solubility enhancement of drug that all parameter give efficient therapeutic response with minimal adverse effect.

[2] INTRODUCTION OF GASTRIC CANCER

[2.1]Introduction

Gastric cancer(GC) one of the common cancer malignancies worldwide. It is on the second rank in death due to cancer worldwide(1,2). This high mortality rate is due to late or advance stage diagnosis of cancer; symptoms are begun at an advanced stage of malignancy. Its five-year survival rate is also less and different in all worldwide countries. Japan is the only country where the five-year survival rate is more than 90%(3). However, in a European country, it is between 10%-30%. Early stage diagnosis and thereby it resection are most impactable factor for 90 % survival rate in japan(3,4).

[2.2]Risk factor

Genetic alteration and environmental factors are responsible for the initiation of gastric carcinoma. Dietary factor or habit has an important impact on gastric cancer. Factors responsible for reduces the risk of GC are high intake of fresh fruit and vegetables, casual alcohol drinking, maintaining proper BMI index, diet with less sodium, different food which are preserved in salt, red and high cured meat. Exposure of nitrogen oxide, N-nitroso compounds, and radiation are most proven to GC. A profession like a fisherman, machine operators, nurses, cooks, dry cleaners has the highest susceptibility of GC(5–8).

In 1982, Marshal and Warren discovered the association between H.pylori and gastritis, and also classified as class 1 carcinogen by the International Agency for Research on Cancer. The reason behind more gastric problem is H. pylori infection often, among them only 10 % are susceptible for more severe problems like GC and peptic ulcer (9).

The one significant risk factor for gastric cardia carcinoma is obesity. In some cases gastrectomy was risk factor for GC, after long time of gastric surgery(10).

[2.3] Classification of gastric cancer

Sporadic gastric cancer

This type of GC is more among all GC, and people over the age of 45 are more susceptible to it. It commonly termed “Sporadic gastric cancer”(SGC). The reason behind the development of SGC there are so many environmental factors. Mostly shows in 60-80 yr age group and male are more capable to develop this cancer than females(11).

Early-onset gastric cancer

GC before the age of 45, known as EOGC, and 10% cases among all GC. The genetic factor plays a vital role in its development(11). Females are more susceptible to EOGC because of hormonal changes. SGC and EOGC both differ at their molecular level(12).

Gastric Stump Cancer

This type of cancer develops in the gastric remnant of peptic ulcer surgery after at least 5 years. GSC represents 1.1% to 7% among all GC. Male are more susceptible to it than females. The risk factor of this cancer includes gastrectomy and H.pylori infection; both are the well-established reason for it. After 15 years of gastrectomy risk of developing GSC increases 4 to 7 fold compared to the healthy population(13,14).

Hereditary diffuse gastric cancer

HDGCs is developed by inherent syndrome, among all gene mutation CDH1 gene is most common, and it encodes E-Catherin. This autosomal condition produces diffuse and poorly differentiate GC, which results in the thickening of the stomach wall without distinct mass development. Its concern with 1%-3% among all GC(15).

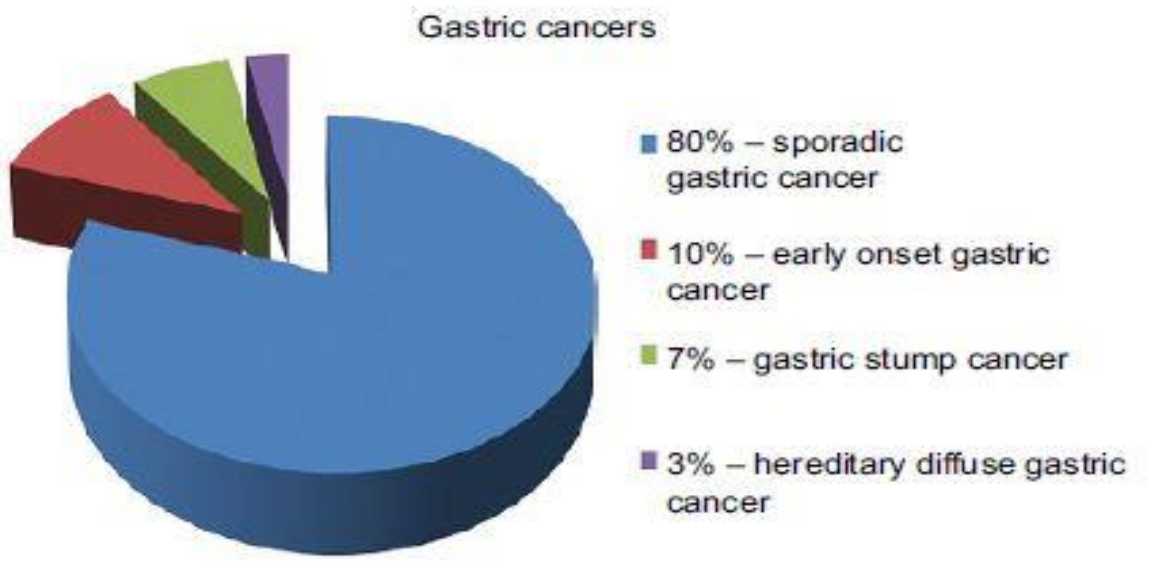


Figure 1:- Classification of gastric cancer(1)

[2.4] Treatment

For the treatment of GC, so many steps has been taking place like chemotherapy; based upon the stage of GC, Gastrectomy is process to remove the cancerous part of stomach, chemotherapy, along with gastrectomy, radiation therapy. The selection of treatment is made by the extent of metastasis.

Chemotherapy most frequently used treatment for majority type of all cancer. Combination of different chemotherapeutic agents shows synergistic effect to kill metastatic cancer cells and also reduces chances of resistance to therapy; however, most common problem associated with cancer cell is to develop resistance to therapy, although it has been addressed by combination of different chemotherapeutic agent. The most commonly used combination therapy are ECF (epirubicin, cisplatin, 5-FU), ECX (epirubicin, cisplatin, capecitabine), EOF (epirubicin, oxaliplatin, 5-FU), and EOX (epirubicin, oxaliplatin, capecitabine).

These all are first-line therapies for GC. Second-line therapies are paclitaxel, irinotecan, docetaxel(16,17).

Chemotherapeutic drug main problem is it gives off-target effect along with cancer cell, that's why it gives life-threatening side effect along with therapeutic effect this result in a decrease in survival rate of patient(21). So many trials has been implemented for site-specific

effect of chemotherapeutic drug. USFDA approved ‘Transtuzumab’ monoclonal antibody, which has site-specificity to HER2 receptors. A combination of trastuzumab with 5-FU has a good effect on HER2 positive GC. Ramucirumab is another site-specific monoclonal antibody that has an affinity to VEGFR-2 receptor responsible for the formation of new vasculature.

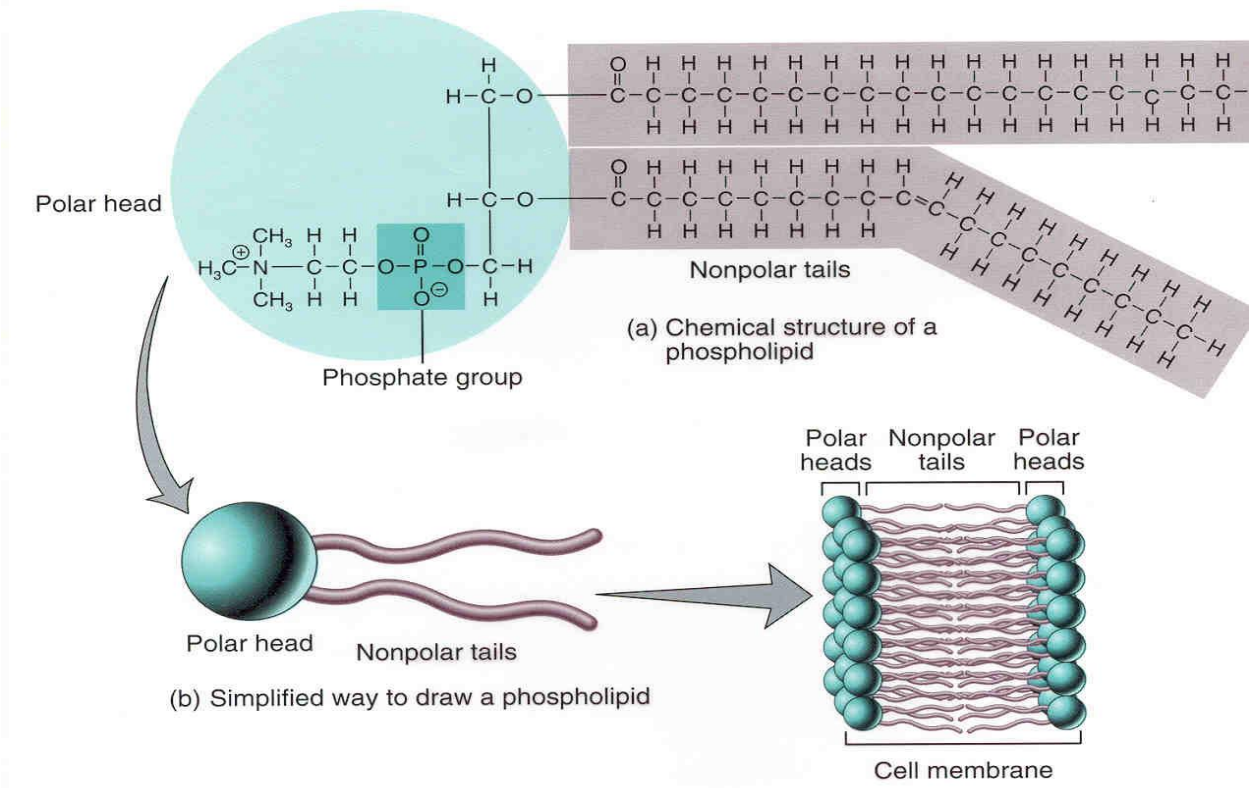
Combination of it with taxel derivatives or irinotecan is mostly used as second-line treatment(18,19).

INTRODUCTION OF LIPOSOME

[3] LIPOSOME

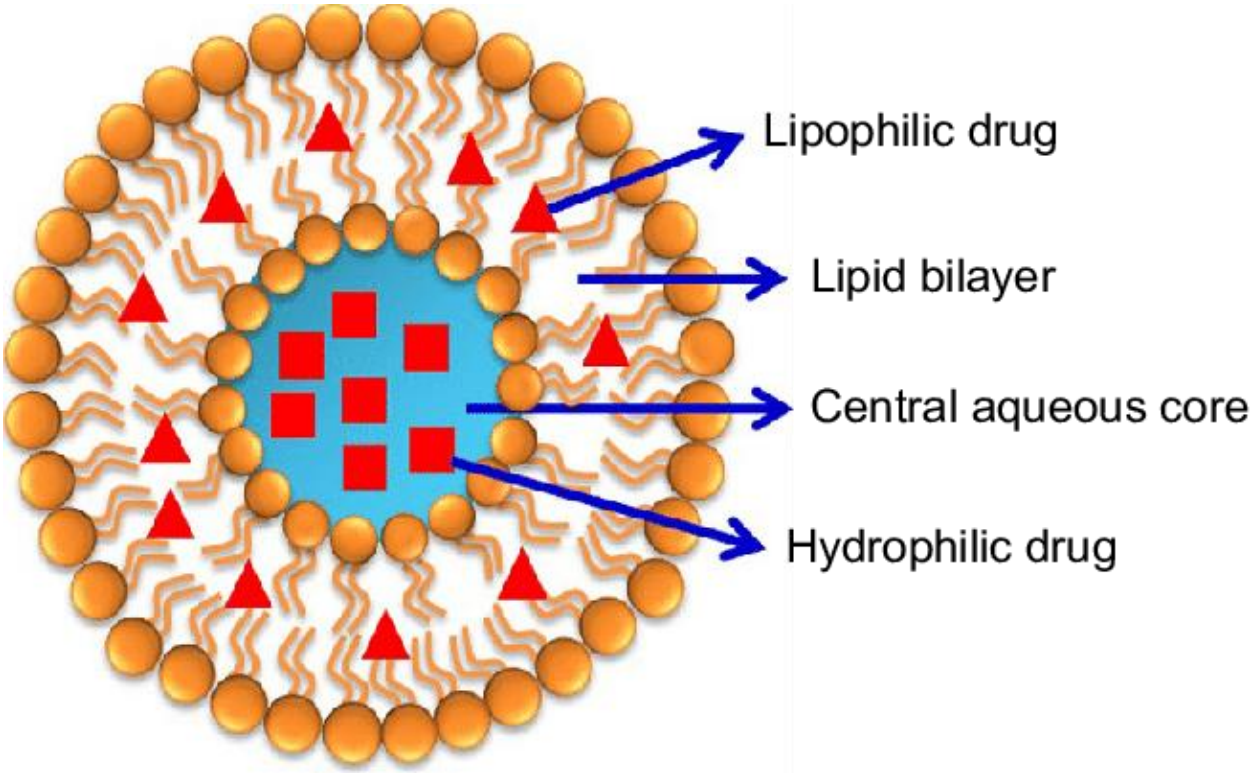
[3.1] Introduction

Liposome are colloidal, vesicular structure made up of different type of phospholipids may one or more in number and able to encapsulate hydrophilic, lipophilic both type of drug. Phospholipids are the same lipid which is in human cell membrane. It has one hydrophilic phosphate head and two hydrophobic chains. Phospholipids always self oriented in bilayer sandwich like structure. Therefore on the basis of number of bilayer liposome are differentiate and divided in different type (20,21).



(http://homepage.smc.edu/wissmann_paul/anatomy2textbook/phospholipids.html)

Figure: structure of phospholipids



(https://www.researchgate.net/figure/Diagrammatic-representation-of-liposom structure_fig2_320243427)

Figure: structure of liposome

When free drug is injected in parenteral route it can able to achieve concentration between therapeutic window for very short period of time, because of it metabolized faster and excreted from body. If drug encapsulated by liposome can able to achieve prolong action of drug as drug get released very slowly from liposome (5, 6).

[3.2] Advantages of liposomes(22)

- Liposomes are bio-degradable, bio-compatible, non-toxic and non-immunogenic.
- Feasible for all type of cargos like hydrophilic, hydrophobic and amphipatic.
- Lipidic coat on drug protect it from external environment.
- It can be target to specific site thereby, we can avoid it exposure to unwanted site and reduce the adverse events.
- Increase the bioavailability of drug and reduce it dosing strength and it frequency also.

[3.3]Disadvantages of liposomes(22)

- Fabrication cost is high.
- Shows short half life.
- Some time leakage of encapsulated drug due to stability problem.

[3.4] Type of lipososmes

Based on structural parameters :-

Unilamellar vesicles

- This type of liposome made up with only one phospholipids bilayer, so it's called unilamellar vesicle.
- Small unilamellar vesicle (SUV): Those types of liposomes are in the size range of 20-40 nm.
- Medium unilamellar vesicle (MUV): Those types of liposomes are in the size range of 40-80 nm.
- Large unilamellar vesicle (LUV): Those type of liposomes are in the size range of 100-1000 nm.

Oligolamellar vesicles

- This type of liposome made up with more than one lipid bilayer mostly 2-10 lipid bilayers.

Multilamellar vesicles

This type of liposomes made up with more than 10 lipid bilayers. This arrangement is resemble to the onion structure. They are in micrometer in size.

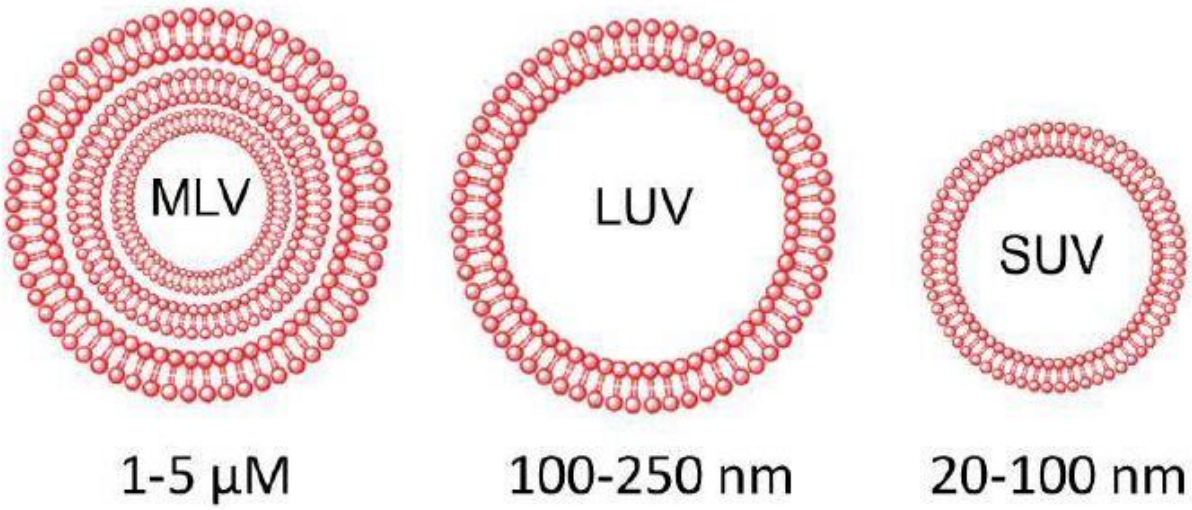


figure: structure of MLV, LUV and SUV with its size range(20)

Based on composition and application

Conventional liposome

That is simple liposome containing phospholipids and cholesterol in its structure and entrapped drug in aqueous or lipiphilic environment. That is also known as first generation liposome. It has neutral or negative charge on it surface.

Long circulating liposome (stealth liposome)

That type of liposomes surface attached with polyethylene glycol (PEG) derivatives. That is responsible for reduce the detection of liposome by reticuloendothelial system (RES) and reduce its clearance from body and increase it circulating time. Those type of liposomes are known as PEGylated liposome.

Cationic liposome

That type of liposome has positive charged surface, positive charge of surface is get by use of specific type of lipid such as DOPE (Dioleoyl phosphotidyl ethanolamine). As we know human body cell membrane is contain negative charge on the surface and liposome has positive charge. Opposite charge induce attraction and that responsible for higher cellular uptake of liposome thereby, high accumulation of liposome in cell which lead to high bioavailability and good therapeutic response.

There is some more type of liposome such as immunoliposome, pH sensitive, Temperature sensitive.

[3.5] Preparation of liposome(22–26)

The liposomal preparation method selection is mostly based on this following parameter .

- Physicochemical properties of lipid from which liposome going to made.
- Physicochemical properties of drug or material to be entrapped.
- Batch size and also possibility for scale up the liposome
- Also compatibility of lipid and drug with manufacturing processing materials.

Preparation of liposome carried out in two method.

- General method
- Solvent dispersion method

General method

The lipid material and cholesterol are dissolved in organic solvent. Organic solvent selection based on solubility of lipid and drug, often chloroform and methanol used in specific ration. On homogeneous mixing of lipid, drug in organic solvent, evaporation is carried out on above glass transition temperature of lipid to prepare thin film at bottom of round bottom flask. After complete drying of film it is hydrated with buffer solution that can also contain drug. Addition of drug is in lipid solvent or buffer solution based on its solubility. Shake the flask till all film gets dispersed in it, that dispersion contains MLV liposome. sonication of this solution lead to formation of SUV. Free drug in liposomal solution is separated by gel chromatography.

Solvent dispersion method

In this method lipid and cholesterol are dissolving in organic solvent furthermore, if drug is lipid soluble than added it in organic solvent mixture. Addition of aqueous solution that can be buffer solution or water for injection and if drug is soluble in aqueous medium than added it in. gradual addition of water in organic solvent tends to form liposome structure.

Main two methods: 1. Ethanol injection method

2. Ether injection method

Ethanol injection method

Lipidic content dissolve in ethanol and it is injected in buffer solution contain drug to be entrapped by needle gradually. This addition leads to formation of liposomal dispersion of SUV (size range about 25nm)

Ether injection method

This method is same as above method but organic solvent used is ether. Disadvantage of this method is high risk of oxidative degradation , long time required for this process and careful control required to add lipid into aqueous solution.

Sonication method

This method use to form SUV from the dispersion of LMV by giving it sonic energy. Mainly two type of sonicator used bath sonicator and probe sonicator. Probe sonication direct give sonic energy into lipid dispersion by probe but due to some disadvantages this method is less applicable. This method is responsible for the heating solution during process due to sonic energy leads to degradation of liposome and also some contamination chances of probe into dispersion. Therefore bath sonicator widely usedin this method place the vessel full of dispersion in to bath sonicator give temperature above lipid glass transition temp and sonicateit for 5-10 min. parameter influence are sonic energy, time, temperature of bath etc.

Extrusion method

This method use for the prepare liposome with homogeneous size distribution from MLV by passing through polycarbonate membrane. Whole process must be handled above $T_{c..}$ Above transition temperature liposome are flexible so size reduction must be easier without damage to liposome. There are different types of polycarbonate membranes are available 0.2µm, 0.05µm, 0.08µm.

[3.6] Available marketed liposome for cancer treatment(20)

Active Ingredient	Liposome Composition	Cancer Type Being Targeted
Doxorubicin	HSPC/DSPE/cholesterol (12.5:1:8.25 molar ratio)	Colorectal (in-vitro)
Doxorubicin	Cholesterol, DSPC, DSPE and DSPE-PEG2000 (10 _mol total phospholipid)	Prostate cancer (in-vivo/in-vitro
Doxorubicin	HSPC: cholesterol: lipid with a PEG head group (DSPE-PEG2000) (molar ratio 56.4:38.3:5.3)	Colorectal (in-vitro)
Doxorubicin	1-Palmitoyl-2-oleoylphosphatidylcholine: cholesterol (molar ratio 55.8:44.2)	Metastatic (clinical trial & in clinic)
Daunorubicin	DSPC:cholesterol (molar ratio 2:1)	Kaposi's sarcoma
All trans retinoic acid	DPPC:cholesterol:1,2-distearoyl-sn-glycero-3-phosphoethanolamine -Methoxy PEG2000 (molar ratio 6:3:1)	Human Thyroid carcinoma (in-vitro)
Mitoxantrone	HSPC: DSPE-PEG2000: cholesterol: anacardic acid (molar ratio 0.55:0.05:0.35:0.05)	Melanoma cell lines (in-vitro)
Paclitaxel	Egg phosphatidylcholine: cholesterol: TPGS1000-TPP (molar ratio 88:3.5:8.5)	Lung cancer cell lines (in-vivo & in-vitro)
Irinotecan		Pancreatic ductal adenocarcinoma

INFORMATION OF API

[4] SORAFENIB

Sorafenib is multitargeted kinase inhibitor of VEGFR 1, 2, 3, EGFR, PDGFR along with pathway inhibitor responsible for GC. Most common problem with the single targeted tyrosine kinase inhibitor is that when give it to tumor cell then one pathway inhibited for progression and metastasis but other one will get pop up for progression and metastasis, therefore no any therapeutic effect seen. That problem can be addressed by giving the multikinase inhibitor. Nevaxar is a tablet dosage form containing sorafenib and approved for the hepatocellular carcinoma (HCC) and renalcellular carcinoma (RCC) by USFDA. Only available dosage form in market. There are number of phase 1 clinical trial in that sorafenib used as single main chemotherapeutic drug including pancreatic cancer. There are several phase I, II, and III clinical trials in that sorafenib used as chemotherapeutic agent with combination effect of other chemotherapeutic agent to get synergistic and high therapeutic efficacy(27–29).

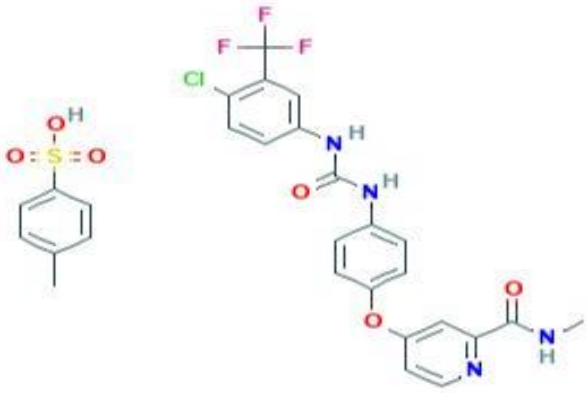
This all evidence give strong rationale to use sorafenib in treatment of GC, despite this drug has several adverse effects and also solubility issue because it has very less aqueous solubility with extremely low oral bioavailability which works as challenges in treatment of cancer by sorafenib. To address this problem we went for nano drug delivery system, righ now in market only tablet dosage form is available, therefore in this study, we went for the preparation of sorafenib loaded liposomes in treatment of pancreatic cancer, by the available literature and experimental work.

liposome had the greatest impact on oncology to date, because of their size, biocompatibility, biodegradability, hydrophobic and hydrophilic characteristic, low toxicity and immunogenicity. Last but not the least, SF had high hydrophobicity and good lipid affinity. Thus, liposome was chosen as the nanovector for the Sorafenib delivery(30).

Chemical formula; $C_{28}H_{24}ClF_3N_4O_6S$

"IUPAC name; 4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methylpyridine-2-carboxamide;4-methylbenzenesulfonic acid"

Structure;



Molecular weight; 637 g/mol

Description

It is tosylate salt of sorafenib, this compound responsible inhibition of growth signaling pathways and also angiogenisis. It is inhibit cell growth by stop the MAF/MEK/ERK pathways by inhibition of RAF kinase enzyme also, furthermore, it can block different VEGFR and PDGFR that are responsible for formation of angiogenesis.

Physical properties

Description:- off-white amorphous powder.

Solubility:- insoluble in water, soluble in methanol and DMSO.

Partition coefficient:- 3.8

BCS class:- 2

Pharmacokinetics

Absorption

When we compare oral tablet bioavailability with oral solution it was found 38-40%. It takes 3 hr to reach at its peak plasma concentration on oral administration. On fasting condition its gives its maximum bioavailability but if cunsuption with high fat meal then bioavailability reduces by 29%.

Metabolism

Liver is the main site for sorafenib metabolism, in it undergoes oxidative metabolism that done by CYP3A4 furthermore, glucoronidation by UGT1A9. Sorafenib accounts for approximately 70-85% of the circulating analytes in plasma at steady- state. There are mainly 8 metabolites have been found in human plasma among them 5 have been detected in human plasma. The pyridinr N-oxide is the main circulating metabolite of sorafenib found in blood. It has same potency os sorafenib in vitro.

Protein binding

99.5% of total drug are bound to plasma proteins.

Rout of elimination

On 100 mg sorafenib administration 96% of its total dose eliminate within 14 day, 77 % from feces and 19 % from urine by glucoronidates metabolites.

Half life

25-48 hr

Clinical pharmacology

Mechanism of action

Sorafenib responsible to block many receptor and kinase such as VEGFR, PDGFR responsible for initiation of angiogenesis, furthermore inhibits RAF kinase that is responsible to regulate pathways for cell growth.

Indication and usage

It is first-line therapy for the treatment of unresectable hepatocellular carcinoma and also advanced renal cell carcinoma with the combination of another chemotherapeutic agent. This is approved by FDA for both cancer treatment.

Adverse effect

Hepatotoxicity

In large number of clinical trials elevated serum aminotransfarase level obsereved , but value greater than 5 times to upper limit was observed in only 1-3 %. If therapy was stopped then recovery shown good as compare to contineuos therapy. Furthermore, some case shown hepatic failure and progression in liver injuries.

Other common adverse events associated with chemotherapeutic agents.

HYPOTHESIS

[5] HYPOTHESIS

For selection of this investigation there are some reasons for that as follows.

[5.1] Current available marketed formulation of sorafenib tosylate

Sorafenib tosylate is currently available in only tablet dosage form.

Brand name:- NEXAVAR

Inventor company: Bayer Healthcare Pharmaceuticals LTD.

Available dosing strength: 200 and 400mg per tablet.

[5.2] Drawbacks of available dosage form:-

- Tablet undergoes first-pass metabolism
- Required higher drug strength to reach therapeutic window
- As increasing drug concentration, increasing adverse events
- Less site specific
- Life threatening side effect due to reach drug at unwanted site of action
- Solubility problem
- Bioavailability problem

Tablet dosage form facing problems mention above, this proposed investigation is for an address such type of drawbacks of conventional dosage form.

Selected dosage form in this investigation is liposome.

[5.3] Reasons for selection of liposome as drug delivery vehicle:-

- Drug sorafenib is highly lipophilic in nature so it has good solubility in a lipid environment, and liposomes are the lipidic nanocarrier. Therefore, solubility issue of drug can be addressed by liposome.
- Liposome consists of high amount of lipid in it structure and this lipids are phosphobilayer lipids, which is biocompatible in nature and non-toxic.
- Here we prepare treatment for gastric cancer, and as we know, all the chemotherapeutic dosage forms must be site-specific to eliminate adverse events. Liposomes can be given to a specific site by its surface functionalization. There is so much evidence available for liposome surface functionalization to give site-specific action.
- Liposome has higher bioavailability, and more in-vivo efficacy compare to an available tablet dosage form, and this thing has been proved.
- Liposome's lipidic environment able to hold a high amount of lipophilic drug, therefore, we can say that drug encapsulation will be more, and it will directly impact dosing strength and frequency.

[5.4] Reasons for selection of sorafenib as a therapeutic agent:-

- Sorafenib is a multi tyrosine kinase inhibitor, which is responsible for the inhibition of more than two tyrosine kinases simultaneously. Such as VEGFR, EGFR, PDGFR, etc. this characteristic of sorafenib gives fewer chances to tumor get resisted with it.
- Sorafenib also responsible for the inhibition of pathways that are responsible for the initiation of gastric cancer, furthermore it induces pathways responsible for cancerous cell apoptosis. Pathways like RAF/MEK/ERK/STAT 3.
- Sorafenib also reposted for reverse the drug resistance to tumor cells (31).

[5.5] Reasons for selection of a parenteral route for drug delivery:-

- As we discuss earlier sorafenib undergoes the first-pass metabolism by the oral route, therefore to achieve therapeutic concentration higher dosing strength and that lead to adverse events, so to address this problem parenteral route is the best choice.
- Furthermore, we can not achieve high bioavailability and site-specificity by the oral route.

The proposed investigation have selected based on these reasons.

LITERATURE SURVEY

[6] LITERATURE SURVEY

Survey on liposomes:-

Meng lie et al(32)***.:-*** has proposed a dual functionalized liposome of paclitaxel with sorafenib to get the synergistic effect on the tumour with reverse multi-drug resistance. They prepared hyaluronic-acid coated liposome that contains TPGS cationic lipid and paclitaxel, sorafenib both as a chemotherapeutic agent. Prepared liposome showed good plasma stability in rat plasma and in the presence of HAase liposome was capable to reversing zetapotential and acidic environment. In vitro study on drug resisted tumour cell line showed that prepared liposome able to inhibit P-gp efflux pump and effectively able to inhibit cell growth. In summary, prepared multifunctional liposome exhibited the excellent potential for treating the MDR tumor by the synergistic effect of PTX and SOR, and it could be a potential nano-carrier for reversing the MDR and improve efficacy of therapy.

Zhang et al.(33) ***:*** They have prepared liposome with dual function for targeting and reverse or reduce the resistance hepatocellular carcinoma acquired by drug. Preparation of liposome was carried out by polymeric nano-biomaterial, therefore liposome can target and reverse the drug resistance acquired by hepatocellular carcinoma. Prerared mitoxatron loaded liposome had 100 nm particle size and entramptment efficiency was 97.3%. The cytotoxic and cellular uptake were evaluated in-vitro on BCRP overexpressed cell line and results were found good with increasing 14.9 fold concentration. Pharmacokinetic study was carried out on rat and it shown prolonged the circulation time of MX liposome compare to free MX and also increased bioavailability.

Olusanya et al.(34) ***:*** This is review article on delivery of anticancer drug in liposomal drug delivery system. Cancer is one of the most life threatening disorder. The first-line therapy of cancer is removal of solid tumour by surgical procedure, thereby radiation therapy and chemotherapy. Most of chemotherapeutic agent are cytotoxic agent and give it effect to both normal cell and cancereous cell that one of the most disadvantage of chemotherapeutic agent. Therefore, its became challenge to target it to only cancer cell and save other normal body cells with its effect. This review article focus on encapsulation of chemotherapeutic agent in to

liposome that can targeted to cancerous cell by the different approaches and that approaches give reduce side effect by specific targeting.

Hong et al. (32) ***:*** They had prepared ginsenoside multifunctional liposome for combination therapy of gastric cancer. Approved gastric cancer treatment has so many disadvantages and it form drug resistance that limits the use of availale approved chemotherapy. Therefore in this research article researcher prepared multifunctional liposome that contain ginsenoside. Ginsenoside it self work as cytotoxic agent, it is able to kill cancereous cell and furthermore, it has structural similarity with cholestrerol and also has stabilizing capacity which cholestrerol has. Ginsenoside has also affinity with glucose related transporter that are over expressed in cancereous cell. They prepared liposome with thin film hydration method. In conclusion they found that prepared PTX loaded liposome shown significantly reduction in GC tumour growth and excellent result compare to reported PTX formulation like Abraxane©, Lipusu©.

Survey on effect of sorafenib on cancer

Huang et al.(31) ***:*** they proposed a article it says sorafenib reverse the resistance of gastric cancer to treatment by cisplatin. Cisplatin already approved agent for GC treatment but it resistance to tumour cell limit its use. To investigate effect of sorafenib they treated SGC7901/DDP cell line with different concentration of sorafenib . in evaluation they found that SRF inhibits the proliferation of GC cell line with and without cisplatin in both cases. Furthermore, level of MDR1, p-Aktand p-ERK were significantly decrease after treatment of sorafenib. In conclusion they confirmed that sorafenib is responsible for down regulation og MDR1 in GC cell thereby it reverse the resistance of drug.

Ibrahim et al. (35) ***:*** This is review article on sorafenib single and combination therapy in treatment of cancer. Sorafenib is multikinase inhibitor which gives it effect by disturbing the tumour microvasculature through anti-proliferative and anti-angiogenic effect. Multiple target can inhibited by sorafenib like Raf serin/threonin kinases, different vasculature endothelial growth factor receptor (VEGFR-1,2,3) and pletlet derived growth factor β (PDGFR). This all receptor are overexpressed in human cancer. Furthermore, responsible for induce cell apoptosis. Therefore sorafenib is agent shows excellent activity in cancer like renal cell carcinoma (RCC), hepatocellular carcinoma and thyroid cancer. Sorafenib is approved by FDA in treatment of RCC and unresectable hepatocellualr carcinoma. Also many clinical trial have been adapted to find effect of sorafenib on different type of cancer.

Janjiagin et al. (36)***:*** This research article of phase 2 clinical trial of sorafenib in refractive metastatic esophageal and gastroesophageal junction cancer. Patients were given 400 mg twice daily sorafenib who's enrolled for esophageal and gastroesophageal junction cancer. In result they found among 34 patients PFS was 61%. Median PFS was 3.6 months and overall survival was 9.7 months. In conclusion they found sorafenib was able to disease stabilization and increase the PFS in patient with esophageal and GE junction cancer.

Zang et al. (37) ***:*** They prepared lipid coated nano-diamonds of sorafenib to improve it efficacy in treatment of resisting metastasis gastric cancer. Prepared nano-diamomds shown great improvement in bioavailability around 14.95 fold. Furthermore, effectiveness of it performed on xenograft model and it evaluation shown that it inhibit tumor growth that was verified by histological examination. Therby, they concluded that lipidic coated nano-diamonds could be a great and effective platform for improve the bioavailability of lipophilic drug.

Material & Methodology

[7] Material

Name of material	Company
Soya phosphotidyl choline	HIMEDIA
Cholesterol	HIMEDIA
Sorafenib tosylate	Intas pharma (Gift sample)

Equipment

Equipment	Company
Uv-visible spectrophotometer	Shimadzu, japan
Optical microscope	Olympus CXZ1ILED, India
Centrifuge	Remi R24, India
Rotary vacuum evaporator	Buchi
Particle size analyzer	Horiba

[8] Methodology

Preformulation studies

Uv-visible spectroscopy

Standard curve in methanol: phosphate buffer saline (pH 7.4)

Prepared 50 ppm stock solution, by dissolving sorafenib in 5ml methanol then volume make up to 100 ml with 7.4 pH PBS solution. From this stock solution further dilution were prepared 2 ppm, 4 ppm, 6 ppm…30 ppm, and take their observation in uv-visible spectrophotometer at 265 nm.

We have used 5% methanol solution because sorafenib is soluble in methanol, and then volume make up with PBS. Ratio 95:05 (PBS:methanol)

Differential scanning calorimetry

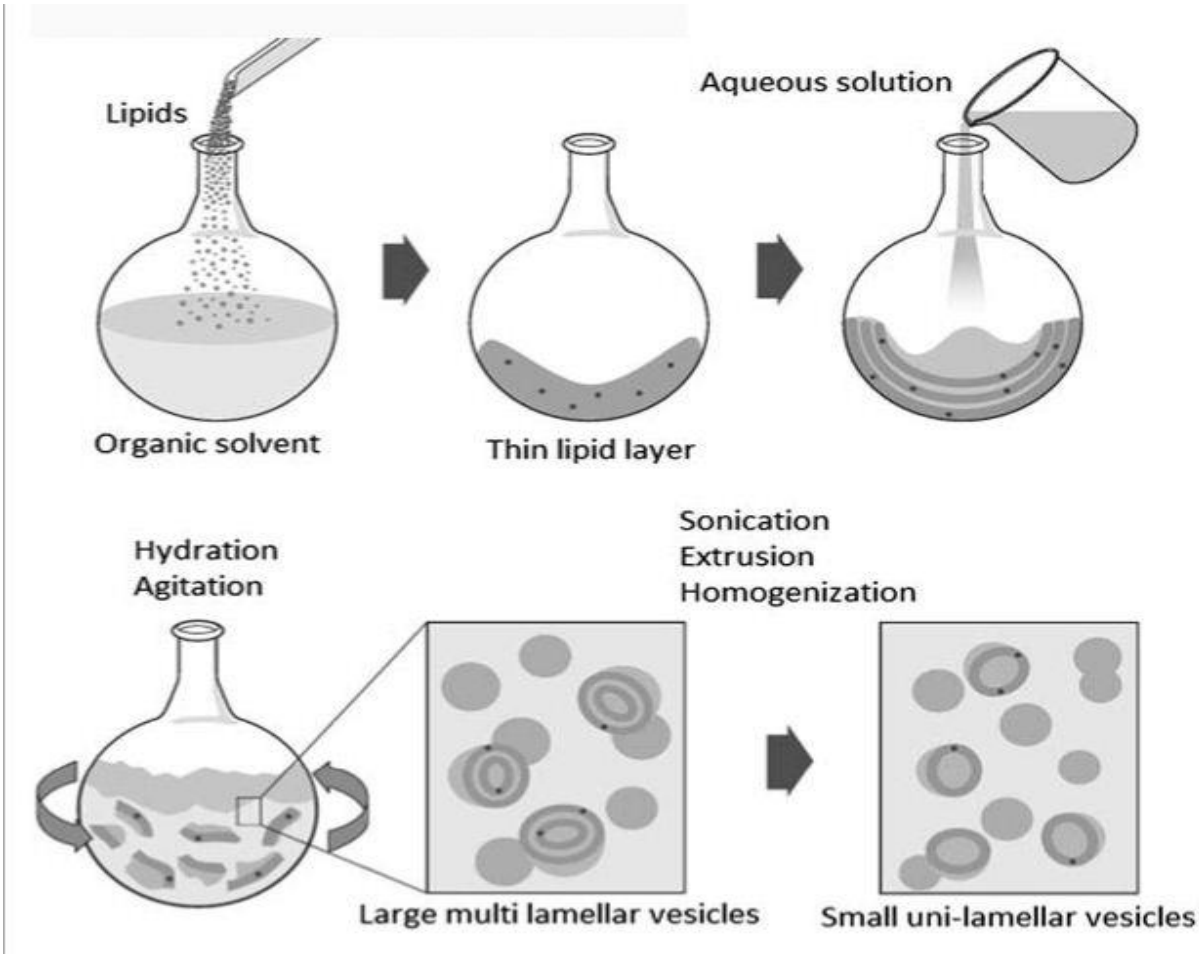
This was carried out to check drug purity and identity by the mean of verifying its melting point. This process was carried out on differential scanning calorimeter.

Solubility study of drug in phospholipids

Selection of phospholipid for liposome is based on literature and drug solubility in it. Solubility studies were carried out by dissolving 2 mg of drug in phospholipid gradually till saturation came. During whole process set temperature was slightly above to its melting point.

Preparation of liposome(38,39)

Preparation of liposome was done by thin film hydration method. Phospholipid and cholesterol were dissolving with mixture of chloroform and methanol (2:1) in 100ml round bottom flask. Shake it till all lipid and cholesterol part get mixed homogeneously. Furthermore, sorafenib is lipid soluble drug and as we have done passive loading so sorafenib was also dissolved in lipid and organic solvent mixture. Now this solvent mixture connected to rotary vacuum evaporator to evaporate all the organic solvent and for formation of thin-film of phospholipid. After complete evaporating of organic solvent film was placed in vacuum chamber for overnight to extract all remain residue of organic solvent. Hydration of film was carried out by PBS pH 7.4, as we have already added drug in lipid solution so no any drug in buffer solution. After complete dispersion of film in buffer solution, it is subject to probe sonication to convert MLV to SUV. During sonication process 2-8 ◦C controlled temperature is required. After sonication liposome are subject to further evaluation.



(https://ebrary.net/61019/engineering/lipid_film_hydration_method_preparation_liposomes)

Processing condition:-

Parameter	Condition
Temperature of water bath	40 ◦C
RPM of rotary vacuum evaporator	25-30
Vacuum of rotary vacuum evaporator	-200 mmHg
Duration	Till dry film form

[9] Characterization

Particle size:-

Horiba SZ 100 particle size analyzer was used to analyze particle size. Firstly, prepared liposome base formulation and then dilution of sample with triple distilled water and evaluated. This system analyzed D10, 50, 90, Z average, polydispersity index.

% Entrapment Efficiency:-

Entrapment efficiency of liposomes was determined using the Sephadex G-50 minicolumn centrifugation method. The unentrapped drug in liposomal formulation was separated using the Sephadex G-50 minicolumn centrifugation method. The separated liposomes were than disrupted using chloroform methanol 1:1 mixture and analyzed for drug content by UV- spectroscopy method.

$$\%EE = \frac{\text{DRUG LOADED}}{\text{TOTAL DRUG ADDED}} \times 100$$

Found absorbance put in equation y = mx + c derive from standard curve and find the concentration of drug loaded in liposome. To estimate %EE put the value of drug loaded and total drug added in above equation.

Differential scanning calorimetry:-

101-400 º C temperature range was done DSC analysis and DSC-60 series equipment by DSC thermogram possible any interaction of API and excipient can determine.

In vitro release study :-

***Diffusion bag method*:**

This method also known as dialysis bag method, and this method was prepared by dialysis membrane tying edges. Dialysis membrane was uses and this membrane molecular weight between 12000 – 14000. Nano Structure lipid carrier dispersion was added to bad deep in release media and 150-200 rpm stirring speed & 32 ºC temperature stirred. Sample was taken different time and analysed by UV spectroscopy.

Experimental work

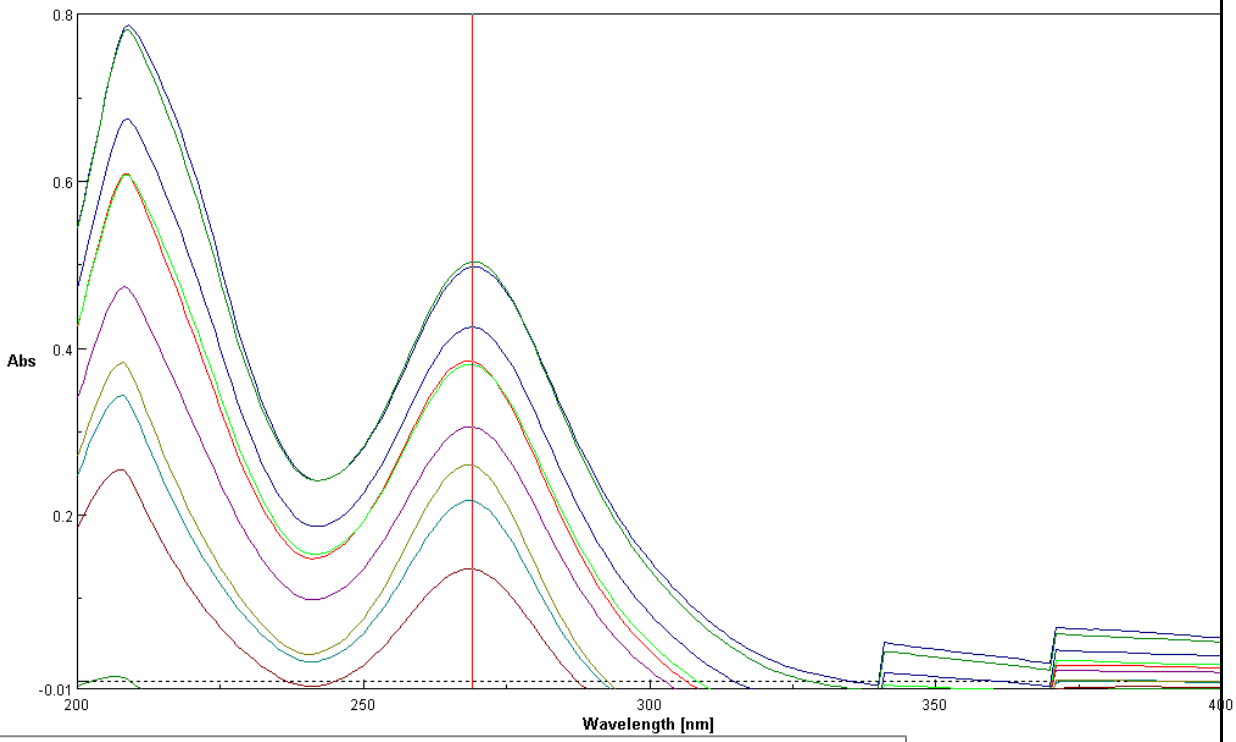
[10]Experimental work

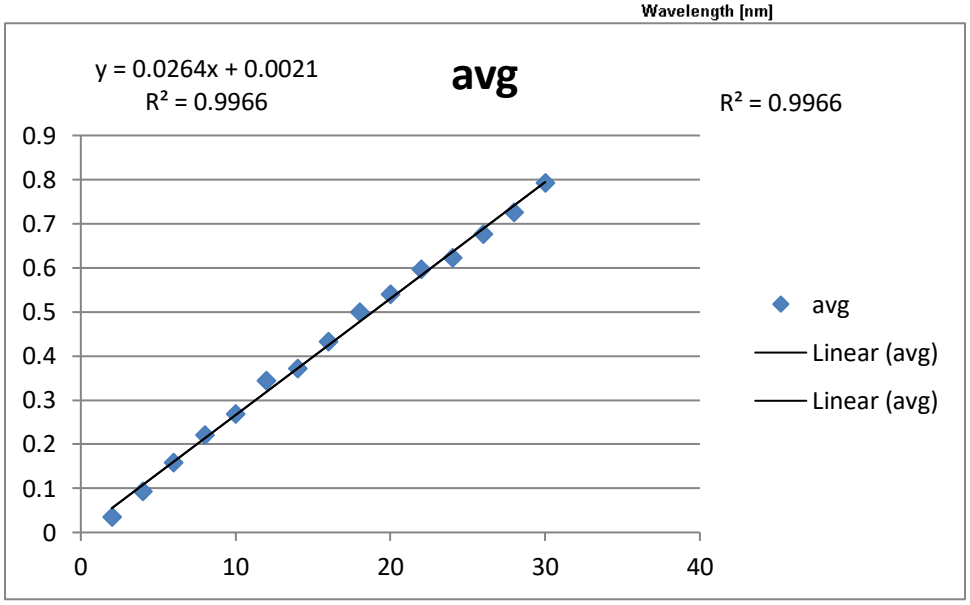
[10.1] Preformulation study :-

Uv-spectroscopy data:-

Standard curve in 5% methanol PBS solution.

Concentration	1	2	3	Average
2	0.0873	0.033	-0.0147	0.0352
4	0.136	0.0918	0.0483	0.092033
6	0.1984	0.164	0.1124	0.158267
8	0.2555	0.2272	0.1775	0.220067
10	0.2986	0.2803	0.227	0.268633
12	0.3586	0.3633	0.3094	0.343767
14	0.3882	0.4076	0.3171	0.370967
16	0.4389	0.4678	0.3888	0.431833
18	0.4919	0.5314	0.4738	0.499033
20	0.5386	0.5906	0.4884	0.5392
22	0.599	0.6444	0.5429	0.595433
24	0.6108	0.6854	0.5704	0.6222
26	0.6872	0.7425	0.597	0.675567
28	0.7087	0.8142	0.6532	0.725367
30	0.7699	0.8622	0.7424	0.7915





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-

Parameter	Value
Slope	0.996
Correlation coefficient	0.026
Intercept	0.002

[10.2] Differential scanning calorimetry data:-

- It was carried out to check drug purity and identity.
- Found melting point is <u>229.2 degree C.</u>
- Melting point of pure sorabenib 229-232 ◦C
- As both melting point same that confirms its identity.

```
Module:            DSC                 Temperature Program:                    Comment:
Data Name:         SRB T                     Cel  Cel  Cel/min  min    s     Operator: Kh
Measurement Date:  26-11-2019  1*          30  240       10    0  0.5     Gas1: N2
Sample Name:       SRB T                                                    Pan: Al
Sample Weight:     2.000 mg                                                 Drug
Reference Name:    Empty
Reference Weight:  0.000 mg
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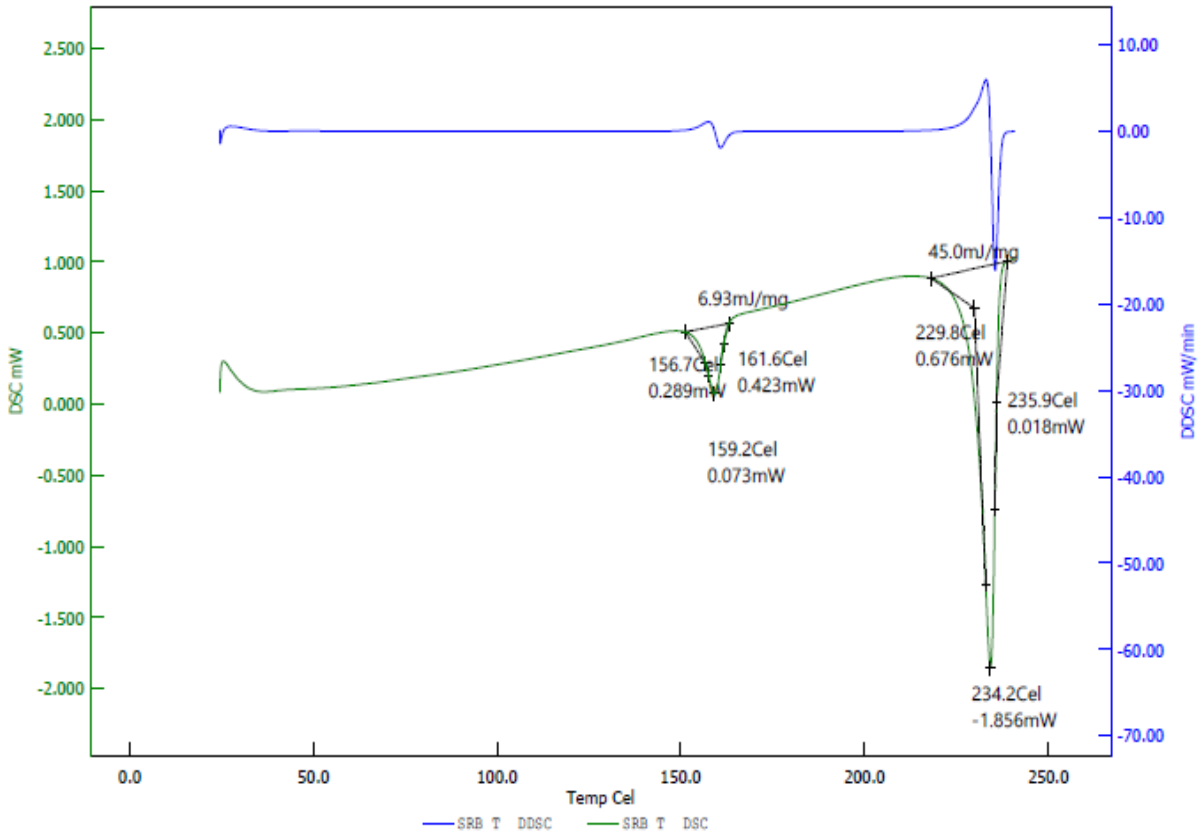


[10.3] Selection of phospholipid & solubility:-

- Selection of phospholipid is based on literature review and availability.
- 25 mg of drug get dissolved in 1 gm of lipid.

Selected phospholipid	Soya phosphotidyl choline
Amt. of drug dissolve in it	25 mg/gm

[10.4] Experimental trials :-

Excepients required for preparation batchs:-

Exepient	Purpose
Soya phosphotidyl choline	Phospholipid
Cholesterol	Stabilizer
Sorafenib tosylate	API
Chloroform	Organic solvent
Methanol	Organic solvent

Trial based on different ratio of phospholipid & cholesterol:

Sr. no	Batch	Parts	
		Soya phosphotidyl choline	Cholesterol
1	B1	9	1
2	B2	8	2
3	B3	7	3
4	B4	6	4
5	B5	5	5
6	B6	4	6

- Each contain total 500mg of lipid and cholesterol + 10 mg drug

Particle size data of above batch:

Sr no.	Batch	PDI	Particle size data (nm)				
			D10	D50	D90	Mean	Z-AVG
1	B1	0.36	168.2	200.8	350.7	286.1	290.5
2	B2	0.285	147.7	203.7	287.7	211.2	189.5
3	B3	0.375	288	359.4	480.8	375.6	400
4	B4	0.581	450.3	509.1	640	533	497.3
5	B5	0.666	390.8	457.3	673.2	506.6	612.1
6	B6	0.542	504.2	652.1	684	613.3	768.8

- As we can see batch B2 has lowest particle size with good PDI so we can optimize this batch for further optimization.

Composition & processing parameter of batch B2:

Material	Concentration
SPC	400 mg
Cholesterol	100 mg
Drug	10 mg
Chloroform	12 ml
Methanol	6 ml

Parameter	Condition
Temperature	40 ◦C
Vacuum	-200 mmHg
RPM	25-30

Time	Till dry film form
Probe sonication	4 cycle of 30 sec with 15 sec pulse at 20% Apt

Particle size data at different probe sonication cycle:

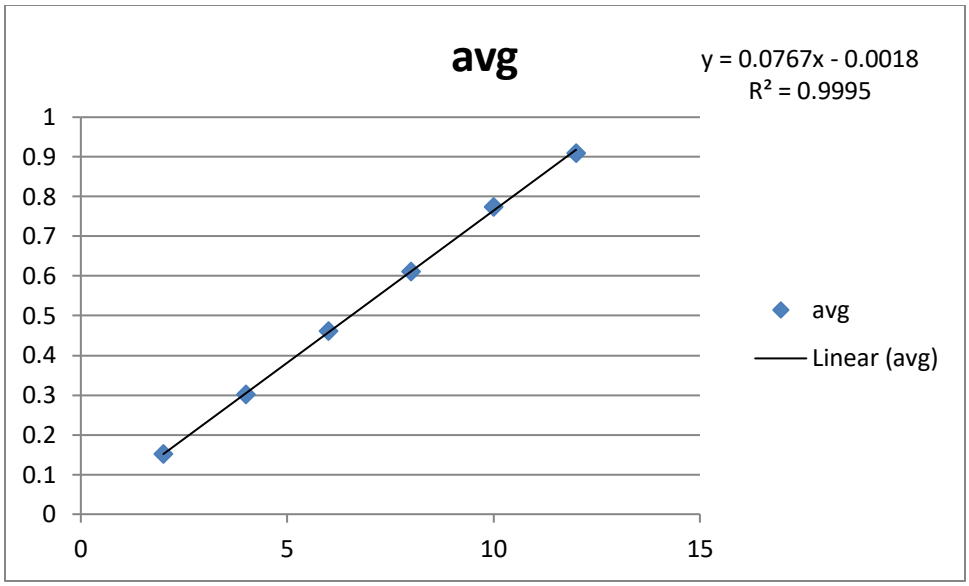
Probe cycle	**PDI**	**Particle size data(nm)**			
		D50	**D90**	**Mean**	**Z-AVG**
Without cycle	0.502	996.9	1643	1043	931.4
2	0.435	320.4	448.8	332.3	310.2
3	0.372	250.1	310.1	225.2	203.6
4	0.285	203.7	287.7	211.2	189.5
5	0.480	420.1	764.3	599.7	550.3

- As we can see cycle 3 and 4 have very less difference but on 5 cycle particle size again increases indicate rupturing of liposome, hence 4 cycle is sufficient for reduce the size without rupturing liposome.

% Entrapment efficiency:-

Standard curve for %EE in Chloroform: methanol (1:1)

Concentration	**1**	**2**	**3**	**Average**
2	0.168	0.146	0.139	0.151
4	0.32	0.29	0.293	0.301
6	0.46	0.462	0.461	0.461
8	0.623	0.596	0.613	0.610667
10	0.791	0.776	0.757	0.774667
12	0.958	0.891	0.881	0.91



Parameter	Value
Slope	0.999
Correlation coefficient	0.076
Intercept	0.001

- Entrapment efficiency of liposomes was determined using the Sephadex G-50 minicolumn centrifugation method. The unentrapped drug in liposomal formulation was separated using the Sephadex G-50 minicolumn centrifugation method. The separated liposomes were than disrupted using chloroform methanol 1:1 mixture and analyzed for drug content by UV- spectroscopy method.
- Found absorbance put in above y=mx+c equation and get concentration of drug loaded. That loaded drug value put in %EE equation and get % of drug entrap in liposome.

Batch no.	% EE
B2	50.3 %

OUTCOME

[11] outcome

As the out come of this project we can get the increased therapeutic efficacy , bioavailability, specific tumor targeting by modifying drug delivery system and the drug loading percentage will also increase with stability, furthermore solubility enhancement of drug that all parameter give efficient therapeutic response with minimal adverse effect.

From this experimental and literature data we can conclude that liposomal delivery of sorafenib tosylate through parenteral rout is more effective than the conventional sorafenib drug delivery system.

We investigated the novel use of sorafenib tosylate for the treatment of GC. To inhibit the overexpressed receptors in GC, liposome of sorafenib tosylate will efficiently work by controlled release of drug and due to the paranteral route of the formulation the dose will reduce which subsequently reduces the adverse effects of the drug and will increase the patient compliance.

Thus, we can conclude that Sorafenib loaded liposome can be used in a novel way to enhance the therapeutic efficacy for the treatment of GC.

FUTURE PERSPECTIVE

[12] Future perpective

Sorafenib tosylate loaded liposome for the treatment of GC through parenteral route is a novel approach. This will overcome the issues related to the conventional form of the drug. But, there are chances of many potential improvements to optimize the formulation and to obtain significant therapeutic efficacy.

The formulation further required optimization to obtaine reproducibility in manufacturing nano sized stable liposome with high entrapment. The scale up process will be also one of the major efforts towards this goal for its FDA approval and clinical application in GC. The in vivo study is another major direction to reduce the dose of the drug which subsequently reduces adverse effects during clinical management.

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