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Research

Formulation And Evaluation Of Indomethacin Liposomes

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Abstract

Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling from inflammation. The objective of the present study was to formulate and evaluate liposomes loaded with Indomethacin. Liposome of Indomethacin was made by thin film hydration method. Phospholipids and cholesterol were used to make multilamellar vesicles. Six batches of liposomes were prepared based on the different weight ratio of Phospholipids and cholesterol. Differential scanning calorimetry (DSC) study conducted to study in any incompatibility. Liposomes were produced by the thin-film hydration method. Six formulations of liposomes were prepared by varying the concentrations of Phospholipids and cholesterol and changing the drug ratio. The obtained liposomes were characterized for surface morphology, FTIR, particle size, zeta potential, drug content, entrapment efficiency, and *in-vitro* diffusion studies. Among the Six formulations of liposomes, F5 was found to be the best formulation with entrapment efficiency of 85.81% and a zeta potential value of -28.4mV. Liposomes followed Peppas release kinetics. Indomethacin loaded liposomes were prepared with good stability and the highest entrapment efficiency.

Keywords: Indomethacin and liposomes, thin-film hydration method, Phospholipids and cholesterol.

INTRODUCTION

Novel Drug Delivery System

Novel Drug Delivery system (NDDS) refers to the approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects. NDDS is a system for delivery of drug other than conventional drug delivery system. NDDS is a combination of advance technique and dosage form which are far better than conventional dosage form1.The aim of NDDS is to provide a

therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration2. NDDS combining polymer science, pharmaceutics and molecular biology.[3]

Advantages of novel drug delivery system[4,5]

Optimum dose at the right time and right location. Efficient use of expensive drugs, excipients and reduction in production cost. Improves the therapy by increasing the duration of action and reducing the side effects. Increases patient compliance and provides convenient route of administration. Achieve the targeting of drugs to specific sites which reduces the unwanted side effects and obtain maximum efficacy. Reduces the dose and thus reduces the side effects of drugs.

Types of novel drug delivery systems[4,5]

There are number of novel drug delivery systems are available. They are

1. Hydrogels
2. Colloidal drug carrier systems
    a) Micelles
    b) Microspheres
    c) Nanoparticles
    d) Liposomes and Neosomes
3. Mucoadhesive
4. Transdermal drug delivery
5. Ocular drug delivery
6. Nasal drug delivery

LIPOSOMES-An Introduction

Liposomes are colloidal, vesicular structure composed of one or more bilayers surrounding an equal number of aqueous compartment[7]. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery[8]. The sphere like shell encapsulated a liquid interior which contain substances such as peptides, protein, hormones, enzymes, antibiotics, anti-fungal and anti-cancer agents.[7]

Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoyl phosphatidylcholine) form a rigid, rather impermeable bilayer structure.[8]

It has been displayed that phospholipids impulsively form closed structures when they are hydrated in aqueous solutions. Such vesicles which have one or more phospholipid bilayer membranes can transport aqueous or lipid drugs, depending on the nature of those drugs. Because lipids are amphipathic (both hydrophobic and hydrophilic) in aqueous media, their thermodynamic phase properties and self-assembling characteristics influence entropically focused confiscation of their hydrophobic sections into spherical bilayers. Those layers are referred to as lamellae[9].

Liposomes particle sizes range from 30 nm to several micrometers. They consist of one or more lipid bilayers surrounding aqueous units, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases. On the other hand, self-aggregation of polar lipids is not limited to conventional bilayer structures which rely on molecular shape, temperature, and environmental and preparation conditions but may self-assemble into various types of colloidal particles[10].

Advantages of Liposomes

Some of the advantages of liposome are as follows [1,8,11, 12]:

1) It can carry both water and lipid soluble drugs.
2) Provides selective passive targeting to tumor tissues (liposomal doxorubicin).
3) Liposomes increased efficacy and therapeutic index of drug (actinomycin-D).
4) Liposome increased stability via encapsulation.
5) Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations.
6) Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol).

7) Liposomes help reduce the exposure of sensitive tissues to toxic drugs.
8) Site avoidance effect.
9) Flexibility to couple with site-specific ligands to achieve active targeting.
10) Improved pharmacokinetic effects (reduced elimination, increased circulation lifetimes).
11) It provides sustained release.
12) It can be administered through various routes.
13) It engenders incorporating micro and macro molecules.
14) It also acts as reservoir of drugs.
15) Liposomes can modulate the distribution of drug.
16) It directs interaction of the drug with cell.

Disadvantages of Liposomes

Some of the disadvantages of liposome are as follows

1) Low solubility.
2) Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction.
3) Short half-life.
4) Leakage and fusion of encapsulated drug/molecules.
5) Production cost is high.
6) Fewer stables.
7) Quick uptake by cells of reticuloendothelial system (R.E.S).
8) Allergic reactions may occur to liposomal constituents.
9) Problem to targeting to various tissues due to their large size.

Application of Liposomes

Liposomes for Brain Targeting

The biocompatible and biodegradable behavior of liposomes has recently led to their exploration as drug delivery system to brain. Liposomes with a small diameter (100 nm) as well as large diameter undergo free diffusion through the Blood Brain Barrier (BBB). However, it is possible that a small uni lamellar vesicle (SUVS) coupled to brain drug transport vectors may be transported through the BBB by receptor mediated or absorptive mediated transcytosis[13].

Liposome in Eye Disorders

Liposome has been widely used to treat disorders of both anterior and posterior segment. The disease of the eye includes dry eyes, keratitis, corneal transplant rejection, uveitis, endophthalmitis and proliferative vitro retinopathy. Retinal diseases are leading causes of blindness in advanced countries. Liposome is used as vector for genetic transfection and monoclonal antibody directed vehicle. The recent techniques of the treatment like applying of focal laser to heat induced release of liposomal drugs and dyes are used in the treatment of selective tumor and neo-vascular vessels occlusion, angiography, retinal and choroidal blood vessel stasis.[13]

Liposome for Respiratory Drug Delivery System

Liposomes are widely used in several types of respiratory disorders. The recent use of liposome for the delivery of DNA to the lung means that a greater understanding of their use in macromolecular delivery via inhalation is now emerging. Much of this new knowledge, including new lipids and analytical techniques, can be used in the development of liposome based protein formulations. For inhalation of liposome the liquid or dry form is taken, and the drug release occurs during nebulization. Drug powder liposome has been produced by milling or by spray drying[13].

Liposomes in parasitic diseases and infections

Since conventional liposomes are digested by phagocytic cells in the body after intravenous administration, they are ideal vehicles for the targeting of drug molecules into these macrophages. The best known examples of this 'Trojan horse-like' mechanism are several parasitic diseases which normally reside in the cell of mononuclear phagocytic system. They include leishmaniasis and several fungal infections[14].

Macrophage activation and vaccination

Some natural toxins induce strong macrophage response which results in macrophage activation. This can be duplicated and improved using liposomes because small molecules with immunogenic properties (haptens) cannot induce immune response without being attached to a larger particle. For instance, liposomes containing muramyl

tripeptide, the smallest bacterial cell wall subunit with immunogenic properties cause macrophage activation. Activated macrophages are larger and contain more granulomae and lysosome material. Their state lasts for a few days during which they show enhanced tumouricidal, virocidal and microbicidal activity.[14]

Liposomes in anticancer therapy

Many different liposome formulations of various anticancer agents were shown to be less toxic than the free drug. Anthracyclines are drugs which stop the growth of dividing cells by intercalating into the DNA and therefore kill predominantly quickly dividing cells. These cells are in tumours, but also in gastrointestinal mucosa, hair and blood cells and therefore this class of drugs is very toxic[14].

MATERIALS AND METHODS

Indomethacin-Provided by SURA LABS, Dilsukhnagar, Hyderabad, Phospholipid -Purchased from Loba Chemie, Mumbai, Cholesterol -Purchased from SD Fine Chem Ltd., Mumbai, Methanol -Purchased from Merck Limited, Mumbai (India), Chloroform -Purchased from Himedia, Mumbai.

Methodology

Identification and Characterization of Drug

Preparation of reagents:

Preparation of 0.2M NaOH Solution

Dissolved 4g of Sodium hydroxide pellets in to 1000mL of Purified water and mixed

Preparation of pH 6.8 Phosphate buffer

Dissolved 6.805 g of Potassium dihydrogen phosphate in to 800mL of purified water and mixed added 112mL of 0.2M NaOH solution and mixed. Diluted to volume 1000mL with purified water and mixed. Then adjusted the pH of this solution to 6.8 with 0.2M NaOH solution.

Determination of absorption maxima

A solution containing the concentration 10 µg/ ml drug was prepared in 6.8 phosphate buffer UV spectrum was taken using Lab India Double beam UV-VIS spectrophotometer (Lab India UV 3000+). The solution was scanned in the range of 200 – 400 nm.

Preparation of calibration graph for Indomethacin: Preparation of calibration curve in pH 1.2, pH 7.4 and pH 6.8 buffer solutions: An accurately weighed amount of Indomethacin100mg was dissolved in small volume of buffer solutions in each of three 100 ml volumetric flask and the volume was adjusted to 100 ml with 1.2 pH buffer in first volumetric flask, 7.4 pH buffer in second volumetric flask and the third one was adjusted to 100 ml with 6.8 pH buffer. A series of standard solution containing in the concentration range from 10 to 50 µg/ml of Indomethacin were prepared for 1.2 pH buffer solution, 7.4 pH buffer solution and 6.8 pH buffer solution separately, absorbance was measured at 230 nm and calibration graph was plotted using concentration versus absorbance.

Organoleptic properties

Take a small quantity of sample and spread it on the white paper and examine it visually for color, odour and texture.

Determination of Indomethacin Melting point

The melting point of Indomethacin was determined by capillary tube method according to the USP. Enough Indomethacin powder was introduced into the capillary tube to give a compact column of 4-6 mm in height. The tube was introduced in electrical melting point apparatus and the temperature was raised. The melting point was recorded, which is the temperature at which the last solid particle of Indomethacin in the tube passed into liquid phase**.**

Determination of Indomethacin Solubility

Determination of solubility of drug by visual observation an excess quantity of Indomethacin was taken separately and adds in 10 ml of different solutions. These solutions were shaken well for few minutes. Then the solubility was observed, and observations are shown in the Table.

Formulation Of Liposomes

Table 1: Formulation of liposomes

INGREDIENTS	FORMULATION CHART					
	F1	F2	F3	F4	F5	F6
Indomethacin	25	25	25	25	25	25
Phospholipids (mg)	50	100	150	200	250	300
Cholesterol (mg)	100	100	100	100	100	100
Methanol (mL)	10	10	10	-	-	-
Chloroform (mL)	-	-	-	10	10	10
Phosphate buffer pH 6.8 (mL)	10	10	10	10	10	10

RESULT AND DISCUSSION

Organoleptic properties

Table 2: Organoleptic properties

S NO.	Properties	Results
1	State	Solid
2	Colour	White
3	Odor	Odorless
4	Melting point	151°C

Solubility studies

Table 3: Solubility studies of drug in different solvents

S NO.	Solvents	Solubility of Indomethacin
1	Water	Insoluble
2	Methanol	Soluble
3	Acetonitrile	Insoluble
4	pH 6.8 Phosphate Buffer	Free soluble
5	Ethanol	Soluble
6	DMSO	Soluble

Initially the drug was tested by UV to know their significant absorption maximum which can be used for the diffusion study of the drug.

Analysis of drug
UV scans
The lambda max of Indomethacin was found to be 230 nm.

Construction of calibration curve:

Table 4: Standard graph of Indomethacin

Concentration (µg/ml)	Absorbance		
	pH 1.2	pH 7.4	pH 6.8
0	0	0	0
2	0.163	0.152	0.176
4	0.308	0.289	0.303

6	0.468	0.451	0.476
8	0.607	0.604	0.608
10	0.787	0.781	0.791

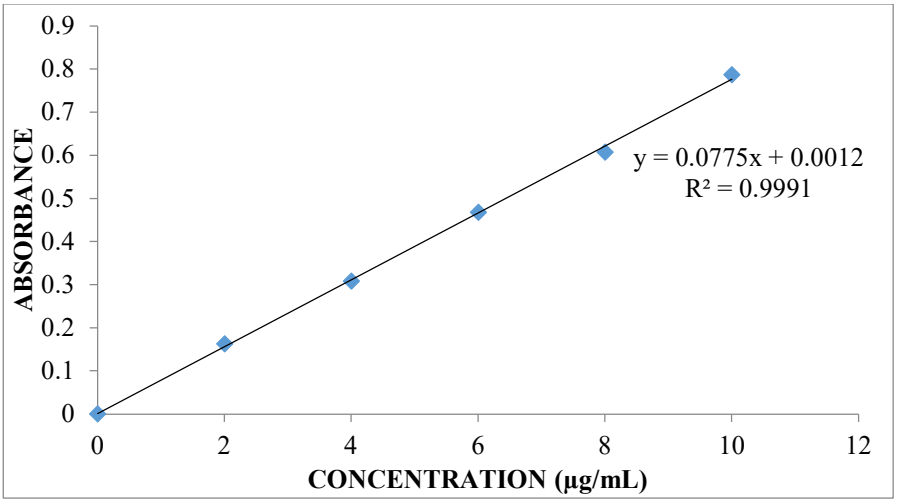


Fig 1: Standard calibration curve of Indomethacin0.1N HCl

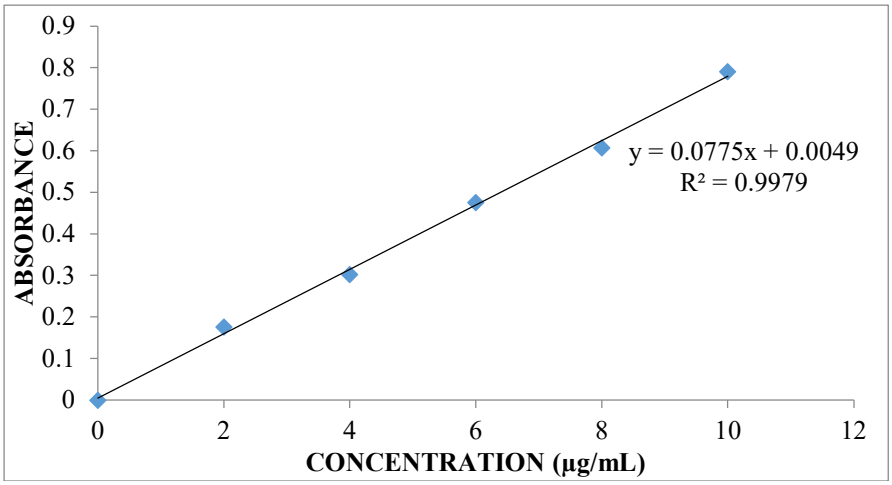


Fig 2: Standard calibration curve of Indomethacin pH 7.4 PB

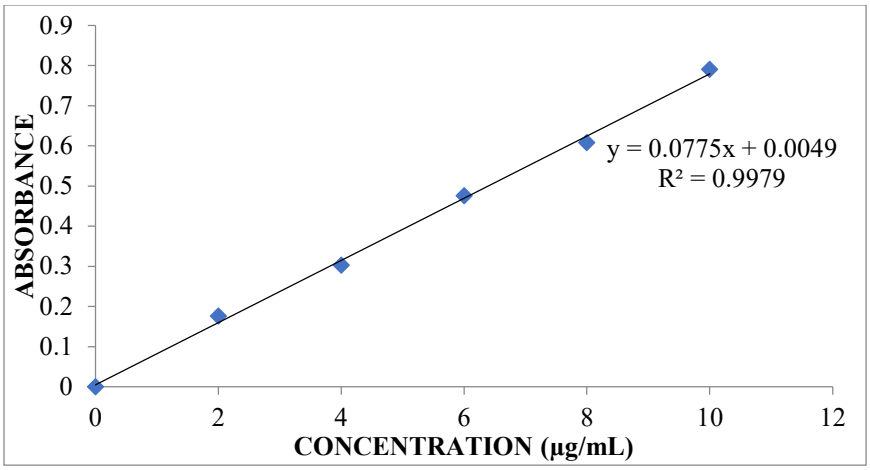


Fig 3: Standard calibration curve of Indomethacin pH 6.8

Characterization of Liposomes

Table 5: % yield, Drug Content, Entrapment Efficiency of all Liposomes formulations

FORMULATION	Particle Sizes	Zeta Potential	Entrapment Efficiency	Drug content
F1	268.5	-20.5	43.21	97.41
F2	269.3	-21.4	49.67	98.46
F3	270.2	-22.7	60.56	99.43
F4	271.1	-26.1	81.72	99.47
F5	272.5	-28.4	85.81	99.44
F6	272.9	-26.6	86.93	98.34

Particle size and entrapment efficiency of the Indomethacin Liposomes (F1- F6) were increased with increasing Phospholipids concentration. This may be due to the high amount of availability of Phospholipids to encapsulate the drug, upon increasing the Phospholipids concentration, number of layers coated the drug was increased; this resulted in increased particle size and entrapment efficiency. Further increase in the Phospholipids concentration (F1 and F6), there is no much increase in the entrapment efficiency due to the availability of the drug to be incorporated is low which is not enough for further encapsulation of drug by Phospholipids. Based on the results of Particle size and entrapment efficiency of the Indomethacin Liposomes (F1- F6), the trial F5 which contains 250 mg of phospholipids concentration was selected as the best formulation.

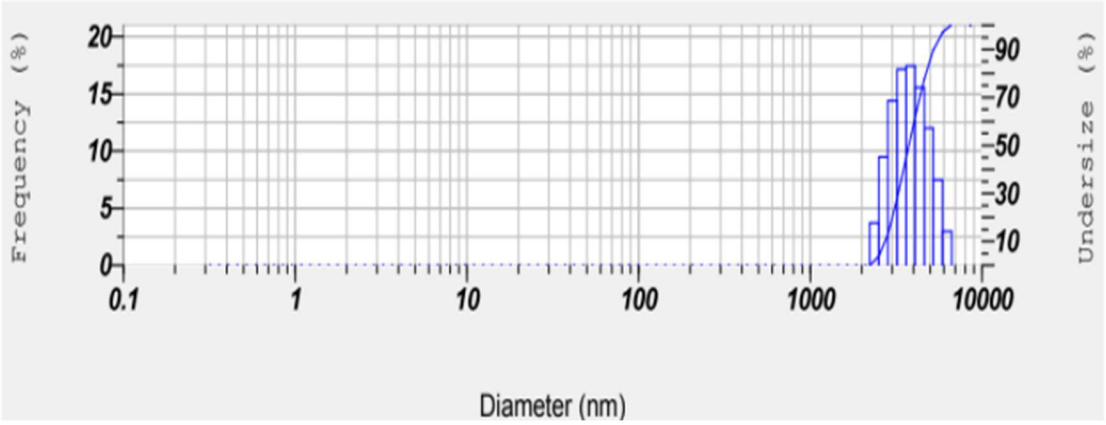


Fig 4: Particle size of optimized Indomethacin liposomes (F5)

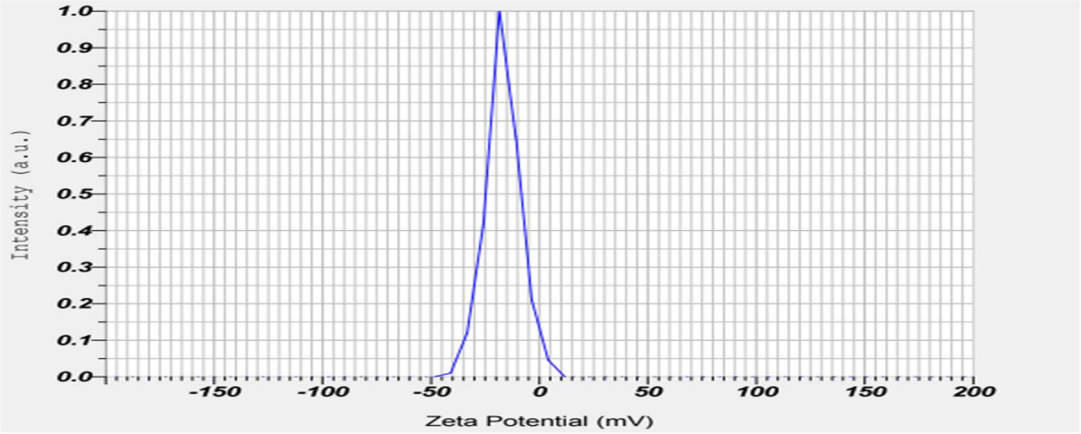


Fig 5: Zeta potential of optimized Indomethacin liposomes (F5)

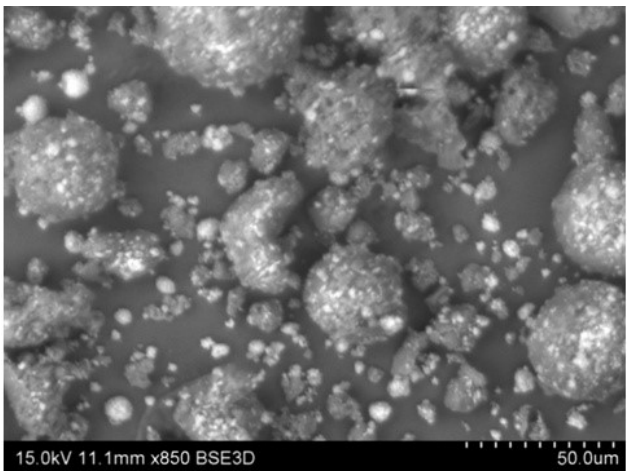


Fig 6: SEM of optimized Indomethacin liposomes (F5)

SEM studies showed that the Indomethacin- loaded liposomes had a spherical shape with a smooth surface as shown in Figure.

% Drug release

Table 6: *In vitro* dissolution studies of F1-F6 liposomes formulations in percentage

Time (hour)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
0.5	22.62	25.83	43.28	56.10	57.54	55.03
1	29.03	39.56	56.21	63.31	66.22	63.19
6	43.92	45.37	63.46	69.92	71.19	68.42
12	49.60	53.21	70.91	75.69	76.59	73.71
16	53.09	63.93	75.29	82.20	83.32	80.86
20	59.10	70.92	84.11	89.12	92.73	89.90
24	65.16	79.99	93.09	94.31	99.81	96.61

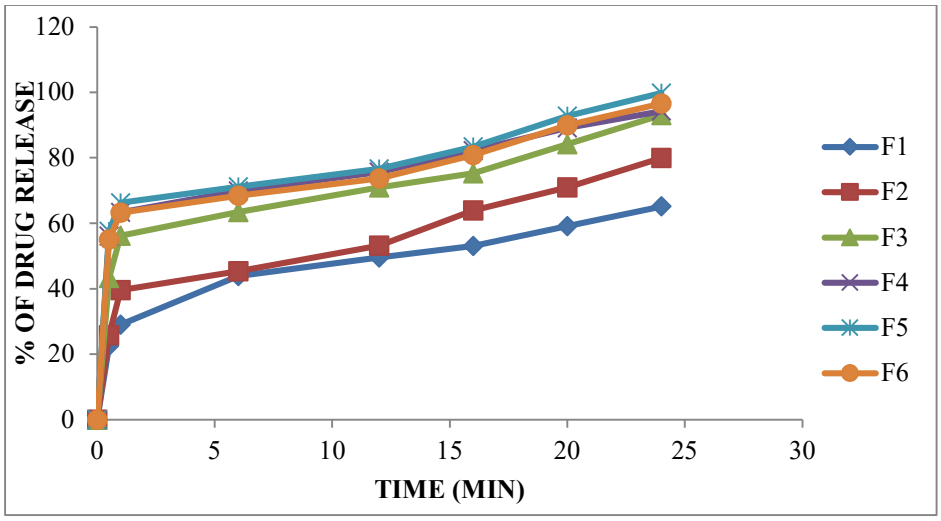


Fig 7: *In vitro* dissolution studies of F1-F6 liposomes formulations

From the *in vitro* drug release study results, the maximum percentage drug release (99.81 %) at the end of 24h was observed with trial F5 which contains 300mg of Phospholipids. From the *in vitro* drug release data for F1-F6, it was observed that increase in Phospholipids concentration delays the drug release due to increased particle size and reduced surface area of the prepared liposomes. From all the formulations, F5 was selected as best formulation due to its ideal particle size (272.5nm), high entrapment efficiency (85.81%) and desirable drug release (99.81% at the end of 24 h).

Release Kinetics

To analyze the drug release mechanism the *in vitro* release was fitted into various release equations and kinetic models first order, zero order, Higuchi and Korsmeyer-peppas. The release kinetics of optimized formulation is shown in Table and in following Figures.

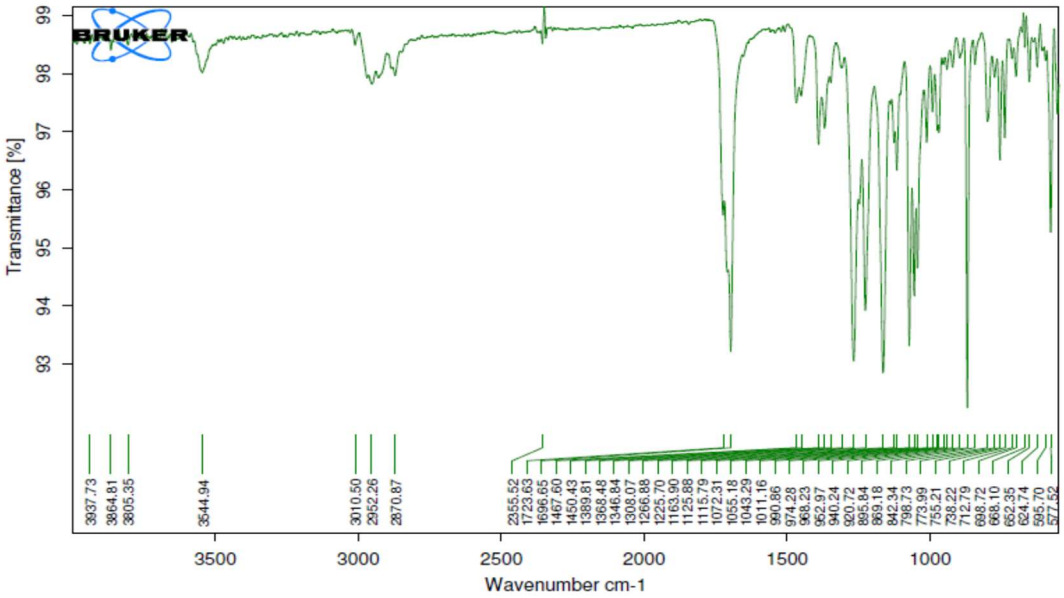
Table 7: Release kinetics of optimized formulation

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
57.54	0.5	0.707	1.760	-0.301	1.628	115.080	0.0174	-0.240	42.46	4.642	3.489	1.153
66.22	1	1.000	1.821	0.000	1.529	66.220	0.0151	-0.179	33.78	4.642	3.233	1.409
71.19	6	2.449	1.852	0.778	1.460	11.865	0.0140	-0.148	28.81	4.642	3.066	1.576
76.59	12	3.464	1.884	1.079	1.369	6.383	0.0131	-0.116	23.41	4.642	2.861	1.781
83.32	16	4.000	1.921	1.204	1.222	5.208	0.0120	-0.079	16.68	4.642	2.555	2.087
92.73	20	4.472	1.967	1.301	0.862	4.637	0.0108	-0.033	7.27	4.642	1.937	2.704
99.81	24	4.899	1.999	1.380	-0.721	4.159	0.0100	-0.001	0.19	4.642	0.575	4.067

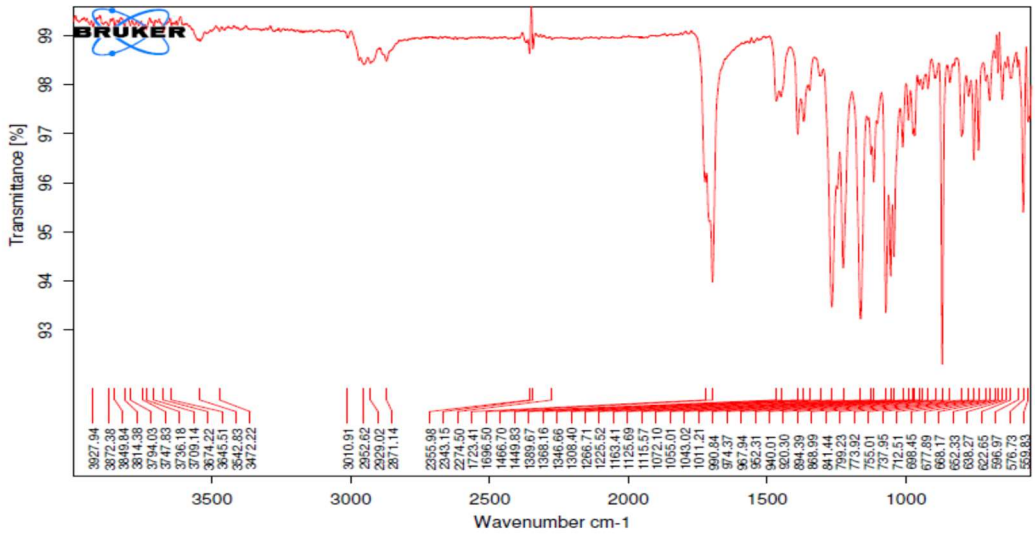
The prepared optimized Liposomes were subjected to drug release kinetics and release mechanism. The formulation was studied by fitting the drug release time profile with the various equations such as Zero order, First order, Higuchi and Korsmeyer pappas. The data revealed a better fit to the Peppas release kinetics.

FT-IR

FTIR spectra of the drugs and the optimized formulation were recorded. The FTIR spectra of pure Indomethacin drug, optimized formulation shown in the below figures respectively. Drugs are also present in the physical mixture, which indicates that there is no interaction between drug and the polymers, which confirms the stability of the drug. There was no disappearance of any characteristics peak in the FTIR spectrum of drug and the polymers used. This shows that there is no chemical interaction between the drug and the polymers used. The presence of peaks at the expected range confirms that the materials taken for the study are genuine and there were no possible interactions.



Graph 1: FT-IR graph of Indomethacin



Graph: FT-IR graph of optimised formulations

CONCLUSION

The active pharmaceutical ingredient Indomethacin was evaluated for its Organoleptic properties and solubility. The results obtained were satisfactory. Indomethacin liposomes were prepared by thin film hydration technique and the phospholipids concentrations were optimized by various trials. In the present study liposomes containing Indomethacin was prepared. The effect of increase in phospholipids concentration in various parameters like particle size and *invitro* release profile was studied. The Indomethacin liposomes were formulated and evaluated for its drug content, entrapment efficiency, particle size analysis, zeta potential and in vitro drug release profile. Based on the results of Indomethacin liposomes formulations (F1- F6) formulation F5 was selected as the best formulation in which the particle size was 272.5nm and the entrapment was 85.81%. The *in vitro* % drug release of F5 formulation was 99.81 % at 24 hrs and it was found to be suitable formulation to manage the condition of fever, pain, stiffness, and swelling from inflammation. Hence it can be concluded that the newly formulated controlled release liposomal drug delivery systems of Indomethacin may be ideal and effective in the management of inflammatory by allowing the drug to release continuously for 24 hrs.

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