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Research Article

FORMULATION AND EVALUATION OF LIPOSOMES OF INDOMETHACIN

Harshita Mishra, Kapil Kumar, Gurleen Kaur*, Deepak Teotia

Global Institute of Pharmaceutical Education and Research, Kashipur, Uttarakhand, India

**Corresponding author: gurleensandhu1991@gmail.com*

ABSTRACT

Liposomes are spherical shaped vesicles having 0.05- 5.0 micrometer in diameter consist of phospholipids and cholesterol. This novel drug delivery system aims to target the drug directly to the site of action. Liposomes are biocompatible and stable and have unique property to entrap both hydrophilic drug and lipophilic drug. The aim of the present study is to formulate and evaluate the liposomes of Indomethacin using HPMC, PVP, Sodium lauryl sulphate which leads to increase the solubility. Various formulations from LS1 to LS7 of Indomethacin Liposomes were developed using different concentrations of polymers like PVP, HPMC etc. The Liposomes were prepared by thin film hydration method. Liposomes were characterized by polymer compatibility by using spectral analysis of drug. The prepared Liposomes were evaluated for solubility, viscosity determination, entrapment efficiency *in vitro* drug release and stability studies. Finally, batch LS1 is concluded as optimum formulation.

Keywords: Liposomes, Target Drug Delivery, Amphiphatic, Controlled Release

1. INTRODUCTION

The evolution of any drug molecule from conventional form to a novel delivery system can improve its performance in terms of patient compliance, safety and efficacy [1]. Its aim is to instigate a therapeutic amount of drug to the proper site to achieve and maintain the desired drug concentration [2, 3]. It basically focuses on selectivity, target specificity, target-ability, safety and efficacy. Their distribution was observed by controlling their release and by modifying the interaction of target molecules and receptor [4].

Liposome were defined as spherical-shaped concentric vesicles, consist of an internal aqueous environment which was entrapped by one or more lipid bilayer.[3] They are more commonly used as carrier to target the specific molecules to specific organ to produce the good therapeutic response [4]. It has been observed that they can increase the drug stability, increase the therapeutic effects, prolong circulation time and enhance the uptake of entrapped drugs to target site by reducing the drug toxicity [5, 6].

Indomethacin is a non-steroidal anti-inflammatory agent (NSAIDS) with anti-inflammatory, analgesic and antipyretic activity. Its pharmacological effect is mediated by inhibition of enzyme cyclooxygenase (COX), the enzyme responsible for catalyzing the rate limiting step in prostaglandin synthesis via arachidonic acid pathway [7]. Indomethacin is the first line of medicine used in the treatment of rheumatoid arthritis and it was first developed in the year 1960. It is an indole derivative drug compound used to reduce spasm. It is soluble in methanol, chloroform, ether and insoluble in water [8]. When the drug remains for long duration in stomach it produces irritating effects which may leads to ulcers [9].

Thus for reducing the side effects of indomethacin, liposomes were prepared along with the addition of polymer to increase their effects for longer duration and further evaluation of different parameters performed.

2. MATERIALS AND METHODS

Indomethacin was obtained as a gift sample from Akums Pvt. Ltd. Haridwar, India. Poly-vinyl pyrrolidone, Cholesterol, Hydroxy propyl methyl cellulose,sodium lauryl sulphate and was purchased from Central drug house Pvt. Ltd. Delhi (IND) and Agron Remedies Pvt. Ltd Kashipur, India.

2.1.Preformulation studies

2.1.1. Angle of Repose

It determines the flow property of powder. In general, the higher is the angle of repose poor is the flow ability of powder [10]. The method used is glass funnel method. It has a given formula:

$$\tan \theta = h/r$$

Where, θ= Angle of Repose, h= Height of the pile, r = Radius of the cone

2.1.2. Bulk Density

It is ratio of mass of bulk volume. It depends on the particle size, shape and tendency of particles to adhere together. Bulk density was determined by taking a known mass of liposomes in a 5 ml graduated measuring cylinder. The cylinder was dropped three times from a height of one inch at an interval of two seconds [11].

2.1.3. Tapped density

It is also used to determine the geometry and flow ability. It was determined by tapping using weighed amount of sample in measuring cylinder [12].

2.1.4. Carr's compressibility index

It determines the uniformity of weight [13].

$$\text{Carr's Index} = \frac{\text{(Tapped density – Bulk density)}}{\text{Tapped density}} \times 100$$

2.1.5. Hausner's ratio

Hausner's ratio less than 1.25 indicates good flow and greater than 1.5 indicates poor flow [14]. It can be given by :

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

2.1.6. Solubility analysis

The solubility of Indomethacin was determined in a set of solvents (methanol, water and ethanol). A small amount of solvent (upto 1-5 ml) was placed in a test-tube and then small amount of drug was added and the solution was kept overnight for complete solubilization. After that, the solution was sonicated for 5 minutes and then 0.1 ml of solution was pipette out from the test-tube and then further dilutions were prepared and absorbance was determined by UV Spectrophotometer against with an appropriate blank solution. The amount of drug which is soluble was calculated with the calibration curve equation [15].

2.1.7. Melting point determination

Melting point determination was performed to determine the purity of drug, if there is any impurity found the melting range deviates from its original readings. Melting point of Indomethacin was determined by using Melting Point Apparatus, the drug is filled (sample amount) in a capillary tube whose one end is sealed with flame and it was placed in the pockets of apparatus attached with thermometer. The heating was started and the point at which drug start melting was noted [16].

2.1.8. Preparation of Calibration Curve

The standard calibration curve of Indomethacin was prepared in phosphate buffer 6.8.

2.1.9. Preparation of phosphate buffer

Place 50 ml of 0.2M potassium di hydrogen phosphate in a 200 ml of volumetric flask. Add 22.4 ml of 0.2M sodium hydroxide and then add water to make up the volume.

2.1.10. Calibration curve of Indomethacin

10 mg of drug was weighed and dissolved in 10 ml of phosphate buffer (pH 6.8), to give a solution of 1000 μg/ml concentration. From this solution 1 ml was taken and diluted to 10ml using Phosphate buffer 6.8 to produce a stock solution of 10 μg/ml. From this stock solution different concentrations were prepared. The absorbance of these solutions was measured at 200-400 nm by UV spectrophotometer. The absorbance was measured at 400 nm by UV spectrophotometer [17].

2.1.11. Excipients compatibility studies: Fourier Transform Infra-Red (FTIR) Spectroscopy

IR analysis was done on IR spectrometer with KBr disc. In IR the spectrum was recorded in the wavelength region of 4000 to 400cm^{-1}. 10mg of drug was mixed with KBr and triturated then it was placed in holder and pressed to form a pellet. It was placed under IR beam and a spectrum was obtained on computer. The IR spectrum of drug exhibit maxima only at the same wavelength as that of similar preparation of the corresponding reference standard, thus IR spectrum of substance being examined should be concordant with the reference spectrum of the drug [18].

2.2. Development of liposomes of Indomethacin by thin film hydration method

Multilamellar vesicles (MLVs) were prepared by this conventional method. In this method, specified quantity of lipid(s) and the drug were dissolved in chloroform in a round-bottom flask. The solvent was evaporated under reduced pressure to obtain a thin film. The flask was stored overnight under vacuum to remove traces of the solvent. The lipid film was hydrated with phosphate-buffered pH 6.8 [19].

Table 1: Formulation of Liposomes of Indomethacin

Formulation code	Drug (mg)	Cholestrol (%)	HPMC (mg)	SLS	PVP (mg)
LS1	25	3%	25	_	_
LS2	25	4%	_	0.25	_
LS3	25	5%	_	_	0.5
LS4	25	3%	10	10	_
LS5	25	5%	_	10	10
LS6	25	2%	10	_	10
LS7	25	3%	_	_	_

2.3. Evaluation Parameters studies for liposomes of Indomethacin

2.3.1. Determination of Percentage yield

The prepared liposome was collected and weighed. The measured weight was divided by the total amount of all non-volatile components, which were used for the preparation of the liposomes [20].

$$\text{Percentage Yield} = \frac{\text{Actual weight of products}}{\text{Weight of drug and excipients}} \times 100$$

2.3.2. Drug Entrapment Efficiency

Liposomes equivalent to 100 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the liposomes and extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured at suitable wavelength against appropriate blank [21]. The amount of drug entrapped in the liposomes was calculated by the following formula:

Drug Entrapment Efficiency = (Amount of drug actually present/ Total amount of drug entrapped) x 100

2.3.3. Determination of Moisture Content

The formulations were subjected to moisture content study by using an IR moisture balance by placing the liposomes at 105 °C for 10 min [20].

2.3.4. Determination of Viscosity

Brookfield Viscometer is used for the determination of viscosity. The angular viscosity got increased by increasing the angular viscosity from 2 to 50 rpm. The Ph of formulation got increased by using hallipathspindle. By using Brookfield viscometer, the rheology of the formulation got increased [22].

2.3.5. In-*vitro* drug release study

The drug release study from liposomes is performed using USP dissolution apparatus Type I in 900 ml of 0.1 N HCl dissolution media (pH-6.8) at 50 rpm and 37°C. 2 ml sample was withdrawn at 1 hr. time interval for 12 hr. and same volume of fresh medium was replaced to maintained sink condition. Withdrawn samples were assayed spectrophotometrically at suitable wavelength. The drug release was analyzed by UV spectro-photometer [23].

3. RESULTS AND DISCUSSION

Preformulation studies were carried out to determine the flow of powder such as angle of repose, bulk density, tapped density and Carr's index, Hausner's ratio.

3.1. Powder properties of Indomethacin

- Angle of Repose -It was found to be 17.42 which comes under the range of < 20 showing excellent flow property of Indomethacin.
- Bulk density - It was found to be 1.08 within limit showing good flow property.
- Tapped density -It was found to be 1.35 which shows the satisfactory results for Indomethacin.
- Carr's Index- In this formulation it was found to be 14.68 which come under the range of 5-15 showing good flow property.
- Hausner's ratio- The range for this is that it should be less than 1.25 and the formulation comes under the reading of 1.23 shows good flowing property.

Table 2: Powder Flow properties of Indomethacin

Powder Properties	Results
Angle of repose	17.42
Bulk density (gm/mL)	1.08
Tapped Density (gm/mL)	1.35
Carr's Index (%)	14.68
Hausner's Ratio	1.23

Table 3: Organoleptic Properties evaluation of sample of drug

Properties	Results
Physical appearance	White to yellow crystalline powder
Odour	Odourless
Taste	Tasteless
Solubility	Soluble in methanol, ether, chloroform and insoluble in water
Melting point	151°C
Moisture content	0.5 %

Table 4: Solubility of Indomethacin in methanol and water

Concentration (µg/ml)	Absorbance	
	Methanol	Water
0	0	0
1	0.139	0.008
2	0.282	0.029
4	0.439	0.043
6	0.716	0.056
8	0.993	0.074

Table 5: Viscosity of different formulations

Formulation code	Viscosity(cps)
LS1	8.15
LS2	8.10
LS3	8.25
LS4	8.21
LS5	7.96
LS6	7.85
LS7	7.90

The drug-polymer interactions show that there were no major shifts in the absorption bands (peaks) in presence of polymer and it was observed that all the characteristics peaks of drug was present in the combination of drug and polymer spectra indicating the compatibility of drug with the polymer used.

From the result we can conclude that as the amount of polymer in the formulation increases, the as a result of increase in drug entrapment.

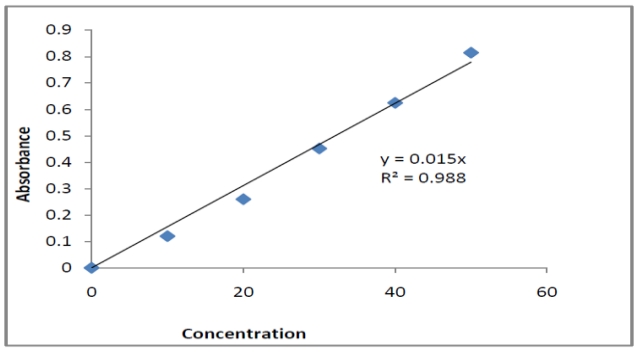

Fig. 1: Standard Curve for Indomethacin in phosphate buffer 6.

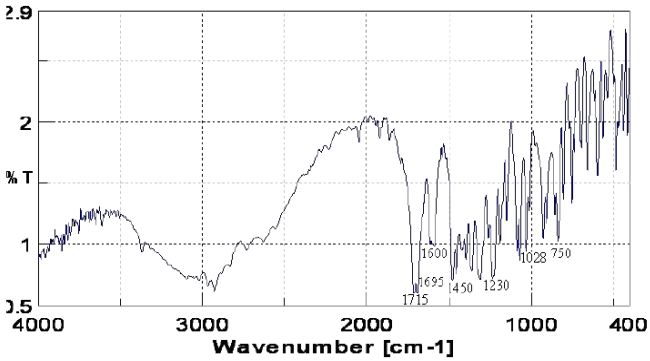

Fig. 2: FTIR spectra of Indomethacin

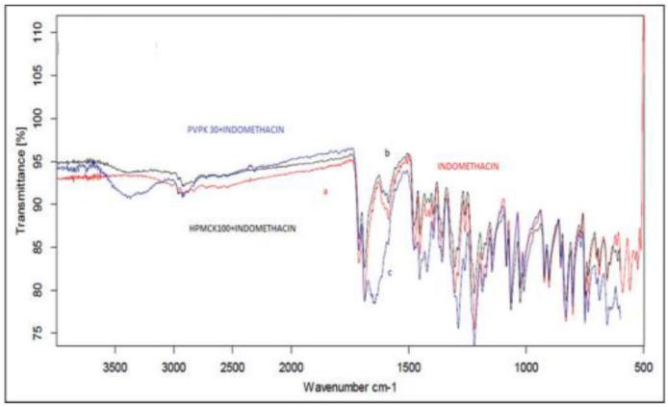

Fig. 3: Drug Excipient compatibility of Indomethacin, HPMC, PVP

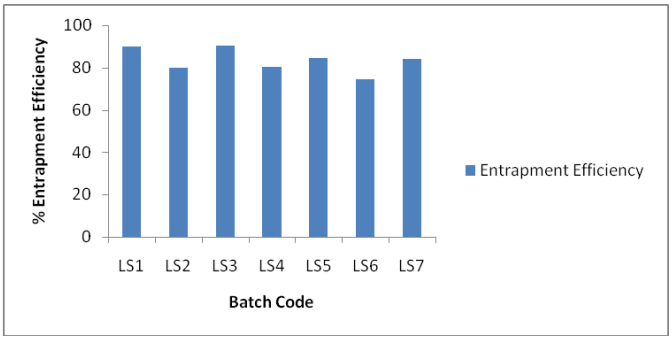

Fig. 4: Comparison of Entrapment Efficiency of different liposomes of Indomethacin

3.2. *In-vitro* release study

Indomethacin liposomes were evaluated by exposing them 6.8 pH phosphate buffer for 12 hrs. The drug release at different time intervals was analyzed by UV double beam spectrophotometer at 400 nm.

Indomethacin liposomes were evaluated by exposing them in phosphate buffer 6.8 pH for 12 hrs. The drug release at different time intervals was analyzed by UV double beam spectrophotometer at 400 nm. In the above formulation, LS1 was found to be optimum among seven formulations.

The release kinetics for all the formulations ie LS1, LS2,LS3,LS4,LS5,LS6,LS7 were studied by plotting graph of zero order, first order, higuchi's plot and then it was found that they follow the zero order kinetics for drug release.

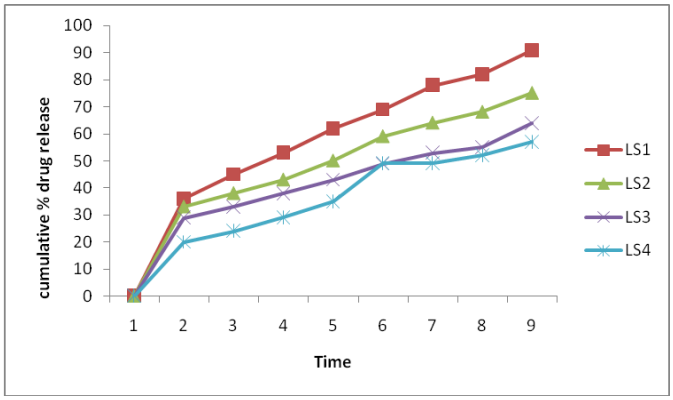

Fig. 5: Percentage of drug released from liposomes of Indomethacin of batch LS1 to LS4.

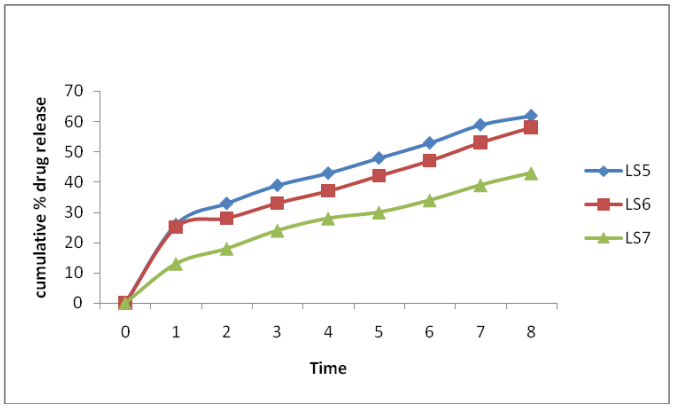

Fig. 6: Percentage of drug released from liposomes of Indomethacin of batch LS 5 toLS7.

4. CONCLUSION

From the present study it has been concluded that the liposomes were formulated to improve the solubility of drug and provide the longer duration of action. The liposomes of Indomethacin were successfully prepared by using HPMC, polyvinyl pyrollidone, cholesterol , sodium lauryl sulphate for oral administration. The polymers were found to be the carrier for Indomethacin drug release. The Liposomes have capability to provide longer duration of action and it is used for the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis.

From the above experiments it has been concluded that Different Preformulation tests were performed on sample Indomethacin and were prepared by using different polymers like HPMC and polyvinyl pyrrolidone. All formulations were found to free from presence of particles. The extrudability of formulations was found to be satisfactory and good. Indomethacin loaded liposomes were prepared by using thin film hydration technique and equipment used was rotator film evaporator. The viscosity and drug entrapment efficiency of optimized formulation was determined i.e. (LS1andLS3).Among all the formulations, the maximum percentage of drug release was found to be in formulation one i.e. LS1. The release kinetics they follow is zero order drug release. Finally, this can be concluded that Indomethacin can be considered as good for the preparation of liposomes which increase the therapeutic effect and provide relief to the pain in various diseases. A number of drug substances which are highly potent and have low therapeutic indication can be targeted to the required diseased site using the liposomal drug delivery system.

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