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FORMULATION AND EVALUATION OF FLURBIPROFEN LOADED STEALTH LIPOSOME USING VARIOUS POLYMERS

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Keywords:

Flurbiprofen, Liposomes, Stealth liposomes, Arthritis, Anti-inflammatory

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ABSTRACT: Flurbiprofen (FP) is a phenyl alkanoic acid derivative and a family of non–steroidal anti-inflammatory drugs used in the treatment of arthritis. Flurbiprofen was formulated as a liposome using soya lecithin and cholesterol by thin film hydration method. It is then formulated as a stealth liposome to increase efficacy and reduce toxicity. Two polymers are used to prepare flurbiprofen as stealth liposomes i.e., PEG and PVP, and their effects are compared. The formulated liposomes and stealth liposomes were evaluated for various parameters like surface morphology, zeta potential, polydispersity index, drug content, percentage drug encapsulation, and *in-vitro* drug release. The optimized formulation (F2) containing a minimum concentration of cholesterol showed moderate stability and good entrapment efficiency. When compared to conventional liposomes, stealth liposomes showed more zeta potential values due to the effect of PEG 4000 and PVP K30.The zeta potential value demonstrated that stealth liposomes had enough charge to prevent liposome aggregation due to electric repulsion. The kinetic data analysis of formulations indicated that it fits the Higuchi model and follows zero-order release kinetics.

INTRODUCTION: An ideal drug delivery system (DDS) delivers the drug at a rate dictated by the body's needs for the duration of the treatment and only delivers the active entity to the site of action [2]. There are different approaches to delivering a therapeutic substance to a target site and one such approach is liposomes. Liposomes as drug carriers are one such approach that can be used in a prolonged controlled release manner. The amphoteric nature of phospholipids and their analogues gives them the ability to form a closed concentric bilayer in the presence of water.

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Liposomes grow when thin lipid membranes are hydrated and the lipid bilayers become loose and bulging. The hydrated lipid sheets are separated during shaking and close to form large multilayer vesicles to prevent the interaction of water with the hydrophobic core of the bilayer [3, 4]. A stealth liposome is a long-circulating liposome achieved by modulating the lipid composition, size, and charge of the vesicle.

They are called 'stealth' because they increase the circulation time of these liposomes by avoiding detection by our body's immune system [5, 6]. Polymers used in stealth liposomes are polyacrylamide, polyvinylpyrrolidone, and polyglycerol [7, 8]. Stealth liposomes are prepared by pre-insertion, post-insertion, and post-modification by chemical reaction [9]. In this study, flurbiprofen is formulated as a stealth liposome to increase efficacy and reduce toxicity, while also targeting

RES organs and thus prolonging its circulating time, thereby reducing clearance waste. Two polymers were used to prepare flurbiprofen as stealth liposomes, PEG, and PVP, and their effects were compared [9].

MATERIALS AND METHODS: The materials used in this study were flurbiprofen, soya lecithin, cholesterol, methanol, chloroform, PEG 4000, and PVP K30.Flurbiprofen drug was identified and a pre-formulation study was done by organoleptic evaluation, determination of melting point, and determination of solubility. Analytical methods used for the determination of drugs were UV spectrophotometry, FTIR, and DSC [10].

Formulation of Liposomes and Stealth Liposomes: Flurbiprofen liposomes were prepared by thin layer hydration technique. The drug, soya lecithin, and cholesterol were dissolved in a solvent system consisting of a mixture of chloroform and methanol (2:1, v/v) in a 250 ml round bottom flask. The organic solvent system was removed by a rotary vacuum evaporator to obtain a thin film on the wall of the flask. During the process, the temperature and speed are adjusted to 55±5°C and 60 rpm, respectively. The flask is left under reduced pressure to completely remove any residual solvent.

The lipid membrane was hydrated with pH 7.4 phosphate-buffered saline at 60 ± 2°C. The resulting suspension was vortexed and left to stand for 2-3 h for the lipid membrane to fully swell. Stealthy liposomes were prepared by injecting 1 ml of PEG 4000 5, 7.5, and 10% w/v and 1 ml 0.5, 1, and 2% w/v PVP K 30 into vesicularly dispersed liposomes. Stir slowly at 100 rpm to ensure an even coating of PEG and PVP around the bags [11].

TABLE 1: FORMULATION DESIGN OF LIPOSOMES

Ingredients (mg)	L1	L2	L3	L4	L5
Flurbiprofen	250	250	250	250	250
Soya Lecithin	400	400	400	400	400
Cholesterol	50	100	150	200	250
Phosphate Buffer Saline	10	10	10	10	10

Evaluation of Liposomes and Stealth Liposomes:

Optical Microscopy: The prepared liposomes were observed under a binocular compound microscope at 10X and 40X magnification to study the shape and surface morphology [12].

Particle Size and Polydispersity Index: The mean particle size and particle size distribution were determined by the Malvern nano zeta sizer instrument [11].

The Yield of Liposome: The prepared liposomes were collected and weighed.

Percentage yield = Actual weight of product / Total weight of the product ×100

Determination of Percentage Drug Content: 1 ml of suspension was pipetted from the dispersion and was further diluted with pH 7.4 phosphate buffer saline and the samples were analysed spectrophotometrically at 293nm [13].

Determination of Percentage Entrapment Efficiency: Centrifugation was used to determine the %EE of the bags. For 20 minutes, the vesicle dispersions were centrifuged. The unentrapped drug supernatant was extracted and quantified using UV spectrophotometry at 293 nm in phosphate-buffered saline at pH 7.4. Every decision was made in triplicate.

Determination of pH of Vesicular Dispersion: The pH of the stealth vesicular dispersion was measured by a pH meter [7].

***In-vitro* Drug Release:** *In-vitro* drug release was measured with a Franz diffusion cell. 50 mg of flurbiprofen containing a liposomal suspension was placed on one side of the egg membrane in a vertical Franz diffusion cell.

The other side of the membrane exposed to the dissolution medium was 22 ml of phosphate-buffered saline with pH 7.4. The soluble complex was placed on a magnetic stirrer at 37°C. Sections of the dissolution medium were taken at different time intervals for 8 hours. The drug concentration in the dissolution medium was determined by UV spectroscopy at 293 nm [7].

Zeta Potential: Zeta potential of the liposomal formulation was determined using a zeta sizer. 1ml of liposome was diluted with water and the sample taken in a clear and cleaned cuvette was placed inside the sample holder for measurement of size [7].

Transmission Electron Microscopy (TEM): A drop of pore dispersant is dripped onto a copper grid covered with a carbon film. Excess dispersion is removed from the mesh with filter paper to form a thin film sample. The sample is then examined under TEM [14].

Release Kinetics: A kinetic study was carried out by fitting the in vitro drug release data into zero order, first order, higuchi model, hixon-crowell cube root law model, and korsmeyer- peppas models. The best outfit model was confirmed by the value of R2 which is near to the kinetic results [15].

Stability Studies: The stability tests were carried out in accordance with ICH recommendations. In sealed glass vials, a sufficient quantity of optimized stealth liposomes and liposomes were retained. It was submitted to a three-month stability investigation at 41°C and 40°C with 75% relative humidity. The physical stability of liposomes and stealth liposomes was evaluated at the beginning and after one month by measuring particle size, % drug content, zeta potential, surface pH, and *in-vitro* drug release [7].

RESULTS AND DISCUSSION:

Identification of Drugs:

Fourier Transform Infrared (FTIR) Study and DSC Study: FTIR spectrum of Flurbiprofen exhibited peak signals at 3073.87cm^{-1}, 2975.94 cm^{-1}, 1690.07 cm^{-1}, 1403.75cm^{-1} and 1213.25,764.24 cm^{-1} due to carboxylic acid, alkane group stretching, C=O stretching of carbonyl group, stretching of amines and presence of halogen. The Differential Scanning Calorimetry studies were carried out for drug [flurbiprofen] and drug-excipients physical mixtures. The recorded DSC thermograms showed the profile of flurbiprofen with a melting point of 117ºC.

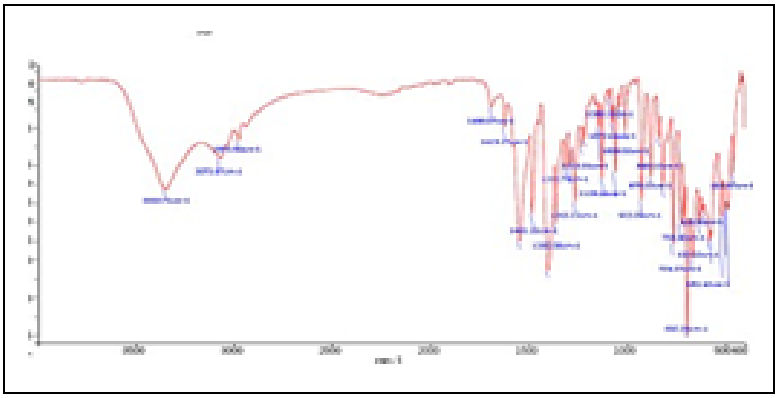


FIG. 1: FTIR SPECTRA FLURBIPROFEN

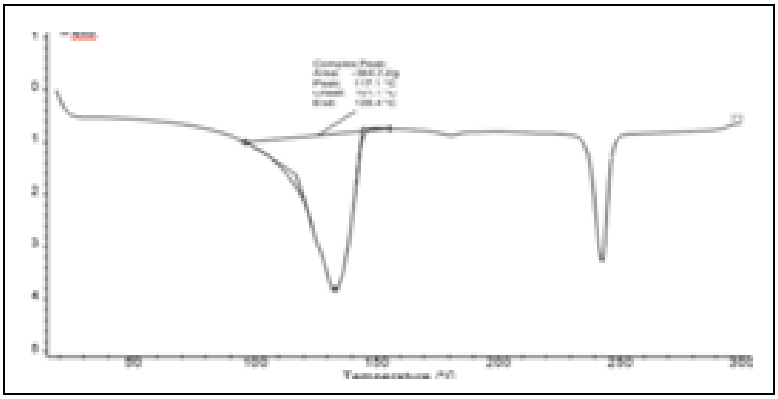


FIG. 2: DSC CURVE OF FLURBIPROFEN EVALUATION OF FLURBIPROFEN LIPOSOMES

Optical Microscopy: Images of liposomes obtained under an optical microscope found that the formed vesicles were spherical in shape. The batches containing non-dispersed lipid film, drug

precipitate, or aggregates were detected and discarded. The microscopic images of stealth liposomes obtained under an optical microscope confirmed the coating of PEG and PVP around the liposome. It was observed that the coated liposomes showed no aggregation.

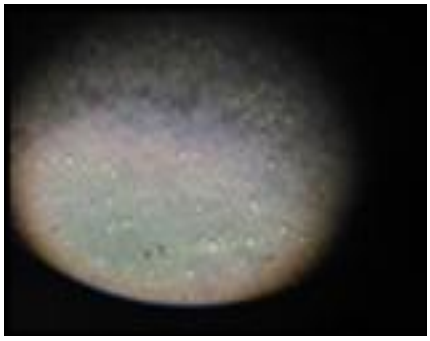


FIG. 3: MICROSCOPIC VIEW OF LIPOSOME

Particle Size and Polydispersity Index Measurement: The particle size of the liposome suspension was measured with a Malvern Nano Zeta sizing device. All vesicles are in the nanometer range with a low polydispersity index, indicating uniformity of particle size.

As cholesterol levels increase, the particle size also increases. The optimal liposome size for slower blood clearance is between 70 nm and 200 nm.

TABLE 2: MEASUREMENT OF PARTICLE SIZE

Formulation code	Particle size (nm)	PDI
L1	157	0.30
L2	178	0.28
L3	187	0.32
L4	230	0.31
L5	244	0.32

% Yield, % Drug Content, % Drug Entrapment, Zeta potential of Liposome: The yield of liposomes increased as the concentration of cholesterol increased. Data on drug composition revealed no significant differences in drug content uniformity between formulations. As a result, flurbiprofen is uniformly dispersed as a vesicular dispersion. The drug entrapment of liposomes was found within the range of 54.51%- 78.12%. The drug entrapment depends on the concentration of cholesterol and lipid. The formulation L2 showed more %EE than that of L1 because cholesterol increases the hydrophobic nature of the membrane bilayer favouring the inclusion of hydrophobic drug molecules. But on further addition of cholesterol, % EE decreases because both cholesterol and the drug prefer to align themselves in the hydrophobic region of the membrane that resulting in lower drug encapsulation with the increasing cholesterol content.

TABLE 3: % YIELD, % DRUG CONTENT, % DRUG ENTRAPMENT, ZETA POTENTIAL OF LIPOSOME

Formulation Code	Yield %	Percentage drug content (Mean± S.D) *	% Drug entrapment (Mean ±S.D)*	Zeta potential (MeV)
L1	72	80.01±0.573	54.51±0.531	-29
L2	80	84.23±0.539	78.12±0.472	-30.7
L3	83	80.15±0.426	70.17±0.812	-24
L4	88.2	81.42±0.814	64.72±0.647	- 22.2
L5	92	82.31±0.512	60.34±0.935	-19.2

Determination of pH of Vesicular Suspension: The pH value of all the prepared liposomal vesicular dispersions ranged from 7.41-7.43. As pH was found to be within the range of blood pH [7.35-7.45], these formulations could be suitable for parenteral drug delivery. The pH values of liposome assured that there will be no irritation. Also, no significant difference was found in the pH of different formulations. This is because of the addition of pH 7.4 phosphate buffer saline.

TABLE 4: MEASUREMENT OF PH OF LIPOSOMES

Formulation Code	pH (Mean± S.D) *
L1	7.41±0.025
L2	7.42±0.02
L3	7.41±0.01
L4	7.43±0.01
L5	7.41±0.025

***In-vitro* Drug Release Studies of Stealth Liposomes:** The result of the *in-vitro* drug release studies showed that with an increase in the concentration of cholesterol, the release rates were found to decrease gradually. The results revealed that the liposome had the ability to extend the

release of flurbiprofen for a duration of 8 hrs. The release rates were graphically represented in **Fig. 4**. The formulation L2 containing 100mg cholesterol showed a maximum of 85.1% drug release at 8 hr compared to other formulations.

TABLE 5: % CDR OF THE FLURBIPROFEN LIPOSOMES

Time (hr)	%CDR				
	L1	L2	L3	L4	L5
0	0	0	0	0	0
1	34.1	42.7	25.8	23.2	20.9
2	39.7	51.6	32.7	32.9	29.8
3	44.5	59.4	43.9	37.4	34.7
4	51.2	64.5	50.2	44.5	42.5
5	57.4	71.2	64.7	54.5	51.3
6	62.3	78.7	72.3	65.5	61.8
7	68.9	82.4	79.4	73.7	67.4
8	72.6	85.1	82.5	79.1	75.1

Transmission Electron Microscopy (TEM): The morphology of the liposomes was revealed using transmission electron microscopy. Liposomes have been found to have a spherical shape.

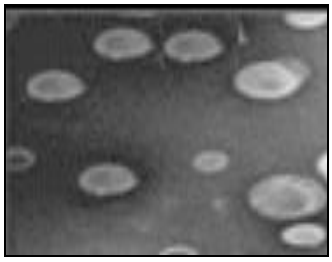
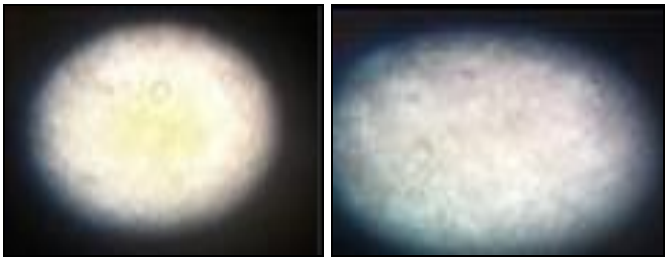


FIG. 4: TEM OF FLURBIPROFEN LIPOSOME EVALUATION OF FLURBIPROFEN STEALTH LIPOSOMES

Optical Microscopy: The microscopic view of stealth liposomes of flurbiprofen was shown in **Fig. 6.** The microscopic images obtained under an optical microscope confirmed the coating of polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP) around the liposome. It was observed that the coated liposomes showed no aggregation.



(A) PEG **(B) PVP**

FIG. 5: MICROSCOPIC VIEW OF STEALTH LIPOSOME

Particle Size and Polydispersity Index: The particle size of the stealth liposomal suspension was measured in the Malvern Nano zeta sizer instrument. The vesicle size of Flurbiprofen-loaded stealth liposomes was shown in **Table 6**. The size of stealth liposomes was slightly enhanced compared to conventional liposomes due to PEG 4000 and PVP forming a thick surface layer on the surface of the liposome vesicles. As the concentration of PEG 4000 and PVP increases, the size of the stealth liposome also increases.

TABLE 6: PARTICLE SIZE AND POLYDISPERSITY OF STEALTH LIPOSOME

Formulation code	Particle size (nm)	PDI	Zeta potential (MeV) (PEG 4000)	Zeta potential (MeV) (PVP K30)
SL1	208	0.24	-59	-57
SL2	220	0.24	-64.2	-60.8
SL3	244	0.23	-60.1	-52.2
SLA	220	0.31	-45	-50
SLB	231	0.25	-58	-51
SLC	250	0.27		

Drug Entrapment Studies and pH Determination of Stealth Liposome: The drug entrapment of stealth liposomes was given in **Table 7**. It was observed that the % drug entrapment of stealth liposomes was similar to that of liposomes. It was revealed that there was no drug loss while the coating of liposomes using PEG 4000 and PVP. The pH of stealth liposomes was also found to be

around pH 7.4 as shown in Table 7. The PEG 4000 and PVP do not change the pH of the stealth vesicular suspensions and are suitable for parenteral drug delivery.

TABLE 7: % DRUG ENTRAPMENT AND PH OF STEALTH LIPOSOME

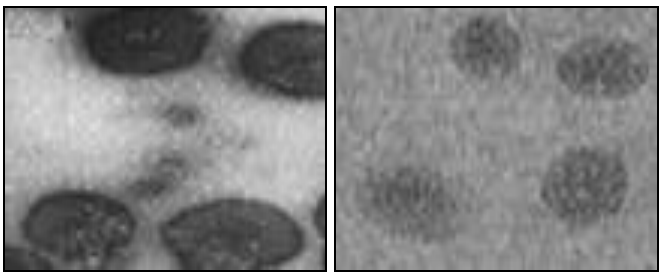
Formulation Code	% drug entrapment	pH* (Mean± S.D)
SL1	78.52±0.714	7.41±0.004
SL2	78.68±0.514	7.41±0.01
SL3	78.78±0.876	7.41±0.01
SLA	77.30±0.632	7.41±0.05
SLB	78.52±0.712	7.41±0.02
SLC	78.14±0.436	7.41±0.02

***In-vitro* Drug Release Studies of Stealth Liposomes:** The results of the *in-vitro* drug release studies revealed that with increasing concentration of PEG 4000, the stealth liposomes showed a more sustained drug release profile than that of PVP K30. The result showed that the stealth liposomes with PEG 4000 had the ability to extend the release of flurbiprofen for a duration of 24 hr than PVP K30 **Table 6**. This result indicated that the stealth liposomes with PEG 4000 showed a sustained release profile than conventional liposomes.

TABLE 8: *IN-VITRO* DRUG RELEASE OF STEALTH LIPOSOME

Time (hr)	%CDR					
	SL1	SL2	SL3	SLA	SLB	SLC
0	0	0	0	0	0	0
1	16.7	20.7	13.7	11.27	17.2	18.4
2	21.4	27.3	18.6	22.2	20.3	24.7
3	29.5	32.8	22.7	28.2	30.4	32.3
4	34.7	46.7	29.8	32.1	35.7	41.2
5	40.9	51.4	37.6	50.3	41.3	49.7
6	48.6	60.7	44.9	69.5	49.1	53.2
7	52.3	69.1	56.1	50.4	54.6	60.9
8	60.8	70.9	65.3	55.3	61.5	66.6
9	69.7	76.1	70.2	60.8	67.7	72.3
10	75.7	80.3	79.4	68.3	74.4	79.2
11	80.1	88.3	87.5	78.4	79.2	82.1
12	89.7	91.5	93.7	83.2	86.7	85.5

Transmission Electron Microscopy (TEM): TEM of Flurbiprofen-loaded stealth liposome is given in **Fig. 7**. TEM confirmed the presence of PEG coating around the liposomes.



(A) PEG **(B) PVP**

FIG. 6: TEM OF FLURBIPROFEN STEALTH LIPOSOME KINETIC STUDY OF THE LIPOSOME AND STEALTH LIPOSOME

To determine the release mechanism that gives the best description of the pattern of drug release, the *in-vitro* release data were fitted to zero-order, first-order, Hixson Crowell equation, and Higuchi matrix model. The release data were also kinetically analysed using the Korsmeyer– Peppas model. The accuracy and prediction ability of the models were compared by calculation of R^2. The model giving R^2 close to unity was taken as the best fit model. The value of 'n' indicates the drug release mechanism. The release kinetics data indicates that the release of drug from stealth liposome best fits zero-order release kinetics. The diffusion data were plotted with the Higuchi equation and the dissolution data were also plotted in accordance with Hixon- Crowell cube root law. To determine whether fickian or non-fickian diffusion existed, data were analysed using the Korsmeyer-Peppas equation. The n value determined lies between 0.5 and 1.0 indicating it follows non-fickian diffusion.

TABLE 9: KINETIC PROFILE OF LIPOSOME AND STEALTH LIPOSOME

Formulation Code	Zero order R2	First order R2	Higuchi model R2	Hixon- Crowell model R2	Korsmeyer-Peppas N
L1	0.87	0.965	0.985	0.944	0.592
L2	0.9906	0.974	0.977	0.942	0.566
L3	0.9606	0.984	0.958	0.989	0.595
L4	0.9706	0.970	0.967	0.982	0.601
L5	0.9758	0.978	0.968	0.987	0.616
SL1	0.874	0.980	0.981	0.965	0.664
SL2	0.993	0.961	0.940	0.914	0.604
SL3	0.984	0.884	0.987	0.977	0.729
SLA	0.906	0.972	0.953	0.990	0.616
SLB	0.853	0.977	0.974	0.949	0.655
SLC	0.792	0.949	0.952	0.906	0.623

Stability Studies: The selected liposome and stealth liposome formulations were subjected to a stability study. Initial and three-month studies were done. There were no significant changes in the particle size, zeta potential, % drug content, surface pH, and *in-vitro* drug release for stealth liposomes at 4±1ºC compared to liposomes stored at 40ºC. So, stealth liposomes were more stable at 4±1ºC temperature. The stability studies will be continued further up to 6 months.

TABLE 10: STABILITY DATA OF LIPOSOME AND STEALTH LIPOSOME AT 40°C

Parameters	Liposomes		Stealth liposomes			
	Initial	After 90 days	Initial		After 90 days	
			PEG 4000	PVP K30	PEG 4000	PVP K30
Particle Size	178nm	160nm	245nm	230nm	250nm	240nm
Zeta Potential	-30.7mV	-20mV	-60.8mV	-64.2mV	-50mV	-40.4Mv
% drug Content	84.2	70.1	84.2	82.7	74	71
Surface pH	7.4	7.1	7.4	7.4	7.3	7.2

TABLE 11: STABILITY DATA OF LIPOSOME AND STEALTH LIPOSOME AT 4±1°C

Parameters	Liposomes		Stealth liposomes			
	Initial	After 90 days	Initil		After 90 days	
			PEG 4000	PVP K30	PEG 4000	PVP K30
Particle Size	178nm	187nm	245nm	230nm	247nm	233nm
Zeta Potential	-30.7Mv	-28Mv	-60.8mV	-64.2mV	-59mV	-60.4Mv
% drug Content	84.2	74.1	84.2	82.7	81.7	82
Surface pH	7.4	7.2	7.4	7.4	7.4	7.4

CONCLUSION: Stealth liposome is a novel dosage form that has prolonged release than conventional oral dosage forms and improves the stability of the drug. The best-formulated liposomes were coated with 10% PEG4000 and 1% PVP K30 to form stealth liposomes and compared the study. RA is a major cause of disability and death and has a high social and economic cost. The most well-known period of the beginning of joint pain is between 30-40 years. The prevalence of RA in India is higher than that reported in China, Indonesia, the Philippines, and Africa, with approximately 7 million patients. The regular liposome has the downside of simple acknowledgment by the mononuclear phagocyte framework and is quickly cleared from the blood. Stealth liposomes are long-circulating liposomes whose hydrophilic polymers modify their surface. The presence of hydrophilic polymers on the outer layer of the liposomal transporter has been displayed to expand blood dissemination time while diminishing phagocyte framework take-up and, in this way, conquering the downsides of the ordinary liposome. Since the stealth liposome has a long, slow release, side effects, and toxicity will be minimized. Although a limited number of PEGylated liposomal formulations have been approved or are undergoing advanced testing, stealth technology is expected to continue developing, providing a targeted delivery system. PEG 4000 and PVP K30 were used to make a stealth liposome loaded with flurbiprofen in this study. In view of the *in-vitro* disintegration concentrate it was cleared that the secrecy liposome

formed with Stake 4000 shows preferable supported discharge over the covertness liposome with PVP K30. Consequently, covert liposomes offer a promising road to satisfy the requirement for a parenteral medication conveyance framework that can further develop plasma soundness and keep up with drug movement for a delayed time frame subsequently permitting a supported activity; improving patient compliance by reducing administration frequency. As a result, the stealth liposome offers an alternative to the standard dosage form.

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CONFLICTS OF INTEREST: Nil

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