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Development and evaluation of metformin liposome formulations

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Abstract

This inquiry focuses on liposomes as its subject matter. Liposomes are an excellent means of increasing the period of time during which an impact is active from hours to months. In the current investigation, liposomal formulations of metformin hydrochloride were created and analysed by the researchers. Because its plasma half-life is much shorter than that of other drugs (t1/2 = 1.5 h), the patient has to take the drug more often in order to keep its concentration in steady state plasma. In this study, liposomal formulations of metformin were tested for drug entrapment, surface characterisation, in-vitro drug release investigations, release kinetics, and their underlying processes. Study concludes that the development of liposomal solutions of metformin hydrochloride was a fruitful endeavour.

Keywords: Liposomes, metformin, in-vitro drug release.

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Introduction

Liposomes are colloidal structures that take the form of vesicles and are made up of one or more bilayers encasing an equal number of aqueous compartments. Liposomes are artificial vesicles that are very tiny and have a spherical form. They may be manufactured from cholesterol and natural phospholipids that are not poisonous. Liposomes are intriguing candidates for use as drug delivery vehicles due to their size, which makes them both hydrophobic and hydrophilic, in addition to being biocompatible. The liquid inside of the sphere-like shell included compounds such as peptides, protein, hormones, enzymes, antibiotics, anti-fungal agents, and anti-cancer agents. The shell was in the shape of a spherical [1-5].

Hydration of phospholipids in aqueous solutions has been demonstrated to instantly initiate the formation of closed structures. [Needs citation] These vesicles with one or more phospholipid bilayer membranes may carry either aqueous or lipid drugs, depending on the makeup of the medications. Hydrophobic and hydrophilic lipid sections in spherical bilayers are captured by entropically focused confiscation because lipids in aqueous solutions are amphipathic, meaning they have both hydrophobic and hydrophilic properties. Each of these layers is referred to as a "lamellae [6-9]."

The particle sizes of liposomes may vary anywhere from 30 nanometers to several micrometres. The polar head groups of the lipids are organised in such a way that they face in the direction of the route that links the interior and exterior aqueous phases, and these lipid bilayers contain aqueous units. A broad range of colloidal particles may be self-assembled from the classic bilayer architectures that rely on temperature, ambient conditions, and the preparation procedures for polar lipid self-aggregation, however [10-12].

The purpose of this work was to develop a method for providing sustained drug administration using liposomes formulated with metformin HCl. After the liposomes were created using two distinct approaches

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(the physical dispersion technique and the ether injection method), they were analysed using a number of different criteria [13].

The following is an explanation of the study's overall purpose:

To overcome the inherent flaws that are associated with the usual dose form of metformin HCl by developing oral metformin HCl liposomes that offer the following benefits:

- Decrease the dosing regularity
- Reduce the side consequence.
- Extend the action of drug
- Deliver sustained drug release.
- Improved patient complince

Physical dispersion method

Liposomes were created by altering the ratios of soy lecithin and cholesterol. Chloroform-dissolved soy lecithin and cholesterol. The solution was left to dry overnight in an empty conical flask at room temperature. To dissolve the medicine, we employed a phosphate buffer solution (pH 6.8). In its most basic form, it's a water-carrier. As soon as the oily layer was saturated with water, a further layer of water was applied. As a result, the flask was tilted to one side and aqueous medium was progressively poured down its side. To finish the hydration, the conical flask was submerged in water and heated to 372°C for two hours. The conical flask was gently shaken to remove the lipid layer from the wall and create a liposome suspension. Once the liposomes had matured, the solution was maintained at 4oC for a further day. The liposome suspension had been centrifuged for 20 minutes at 15,000 rpm and was ready. When it was all done, the precipitate had been prepared for further study by dissolving it in distilled water. In agreement with the overall strategy mentioned above, the liposomes in Table 5 reveal their composition [14,15].

Ether injection method

Different ratios of soya lecithin and cholesterol were used in the ether injection procedure to manufacture the products. Ethyl methanol were used to dissolve the cholesterol and soya lecithin. The phosphate buffer pH 6.8 was used to dissolve the medication. It functions as a water-holding medium. The temperature of the aqueous medium was raised to 60 degrees Celsius. A primary unilamellar liposome is formed when ether evaporates and comes into contact with the water phase. After that, the product was collected and kept at 4°C to allow the liposome to mature. After that, the liposomal solution was centrifuged for 20 minutes at 15,000 rpm to remove any remaining particles. This water was used to do assessment studies [16,17].

Table 01: Composition of Metfonnin HCI liposomes

S. No	Ingredients	Physical dispersion method	Ether injection method				
		F1	F2	F3	F4	F5	F6
1.	Cholesterol	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
2.	Lecithin	100 mg	200 mg	300 mg	100 mg	200 mg	300 mg
3.	Metfmmin HC	10 gm	10 gm	10 gm	10 gm	10 gm	10 gm
4.	Ether	-	-	-	7 ml	M 1	71
5.	Methonol	-	-	-	3 ml	3ml	3ml
6	Choloroform	5ml	5ml	5ml	-	-	-
7	Phosphate buffer pH 608	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Evaluation of Liposomes [42, 43]

1. **The percentage of drug entrapment efficiency that may be determined**

Centrifugation was used to determine the efficacy of drug entrapment. The liposome suspension was centrifuged at 15,000 rpm for 20 minutes after being diluted to 10 ml. After collecting and diluting the supernatant liquid, it was ready to be used. As a control, a pH 6.8 solution was used in a UV double beam spectrophotometer to measure absorbance at 233 nm. The following formula was used to determine the drug

entrapment efficiency [18-21].

$$\text{Total entrapment efficiency} = \frac{\text{Amount of drug in supernatant liquid}}{\text{Amount of drug}} \times 100$$

In vitro drug release study:

In phosphate buffer p H 6.8, all of the Metformin HCl liposome formulations were tested for 8 hours in vitro. A temperature of 37°C (0.5°C) and a speed of 50 rpm were used in the experiments, which were conducted in the USP dissolving apparatus II (Paddle). As a dissolving media, phosphate buffer pH 6.8 was utilised. In a dissolving jar, a liposome containing 100 mg of Metformin HCl was taken and the paddle was spun at 50 rpm. Every 30 minutes for up to 480 minutes, 1 ml of samples were extracted and made up to 10 ml with a pH of 6.8 and evaluated using a double-beam UV double-beam spectrophotometer79 for the Metformin HCl concentration [22].

Particle size determination:

In order to determine the particle size, the Malven particle size analyzer is employed. The measuring unit has a flow cell, and the sampler has a dispersion bath. To evaluate the dispersion of particle groups in a liquid media, a flow cell and dispersion bath are used. The dispersion bath is equipped with both a stirrer and an ultrasonic sonicator. A pump is used to bring the scattered suspension to the flow cell. In addition to the liquid medium, the pump's unusual design allows it to circulate small particles as well. It may be operated from a personal computer, making it easy to use. Organic solvents are one kind of dispersion medium [23].

Kinetics of drug release

Many theories and kinetic models describe how immediate and modified release medicines dissolve. Modeling drug dissolution with f (t). Drug dissolution

Using a common equation to quantify the dissolution curve function of several medicinal dose forms facilitates quantitative interpretation. Q is a function of t or Q in drug dissolution kinetic models (t). These include the Higuchi, Korsmeyer-Peppas, Hixson-Crowell, and Weibull models. These models can predict medication disintegration and release [24, 25].

Zero order kinetics

This model shows the pharmacologically prolonged release profile. Zero order release is drug release regardless of drug quantity in delivery method (that is, a constant release rate).

Stability studies

After a month of testing, researchers found that the liposome could keep the medication within for up to two degrees cooler (refrigerator RF) than the ambient temperature of 25 degrees Celsius (plus or minus two degrees Celsius). It was necessary to keep the liposomal compositions in vials with tight-fitting covers. For the evaluation at day 30, we followed up on the sections on drug encapsulation efficiency and in vitro drug release. Study number 81 also looked at the liposome's form as well [26].

Result

Liposomal metformin HCl was the focus of this study in order to make it last longer than eight hours before it loses its effectiveness. The liposomes were prepared using both the physical dispersion technique and the ether injection method. Encapsulating the drug with soya lecithin and cholesterol allowed for a more slow and regulated release of the active substance. Chloroform, ether, and methanol were utilised as solvents. Hydration and loading of the medicine were accomplished by using phosphate buffer, which has a pH of 6.8.

Table 02: Particle size and Zeta potential of formulations.

S.No.	Formulation code	Size (nm)	Zeta Potential	PDI
1	F1	67.6	-08.1	0.57
2	F2	61.42	-08.6	0.61
3	F3	59.56	-08.9	0.59
4	F4	76.26	-10.5	0.51
5	F5	71.36	-09.8	0.53
6	F6	69.11	-10.4	0.49

Table 03: Evaluation parameters liposome formulations.

S No.	Code	% Yiedl Mean ±SD	% Content	% entrapment efficiency
1	F1	46.4±2.72	87.64±2.72	81.64±1.72
2	F2	74.4±1.39	90.47±1.39	82.47±1.30
3	F3	72.84±0.84	89.84±0.84	81.84±0.84
4	F4	78.33±0.93	88.84±0.93	84.84±0.93
5	F5	80.64±1.12	92.63±1.12	89.63±0.12
6	F6	79.64±1.10	91.26±1.10	91.26±0.10

Table 10% Durg content of formulations

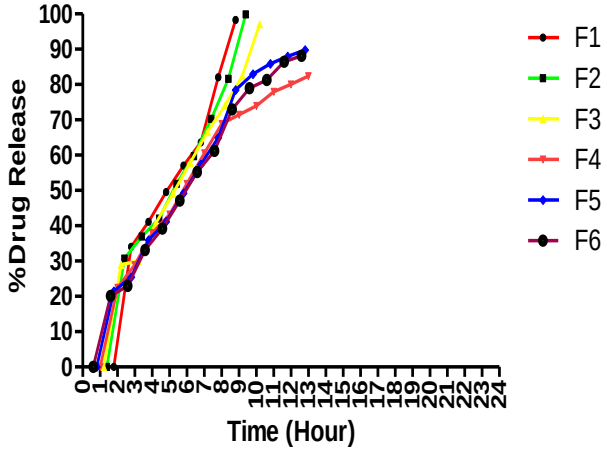

Figure 01: Cumulative Invitro drug release of liposome Formulation

Table 04: Model fitting data for in-vitro release kinetic parameters of Liposome formulations.

Formulation Code	Zero order [r]	First order [r]	Higuchi [r]	Hixson Crowell [r[	Korsmerer-peppas [r]	'n' Values
F1	0.923	0.953	0.991	0.804	0.651	0.520
F2	0.971	0.884	0.991	0.815	0.704	0.6492
F3	0.981	0.910	0.981	0.830	0.746	0.74
F4	0.990	0.860	0.926	0.933	0.946	0.902
F5	0.993	0.822	0.935	0.921	0.951	1.0799

Table 05: Stability Study for F5 formulation

S. No	Formulations	Before storage	Stored at 40oCand 75% ±5%RH		
			1st month	2nd month	3rd month
1	F5	92.63±0.74	91.63±0.74	90.63 ±0.74	89.63±0.74

Discussion

A particle size analyzer was utilised to check the particle size of each liposome formulation that was manufactured. All of the produced liposomes had particle sizes between 30.617 m and 0.031 m, as demonstrated in Table No. 7 and clearly in Figures Nos. 15 through [20]. All formulations of Metformin HCl liposomes produced using either technique showed a negative connection between increasing the concentration of soya lecithin and a decrease in particle size. F 3 and F 6 liposomes have lower particle sizes than previous formulations of Metformin HCl liposomal drugs. This might be due to the higher concentration of soy lecithin in these formulations [27].

An optical microscope (Olympus Opto System, India) was utilised to explore the morphological aspects of liposomes, and digital cameras were employed to capture the images that were produced as a consequence of this optical microscope. Figures 9-14 show microscopic images of the formulations F 1, F 2, F 3, F4, F 5, and F 6. These images were taken from the formulations. Characters of prepared liposomes F 1 through F 6 may be clearly characterised in terms of their shape. The maximum drug content readings for the F5 are 92.63 percent. the percentage entrapment efficiency, with the F5 at 89.94 percent. Because the viscosity of the solution increased as the polymer content was raised, A greater percentage of the substance was successfully trapped. Drug entrapment efficiency is greater in solvent-soluble medicines than it is in solvent-dispersed pharmaceuticals, according to the results of the present investigation. Liposome entrapment efficiency improved as the concentration of polymer utilised in the liposome increased, resulting in a rise in the quantity of polymer required in the manufacturing process.

The drug entrapment efficiencies of formulations F 1, F 2, and F 3 were 86.60%, 79.90%, and 73.10%, while F 4, F 5, and F 6 were 30.47%, 39.58%, and 39.69%. Physical dispersion may be more effective than ether injection for drug entrapment. A USP dissolving apparatus Type I was used to conduct dissolution investigations on all six formulations of metformin. In vitro drug release findings are shown in Table 04 and Figure 01 for all formulations. The cumulative percent drug release for F5 was determined to be 89.83 percent after 12 hours. The cumulative drug release decreased considerably as the polymer content rose. The density of the polymer matrix rises with higher concentrations, resulting in a longer diffusional route. As a consequence, the total release of drugs from the polymer matrix may be lowered. More surface area of the dissolving solution is exposed to the smaller liposme that are produced at

lower polymer concentrations. In order to find an equation that best suited the data obtained from in vitro dissolution testing, we utilised the zero-order, first-order, and Korsemeyer-Peppas equations. It was found out that the formulations F1, F2, F4, and F5 all showed first order release kinetics when the data on the release rate was fitted to the different models28.

Drug release is substantially slower than polymer relaxation in the Fickian release mechanism (erosion). As a result, drug release is mostly influenced by matrix diffusion. The combined effects of drug diffusion and polymer relaxation determine the rate of drug release in the non-Fickian (anomalous) situation. The polymer relaxation is known as Case II release. For formulations F1 through F5, the n values ranged from 0.520 to 1.079, indicating non-Fickian or anomalous release (0.5 n 1). Polymer relaxation (erosion) and diffusion were both controlling drug release from microspheres, according to the n values F1 to F5. The results demonstrated that the F5 formulation remained stable over a three-month period with no significant changes in drug content. F5 was tested for 3 months at 400 degrees Celsius and 75% relative humidity to find the ideal formulation. Under accelerated stability settings, the microspheres' efficacy was between 90 percent to 110 percent of the label promise. The spheres were chemically stable for more than 3 months, according to the findings of the stability tests

Conclusion

According to the results of this study, it was possible to successfully produce a liposomal drug delivery system that included metformin HCl by using a range of approaches, such as the physical dispersion approach and the ether injection method.

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