ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.3.p7-13

International Journal of Life science and Pharma Research

Research Article

Pharmacy for better health

Development of optimized formulation of liposome using 3-factor Box-Behnken Design

MahendraChouhan[1], Rajesh Sharma[1*] and Kamlesh Dashora[2]

[1]*School of Pharmacy Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India*

[2]*Institute of Pharmacy, Vikram University, Ujjain Madhya Pradesh, India*

Abstract: The objective of our present study is to optimize Chitosan coated liposomes formulation by Response surface methodology using 3-factor Box-Behnken Design. Different polymers based liposomes used for delivery of stable pH dependent formulation. Chitosan has been used as a pH sensitive polymer coating to target nanoparticles specifically to tumors which have a slightly acidic pH. Closed membrane system can accommodate amphiphilic or lipophilic drugs in vesicles. The optimized batch was formulated as a liposome delivery system and evaluation was done. To evaluate the unentrapped drug Shimadzu UV-Spectrometry at 228 nm was used and absorbance was noted. Response surface graph was prepared to predict value and the optimized formulation (Chitosan coated liposomes) can be used for loading of bio-active.The values of percent drug entrapment and average vesicle size were presented and found formulation F3&F5 were optimized for further evaluating on basis of particle size and drug loading.

Keywords: Chitosan, liposomes, Box-Behnken Design, lipophilic, nanoparticles, optimization

***Corresponding Author**

Rajesh Sharma , School of Pharmacy Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India

Recieved On 01 June 2020
Revised On 20 January 2021
Accepted On 23 January 2021
Published On 05 May 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Mahendra Chouhan, Rajesh Sharma*, Kamlesh Dashora , Development of optimized formulation of liposome using 3-factor Box-Behnken Design..(2021).Int. J. Life Sci. Pharma Res.11(3), p7-13 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.3.p7-13

1. INTRODUCTION

Liposome, are defined as the particulate drug carriers, prepared by dispersion of phospholipids in aqueous media and is based on Response surface methodology using 3-factor Box-Behnken Design.[1]The aim of study is to design different polymers based liposomes fordeliveryof stable pH dependent formulation. Chitosan has been used as a pH sensitive polymer coating to target nanoparticles specifically to tumors which have a slightly acidic pH. Closed membrane system can accommodate amphiphilic or lipophilic drugs in vesicles. The objective of our present study is to optimize Chitosan coated liposomes formulation by Response surface methodology using 3-factor Box-Behnken Design. The optimized batch was formulated as a liposome delivery system and evaluation was done. The resulting closed membrane structures can accommodate amphiphilic or lipophilic drugs incorporated into or associated with the lipid bilayer, as opposed to direct encapsulation or active entrapment of hydrophilic compounds within the aqueous inner compartment of the vesicles.[2] Bingham's observation on hexagonal liquid crystals, that they are perm selective to the ions in manner similar to biomembrane, led to discovery of an artificial vesicular system based on phospholipids amphiphiles. Liposomes can transferlipophilic drugs to biological systems system. Liposomes composed of glycerolipids or phospholipids which have good biodegradability as well as biocompatibility property system. However, the vesicular structures are chemically and physically unstable. Liposomes have the separate advantages of biodegradable & non-toxicity as they are made up of natural substances. Biologically active liposome protects drugs as well as safely sends drugs to targeted organs. The unique ability of liposomes to trap the drugs in the aqueous phase as well as in lipid phase is an needed for the drug delivery systems.[3-5] The liposomes were composed of different polymer such as Eudragit, Carbopol, HPMC, pectin, alginate, cellulose, polyacrylic acid polyacrylamide and chitosan for delivery purpose by taking advantage of their pH dependent stability. The chitosan coating for the preparation of liposomesis useful in increasing the entrapment efficiency of the drug in the vesicle in comparison with the other polymer. Among the suggested polymers, the entrapment efficiency and mucoadhesiveness of liposomes coated with chitosan showed effective drug release in the stomach pH sensitive area. The chitosan coated liposome may have a promising future in the oral delivery system of various drugs. Hajimee*t al*[6]Prepared a multi unithighly-loaded gastro-floating system of clarithromycin and saw that it enhanced the eradication of *H. pylori*.Likewise Lin et al.[7] prepared water-in-oil emulsification system to prepare a positively charged nanoemulsion particle composed of amoxicillin, chitosan, and heparin, they evaluated that the amoxicillin loaded in the primary phase works as a good device to deliver the drug. Karnet al.[8]prepared and compared the Mucoadhesive power of liposomal preparation and concluded that the liposomes are the most suitable Mucoadhesive delivery systems. Arora et al.[9] studied about Mucopenetrating Chitosan-alginate complex nanoparticles containing amoxicillin release System for *H. Pylori* they found apart from mucoadhesionmucus penetration is also one of the choice bale approaches for the researchers. Faivreet al[10] took cholesteryltetraethylene glycol side as model ligands for targeting the *H. Pylori* and confirmed its orientation towards the infection. Same as various approaches are in progress to delivery of a*ntiH.pylori*drugs with help of Liposome to achieve optimized formulation. Chitosan is been used as a pH sensitive polymer coating to target nanoparticles specifically to tumors which have a slightly acidic pH than the surrounding environment.[11]Low molecular weight chitosan (LMWC) could be used as an alternative stealth coating, responsive to the pH of target tumors. Here, LMWCs with various molecular weights (MWs) were covalently conjugated to poly (lactic-co-glycolic acid) (PLGA). The novelty behind our work is that the nanoparticles prepared with the PLGA-LMWC conjugates were tested with respect to the physical and chemical properties, pH-sensitivity in cell-NP interaction drug delivery, and the potential to provide a stealth surface on NPs, in comparison with PLGA NPs with bare surface and/or PLGA NPs covered with PEG.[12] Existing studies have shown that to optimize the characteristics and stability of LPs, they can be coated by Chitosan which is naturally occurring linear polymer. It is a cationic polysaccharide known to be non-toxic, bioabsorbable, and biocompatible.[13]

1.1 Optimization of Chitosan coated liposomes

1.1.1 Box-Behnken experimental design

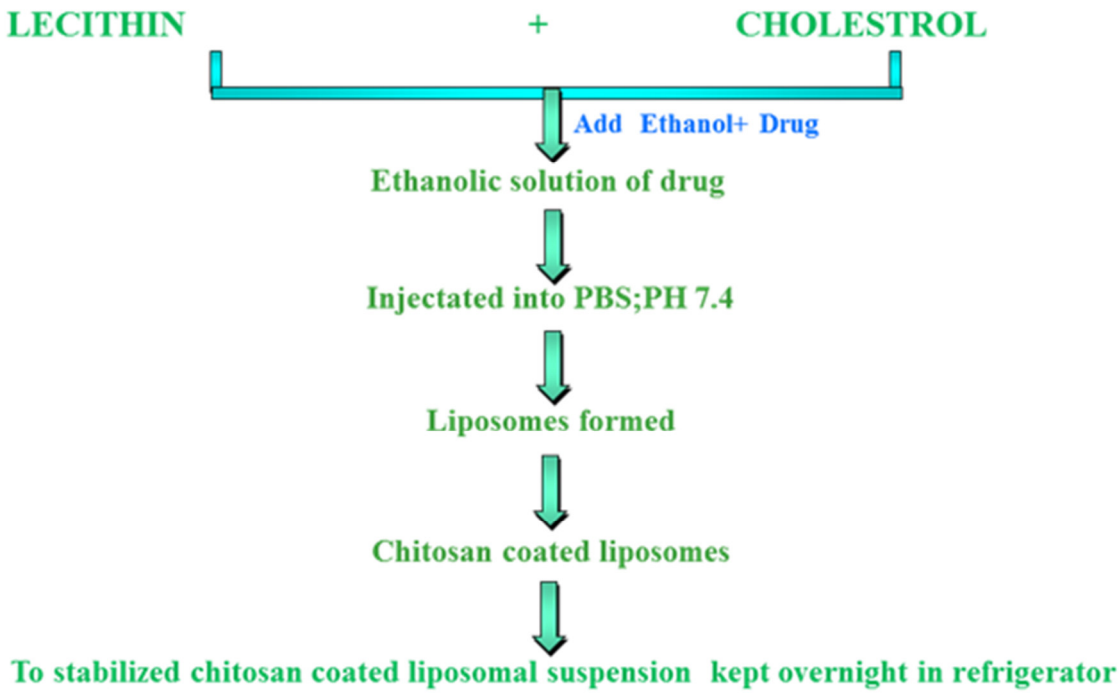
For coating of liposomes and surface modification, Chitosan was used, which has mucoadhesive properties. This increases resident time and absorption at the mucosal site. The purpose of study was to prepare and optimize the Chitosan-coated liposomal formulation using Box-Behnken Design based on RSM design principle using 3-factors on response, through which the optimum formulation was prepared.[14-17]

2. MATERIALS AND METHODS

All chemicals and reagent used in present work is of analytical grade without further purification. Lecithin was obtained as a gift sample and other solvents are of HPLC grades, Chitosan (Fisher Company), Ethyl cellulose are of analytical grade. In this work, we used purified water for preparation and analysis.

2.1 Preparation of Liposomes

Liposomes were prepared by modified ethanol injection method as reported by karn et al, 2011.[8]Briefly, the Lecithin: Cholesterol was taken in a round bottom flask and dissolved using different ratios of anaqueous and organic phase. A thin film was casted by rotating the flask and it was kept overnight for complete removal of solvents. The film was hydrated using PBS pH 7.4 Liposome's coating process composed of(Sentence incomplete)0.1% to 0.3% (w/v) chitosan solutions in 0.1%(v/v) glacial acetic acid. A volume of 3.0 mL of Chitosan solution was added dropwiseinto 3.0 mL of liposomes under magnetic stirring at room temperature for 1 hour, followed by incubation in the refrigerator overnight. The rate of stirring was kept constant for all preparations by this method.[8-11]



2.2 *Experimental Design*

The optimum levels of the independent variables including lecithin amount, cholesterol amount, and ratio of aqueous and organic concentration and Chitosan concentration.[18-19] Table 1 shows the preferred factors and setting of factors levels on the bases of low, medium and high as 1,0 and +1.[20]

Table 1: Independent variable and their levels for Box Behnken Design Expert

Factors	Range and levels			
	units	-1	0	+1
Lecithin: Cholesterol ratio	mg	1:2	1:1	2:1
Ratio of aqueous and organic phase	ml	3.33:1	5:1	10:1
Percentage of Chitosan solution for coated liposomes	%	1%	2%	3%

Box-Behnken design was carried out 3 level and 3- factors using design expert software and there are a total 13 batches were prepared as given by software. (Table 2)

Table 2: Levels of different factors

Run Batch Number	L:C	A:NA	C:C
F1	2:1	5:1	1%
F2	2:1	5:1	3%
F3	1:1	10:1	1%
F4	1:2	10:1	2%
F5	1:1	5:1	2%
F6	1:2	5:1	3%
F7	1:1	3.33:1	1%
F8	2:1	10:1	2%
F9	1:2	5:1	1%
F10	2:1	3.33:1	2%
F11	1:1	3.33:1	3%
F12	1:1	10:1	3%
F13	1:2	3.33:1	2%

2.3 *Characterization of Prepared Liposome*

The 13 batches of Chitosan coated liposome formulations were characterized by their particle size and various functionalized Chitosan coated liposomes was determined by their zeta potential (ζ), calculated according to the Helmholtz-Smoluchowski equation from their electrophoresis mobility.[12-14]

$$Z = 4\pi\mu\eta/D$$

where, μ is the electrophoretic mobility, η is viscosity, D is dielectric constant.

2.3.1 *Particle size determination*

The particle size was determined by Malvern Zetasizer (Malvern Instrument, UK). The particle size of various Chitosan coated liposomes were determined with 0.05 mg/ml concentration suspended in double deionized water (pH 7.0), acidic medium (pH 4.0) and alkaline medium (pH 9.0). The acidic and alkaline media were adjusted with the HCl and NaOH, respectively. In each case, the measurement was carried out in triplicate (n=3).

2.3.2 *Drug encapsulation efficiency*

Two ml of the formulations were taken and separately dialyzed using the dialysis membrane against 50 ml Distilled water for 15 min. One ml of distilled water was withdrawn in a 10 ml volumetric flask and the volume was made up to 10 ml with fresh distilled water. The absorbance was noted on Shimadzu UV-Spectrometry at 228 nm and used to evaluate the entrapped drug. Further the quantity of the drug entrapped was evaluated. Drug encapsulation efficiency of various Chitosan coated liposomes were determined and calculated by the formula.[15-20]

$$DEE\% = \frac{Weight\ of\ encapsulated\ drug}{Weight\ of\ encapsulated\ drug + free\ drug} \times 100$$

3 RESULTS

The particle size was found in the range of 197.91±02.43 (fig2) for Uncoated Chitosan coated liposomes and 262.5±04.23nm (Fig 1) for 2% Chitosan coated liposomes.

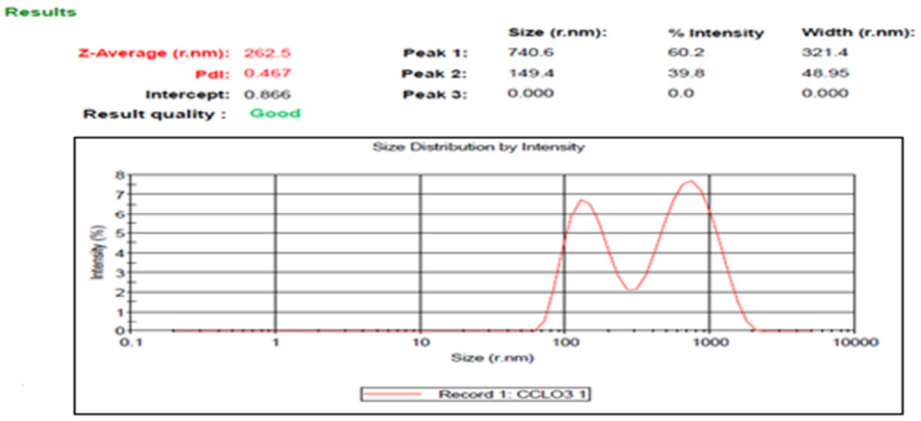


Fig 1: Chitosan coated liposomes(CCLO3)

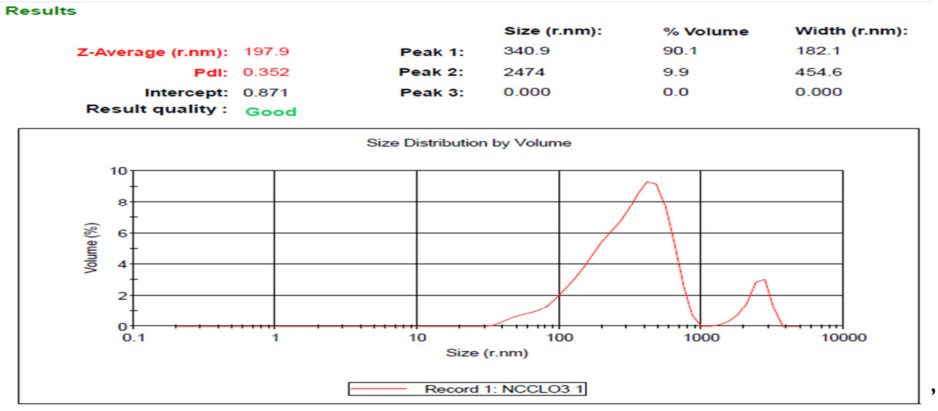


Fig 2: N-Chitosan coated liposomes (NCCL03)

The experimental design used for this study was Box-Behnken experimental design with three independent variables at three different levels Behnken experimental design to full factorial design is that it requires fewer experimental batches.[21]The values of percent drug encapsulated and average vesicle size is shown in Table 3.

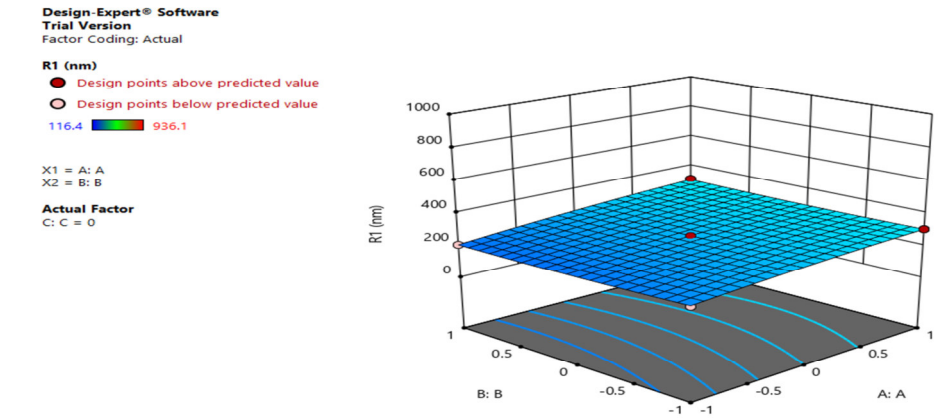
Table 3: Results of response surface method

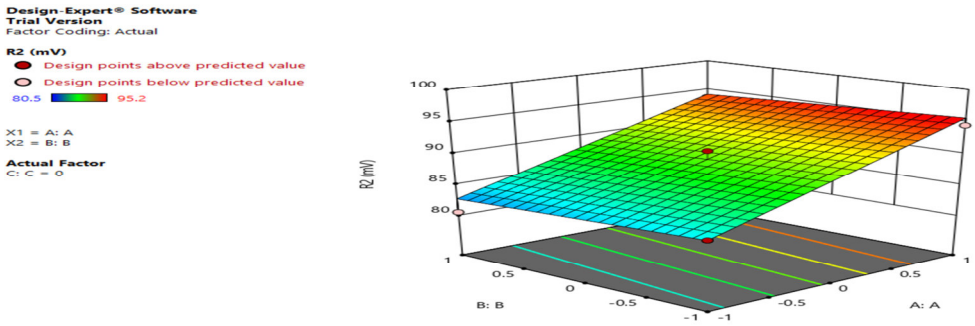
Formulation code	Chitosan conc. (% W/V)	Particle size (nm) R1	Percentage drug encapsulated R2
F1	1%	156.47±02.94	95.24±03.24%
F2	3%	936.12±03.47	93.56±03.46%
F3	1%	197.91±02.43	91.23±01.64%
F4	2%	205.63±03.48	80.51±01.97%
F5	2%	262.5±04.23	90.52±3.92%

F6	3%	811.2±02.93	82.71±03.26%
F7	1%	185.41±04.42	94.64±02.52%
F8	2%	311.1±03.94	93.74±02.52%
F9	1%	116.4±01.91	83.6±04.32%
F10	2%	303.61±01.36	94.52±01.92%
F11	3%	872.50±03.93	89.05±04.12%
F12	3%	829.20±02.41	88.06±02.27%
F13	2%	282.50±03.92	84.06±05.32%

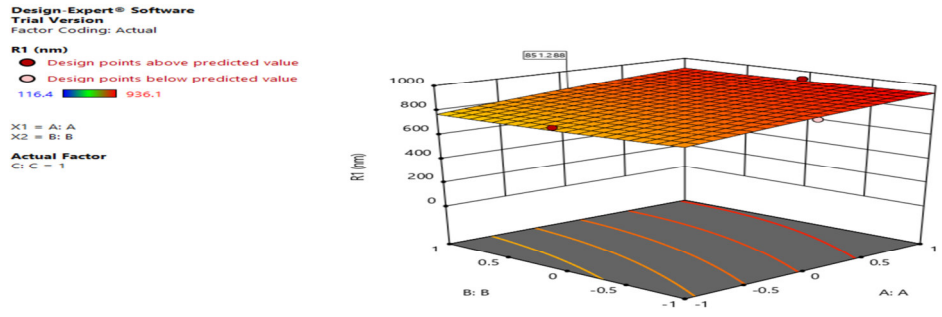
Values are mean ± SD; (n=6)
P<0.01 when compared with control formulation

The 3D response surface plots showed particle size (R1) and percentage drug encapsulated (R2) at the different concentration of Chitosan, technique enumerates the functional relationship between the variables which are measurable at constant level and mathematical analysis indicated the observed responses.[20-21] (Fig 3.)





R1 Particle size, R2 Percentage drug encapsulated



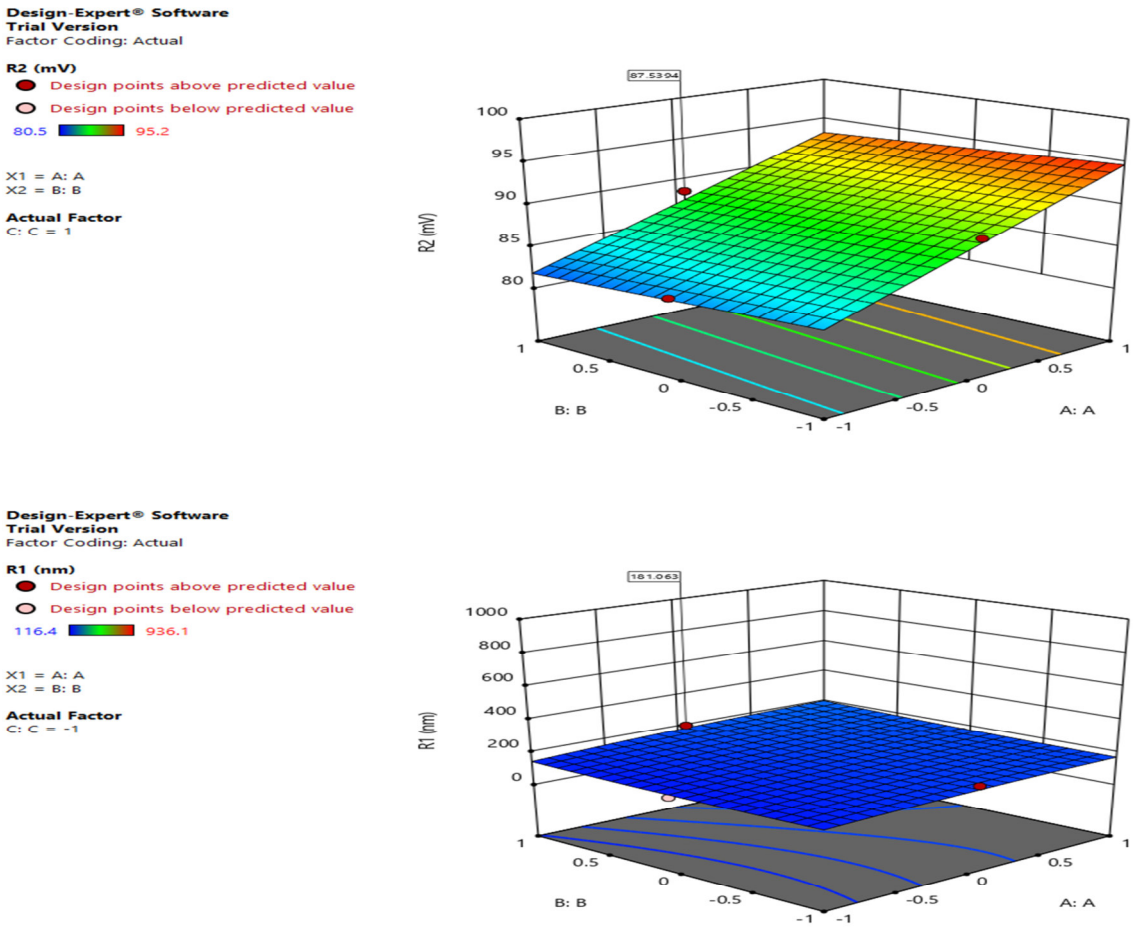


Fig 3: 3D response surface plots showing particle size (R1) and percentage drug encapsulated (R2) (A:A-Lecithin: Cholesterol ratio, B:B -Ratio of aqueous and organic phase)

4. DISCUSSION

The optimum levels of the independent variables including lecithin amount, cholesterol amount, and ratio of aqueous and organic concentration and Chitosan concentration.[18-19] Table 1 shows the preferred factors and setting of factors levels on the bases of low, medium and high as 1,0 and +1. The 13 batches of Chitosan coated liposome formulations were prepared and characterized by their particle size. The various functionalized Chitosan coated liposomes was determined by their zeta potential (ζ), calculated according to the Helmholtz-Smoluchowski equation from their electrophoresis mobility. The formulation started with Cholesterol which was taken in a round bottom flask and dissolved using a different ratio of aqueous and organic phase. The different batches with different concentration prepared and optimized batches was formulated as a liposome delivery system and evaluation was done.[10-12] Modified ethanol injection method was used to prepare thirteen batches of liposomes and then a 3-factor box behnken design for maximum response was applied to the Response surface method. For evaluation of entrapped drugs, two ml of the formulations were taken and separately dialyzed using the dialysis membrane against 50 ml Distilled water for 15 min.[10-17] One ml of distilled water was withdrawn in a 10 ml volumetric flask and the volume was made up to 10 ml with fresh distilled water. The absorbance was noted on Shimadzu UV-Spectrometry at 228 nm and used to evaluate the entrapped drug. Further the quantity of the drug entrapped was evaluated. The values of percent drug entrapment and average vesicle size presented. Formulation F3 & F5 were optimized on bases of particle size and drug encapsulation. Response surface graph was to predicted value and the Chitosan coated liposomes related optimized formulation can be used for loading of bio-active.[19-21]

5. CONCLUSION

The aim of this research work is to optimize Chitosan coated liposomes for delivery of different drugs by surface response technology using design expert software. The model drug was used to prepare liposomes & coating was done by Chitosan. The different batches were selected by Box-Behnken experimental design and response surface graphs were obtained. The observed result shows closeness with predicted value and F3 & F5 were optimized as coated liposomes formulation. This optimized formulation may be used for drug loading of different drugs and can be a better delivery system.

6. FUNDING ACKNOWLEDGEMENT

The authors are thankful to the Head, School of Pharmacy, Devi Ahilya University, Indore, Madhya Pradesh, India, for providing facilities and financial assistance.

7. AUTHORS CONTRIBUTION STATEMENT

Mr. Mahendra Chouhan collected the data, formulated a liposome delivery system and further evaluation done with various techniques. Dr. Rajesh Sharma and Dr. Kamlesh

Dashora analyzed these data and provided suggestions to improve the designing of coated liposome formulation. All authors collectively contributed to methodology and resulted in parts of the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCE

1. Rui Li, FengzhenW,et al. Preparation, optimization, and characterization of chitosan coated solid lipid nanoparticles for ocular drug delivery. The Journal of Biomedical Research, 2018 32(6):411–423.
2. Kulkarni PR, Yadav J, Vaidya KA. Liposomes: A Novel Drug Delivery System. Int. J. Curr. Pharm. Res 2011;2:10-18.
3. Deamer D, Uster P. Liposome preparation methods and monitoring liposome fusion In Baserga R ,Croce c, and Royeza G. (Eds.), Introduction of Macromolecules into viable Mammalian Cells, Alan R. Liss, New York 1980:205 -220.
4. Marie S, Janknegt R, Bakker-Woudenberg, I.A.J.M. Clinical use of liposomal and lipid-complexed amphotericin B. J Antimicrobial Chmother.1994; 33:907-916.
5. Shargel Leon. Vice President, Biopharmaceutics Eon Labs, Inc. Wilson, North Carolina, Applied Biopharmaceutics and Pharmacokinetics.7th ed. 2016. p. 214-218.
6. Hajime A., Yasunori I., Midori M., Shuji N., Shigeru I. Clarithromycin highly loaded gastro-floating fine granules prepared by high-shear melt granulation can enhance the efficacy of Helicobacter pylori eradication. European Journal of Pharmaceutics and Biopharmaceutics.2015; 92:22-27.
7. Lin Y H, Chiou S F, Lai C H, Tsai S C, Chou C W, Peng S F, He Z S. They were formulated and evaluated of water-in-oil amoxicillin-loaded nanoemulsions using for *Helicobacter pylori* eradication. Process Biochemistry 2012; 47:1469–1478.
8. Karn P R, Vanic Z, Pepic I, Basnet NS. The mucoadhesive liposomal delivery systems: the choice of coating material.Drug Development and Industrial Pharmacy 2011;37:482–488.
9. Arora S, Gupta S, Narang R K, Budhiraja RD. Amoxicillin Loaded Chitosan–Alginate Polyelectrolyte Complex Nanoparticles as Mucopenetrating Delivery System for *H. Pylori*. ScientiaPharmaceutica2011; 79:673–694.
10. Faivre V, Bardonnet PL, Boullanger P, Ollivon M, Falson F. The glycosylated liposomes against *Helicobacter pylori*:Behavior in acidic conditions. Biochemical and Biophysical Research Communications 2009; 383: 48–53.
11. S. Mansouri, Y. Cuie, F. Winnik, Q. Shi, P. Lavigne, M. Benderdour, E. Beaumont, J.C. Fernandes. Characterization of folate-chitosan-DNA nanoparticles for gene therapyBiomaterials.,2006, 27, 2060-2065.
12. Z. Amoozgar, J. Park, Q. Lin, Y. Yeo. Low molecular-weight chitosan as a pH-sensitive stealth coating for tumor-specific drug delivery. Molecular pharmaceutics., 2012, 9, 1262-1270.
13. W. Thein-Han, R. Misra. Biomimetic chitosan–nanohydroxyapatite composite scaffolds for bone tissue engineering. ActaBiomaterialia., 2009, 5, 1182-1197.
14. Sriram P**,** Yamsani M, YamsaniS.k, Use of Box-Behnken Experimental design for Optimization of process Variables in Iontophoretic delivery of Repaglinide, Journal of Young Pharmacists,2016; 8(4): 350-355
15. Hao J, Fang X, Zhou Y, et al. Development and optimization of solid lipid nanoparticle formulation for ophthalmic delivery of chloramphenicol using a Box-Behnken design., Int J Nanomedicine, 2011, 6: 683–692.
16. Motwani SK, Chopra S, Talegaonkar S, et al. Chitosan-sodiumalginate nanoparticles as submicroscopic reservoirs for ocular delivery: formulation, optimisation and in vitro characterisation. Eur J Pharm Biopharm, 2008, 68(3): 513–525.
17. Singh B, BhatowaR, et.al. Developing micro-/nanoparticulate drug delivery systems using design of experiments" International Journal of Pharmaceutical Investigation, 2011(2)75-87.
18. Ravikumar K, Pakshirajan K, Swaminathan T, Balu K. Optimization of batch process parameters using response surface methodology for dye removal by a novel adsorbent. ChemEng J. 2005;105:131-8.
19. Kiran B, Kaushik A, Kaushik CP. Response surface methodological approach for optimizing removal of Cr (VI) from aqueous solution using immobilized cyanobacterium. ChemEng J. 2007;126(2):147-53.
20. Singh KS., Dodge J et.al. Optimization and characterization of controlled release pellets coated with an experimental latex-I: anionic drug. International Journal of Pharmaceutics.,125,1995:243-255
21. Sriram P**,** Yamsani M, YamsaniS.k, Use of Box-Behnken Experimental design for Optimization of process Variables in Iontophoretic delivery of Repaglinide. Journal of Young Pharmacists,2016; 8(4): 350-355