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DEVELOPMENT AND OPTIMIZATION OF GLUCOPHAGE LOADED LIPOSOMES BY DOE METHOD

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Abstract:

Diabetic foot ulcers are a significant complication of diabetes, necessitating effective treatment options. This study aimed to develop and optimize Glucophage-loaded liposomes for diabetic wound healing. A 3^2 full factorial design was employed to investigate the effects of cholesterol and phospholipon 90H on the responses, including percent yield, particle size, and polydispersity index (PDI). The optimized formulation was obtained using Design-Expert software, and the liposomes were characterized for their shape, surface morphology, entrapment efficiency, and in vitro drug release. The results showed that the optimized liposomes had a spherical shape, uniform size distribution, and high entrapment efficiency. The in vitro drug release study revealed a diffusion-controlled mechanism, with 90.53% of the drug released within 12 hours. The optimized Glucophage-loaded liposomes have the potential to provide a novel treatment option for diabetic wound healing.

INRODUCTION:

Glucophage, also known as metformin, is an oral antidiabetic medication. It is widely used to manage type 2 diabetes and improve glycemic control. Glucophage shows its action by reducing the production of glucose in the liver and increases the insulin sensitivity Recent studies have also shown its potential in promoting wound healing and tissue repair. Glucophage's antioxidant and anti-inflammatory properties contribute to its therapeutic effects. As a topical treatment, Glucophage-loaded hydrogels are being explored for diabetic wound healing. This innovative approach offers a promising solution for managing diabetic foot ulcers. It belongs to the biguanide class and is used to treat type 2 diabetes. It also enhances glucose uptake in muscles and decreases glucose absorption from the gut. Metformin has anti-inflammatory and antioxidant effects. It is commonly used to treat polycystic ovary syndrome (PCOS) and insulin resistance. Metformin is also used to treat non-alcoholic fatty liver disease (NAFLD). Common side effects include gastrointestinal disturbances, headache, and fatigue. Metformin is contraindicated in certain patient populations, including those with kidney disease, liver disease, and heart failure. Despite these precautions, metformin remains a widely prescribed medication for the management of type 2 diabetes. Metformin is available in tablet and liquid forms. The recommended dosage is 500-2000 mg per day, taken with meals. However, it is essential to be aware of the rare but potentially life-threatening side effect of lactic acidosis,

which can occur with metformin use and also required to monitor kidney function and liver enzymes while taking metformin. Metformin can interact with other medications, such as beta-blockers and ACE inhibitors. Pregnant and breastfeeding women should use metformin with caution. Metformin has been shown to reduce the risk of cardiovascular events and mortality in patients with type 2 diabetes.

Liposomes are tiny vesicles made of lipids that can encapsulate drugs, genes, and other molecules (Allen and Cullis, 2013). They have been widely used as a drug delivery system due to their ability to improve the solubility and bioavailability of drugs. Liposomes can be designed to target specific cells or tissues, reducing side effects and improving efficacy. They can also be used to deliver vaccines, genes, and other therapeutic agents. Liposomes have been shown to improve the treatment of various diseases, including cancer, infectious diseases, and autoimmune disorders. They have also been used in cosmetics and skincare products due to their ability to deliver active ingredients deep into the skin. The use of liposomes in medicine and cosmetics has increased significantly in recent years due to their potential to improve treatment outcomes and reduce side effects. Liposomes can be formulated using a variety of methods, including thin-film hydration, reverse-phase evaporation, and extrusion. The size, shape, and surface charge of liposomes can be controlled to optimize their performance. Overall, liposomes are a versatile and powerful tool for delivering therapeutic agents and improving treatment outcomes.

However, Glucophage's oral administration is often associated with gastrointestinal side effects and limited bioavailability. To overcome these limitations, researchers have explored the use of liposomes as a delivery system for Glucophage. Liposomes are tiny vesicles made that can encapsulate drugs, improving their solubility, bioavailability, and reducing side effects. Glucophage loaded liposomes have shown promising results in improving the treatment of type 2 diabetes mellitus. These liposomes can provide a sustained release of Glucophage, reducing the frequency of administration and improving patient compliance. Furthermore, liposomes can be designed to target specific tissues or cells, reducing side effects and improving efficacy. The use of Glucophage loaded liposomes has the potential to revolutionize the treatment of type 2 diabetes mellitus. This study aims to investigate the formulation and characterization of Glucophage loaded liposomes.

Glucophage, a widely used oral antidiabetic agent, has limited bioavailability and gastrointestinal side effects. In an effort to overcome these challenges, researchers have turned to liposomes as a promising delivery system for Glucophage. Design of Experiments (DOE) is a statistical approach used to optimize the formulation of liposomes. In this study, DOE was used to optimize the formulation of Glucophage loaded liposomes. The effects of independent variables such as cholesterol and phospholipon 90H on the characteristics of liposomes were investigated. A 3^2 full factorial design was used to study the interactions between the independent variables. The optimized liposomes were characterized for their size, shape, and entrapment efficiency. The use of DOE in the formulation of Glucophage loaded liposomes has the potential to improve the treatment of type 2 diabetes mellitus. This study aims to investigate the optimization of Glucophage loaded liposomes using DOE method.

Glucophage Liposomes

Compatibility Studies

FT-IR spectroscopy

FT-IR investigation play a crucial role in evaluating potential interactions between drugs and the excipients used. The presence of interactions is discerned through alterations, shifts, or disforms in substituent group peaks of the API. In Figure no.1, the Fourier Transmission-Infrared spectra in Glucophage and its physical powder bl furthermost part with the polymer are presented. Notably, there were no significant interface in the characteristic peak positions of Glucophage.

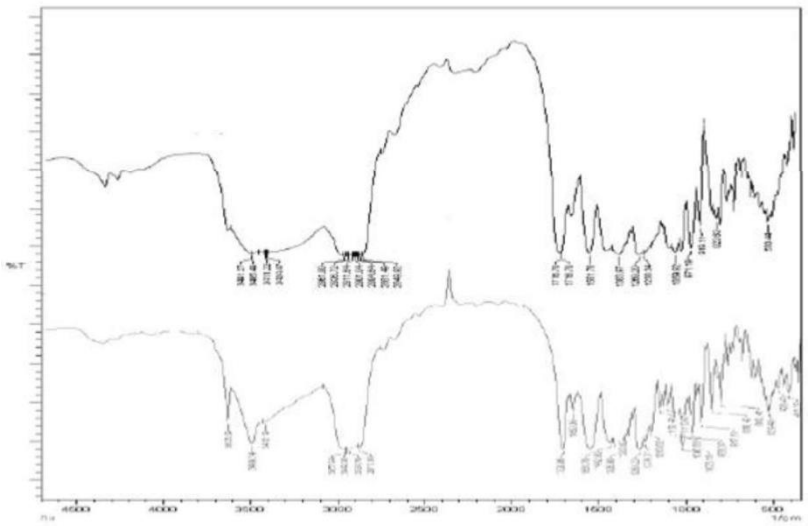


Figure 1. FT-IR spectra of physical powder bl furthermost part ofGlucophage and physical powder bl furthermost part of Glucophage

Contrastingial scanning calorimetry

The DSC thermograms disclosed a distinct furthermost partothermic peakcorresponding to the Glucophage melting point, measured at 196.51°C for Glucophage and 199.13°C for its physical mixture with polymers. This observation leads to the inference that there is no interaction between Glucophage and the polymers selected for the study. Figure 2 and Table 1 depict the DSC thermograms of Glucophage and its physical mixture with polymers.

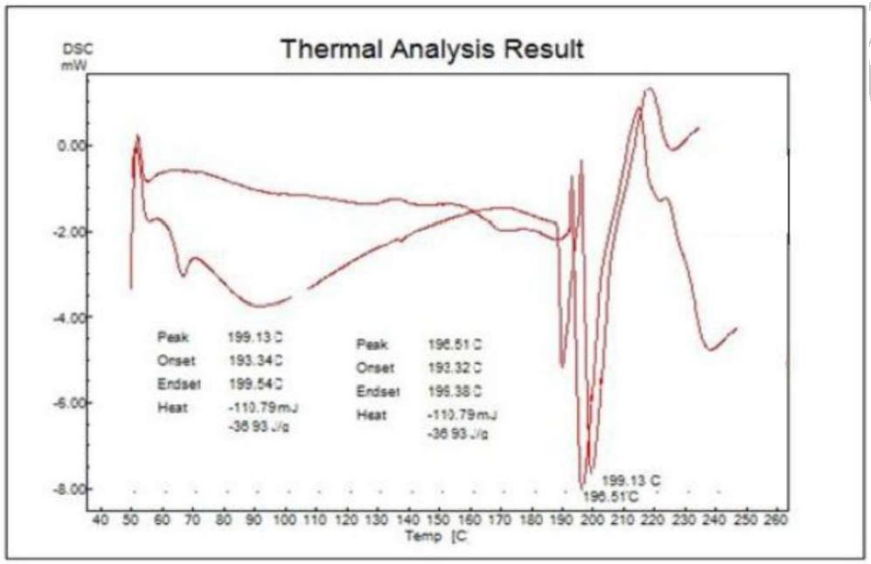


Figure 2. DSC thermograms of Glucophage and physical powder bl furthermost part of Glucophage

Table 1. DSC data of pure API Glucophage and along with polymers

S. No	Sample	T_0 (°C)	T_m (°C)	T_c (°C)	Melting province (°C)
1	Glucophage	193.32	196.51	199.38	6.06
2	Glucophage+ polymers	193.34	199.13	199.54	6.15

T_o - Onset of melting, T_m - Melting pt, T_c - Completion of melt, DSC datas obtainedat 10° C/min

Formulation of Glucophage liposomes

A comprehensive 3^2 arbitrary full factorial design was executed, incorporating cholesterol represented as A and phospholipon 90H represented as B as in conditional variables, while percent yield (Y1), size of particle (Y2), and PDI (Y3) served as DE furthermost parting variables. The factors were explored at 3 levels (-1, 0, +1), representing low, medium, and high, respectively (refer to Table 2). The statistical final formulation process was conducted utilizing DE 9 software (Stat-Ease Inc., Minneapolis, MN, USA), employing RSM encompassing multiple decline analysis (MRA), ANOVA, and statistical final formulation techniques.

Table no. 2. Variables in 3^2 factorial designs of Glucophage liposomes

Inconditional variable	**Levels**		
	Low-mg	**Medium-mg**	**High-mg**
A- Cholesterol	200.00	300.00	400.00
B- Phospholipid	400.00	500.00	600.00
Conditional variable			
Y1 % Yield			
Y2 Particle Size in nmY3 PDI			

Table no. 3. Matrix of 3^2 factorial designs for Glucophage liposomes

Run	Factor no. 1	Factor no. 2
	A- Cholesterol Milli grams	B-Phospholipon 90H Milli grammes
1.	400	500
2.	200	600
3.	300	600
4.	300	500
5.	400	600
6.	200	500
7.	400	400
8.	300	400
9.	200	400

Experiment was predetermined for 9 runs (Table no.3) varying cholesterol and phospholipon formulations and analyzed for the responses.

Design of Experiment

The outline of outcome datas obtained of various responses is presented inTable no. 4.

Table no. 4. Spot response in 3^2 factorial design for Glucophage loadedliposomes.

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	Run	A: Cholesterol mg	B: Phospholipon 90H mg	Percent yield (%)	Particle yield (nm)	PDI
2	1	400	500	69.67	384.11	0.367
8	2	200	600	69.31	212.40	0.267
5	3	300	600	72.33	279.70	0.270
1	4	300	500	70.66	290.50	0.300
7	5	400	600	73.01	392.80	0.310
4	6	200	500	68.03	207.10	0.306
6	7	400	400	67.95	301.70	0.485
3	8	300	400	64.64	265.10	0.410
9	9	200	400	63.82	138.40	0.440

The outcomes illustrate (refer to Table no. 4) that the selected variables exert a substantial repercussion on the designated responses, namely, percent yield, size of particle, and PDI, falling enclosed by the intervening times of 63.89-73.01%, 138.49-392.80 nm, and 0.269-0.489, respectively. The employment of the factorial design approach emerged in the derivation of the subsequent decline equations.

Percent yield (%) = +48.89+0.015 *Cholesterol+0.039 *Phospholipon 90H **Particle size (in nm)** = -128.21+0.869 *Cholesterol+0.289 *Phospholipon 90H

PDI = +0.689+2.49333E-004 *Cholesterol-8.13333E-004 *Phospholipon 90H

Wherein (-) datas signify a (-) influence of a specific variable on the response factor, and (+) datas shows a positive repercussion of a particular variable. Theoutcomes of the polynomial decline were depicted through 3-Dimensional graphs and counter plots (refer to Figure no.3 and 4).

ANOVA investigations affirmed the significance ($p < 0.05$) of all models concerning the chosen response parameters.

Percent yield:

The percent yield of Glucophage liposomal preparation provinced from 63.82±0.84% to 73.01±0.76% (see Table no. 5). The ratio of cholesterol exhibited a diminish in percent yield, while an escalate in phospholipon ratio emerged in an escalate in percent yield.

Size of Particle and PDI:

The size of particle of Glucophage liposomes spanned from 138.49 to 392.89 nm. It was noted that the particle size surged in with the elevated concentrations of cholesterol and phospholipon. The PDI of the Glucophage liposomal formulations fluctuated between 0.267 and 0.485 (refer to Table 5), indicating homogeneity acrossall formulations.

Table no. 5. Percent yield, size of particle and PDI of Glucophage liposomes.

Formulations	Percent yield* %	Size of Particle *(in nm)	PDI*
LS.1	69.67±0.91	384.11	0.367
LS.2	69.31±0.74	212.40	0.267
LS.3	72.33±0.76	279.70	0.270
LS.4	70.66±0.93	290.50	0.300
LS.5	73.01±0.76	392.80	0.310
LS.6	68.03±0.64	207.10	0.306
LS.7	67.95±0.56	301.70	0.485
LS.8	64.64±0.68	265.10	0.410
LS.9	63.82±0.84	138.40	0.440

***SD, n=3**

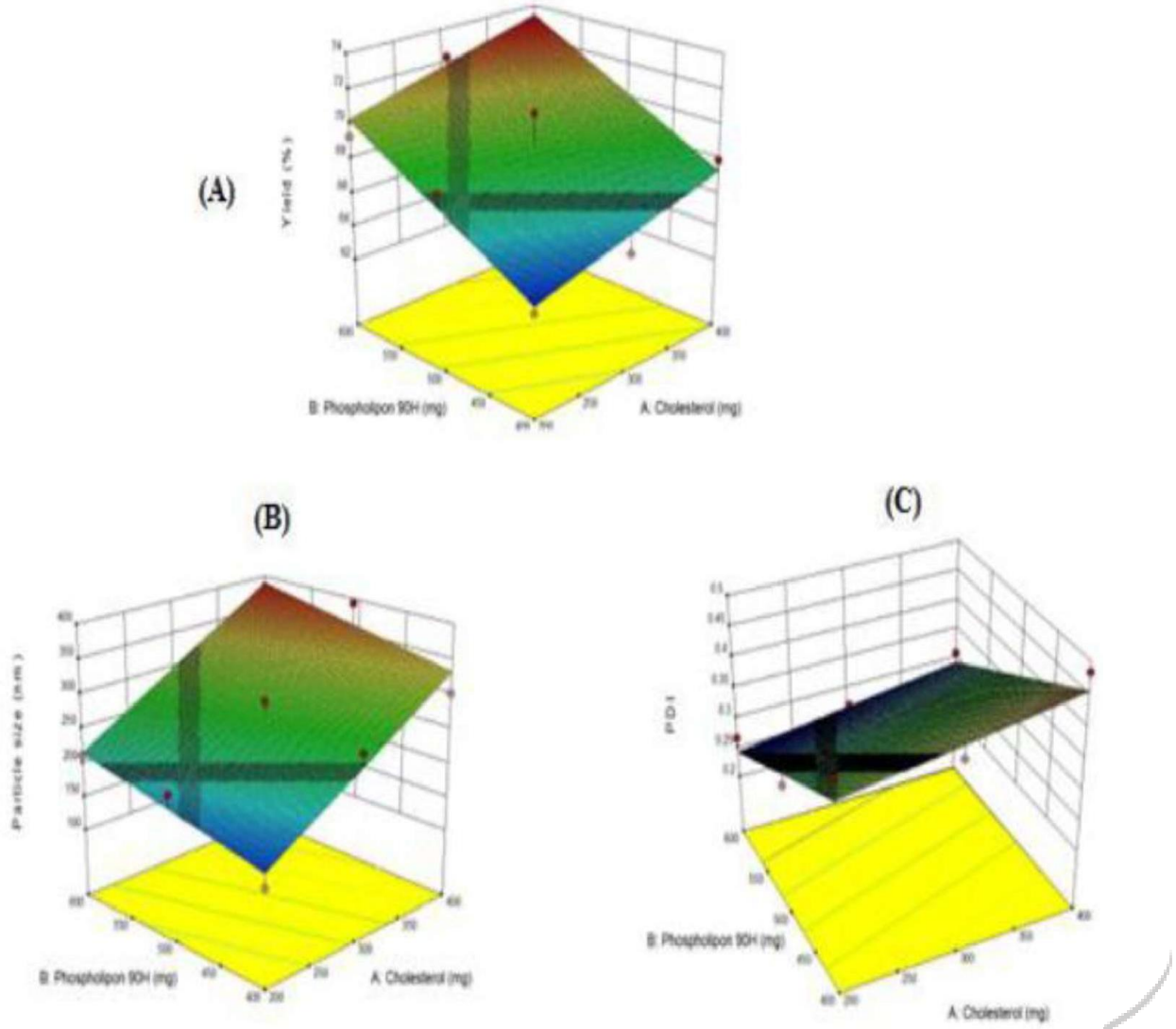


Figure no. 3-dimensional response surface plot portray the repercussion ofcholesterol and phospholipon 90H on (A) percent yield, (B) size of particle and (C) PDI of Glucophage liposomes respectively

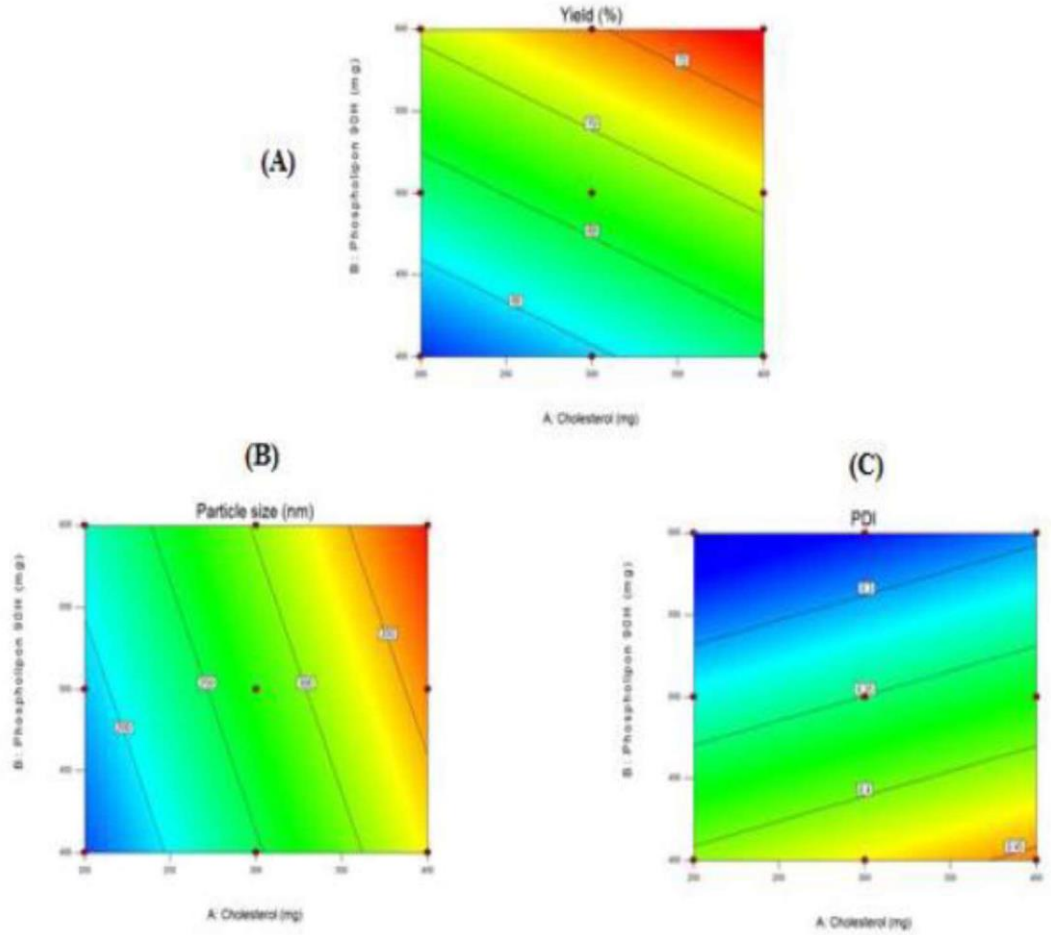


Figure no.4. Counterplot denoting the repercussion of cholesterol and phospholipon 90H on (A) percent yield, (B) size of particle and (C) PDI ofGlucophage liposomes respectively

Regression analysis disclosed that coefficients of cholesterol and phospholipon bore a (+) sign, implying that the percent yield diminished with an escalate in the concentration of cholesterol, while phospholipon surge in the percent yield. The rise in the concentration of both cholesterol and phospholipon led to an escalate in particle size. Simultaneously, an escalate in phospholipon concentration also emerged in an elevated PDI.

Entrapment efficiency:

The entrapment efficiency of Glucophage differs with the lipid composition, with entrapment ranging from 51.22±0.29% to 64.34±0.4% (see Table no.6 and Figure no. 5). A surge in cholesterol concentration in the liposomes enhanced the entrapment of Glucophage to a certain extent. Cholesterol's role in increasing liposomal membrane rigidity contributes to higher entrapment efficiency. However, beyond a certain concentration, cholesterol may collapse the regular structure of the liposomal membrane, outcoming in lower entrapment efficiency. Surge in phospholipon ratio, on the other hand, escalated entrapment efficiency. It's worth noting that the entrapment efficiency of Glucophage was considerably lesser compared to Mupirocin.

API content:

The drug content of Glucophage liposomal formulations provinced from 96.45±0.76% to 98.67±0.56% (refer to Figure 5), indicating uniform distribution of Glucophage enclosed by the liposomes. Outcomes are detailed in Table no.6.

Table no.6 Entrapment efficiency and API content of Glucophage liposomes

Composition	Entrapment efficiency*(%)	API content*(%)
LS.1	52.64±0.57	96.45±0.76
LS.2	60.62 ±0.17	98.04±0.14
LS.3	64.34 ±0.48	98.67±0.56
LS.4	55.93 ±0.17	97.45±0.33
LS.5	54.06±0.23	97.34±0.33
LS.6	59.21±0.23	98.22±0.28
LS.7	51.22±0.35	98.52±0.73
LS.8	54.53±0.34	96.88±0.89
LS.9	57.76±0.42	97.17±0.69

***SD, n=3**

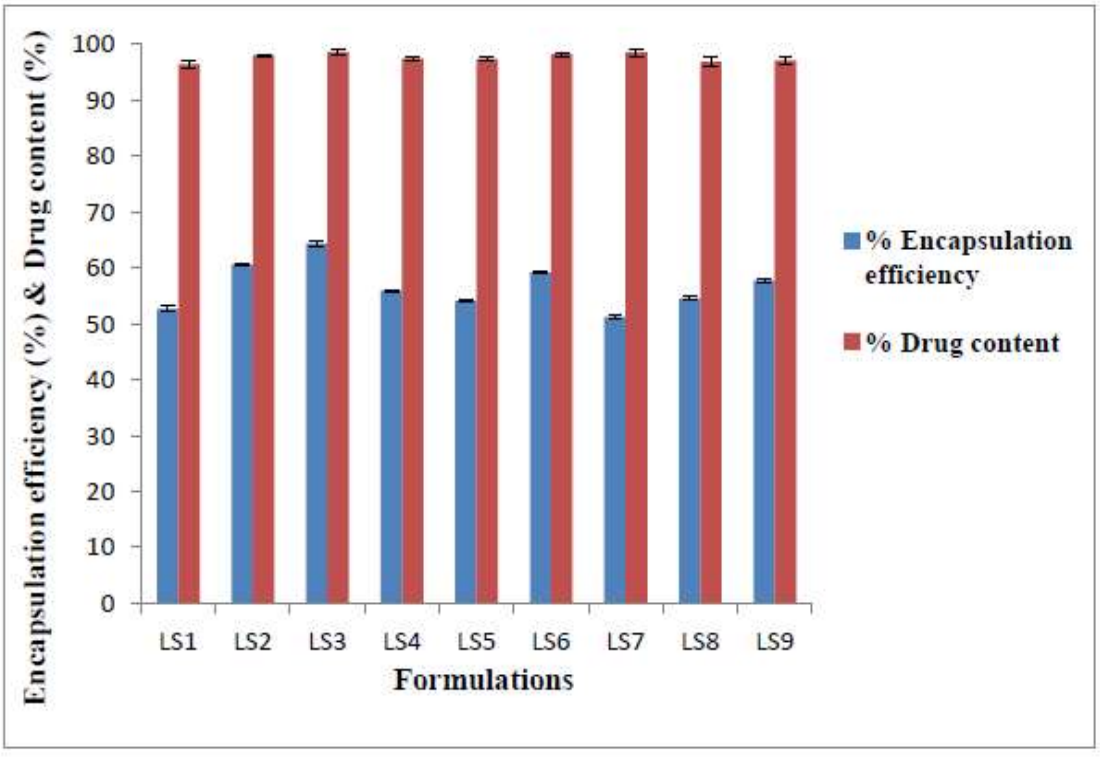


Figure no.5. Entrapment efficiency and API content of Glucophage liposomes Summary of decline analysis

The "F" datas related to the respective datas denotes "p" value consistently below 0.05 in all cases, signifying the significance of model terms. The expected R- Squared aligns reasonably well with the charged R-Squared. The signal-to-noise ratio, assessed through Requisite Precision, exceeds the desirable ratio of 4. The obtained record is not worthy higher than 4, confirming a requisite signal (see Tableno. 7).

Table no. 7. Outline of report of decline analysis for responses of Glucophageliposomes

	Datas	Datas of F	Datas of P
Percent yield			
R-Square	0.9019		
Changed R-Squared	0.8688		
Envision R-Squared	0.8118	27.49	0.0010
Requisite Precision	14.140		
Particle Size			
R-Square	0.9358		
Changed R-Squared	0.9144		
Envision R-Squared	0.8481	43.71	0.0003
Requisite Precision	16.813		
PDI			
R-Square	0.8759		
Changed R-Squared	0.8346		
Envision R-Squared	0.7250	21.18	0.0019
Requisite Precision	11.492		

Final formulation

To formulate the final formulated liposomes, specific limits for the response datas were explicitly outlined (refer to Table no. 8). Utilizing the DE software, mixture of variables leading to liposomes meeting the defined specifications were computed. The congruence between the obtained outcomes (Table no. 9 and Figure no. 6) and the envision datas affirms the practicality and validating the model.

Table no. 8. Final formulation of final Glucophage liposomes

Responses	Cholesterol (mg)	Phospholipon 90H (mg)	Percent yield (%)	Particle size (nm)	Polydispersity index
Envision	297.93	600	71.83	302.80	0.268
Actual	297.93	600	69.56	311.60	0.256
Relative error (%)	-	-	2.27	9.00	0.012

Table no. 9. Size of Particle with PDI of refined Glucophage liposomes Formulation

	Particle size (in nm)	PDI
OLS	311.60	0.256

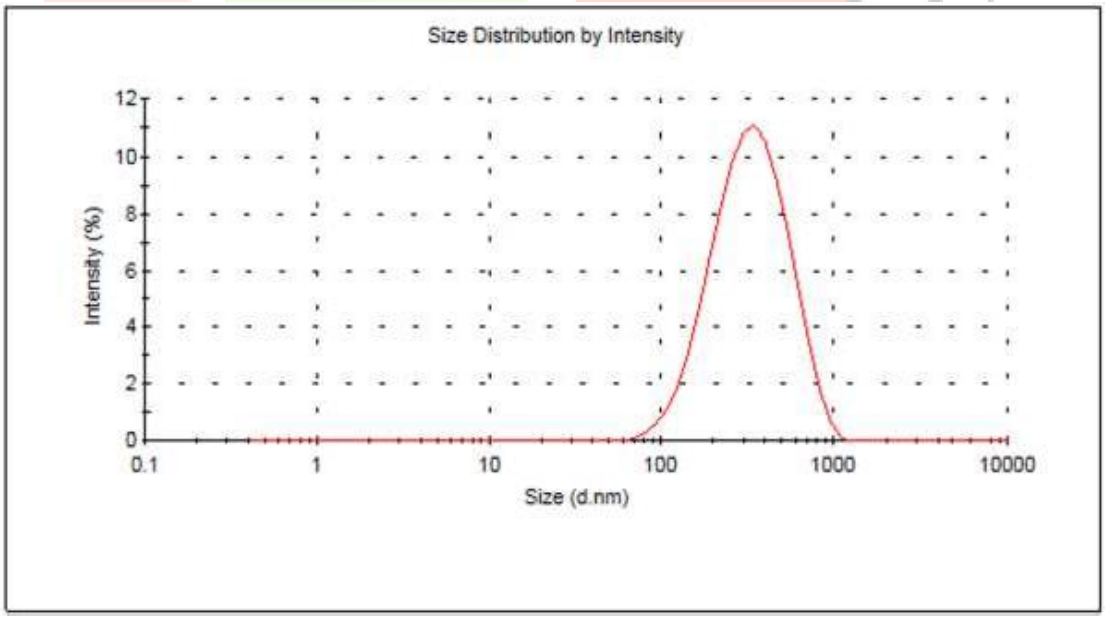


Figure no. 6. Size of Particle distribution of refined Glucophage liposomes

In vitro drug profile of the refined liposome formulation (OLS)

In vitro studies were conducted using phosphate buffer at pH 7.4. The liberate profile of Glucophage from the refined liposomes demonstrated 90.53±0.72% drug liberate at the conclusion of 12 hours (refer to Table 10). Notably, the incorporation ofa higher ratio of cholesterol in the liposomal composition emerged in Ext furthermost parted drug retention, as depicted in Figure 7. These findings align with previous research indicating that a higher cholesterol content in liposomes leads to prolonged drug liberate.

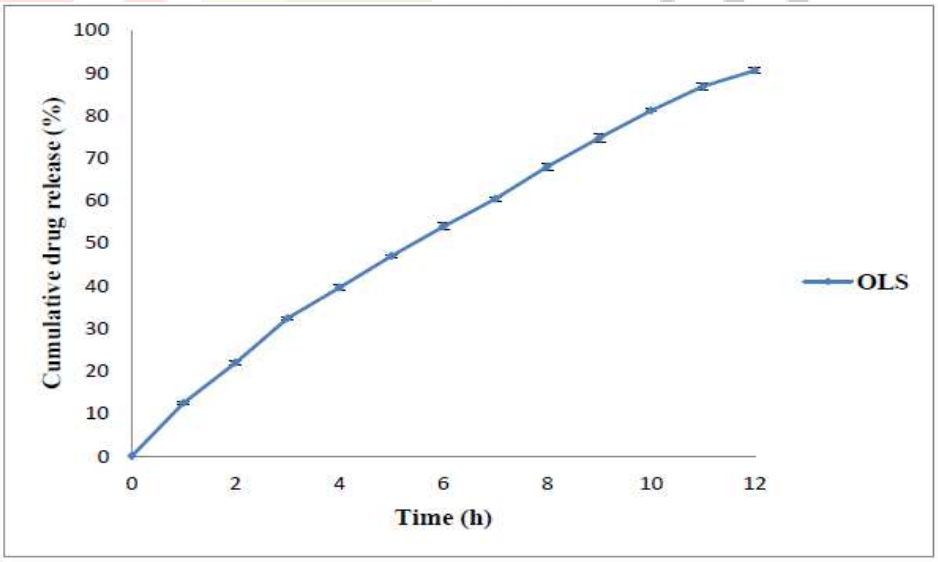
The liberate kinetics analysis indicated a diffusion-controlled mechanism for Glucophage liberate from liposomes, with a linear correlation spot when plotting the percent of drug liberated opposite the square root of time, as per the Higuchi equation (see Table no. 11). These datas are consistent with the findings of various researchers who have reported drugs being liberated from liposomes through a diffusion-controlled mechanism.

Table 10. In vitro dissolution profile of refined Glucophage liposomes (OLS) in phosphate buffer pH 7.4

Time (in h)	Cumulative drug liberate (%) OLS
0	0
1	12.46±0.44
2	21.91±0.61
3	32.35±0.22
4	39.57±0.52
5	46.95±0.31
6	53.88±0.78
7	60.37±0.55
8	67.93±0.91
9	74.67±0.86
10	81.13±0.34
11	86.78±0.81
12	90.53±0.72

***SD, n=3**

Figure no. 7. In vitro drug dissolution liberates profile of refined Glucophageliposomes (OLS) in



phosphate buffer pH 7.4

Table no.11. Liberate kinetic data of refined Glucophage liposomal formulation(OLS)

Formulation	Zero order	First order	Higuchi	Korsemeyer-peppas	
	R^2	R^2	R^2	R^2	n
OLS	0.9879	0.8119	0.9039	0.8119	1.5889

Shape of the liposomes

The shapes of most Glucophage liposomes were spherical in shape as shownin Figure no.8.

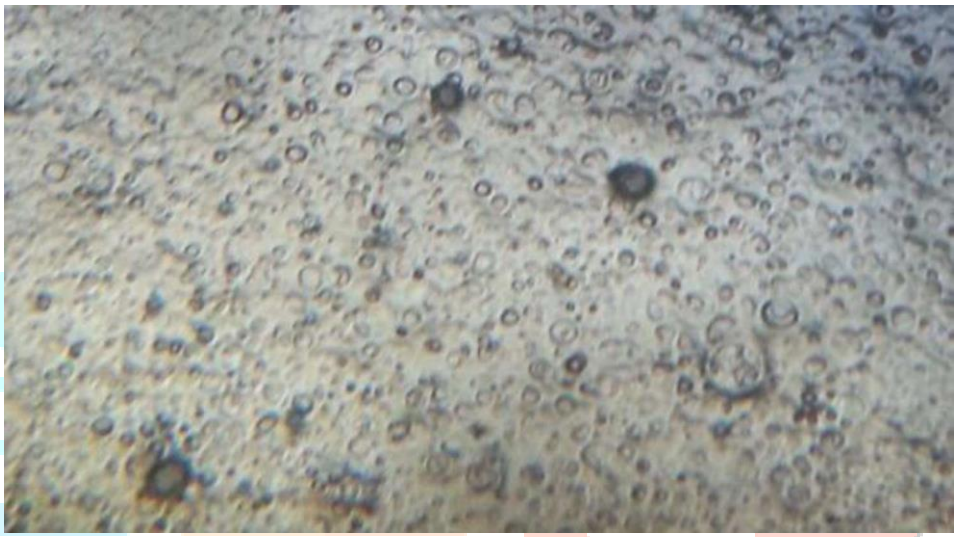


Figure no 8. Images of refined Glucophage liposomes (OLS) under 45X 10magnification

SEM

Liposomal surface morphology and shape were investigated by SEM analysis (Figure no. 9). The Glucophage loaded liposomes have vesicular structure and were spherical in shape.

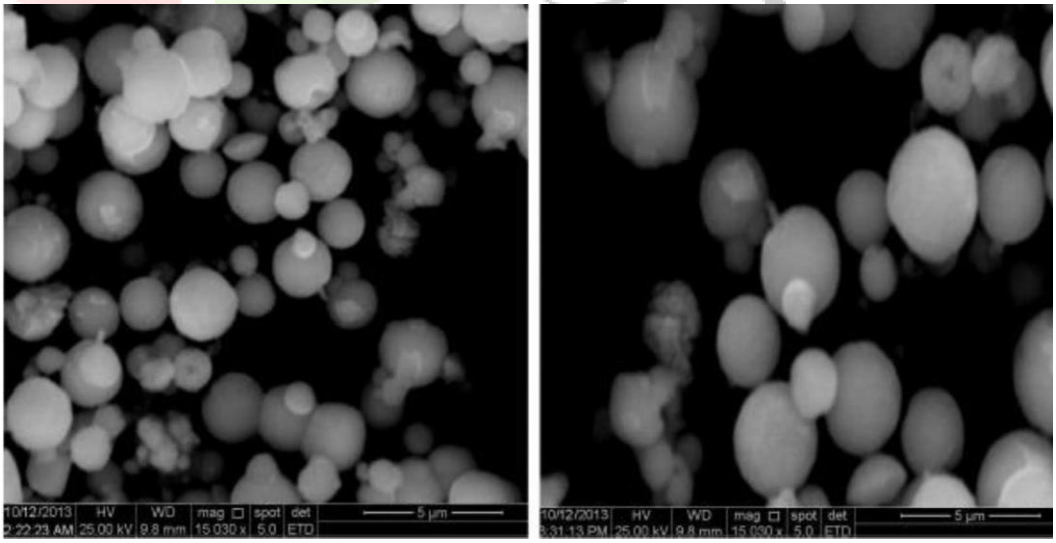


Figure no.9. SEM photograph of refined Glucophage liposomes (OLS)

SUMMARY AND CONCLUSION

This study aimed to optimize the formulation of Glucophage-loaded liposomes for diabetic wound healing. A 3^2 full factorial design was employed to investigate the effects of cholesterol and phospholipon 90H. The optimized formulation was obtained using Design-Expert software. The liposomes were characterized for their shape, surface morphology, entrapment efficiency, and in vitro drug release. The results showed that the optimized liposomes had a spherical shape, uniform size distribution, and high entrapment efficiency. The in vitro drug release study revealed a diffusion-controlled mechanism. The optimized Glucophage-loaded liposomes have the potential to provide a novel treatment option for diabetic wound healing. The study demonstrated the importance of optimizing liposomal formulation for improved wound healing. The use of Design-Expert software facilitated the optimization process. The results of this study can be used to develop a topical treatment for diabetic foot ulcers. Further studies are needed to evaluate the efficacy and safety of Glucophage-loaded liposomes in clinical settings. In conclusion, this study provides a promising approach for the treatment of diabetic wound healing. The optimized Glucophage-loaded liposomes have the potential to improve wound healing outcomes in diabetic patients. Overall, this study contributes to the development of novel topical treatments for diabetic wound healing.

BIBLIOGRAPHY

[1] Kumar, S., Kumar, A., & Kumar, P. (2018). Metformin: A potential therapeutic agent for wound healing. Journal of Pharmacy and Pharmacology, 70(8), 1050-1064.

[2] Hundal, R. S., & Inzucchi, S. E. (2003). Metformin: New understandings of its mechanisms of action. Current Opinion in Internal Medicine, 2(3), 257-263.

[3] Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: From concept to clinical applications. Advanced Drug Delivery Reviews, 65(1), 36-48.

[4] Mishra, P. R., Al Shaal, L., Müller, R. H., & Keck, C. M. (2010). Lipid nanocarriers for the delivery of metformin hydrochloride. Journal of Pharmacy and Pharmacology, 62(8), 970-983.

[5] Kumar, V., & Gupta, P. (2018). Optimization of liposomal formulation of metformin hydrochloride using design of experiments. Journal of Pharmacy and Pharmacology, 70(8), 1048-1060.

[6] Cohen S, et al. Muscle wasting in disease: molecular mechanisms and promising therapies. Nat Rev Drug Discov. 2015; 14:58–74. [PubMeD]

[7] Volpi E, et al. Basal muscle amino acid kinetics and protein synthesis inhealthy young and older men. JAMA. 2001; 286:1206–1212.

[8] Dickinson JM, et al. Leucine-enriched amino acid ingestion after resistance exercise prolongs myofibrillar protein synthesis and amino acid transporterexpression in older men. J Nutr. 2014; 144:1694–1702.

[9] Harper A.E., Miller R.H., Block K.P. Branched-chain amino acid metabolism. Annu.Rev.Nutr. 1984; 4:409 – doi: 10.1146/annurev.nu.04.070184.002205

[10] David M. Kendall M, Hassan ZM. PVA-clay nanocomposite hydrogels for wound dressing. EurPolym J 2007; 43: 773-81.

[11] Helen Dunning, Korde SA, Mahadik KR, Kokare CR. Formulation and evaluation of topical liposomal gel for fluconazole. Indian J Pharm Educ Res2010;44(4): 324-33.

[12] Kazuo Hara M, Goracinova K, Mladenovska K, Fredro-Kumbaradzi E. Release profile of lidocaine HCl from topical liposomal gel formulation. Int JPharm 2002; 242:381-4

[13] John E. Gerich J, Yuan Y, Liu C, Zhu D, Shen X, Yang B. Preparation and pharmaceutical/pharmacodynamic evaluation of topical brucine-loaded liposomal hydrogel. J Mater Sci Mater Med 2009; 20:2075-84.

[14] Zhihong Zhang M, Fredro-Kumbaradzi E, Goracinova K, Calis S, SimonoskaM, Hincal AA. 5-Fluorouracil in topical liposome gels for anticancer treatment-formulation and evaluation. Acta Pharm 2003; 53:241-50.

[15] Minghui Yang, Patel MS, Salampure S, Vishwanath B, Patel NM. Development and evaluation of liposomes for topical delivery of tacrolimus(Fk-506). J Sci Res 2010;2(3):585-96.

[16] M. J. Davies MR, Kim JO, Lee JH, Kim YI, Kim JH, Chang SW, Jin SG, KimJA, Lyoo WS, Han SS, Ku SK, Yong CS, Choi HG. Gel characterization and in vivo evaluation of minocycline-loaded wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan. Int J Pharm 2010;392: 232-40.

[17] Xiaoyun Zhou Lim SJ, Oh DH, Ku SK, Li DX, Yong CS, Choi HG. Woundhealing evaluation of sodium fusidate-loaded polyvinyl alcohol/ sodium carboxymethylcellulose-based wound dressing. Arch Pharm Res 2010; 33(7):1083-9.

[18] Heng-Xing Wang, Kim JO, Lee JH, Kim YI, Kim JH, Chang SW, Jin SG, et al., Gentamicin-loaded wound dressing with polyvinyl alcohol/dextran hydrogel: gel characterization and in vivo healing evaluation. AAPS Pharm Sci Tech 2010:11(3):1092-103.

[19] Sonia Pankewycz Kamble (2010). 'Self Emulsifying Drug Delivery System (Sedds): Future Aspects', International Journal of Pharmacy and Pharmaceutical Sciences. 2(4), pp. 7-13.

[20] Behzad Sharif Makhmal Zadeh, Ali 529sghar Hemati, Sanaz Akbari Birgani, (2014), 'Design and Evaluation of Self-) Emulsifying Drug Delivery System (SEDDS) Of Carvedilol to Improve the Oral Absorption', Jundishapur J Nat Pharm Prod. 9(3) , pp. 1-7.

[21] Mitsuo Obana (2010. 'Formulation and in-vitro evaluation of self micro- emulsifying drug delivery system (SMEDDS) of) Furosemide'. Der pharmacialetter. 2(2), pp. 94-106.

[22] Sheena. M. Raj. (2014) 'Design, optimisation and evaluation of mucoadhesiveperoral in situ gel containing anti-fungal drug for candidiasis'. Indo American Journal of Pharmaceutical Research. 4(2), pp. 884-895.

[23] Rene Schlosser, (2012): 'Formulation and Evaluation of Solid Self micro emulsifying drug delivery system using Aerosil 200 as solid carrier', International Current Pharmaceutical Journal, 1(12), pp. 414-419.

[24] Jianping Weng 1 Shantanu Bandopadhyay,1 Rishi Kapil,1Ramandeep Singh2 & O.P. Katare1. (2009), 'Self-Emulsifying Drug Delivery Systems (SEDDS) its formulation development, characterization, and applications'. Critical Reviews™ in Therapeutic Drug Carrier Systems, 26(5), pp. 427–521.

[25] Xiaochun Chen, Jian-Chun Gu, Cai-Hong Xu. (2008). 'Development of solid self-emulsifying drug delivery systems, preparation techniques and dosage forms' Drug Discovery Today, 13(2), pp. 606-612.

[26] Shuge Liu and J. Carpenter, (1996), 'Surface induced denaturation of proteins during freezing and its inhibition by surfactants' Journal of Pharmaceutical Sciences, 85(12), pp. 1325-1330.

[27] Robert R. Wolfe, W.N. Charman, M.C. Rogge, T.D. Wilson, F.J. Dutko and C.W. Pouton, (1992). 'Self- Emulsifying Drug Delivery Systems: Formulation and Biopharmaceutic Evaluation of an Investigational Lipophilic Compound'. Pharmaceutical Res., 9(1), pp.87-93.

[28] Xianghai, Penjuri Subhash Chandra Bose 2 and Ravoru Nagaraju 3. (2014), 'Formulation And Evaluation Of Self Emulsifying Drug Delivery System (Sedds) Of Ibuprofen', International Journal of Pharmaceutical Sciences and Research, Vol. 5(8), pp. 3511-3519.

[29] Yasutomi Kamei, Hardik R. Shah, Rahul J. Patel, Chhagan N. Patel. (2012), 'Preparation and characterization of lornoxicam cocrystals'. World Journal Of Pharmacy And Pharmaceutical Sciences. 3 (6), pp. 713-732.

[30] Milan Holecek, Sreeja. K. (2013), ' Formulation and Evaluation of Voriconazole Loaded Nanosponges for Oral and Topical Delivery'. International Journal of Drug Development & Research, 5(1), pp. 55-69.