RESEARCH ARTICLE

Optimization of Beta Sitosterol Phytosome Formulation using Box Behnken Design for Enhanced Bioavailability

Srikala Kamireddy, Shanmuga Sundaram Sangeetha*

Department of Pharmaceutics, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur-603203, Tamil Nadu, India

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ABSTRACT

Beta-sitosterol, a plant-derived phytosterol, holds promise as an anti-obesity medication. However, its limited bioavailability and low aqueous solubility hinder its therapeutic application. This research aimed to utilize phytosome technology, developed and optimized using Box-Behnken Design (BBD), to enhance the bioavailability of beta-sitosterol. L-α-phosphatidylcholine was used as the lipid carrier in the thin-film hydration process to create phytosomes. Entrapment efficiency (EE%), particle size, zeta potential, and polydispersity index (PDI) were the four dependent responses, and the effects of three independent variables, such as molar ratio, reaction time, and temperature, were systematically assessed. The improved formulation demonstrated robust drug encapsulation, nanoscale size, good colloidal stability, and uniformity with an entrapment efficiency of 86.41%, particle size of 163.53nm, zeta potential of -30.06mV, and Poly dispersity index of 0.2148. When compared to the pure drug, *in vitro* drug release experiments showed a noticeable, improved and prolonged release profile of beta-sitosterol from the phytosomal formulation. The ideal formulation parameters, determined at a molar ratio of 2:1, a time taken for the reaction of 74.79 minutes at an operating temperature of 38.26^0C and they were validated by overlay plot analysis. These results demonstrate the potential of this phytosomal strategy to enhance the administration of poorly soluble phytoconstituents, including beta-sitosterol, for anti-obesity treatment and validate the effective use of BBD in modifying phytosomal formulations.

Keywords: Zeta Potential, Entrapment Efficiency, Beta-sitosterol, Phytosome, Box-Behnken Design, Bioavailability, Obesity.

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INTRODUCTION

The excessive accumulation of bodily fat is called obesity. It is characterized by a body mass index (BMI) of 30 or higher[1]. In other words, it is a disorder that arises when calorie intake and utilization are not balanced, resulting in excessive fat storage[2]. Being overweight can be caused by excess water, bone, or muscle[3-12]. The literature evaluation revealed that over 10% of adults were obese globally[13-15]. These alarming figures prompt us to begin new studies utilizing natural substances to combat the worldwide health issue (obesity)[16].

The potential of β-sitosterol as an anti-obesity drug is becoming more widely acknowledged[17]. Phytosomes shield these beneficial compounds from enzymatic breakdown and the harsh gastrointestinal tract by encapsulating plant extract in phospholipid complexes[18].

MATERIALS AND METHODS

Sima-Aldrich private limited in Bangalore, India, supplied beta-sitosterol and L-α-phosphatidylcholine.

The formulation of β-sitosterol phytosomes were optimized by the use of Box-Behnken Design (BBD). Each of the three independent variables was analyzed at three levels: A: Phospholipid ratio (1:1, 1:2, 2:1), B: Stirring temperature (40, 60, and 80 degrees Celsius), C: Stirring times (60, 120, and 180 minutes). The statistical Design Expert Software® was used to build the BBD matrix. The dependent responses were Entrapment Efficiency (%) and Particle Size (nm), zeta potential (mV) and poly dispersity index as shown in Table 1.

Preparation of Phytosomes

Beta-sitosterol phytosomes were made by thin-film hydration. Beta-sitosterol and L-α-phosphatidylcholine dissolved in a 2:1 $_{w/v}$ methanol and chloroform solution in a round-bottomed flask. A rotary evaporator was used to evaporate the solvent at a lower pressure in order to produce a thin lipid coating. The film was hydrated with phosphate buffer saline (PBS) and then sonicated to shrink its size.[19]

Experimental Setup

A Box Behnken Design (BBD) was utilize to analyze three independent variables[20]:

drug to L- alpha phosphotidylcholine ratio (A), the hydration time (B), and the sonication duration (C). The observed variables being examined served as entrapment efficiency (Y1), particle size (Y2) zeta potential (Y3), poly dispersity index (Y4)

Characterization of Phytosomes

Entrapment Efficiency

**Author for Correspondence: sangeets2@srmist.edu.in*

Table 1: Box-Behnken Design Matrix with corresponding experimental results for beta sitosterol phytosomal formulation optimization

Run	Factor-1 Molar ratio	Factor-2 Reaction time minutes	Factor-3 Reaction temperature efficiency	Reaction 1 Entrapment efficiency (EE%)	Reaction 2 Particle size (nm)	Reaction 3 Zeta potential (mV)	Reaction 4 Poly dispersity index
1	2	90	50	85.6	161.6	-30.6	0.26
2	2	60	40	88.7	158.2	-31.2	0.18
3	1	60	30	86.3	163.2	-28.9	0.24
4	3	90	40	89.2	162.6	-32	0.19
5	3	60	30	82.5	166.4	-26.7	0.29
6	2	90	30	84.3	165.9	-27.9	0.27
7	2	60	40	84.6	160.8	-29	0.22
8	3	60	50	87.9	166.6	-30.5	0.2
9	2	30	50	81.4	161.6	-25.4	0.32
10	2	60	40	85.6	161.2	-29.1	0.23
11	3	30	40	84.2	161.4	-29.6	0.26
12	2	60	40	85.3	164.6	-30.8	0.2
13	1	90	40	87.5	162.9	-30.2	0.21
14	2	60	40	87.2	165.6	-29.9	0.22
15	2	30	30	86.3	165.8	-30.1	0.21
16	1	30	40	86.9	160.2	-30.4	0.2
17	1	60	50	87.2	159.5	-30.3	0.21

Using the ultracentrifugation method, the entrapment efficiency (EE%) of beta-sitosterol in the phytosome formulation was established.[20]

A chilled centrifuge (Remi, India) was used to centrifuge a known amount of phytosomal suspension (equal to 10 mg of beta-sitosterol) at 15,000 rpm for 45 minutes at 4^0C. Utilizing a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan), the supernatant containing the unentrapped (free) beta-sitosterol was collected and subjected to spectrophotometric analysis at 206 nm. A standard calibration curve was used to calculate the amount of drug that was not entrapped[21]. The following formula was then used to get the Entrapment Efficiency (EE%):

$$EE\% = \frac{Entrapped\ drug}{Total\ drug} X100$$

The results were presented as mean $\pm$ standard deviation (SD), with each measurement being carried out in triplicate. The effectiveness of drug loading into phytosomal vesicles is efficiently measured by this technique[22].

Transmission Electron Microscopy (TEM) Analysis

Transmission electron microscopy was employed to investigate the surface appearance and structural features of

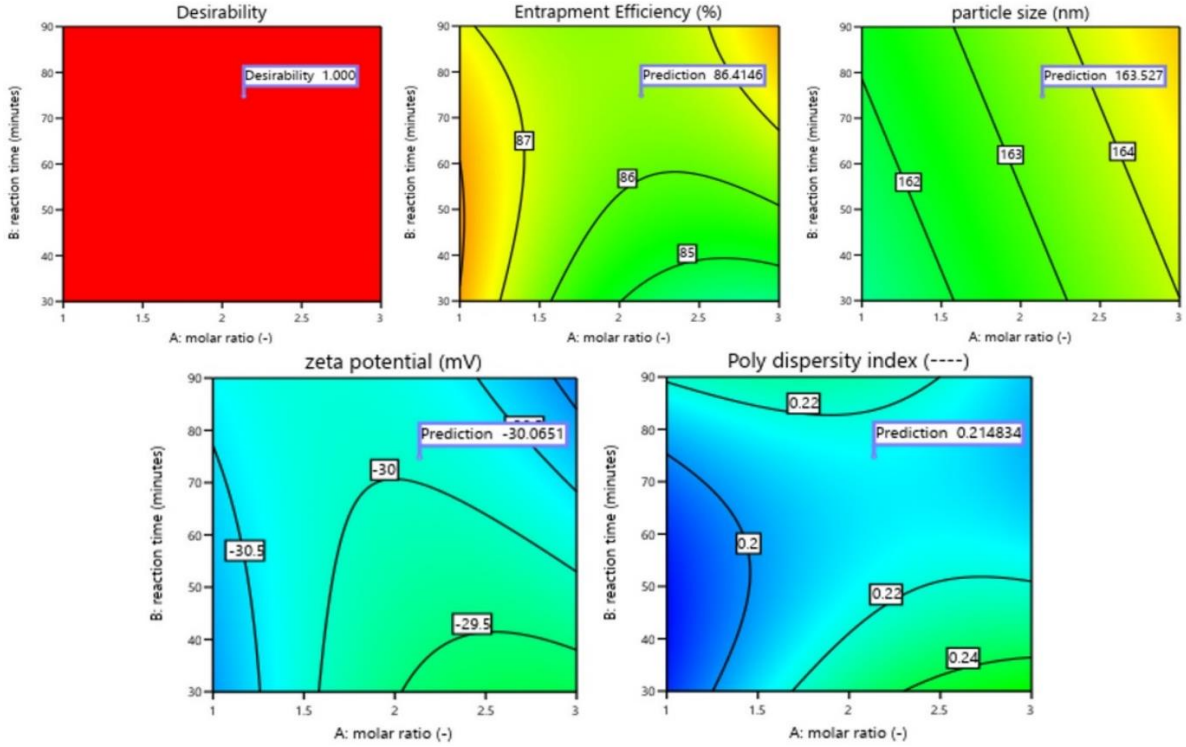

Figure 1: Contour plots of Beta sitosterol phytosomes

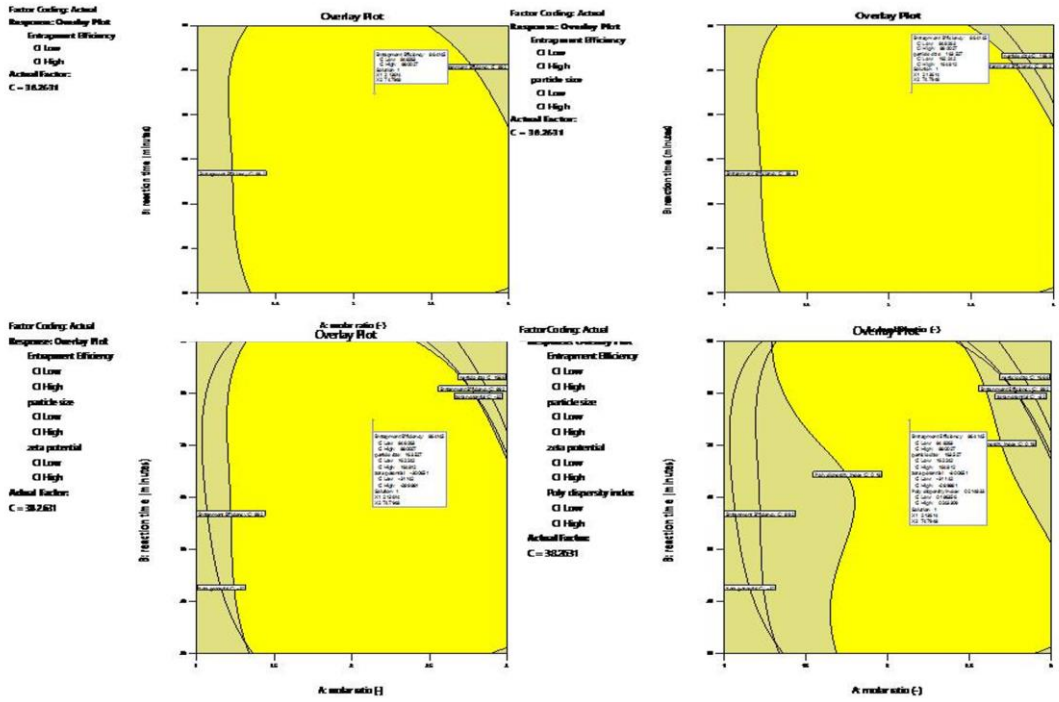

Figure 2: Overlay plots of Beta sitosterol Phytosome

the improved beta-sitosterol formulation[23]. A drop of newly made phytosomal suspension was put onto a 200mesh copper grid covered with carbon and left to settle for one to two minutes. A piece of filter paper was used to gently remove the extra fluid without causing any damage to the film.

For better contrast, the sample was subsequently negatively stained with either 1%W/V uranyl acetate or 1% phosphotungstic acid (PTA, pH~7.0). Prior to examination, the grid was allowed to air dry at room temperature for ten to fifteen minutes following staining. A TEM apparatus (Hitachi H 7500) operating at accelerating voltage of 80 to 200Kv, depending on the equipment parameters, was used to image the dried grid after it had been placed into the sample holder. Several pictures were taken at different magnifications in order to observe: morphology and shape, dispersion of sizes, surface properties, and integrity of the vesicle[24,25].

Zeta Potential

Zeta potential quantification. A 633 nm laser equipped Malvern Zetasizer Nano Z5 (Malvern Instruments Ltd. Worcestershire, UK) was utilized to assess the Beta-sitosterolPhytosome formulation. The instrument was calibrated using a standard measurement (the Zeta potential, Malvern Instruments) before each set of readings. First, distilled water was used to dilute the phytosome samples 100 times in order to get the desired particle concentration. Once the pH was brought down to 7.0 using 0.1 M NaOH, the samples were filtered through a 0.45µmsyringe filter to remove any large aggregates. In addition to measuring zeta potential at 25°C, electrophoretic mobility was studied at a scattering angle of 173. The measurements were taken three times[26].

Each formulation was evaluated in triplicate, with data presented as the average ± standard deviation.

Determination of Poly Dispersity Index

The polydispersity index of the phytosomal formulation was determined using a Dynamic light scattering (DLS) Technique with zetasizer nano ZS90 (Malvern Instruments, UK). The samples suitably diluted with the help of distilled water to avoid the multiple scattering effects and transferred into disposal cuvettes, measurements were carried out at 25 ± 1° C with a scattering angle of 90° and a wavelength of 633nm.

Triplicate readings were taken for every sample, and the average was recorded. The size distribution of the particles in the dispersion is shown by the PDI value. A PDI value of <0.3 was noted to indicate a narrow size distribution and uniformity of the phytosomal vesicles[27].

In-vitro Drug Release Study

A USP Type 2 (paddle) dissolution apparatus (Electro lab, India) was used for the study. The drug samples were contained within a pre-activated cellulose dialysis membrane[28]. To replicate gastrointestinal circumstances, the release research was conducted in two phases: for the first two hours, simulated gastric fluid (SGF, pH1.2) was employed initially, followed by simulated intestinal fluid (SIF, pH6.8) for the subsequent 5 hours.

With a paddle speed of 50 rpm, the dissolving medium's overall volume was kept at 500ml (250ml for each stage) and at 37± 0.5°C. The sample was suspended in the dissolution media and enclosed inside the dialysis membrane in an amount equal to 10mg of beta-sitosterol. To maintain sink conditions, 5ml aliquots were taken out and replaced with an equivalent volume at predefine intervals of 0,15,30,60,120,140, and 480 minutes.

After filtering the obtained samples a UV- Visible spectrophotometer (Shimadzu UV- 1800, JAPAN) was used to detect the absorbance at 206nm.

A calibration curve that had already been established was used to determine the drug concentration. Plotting the cumulative portion of drug release against time was done.

Table 2: Model summary and fit statistics of Beta sitosterol phytosome

Response	R^2	Adjusted R^2	p-value	Adequate Precision	Significance
EE (%)	0.965	0.921	$p < 0.0001$	19.45	Significant
Particle Size (nm)	0.957	0.905	$p = 0.0003$	17.82	Significant
Zeta Potential	0.942	0.891	$p = 0.0012$	16.74	Significant
Polydispersity Index	0.938	0.886	$p = 0.0015$	16.22	Significant

RESULTS AND DISCUSSION

Statistical Optimization via Box- Behnken Design Approach

Formulation parameters affecting the beta-sitosterol phytosomes were optimized employing the Box-Behnken Design. The primary responses assessed were Entrapment Efficiency (EE%), Particle Size, Zeta Potential, and Poly dispersity Index (PDI). The independent parameters under investigation were molar ratio (A), reaction duration (B), and reaction temperature (C).

Model Summary and Fit Statistics

The following responses (Table 2) were examined: Entrapment efficiency (%). Particle size, zeta potential and polydispersity index

For each of the three replies, these numbers show good model fits and predictive skills.

This attests to the BBD optimization as shown in Table 2.

Polynomial Equations

Below are the derived second-order polynomial equations for each response:

Entrapment Efficiency (EE%)

$EE\% = -61.272 + 45.382A + 55.727B + 26.812C - 11.890A^2 - 2.050AB + 3.698AC - 12.741B^2 + 2.858BC - 8.583C^2$

This equation emphasizes that EE% increases with increasing the molar ratio(A), reaction time (B), and temperature (C) up to a certain point. A^2, B^2, C^2 indicates exceeding optimal values leads to a decrease in entrapment efficiency. Moderate interaction is observed especially between B and C.

Particle size (PS)

$PS = 897.682 - 181.075A - 202.651B - 61.673C + 37.210A^2 + 35.064B^2 + 13.278BC + 7.050C^2$

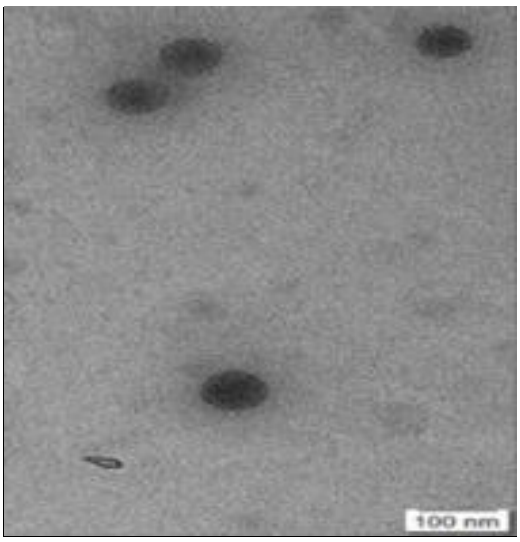

Figure 3: Transmission electron micrographs of Beta Sitosterol Phytosome

Table 3: Drug release kinetics of beta sitosterol phytosomes from F1-F17

Formulation code	Zero order (R^2)	First order (R^2)	Higuchi plot (R^2)
F1	0.951	0.963	0.912
F2	0.954	0.950	0.901
F3	0.948	0.946	0.909
F4	0.962	0.921	0.895
F5	0.950	0.943	0.911
F6	0.943	0.961	0.925
F7	0.953	0.941	0.910
F8	0.960	0.934	0.899
F9	0.949	0.950	0.905
F10	0.48	0.957	0.911
F11	0.945	0.960	0.918
F12	0.951	0.942	0.906
F13	0.946	0.944	0.908
F14	0.950	0.948	0.915
F15	0.942	0.938	0.904
F16	0.955	0.947	0.920
F17	0.964	0.928	0.902

Particle size decreases with increase in molar ratio and reaction time indicating that more efficient mixing and reaction conditions leads to the formation of smaller particles. Exceeding limits causes aggregation effects and increase in the size of particle as shown in the positive quadratic values.

Zeta potential= -98.054 + 24.247A + 17.186B + 5.786C - 4.263A2-2.933B2-2.685C2+1.246AB+0.789AC+1.013BC

Physical stability of the phytosome increased by more negative zeta potential. In the above equation, positive linear coefficient shows that all three factors include in progressing the zeta potential and also quadratic terms in the above equation shows that extremely high levels may reduce stability.

PDI=0.715-0.167A-0.123B-0.071C+0.039A2+0.033B2+0.018C2+0.014AB+0.009AC+0.011BC

PDI values closer to 0 indicates a uniform size distribution. This equation shows that increasing A, B, C reduces PDI. However, the positive quadratic terms indicates a narrow optimal range, above that PDI may slightly increase.

Entrapment Efficiency

The drug entrapment was shown to be considerably influenced by formulation conditions, as seen by the entrapment efficiency, which varied from 81.4% to 89.2%. With a confidence interval of 84.82 – 88.00% the optimized condition forecasted an EE% of 86.41%. A molar ratio of 3:1, a reaction duration of 90 minutes, and a temperature of 40^0C produced the maximum EE. The significant impact of molar ratio and reaction time on drug encapsulation was validated by statistical modeling.

Particle Size

The diameters of the phytosomal particles ranged from 158.2 nm to 166.6 nm, suggesting a comparatively small size distribution that promotes better absorption. The expected particle size of 163.5 nm, which nearly matched the measured range, was displayed by the improved formulation. Reaction duration and molar ratio had a substantial effect on particle size, indicating that improved control over these variables can result in uniform vesicle formation.

Zeta Potential

Zeta potential measurements ranged from -25.4 mV to -32 mV, indicating that the phytosomal suspension had acceptable colloidal stability. With a predicted Zeta potential of -30.06 mV, the improved formulation demonstrated adequate electrostatic repulsion to inhibit aggregation and guarantee formulation stability.

Polydispersity Index (PDI)

The improved formulation displayed a PDI of 0.2148, indicating a limited size distribution and homogeneity, while the other PDI values fluctuated from 0.18 to 0.32. The homogeneity of the vesicles, which is essential for reliable drug release, is shown by a PDI value less than 0.3.

Contour Plot and Overlay Plot Analysis

To maximize several replies at once, overlay plots were created. The following was found to be the ideal area for maximizing EE% while lowering particle size and PDI, Reaction Time was 74.79 minutes, Molar Ratio was 2:1, Reaction Temperature is 38.26^{0}C.

The most desired formulation properties were predicted by these conditions: EE percentage: 86.41%, Size of particle: 163.53 nm, Zeta potential: -30.06 mV, PDI: 0.2148, as shown in figs 1 and 2.

These experimental results support the successful of formulation components using the Box-Behnken Design and corelate the models predictive validity under response surface methodology.

TEM Analysis

Figure 3 shows Transmission Electron Microscopy (TEM) of beta sitosterol. From the image, it is verified that phytosomal vesicles had a consistent, spherical shape. Successful encapsulation and structural stability of beta-sitosterol were indicated by the vesicles appearance of being evenly distributed without aggregating and their preservation of structural integrity.

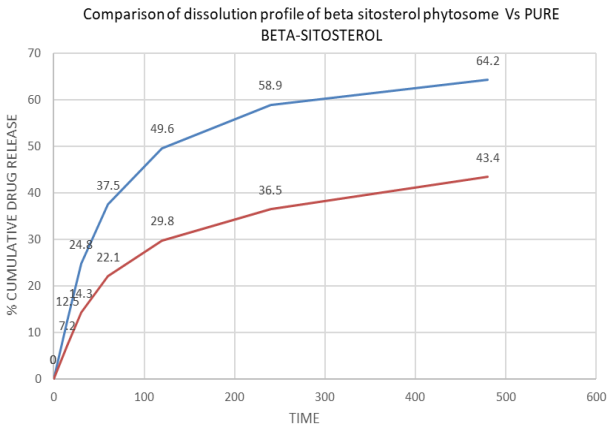

Figure 4: *In-vitro* drug release graph comparing Beta-sitosterol phytosomes with pure Beta-sitosterol

In vitro Drug Release Study

In the *In-vitro* release investigation, dialysis diffusion was stimulated with simulated gastric fluid (SGF) and simulated intestinal fluids (SIF) which was used to compare the phytosomal formulation to pure beta-sitosterol. The phospholipid matrix's improved solubility and protection were responsible for the phytosomal formulation's noticeably higher and longer-lasting release, with 78.2% at the end of 480 min, as pure drug releases only 48.5% as shown in Fig 4. Beta-sitosterol phytosome enhanced gastrointestinal absorption and bioavailability are supported by the regulated release pattern.

Drug Release Kinetics

The *in vitro* drug release behaviour of formulations F1-F17 was analyzed using established kinetic models, including zero-order, first-order, and Higuchi equations. Release data recorded at intervals from 0 to 480 minutes revealed characteristic biphasic profiles, with an initial drug release (15 -30 minutes) followed by sustained release up to 8 hours.

The optimized formulation (F17) showed a gradual and controlled release, reaching 64.2% at 480 min, indicating a diffusion-controlled release mechanism. Several formulations such as F4, F17 and F8 showed strong alignment with zero-order kinetics, reflecting a consistent rate of drug release as shown in table 3. Meanwhile, F1, F11 and F6 demonstrated slightly better correlation with first-order and Higuchi models, suggesting involvement of both concentration-dependent and matrix-diffusion mechanisms. The R^2 values calculated from kinetic model fitting are presented below, confirming that the majority of formulations favour zero-order release, particularly for sustained delivery systems.

CONCLUSION

Beta-sitosterol's phytosomal formulation was effectively studied through the use of Box-Behnken Design. High entrapment efficiency, suitable particle size, acceptable stability, and prolonged drug release were all displayed by the improved formulation. These results support additional research for therapeutic application in the management of obesity and highlight the potential of phytosome technology in overcoming the low bioavailability of phytoconstituents like beta-sitosterol.

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REFERENCES

1. Mohajan D, Mohajan HK. Body mass index (BMI) is a popular anthropometric tool to measure obesity among adults. Journal of Innovations in Medical Research. 2023 May 5;2(4):25-33.
2. Awuchi CG, Igwe VS, Amagwula IO. Nutritional diseases and nutrient toxicities: A systematic review of the diets and nutrition for prevention and treatment. International Journal of Advanced Academic Research. 2020 Jan;6(1):1-46.

3. Rinonapoli G, Pace V, Ruggiero C, Ceccarini P, Bisaccia M, Meccariello L, Caraffa A. Obesity and bone: a complex relationship. International journal of molecular sciences. 2021 Dec 20;22(24):13662.
4. Krzysztoszek J, Laudanska-Krzeminska I, Bronikowski M. Assessment of epidemiological obesity among adults in EU countries. Annals of Agricultural and Environmental Medicine. 2019;26(2).
5. Ahmed B, Konje JC. The epidemiology of obesity in reproduction. Best Practice & Research Clinical Obstetrics &Gynaecology. 2023 Jul 1;89:102342.
6. Güngör NK. Overweight and obesity in children and adolescents. Journal of clinical research in pediatric endocrinology. 2014 Sep 5;6(3):129.
7. Aronne LJ. Classification of obesity and assessment of obesity-related health risks. Obesity research. 2002 Dec;10(S12):105S-15S.
8. Salam MM, Yousuf R, Salam MW, Haque M. Obesity and overweight: A global public health issue. Advances in Human Biology. 2023 Jan 1;13(1):154-6.
9. Phelps NH, Singleton RK, Zhou B, Heap RA, Mishra A, Bennett JE, Paciorek CJ, Lhoste VP, Carrillo-Larco RM, Stevens GA, Rodriguez-Martinez A. Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. The Lancet. 2024 Mar 16;403(10431):1027-50.
10. Lim HJ, Xue H, Wang Y. Global trends in obesity. Handbook of Eating and Drinking: Interdisciplinary Perspectives. 2020:1217-35.
11. Mohajan D, Mohajan HK. Obesity and its related diseases: a new escalating alarming in global health. Journal of Innovations in Medical Research. 2023 Mar 23;2(3):12-23.
12. Parikh NI, Pencina MJ, Wang TJ, Lanier KJ, Fox CS, D'Agostino RB, Vasan RS. Increasing trends in incidence of overweight and obesity over 5 decades. The American journal of medicine. 2007 Mar 1;120(3):242-50.
13. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. Jama. 2010 Jan 20;303(3):235-41.
14. Khan S. *Risk factors of periodontitis in Australian adults* (Doctoral dissertation, University of Tasmania).
15. Jaacks LM, Vandevijvere S, Pan A, McGowan CJ, Wallace C, Imamura F, Mozaffarian D, Swinburn B, Ezzati M. The obesity transition: stages of the global epidemic. The lancet Diabetes & endocrinology. 2019 Mar 1;7(3):231-40.
16. Ye Q, Zou B, Yeo YH, Li J, Huang DQ, Wu Y, Yang H, Liu C, Kam LY, Tan XX, Chien N. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. The lancet Gastroenterology & hepatology. 2020 Aug 1;5(8):739-52.
17. Khan Z, Nath N, Rauf A, Emran TB, Mitra S, Islam F, Chandran D, Barua J, Khandaker MU, Idris AM, Wilairatana P. Multifunctional roles and pharmacological potential of β-sitosterol: Emerging evidence toward clinical applications. Chemico-biological interactions. 2022 Sep 25;365:110117.
18. Abo-Zaid OA, Moawed FS, Ismail ES, Farrag MA. β-sitosterol attenuates high-fat diet-induced hepatic steatosis in rats by modulating lipid metabolism, inflammation and ER stress pathway. BMC Pharmacology and Toxicology. 2023 May 12;24
19. Ding J, Zhang R, Ahmed S, Liu Y, Qin W. Effect of sonication duration in the performance of polyvinyl alcohol/chitosan bilayer films and their effect on strawberry preservation. Molecules. 2019 Apr 10;24(7):1408.
20. Tai K, Rappolt M, He X, Wei Y, Zhu S, Zhang J, Mao L, Gao Y, Yuan F. Effect of β-sitosterol on the curcumin-loaded liposomes: Vesicle characteristics, physicochemical stability, *in vitro* release
21. Lv Y, He H, Qi J, Lu Y, Zhao W, Dong X, Wu W. Visual validation of the measurement of entrapment efficiency of drug nanocarriers. International journal of pharmaceutics. 2018 Aug 25;547(1-2):395-403.
22. Pandey R, Patil PO, Patil MU, Deshmukh PK, Bari SB. Quantitative estimation of diacerein in bulk and in capsule formulation using hydrotropic solubilizing agents by UV-spectrophotometry and the first order derivative using the area under curve method. Pharmaceutical methods. 2012 Jan 1;3(1):4-8.
23. Alhalmi A, Amin S, Beg S, Al-Salahi R, Mir SR, Kohli K. Formulation and optimization of naringin loaded nanostructured lipid carriers using Box-Behnken based design: *In vitro* and *ex vivo* evaluation. Journal of Drug Delivery Science and Technology. 2022 Aug 1;74:103590.
24. Moolakkadath T, Aqil M, Ahad A, Imam SS, Praveen A, Sultana Y, Mujeeb M. Preparation and optimization of fisetin loaded glycerol based soft nanovesicles by Box-Behnken design. International journal of pharmaceutics. 2020 Mar 30;578:119125.
25. Sharma M, Sharma G, Singh B, Katare OP. Systematically optimized imiquimod-loaded novel hybrid vesicles by employing design of experiment (DoE) approach with improved biocompatibility, stability, and dermato kinetic profile. AAPS PharmSciTech. 2019 May;20:1-4.
26. Brito Raj S, Chandrasekhar KB, Bhaskar Reddy K. Design, development and *in vivo* pharmacokinetic evaluation of cardiovascular drug-loaded nanostructured lipid carrier system. Int J Drug Deliv Technol. 2018 Jul–Sep;7(3):190–209.
27. Senthamarai R, Thota S. *Carvacrol-Loaded Phytosomes for Enhanced Wound Healing: Molecular Docking, Formulation, DoE-Aided Optimization, and in vitro/in vivo Evaluation*. *Int J Nanomedicine*. 2023 Dec 31;18:5749–60. doi:10.2147/IJN.S123456.
28. Kamireddy S, Sangeetha S, Roy H. An approach to development and *in-vivo* evaluation of beta-sitosterol phytosomes by Taguchi orthogonal array design. *Int J Drug Deliv Technol*. 2024 Jul;14(3).