Section A -Research paper
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Optimisation and evaluation of emulsomes containing stigmasterol isolated from flower extract of Cristata Sciata

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Abstract:

Emulsomes formulation becomes the emerging delivery system for the active constituents isolated from the plant extract. Emulsome provide the enhanced entrapment efficiency as well as sustainability of the drug at the site of action. In the present study the emulsomes formulation optimised via Box Behnken design expert software. Stigmasterol isolated compound from the flowers extract of *Cristata Sciata* loaded in the optimised emulsomes formulation. Additionally it was compared with the standard Active constituents. This formulation was further analysed and characterised via entrapment efficiency, morphological study (SEM) , drug release and so on. As result it was concluded that isolated compound was equivalent active as the standard compound.

Keywors : Emulsomes, Stigmasterol, Cristata Sciata, NDDS, Flower extract etc.

Introduction

When amphiphilic building blocks self-assemble in the presence of water, vesicular drug delivery systems, which are highly ordered assemblies made up of one or more concentric bilayers, result. Significant work was done in past years to create the Novel medication Delivery System (ND1DS), which satisfies the requirements that it should channel active entity at site of action and give medication at a rate determined by body need over the course of therapy (**V.H.K et al., 1987**;**Namdeo et al.,2014**).The highly structured assemblies of one or more concentric lipid bilayers that form vesicular systems when specific amphiphilic building components are exposed to water. Vesicles can be built from a wide range of amphiphilic materials (**DPet al.,2010**;**AD et al.,1965**;**K. Sashi et al.,2012**). The Vesicular medication Delivery System has vesicles as its preferred medication delivery method. For instance: Liposomes, Niosomes, Pharmacosomes, etc. (**Riaz, et al.,1966**;**Riaz et al.,1966: S.**

Pandey et al.,2009). Due to the limited medication penetration into cells, conventional chemotherapy is ineffective for treating intracellular infections. To eliminate adverse side effects of traditional and controlled release drug delivery methods, enhance bioavailability at the site of illnesses, and resolve the issue of drug degradation and/or drug dose **(F.Volkering et al.,1995).** Emulsomes are a brand-new type of lipoidal vesicular system that have a phospholipid bilayer covering a solid fat core inside. Emulsomal compositions with a soy lecithin and cholesterol-stabilized solid lipid core ingredient. To create miniature emulsomes, the medication is loaded and then sonicated. Emulsomes are anticipated to behave similarly to chylomicrons, the body's natural lipoproteins, given their structural similarities. These tiny lipid-like particles are commonly ingested through the GIT tract's enterocytes using an endogenous lipid absorption process (**Gupta et al.,2006**). Lipid-based excipients can affect oral absorption through a variety of physiological processes, including delayed stomach emptying, increased bile flow, pancreatic juice secretion, and increased membrane lipid fluidity, or by directly affecting enterocytes-based drug transport and disposition (**Bhawandeep et al.,2012; Rajesh et al.,2013**).

Stigmasterol is a 3beta-sterol that consists of 3beta-hydroxystigmastane having double bonds at the 5,6- and 22,23-positions. The primary aim of this study was to determine the efficacy of a low-fat spread enriched with plant sterols in reducing total and low density lipoprotein-cholesterol (LDL-C) concentrations in primary hypercholesterolemia. The secondary objective was to evaluate whether patients receiving a lipid-lowering drug (fibrate) might differ in their response to plant sterols. Emulsome represents lipid-based drug delivery systems with broad variety of therapeutic applications particularly for drugs that are poor aqueous soluble. When amphiphilic building blocks self-assemble in the presence of water, vesicular drug delivery systems, which are highly ordered assemblies made up of one or more concentric bilayers, result(**V.H.K et al.,1987**).The highly structured assemblies of one or more concentric lipid bilayers that form vesicular systems when specific amphiphilic building components are exposed to water. Vesicles can be built from a wide range of amphiphilic materials (**DPet al.,2010**). Due to the limited medication penetration into cells, conventional chemotherapy is ineffective for treating intracellular infections. To eliminate adverse side effects of traditional and controlled release drug delivery methods, enhance bioavailability at the site of illnesses, and resolve the issue of drug degradation and/or drug dose **(F.Volkering et al.,1995).** Emulsomes are tiny lipid assemblies with polar centres that carry pharmaceuticals that are insoluble in water in solution form without the need for a surface

activator or co-solvent. Emulsomes are anticipated to behave similarly to chylomicrons, the body's natural lipoproteins, given their structural similarities (**Gupta et al.,2006**).

Materials and methods

Method of preparation

Preparation of Stigma sterol emulsomes

Various batches of Stigma sterol loaded emulsomes were prepared by thin film hydration method as described by **Amselem *et. al.,* 1994** with slight modification as per laboratory set up.

Preliminary optimization of process parameters

During preparation of emulsomes, process variables, such as selection of lipids, vacuum conditions for dry film formation, speed of rotation of flask during film formation and hydration, were optimized for desired results. The effect of one variable was studied at a time keeping other variables constant.**(Kumar *et.al.*, 2012)**

Table 1 : Selection of preliminary process parameters

Process Parameters	**Level**
Vaccum	350 mm Hg
Speed of rotation	100 rpm
Hydration time	1.5 hr
Speed of rotation during hydration	60 rpm

Optimization by Box Behnken Design (DOE)

For optimization of formulation Response Surface Methodology (RSM) used to study quantitative aspects of the impacts and interactions between key formulation factors of emulsomes. To inoculum the different process parameters at three levels (low, medium, and high, coded as -1, 0, and+1), a Box-Behnken Design with a total of 17 experimental runs was used. The DPPC: GMS ratio (A), lipid: isolated compound stigma sterol (B), and sonication duration (C) were used as independent factors, and their effects on size (Y1) and percentage entrapment (Y2) were evaluated as dependent variables. The optimized batch was selected on the basis of desirability criteria. % prediction error of the prepared batch was calculated in order to evaluate the reliability of developed mathematical models.

Values of Variables

Name	Goal	Lower Limit	Upper Limit	Importance
A:DPPC	is in range	0.1	0.5	3
B:GMS	is in range	0.5	1	3
C:Lipid	is in range	10	15	3
Particle size	none	188.2	387.5	3
EE	none	63.8	78.4	3

Characterization of emulsomes

Determination of particle Size (PS) and Zeta Potential (ζ)

Mean Particle size was determined by using dynamic light scattering mehod (Zetasizer Nano ZS, Malvern, and Worcestershire, UK). The Zeta Potential of emulsomes were measured using the laser Doppler method. **(Gill *et. al.*, 2014)**

Entrapment Efficiency (EE)

The efficiency of stigma sterol (standard) and isolated compound entrapment in the emulsomes was determined by analyzing the amount of drug content of emulsomes in comparison to the total amount added. The following formula was used to calculate the entrapment efficiency **(Ucisik, *et. al.*, 2015).**

Entrapment efficiency (%) = (Determined drug content) / (Total drug added) X100.

Shape and morphology

The shape and surface morphology of the prepared emulsomes was studied by both scanning electron microscopy (JEOL, JSM-6100).

***In-vitro* drug release**

The *in-vitro* drug release profiles of stigma sterol (standard) and isolated compound from different emulsomal formulations were determined using a dialysis tube (Sigma, MO, USA) method. The samples were analyzed for stigma sterol spectrophotometrically**.(Amselem *et. al.*, 1994)**

RESULTS

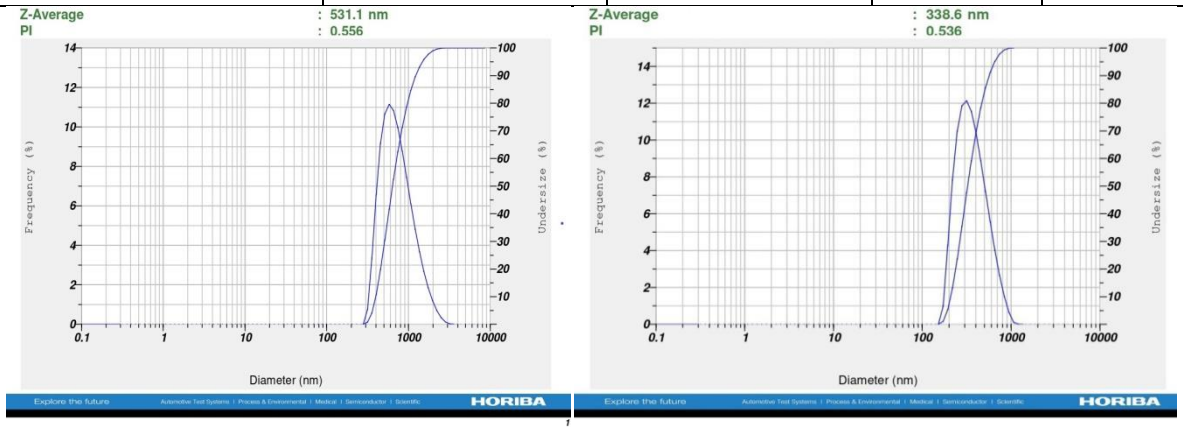
Zeta Potential (ζ) and Particle sizer (PS) of optimized formulation

Characterization of emulsomes

Zetasizer (Particle sizer) Nano ZS (Malvern Instruments Ltd, UK) determined the diameter often distinct formulations. Size distribution curve indicated a variationin particle size in a range of 75–550 nm. Similarly, the diameter of isolated compound stigma sterol loaded emulsomes was found as 531.1 nm, with an average PDI value of 0.556 and the diameter of standard stigma sterol loaded emulsomes was found as 338.6 nm, with an average PDI value of 0.556 . The zeta potential of the isolated and standrad stigma sterol was -72.4 mV and -0.3mV.

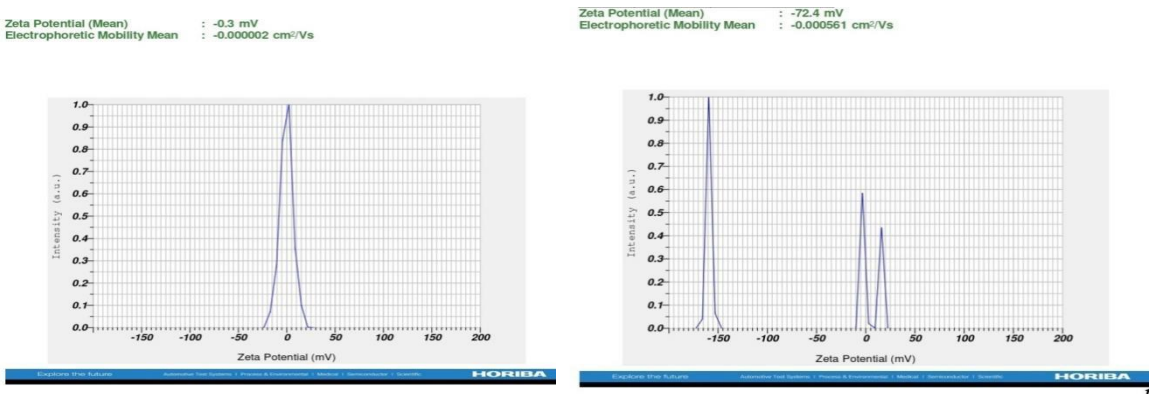
Table 2: Particle sizerand zeta potential study of the isolated and standard stigma sterol

Formulation	Formulation code	Particle sizer	PDI	Zeta potential
Emulsome Formulation containing standard compound stigma sterol	**ESS-1**	338.6 nm	0.536	-0.3mV
Emulsome Formulation containing isolated stigma sterol	**EIS-1**	531.1 nm	0.556	-72.4 mV



(a) **(b)**

Graph 1: (a)Particle sizer of the optimised formula of isolated compound stigma sterol (b) Particle size of the optimised formula of standard compound stigma sterol



(a) (b)

Graph 2(a) Zeta potential of the optimised formula of isolated compound stigma sterol(b) Zeta potential of the optimised formula of standard compound stigma sterol

Entrapment Efficiency (EE) of the optimized emulsomes formulation

The EE of the isolated compound loaded emulsomes and standard stigma sterol loaded emulsomes were shown in the below table: through the result it was found that isolated compound stigma sterol shows the equivalent entrapment efficiency like the standard stigma sterol.

Formulation	Formulation code	Entrapment efficiency %
Emulsome Formulation containing isolated compound stigma sterol	EIS-1	73 ±2.081
Emulsome Formulation containing standard stigma stertol	ESS-1	74 ±1.063

Shape and morphology

The morphological study of the optimized formulation of emulsomes loaded isolated compound stigma sterol and standard stigma sterol were performed by the SEM analysis (JOEL) and result shown the spherical shape of emulsomes with different sizes and result shown in the table and **figure 1**.

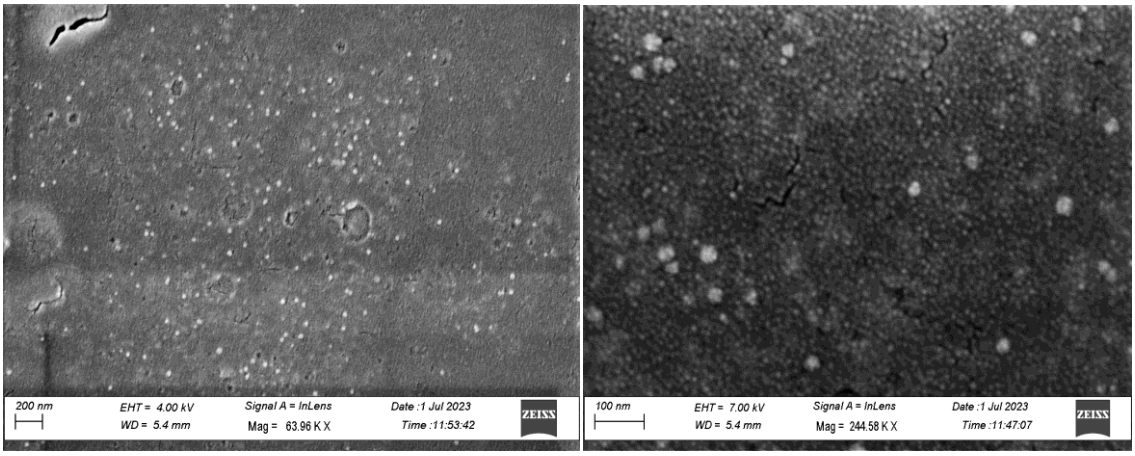


Figure 1 : (a) showing the SEM image of the isolated compound stigma sterol loaded emulsomes (EIS-1) (b) showing the SEM image of the standard stigma sterol loaded emulsomes (ESS-1)

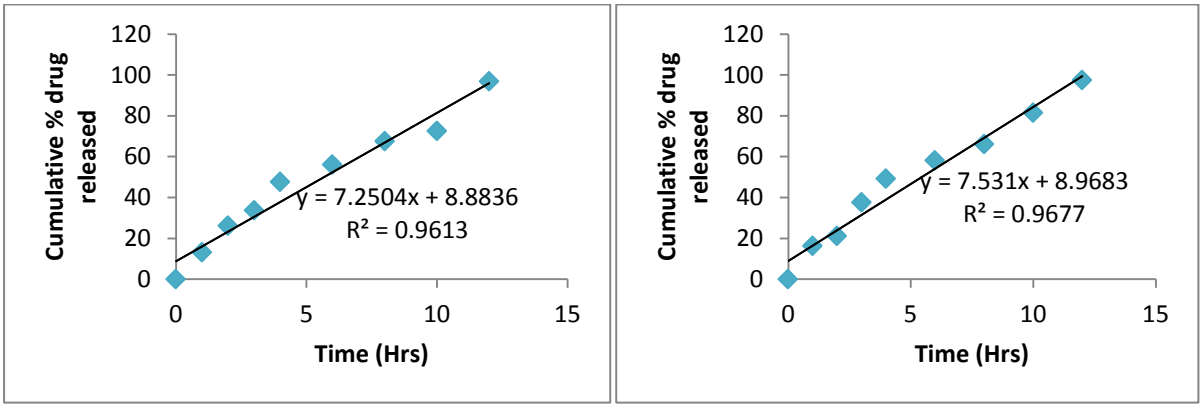
In-vitro drug release

In-vitro drug release of optimized formulation emulsomes loaded standard stigma sterol:

The *In-vitro* drug release of the emulsomes formulation coded ESS-1 shows 97.82 % active release at 12 hrs and its kinetic release study graph of zero order , first order , Higuchi and Korsmeyer peppas shows the regression R2 = 0.961 , R^2 = 0.709, R^2 = 0.960 and R^2 = 0.701with the slope value y = 7.250x + 8.883, y = -0.139x + 2.303, y = 27.28x - 8.790 and y = 1.249x + 0.749.

In-vitro **drug release of optimized formulation emulsomes loaded Isolated compound stigma sterol (EIS-1):**

The *In-vitro* drug release of the emulsomes formulation coded **EIS-1** shows 97.51 % active release at 12 hrs and its kinetic release study graph of zero order , first order , Higuchi and Korsmeyer peppas shows the regression R2 = 0.967,R^2 = 0.733, R^2 = 0.963, and R^2 = 0.689 with the slope value y = 7.531x + 8.968, y = -0.154x + 2.344, y = -0.154x + 2.344 and y = 1.243x + 0.765



(a) **(b)**

Graph 3: (a)Zero order kinetic model of standard Stigmasterol (ESS-1) (b) Zero order kinetic model of Isolated compound stigma sterol (EIS-1)

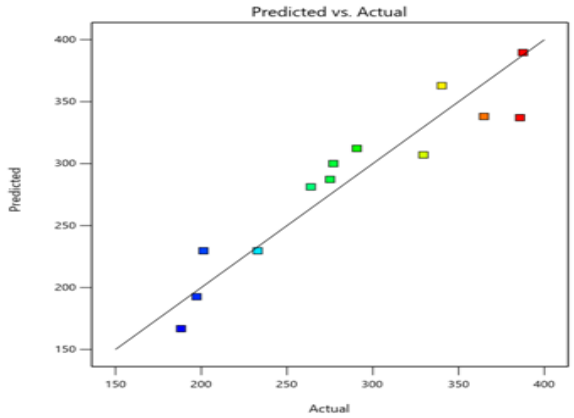
Optimization of formulation via DOE

Design of Expert software version 11.2.2 - Box-Behnken Designs, STAT 503 used for the optimization of the formulation. Below tables shown the result and optimization of formulation as per Box-Behnken Designs through response surface methodology , after the selection of optimized formulation, isolated compound stigmasterol and standard stigmasterol actively loaded in the formulation.

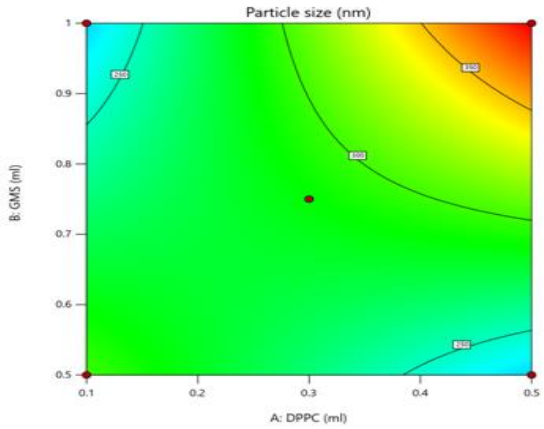
Table 3: Different formulation variables

Run	Factor 1 A: DPPC (ml) X1	Factor 2 B: GMS (ml)X2	Factor 3 C: (Lipid)X3	Response 1 Particle size (nm)Y1	Response 2 (%)Y2
1	0.1	1	12.5	201.3	71.3
2	0.5	0.75	15	290.7	78.4
3	0.3	0.75	12.5	275.1	69.4
4	0.3	1	10	263.9	64.9
5	0.3	0.5	10	340.2	66.2
6	0.1	0.5	12.5	277	70.2
7	0.3	1	15	364.8	75.3
8	0.1	0.75	15	197.3	76.3
9	0.5	0.75	10	329.5	63.8
10	0.3	0.5	15	188.2	74
11	0.5	0.5	12.5	232.9	70.2

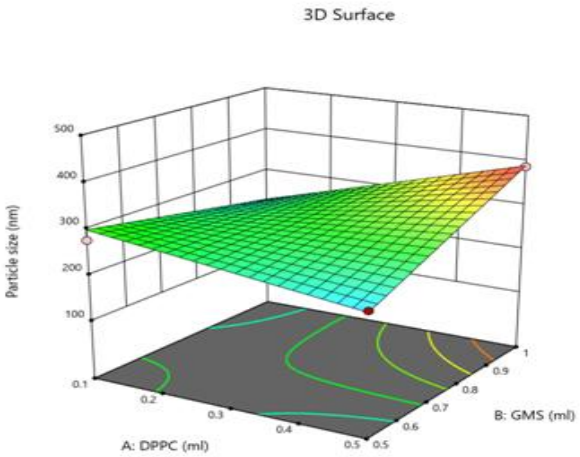
12	0.1	0.75	10	385.8	68.1
13	0.5	1	12.5	387.5	71.7



(A)

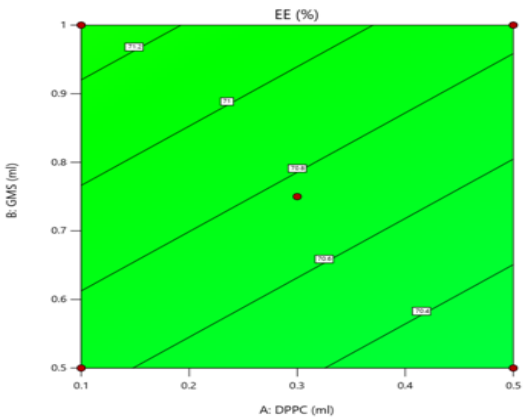


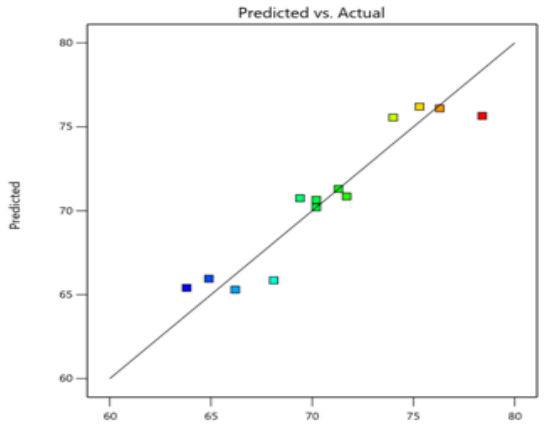
(b)

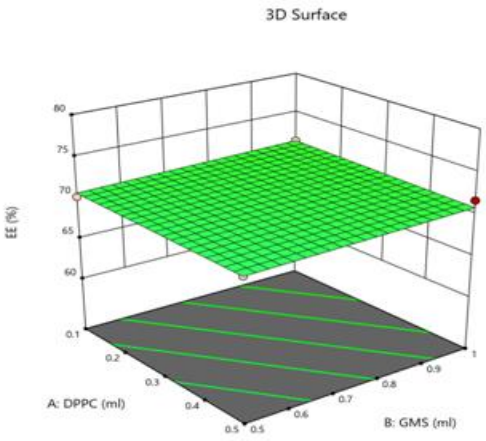


(c)

Graph 4 : A,B, and C , Response surface plot showing combined effect of DPPC and GMS on Particle size of formulation



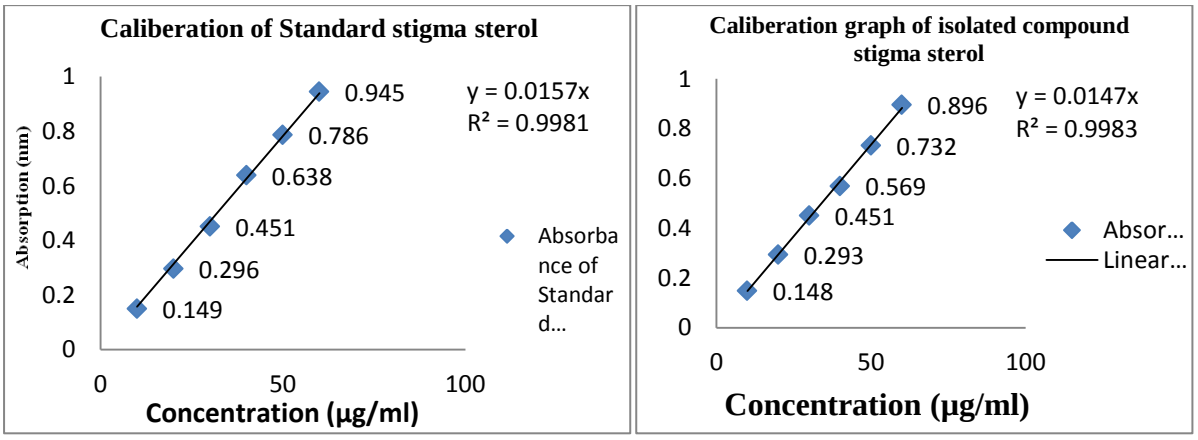




Graph 5 : Response surface plot showing combined effect of DPPC and GMS on Entrapment efficiency of formulations.

Calibration study of isolated compound stigma sterol and standard stigma sterol via UV spectroscopy

The linearity of the proposed method was established by least squares linear regression analysis of the calibration curve. The regression equation for standard stigma sterol and isolated stigma sterol were obtained by plotting absorbance versus concentration of both the compound in the range of 10-60 µg/ml. The regression equation were y = 0.015x (standard stigma sterol) and y = 0.014x (isolated compound). The regression coefficient of standard stigma sterol and isolated compound were R2 = 0.998 calculated. Six points calibration curve were obtained in concentration range from 10-60 µg/ml for sample.

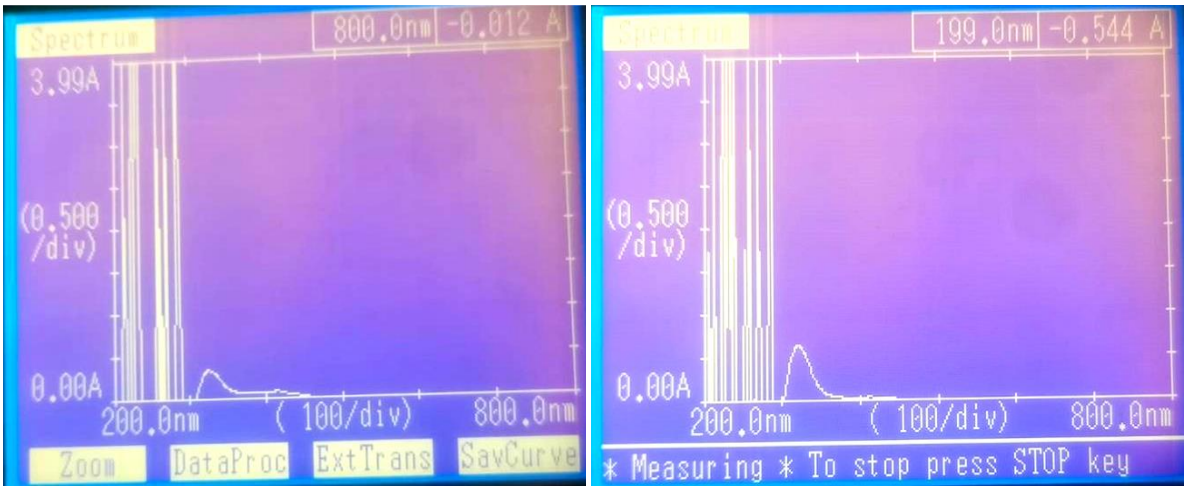


(A) **(B)**

Graph 6 (A) calibration graph of the standard stigma sterol (B) calibration graph of the isolated stigma sterol

UV estimation For Stigma sterol (standard) and isolated compound stigma sterol

UV spectra of the isolated compounds were recorded in n hexane: Acetone (8: 2) over a scanning range of 200-800 nm and λmax of compounds were determined. Spectra were recorded with a Shimadzu 1700 double beam-UV-VIS spectrophotometer. The Blank was n-Hexane: Acetone (8: 2) and the wavelength of active constitute was found out 307 nm. On comparing with the standard stigma sterol the wavelength of this in the same solvent ratio was 307 nm.



(A) **(B)**

Graph 7: (A)UV spectroscopy graph of the isolated compound stigma sterol (B) UV spectroscopy graph of the isolated compound stigma sterol

DISCUSSION

On comparing the organoleptic properties of the isolated compound stigma sterol with standard stigma sterol shows the slightly changes in the color but it was permissible. Solubility study reveals that isolated compound freely soluble in hexane while stigma sterol in both hexane and methanol. The melting point and partition coefficient of the isolated stigma sterol and standard stigma sterol were 163°C, 11.10 (K) and 164°C, 11.49 (K). UV spectra of the isolated compounds were recorded with a Shimadzu 1700 double beam-UV-VIS spectrophotometer. The regression equation were y = 0.015x (standard stigma sterol) and y = 0.014x (isolated compound) and regression coefficient of both were R2 = 0.998 calculated. All the spectra were analysed at the 307 nm. Ruggedness of the isolated compound stigma sterol was found 0.00116±0.023 for analyst-1 and 0.002±0.020 for analyst-

2 whereas the value of % RSD was recorded 0.39 and 0.7 respectively and standard deviation and relative standard deviation and it was found 0.0039±0.013 for analyst-1 and 0.0042±0.015 for analyst-2 whereas the value of % RSD was recorded 1.3 and 1.4 respectively. Similarly the Robustness of isolated compound stigma sterol was assessed with changes in the analytical temperature. The results were expressed as standard deviation and relative standard deviation and were recorded 0.0048 and 0.0076 whereas % RSD was found 1.63 and 2.6 respectively. Optimisation and selection of the ratio for the preparation of emulsomes performed by the Design of Expert software version 11.2.2 - Box-Behnken Designs, STAT 503 , after the selection of optimized formulation, EIS-1 and ESS-1 actively loaded in the formulation . The particle size of the isolated compound stigma sterol loaded emulsomes show 531.1 nm which was more than the standard stigma sterol (338.1 nm)The EE of the isolated compound loaded emulsomes and standard stigma sterol loaded emulsomes concluded that the EE of the EIS-1 AND ESS-1 were shows that the EE of the EIS-1 is equivalent with the ESS-1.After the morphological study of the optimized formulation of emulsomes loaded isolated compound stigma sterol ESI -1 and standard stigma sterol ESS-1 were shows 200 nm and 100nm . The In-vitro drug release of the emulsomes formulation coded ESS-1 shows 97.82 % active release at 12 hrs while the EIS-1 shows 97.51 which was slightly less than the standard**.** After the stability result concluded that there was no significant change in the properties of optimized emulsomes formulation during the stability period. There was a slight decrease in particle size for the stored formulation, but it was well within the acceptable limit.

Conclusion

From the present study it was concluded that the isolated compound was equivalent active as the standard compound and stability study of the emulsomes loaded with the isolated compound not shown any significance changes this reveals that the active constituents containing formulation was stable.

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