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Preparation and Evaluation of Norbixin Phytosomes

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ABSTRACT

Annatto extract (Norbixin) is reported to a natural carotenoid, has been employed in the food industry as an important colorant, mainly in dairy products such as cheese and butter. It has been considered safe for human consumption and treatment of several health disorders such as constipation, fevers, heartburn, asthma, scabies, ulcers, diarrhea, stomach upset, skin diseases, etc. Norbixin has an enormous number of applications in coloring and bleaching of dairy food products especially bakery products, cream desserts, buttermilk deserts, rice flour, and corn starch. Norbixin has low bioavailability because it is less soluble in water and it is rapidly eliminated from the body. This study aimed to prepare the phytosome of Norbixin and evaluate it. The phytosomes containing a molar ratio of 1:1, 1:2, 2:1 and 2:2 of norbixin and soya lecithin were prepared by the anti-solvent precipitation technique. The phytosome was characterized by SEM, DSC, and FTIR. DSC data showed that phytosome has irregular size vesicles consisting of soya lecithin and norbixin was found to be intercalated in the lipid layer. FT-IR spectrum of the phytosome confirmed the formation of cell-like structure through interaction with soya lecithin. SEM data has shown the irregular particle size and crystalline shape of the prepared phytosome of Norbixin. The *in-vitro* drug release rate of the prepared phytosome was evaluated.

INTRODUCTION

Considerable attention has been focused on the development of novel drug delivery systems (NDDS) for herbal drugs in the past few decades. The novel carriers should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, throughout treatment. Secondly, it should channel the active entity of herbal drug to the site of action. Conventional dosage forms including prolonged-release dosage forms are unable to meet none of these.[1]

Plant-derived drugs have gained immense popularity and access to the medicine markets throughout the globe as safer and effective substitutes of modern synthetic medicines which are considered to be full of adverse and toxic interactions. In underdeveloped and developing nations all over the world plant drugs in traditional form or as alternative medicine have been supposed to satisfy the primary healthcare needs of about 80% of the population and even in developed nations, these medicines are being utilized by about 65% of the population.

Currently, as many as one-third to approximately one-half of all the drugs available are derived from plants or other natural sources. The plant drug formulations of traditional systems of medicine like the Chinese and Indian systems usually contain crude extracts of different plants which incorporate in them unwanted and many times harmful principles along with the active principles. With the developments in the field of phyto and analytical chemistry, specific ingredients or a group of similar ingredients from plants are being extracted, isolated and tested for their different medicinal applications. However, the bioavailability of active principles of plants has become an issue of concern for researchers because of poor oral bioavailability of many of them specifically those containing polyphenolic rings in their structures such as flavonoids and other water-soluble constituents like terpenoids and tannins. Some of the basic reasons for the poor bioavailability of these substances are low aqueous or lipid solubility, high molecular weight/size, and poor plasma membrane permeability. Moreover, the standardized extracts when administered orally lose some of their constituents in the presence of gastric fluids. This has restricted the use of pharmacologically effective polyphenolic plant actives for treating different disorders.

To counter these problems and to make herbal therapy more effective these drugs have been incorporated into several novel delivery systems in recent times. Some of the approaches for bioavailability enhancement are formulating at the nanoscale as nanoparticles, binding with

lipids as liposomes or herbosomes/phytosomes, delivery in the form of microemulsions, modification in chemical structures, delivery as prodrug and complexation with cyclodextrins, etc. have several advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improving tissue macrophages distribution, sustained delivery, protection from physical and chemical degradation, etc. Thus the nano-sized novel drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines.[2]

Norbixin has low bioavailability because it is less soluble in water and it is rapidly eliminated from the body.

MATERIALS AND METHODS

Norbixin was purchased from Sigma Aldrich Pvt Ltd, Bangalore Pvt. Ltd., Bangalore, India. Soya Lecithinwas obtained from Himedia Laboratories Pvt Ltd, Mumbai. Whereas all other reagents used were of the highest quality and commercial grade.

Pre-formulation Studies:

Organoleptic Properties

The sample of plant drug norbixin was studied for organoleptic characters such as color, odor, and appearance.

Melting Point

The melting point of norbixin was determined by a capillary method using melting point apparatus. For assurance of melting point, the drug was taken in a glass slim capillary whose one end was fixed by wire. The capillary containing drug was dipped in liquid paraffin inside the melting point apparatus which was outfitted with magnetic stirring facility.[3]

Physical Compatibility Test

The selection of drugs and phospholipid to prepare the phytosome is mainly based on the physical compatibility studies and solubility study. The pre-formulation study was carried out with potential formulation phospholipids or polymer to determine drug-phospholipids interaction/compatibility.

FTIR Spectroscopy

FT-IR range of the drugs was acquired using Shimadzu-8400S FT-IR Spectrophotometer (Tokyo, Japan) by the KBr pellet method. The dry sample of norbixinwas separately blended with IR grade KBr in the proportion of 1:100 and the active was also mixed with excipients to check compatibility. Each blend was compacted in the form of a pellet by applying 10 tons of pressure in a hydraulic press. The pellets were scanned over a wavenumber range of 4000 to 400 cm^{-1} in the Fourier Transform Infrared instrument and spectral analysis was done. Software used for the data analysis was Perkin-Elmer Spectrum 5.3.[4]

Differential Scanning Calorimetry Studies

Thermogram of norbixinwas obtained using Shimadzu DSC-60 Differential scanning calorimeter (Shimadzu DSC-60, Japan) using aluminum pans. The dry samples of the drug (2.00-10.00 ± 5 mg) were separately weighed, fixed in aluminum pans hermetically and warmed at an examining rate of 10°C/min between 30°C to 300°C. The ideal environment was provided by cleansing nitrogen stream at the rate of 40 mL/min.[5]

Determination of Solubility

Saturation solubility of norbixinwas determined in acidic pH 1.2 (0.1 N HCl), phosphate buffers pH 6.8 and 7.4, organic solvents such as acetone, acetonitrile, dimethyl sulfoxide (DMSO) dichloromethane (DCM), ethanol (EtOH), methanol (MeOH) and distilled water (DW). The abundance measure of each drug was separately added exclusively to 5 ml of every media in screw-capped tubes. A vortex mixture was utilized to encourage the solubilization. After 48 h, 1 ml of aliquots were taken out from each sample through filtration using Whatman filter paper No 41. Absorbance was estimated in the range of 200-400 nm on UV Visible Spectrophotometer (Shimadzu-1800, Japan) and calculations for solubility were done.[6-7]

UV Spectroscopy Study (Determination of λ max)

The standard stock solution of the drug was filtered between 200-400 nm using a UV spectrophotometer (Shimadzu-1800, Japan) in acidic pH 1.2 (0.1 N HCl), phosphate buffers pH 7.4, organic solvents such as acetonitrile and methanol (MeOH).

Formulation and Development:

Selection of plant drugs

A natural coloring carotenoid Phyto-constituents was selected for the preparation of phytosome.

Selection of phospholipids

The selection of a phospholipid for the preparation of the phytosome was based on compatibility and interaction with the drug.

Selection of method [8-16]

The following methods were tried in a trial or error base to prepare the phytosome:

1. Anti-solvent precipitation method (AP)

2. Solvent evaporation method (SE)

3. Rotary evaporation technique (Film hydration technique) (RE)

Anti-solvent precipitation method

The required quantities of norbixin and phospholipids were separately taken in a 100 ml round base flask and refluxed with 30 ml of methanol at a temperature not surpassing 60^{o}C for 2 h. The blends were concentrated to 5 ml and n-hexane (20 ml) was added deliberately with constant mixing to get the precipitate which was sifted, gathered and put away in vacuum desiccators for overnight. Powdered phytosomes formulations were set in a golden shading glass bottle and put away at room temperature.

Preparation of preliminary trial batches for selection of method:

The preliminary batches of phytosomes of drugs with phospholipid of 1:1 molar ratio were prepared by the mentioned methods in Table 1.

Table No. 1: Preliminary Batches of Phytosome of Drugs

Sr. No.	Methods	Drug	Phospholipids	Molar ratio	Solvents
1.	AP	Norbixin	Soya lecithin	1:1	Methanol + n-hexane
2.	SE	Norbixin	Soya lecithin	1:1	Tetrahydrofuran
3.	RE	Norbixin	Soya lecithin	1:1	Ethanol + n-hexane

Evaluation of preliminary batches for selection of working method:

For the selection of the working method, the preliminary trial batches of phytosomes of drugs were characterized and evaluated for shape using microscope, % yield, % drug loading and % entrapment efficiency and particle size.

Shape of the phytosomes

An optical microscope was used for the characterization of the formulations. The formulations were separately suspended in phosphate buffer pH 7.4 and a drop was set on a slide and secured with a coverslip. Microscopic view of the complex was observed at a magnification of 10x10.[17-18]

Determination of % yield

Assurance of % yield of formulations was calculated by the accompanying equation: [19]

$$(\%)\ \text{Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Determination of particle size

The mean diameter of each formulation was estimated by dynamic light scattering (DLS) using particle size analyzer (Zetasizer 2000, Malvern Instruments Ltd., UK) at a settled scrambling point of 90ºCat 25ºC. [20-22]

Determination of entrapment efficiency and drug loading

The entrapment efficiency and drug loading of formulations were dictated by the centrifugation method (RemiElektroTechnik Ltd, Vasai, India). The formulations were separately centrifuged with a 10 ml volume of methanol at 5000 rpm for 10 min. The free

amount of the drug in the filtrate was determined by UV/Vis spectroscopy (Shimadzu-1800, Japan) at 283 nm. Estimations were performed in triplicate. The entrapment efficiency and drug loading were figured by the accompanying formula: [23-25]

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{amount of free drug}}{\text{Total amount of drug}} \times 100$$

$$\text{Drug loading (\%)} = \frac{\text{Weight of the entrapped drug}}{\text{Weight of the Formulation}} \times 100$$

Preparation of final batches of Phytosomes

The different molar ratio of the phytosome of the drug was prepared by the selected optimized working method as mentioned in Table 2.

Table No. 2: Final Batches of Phytosome of Drugs

Sr. No.	Norbixin Phytosomes	Molar ratios	
		Norbixin	Soyalecithin
1.	NOP1	0	1
2.	NOP2	1	1
3.	NOP3	1	2
4.	NOP4	2	1
5.	NOP5	2	2

#NOP1 (Blank phytosomes)

Evaluation of final batches of phytosomes:

The phytosomes of norbixin were characterized by physical appearance, FT-IR and DSC compatibility study and evaluated for % yield, % entrapment efficiency, % drug loading, particle size, zeta potential and *in vitro* drug release.

Physical Appearance

All the prepared phytosomes of norbixin were visually inspected for color, odor and physical state.[27]

FT-IR compatibility study

The prepared phytosome of norbixin were characterized using Shimadzu-8400S FT-IR Spectrophotometer (Tokyo, Japan) by KBr pellet method.[28]

Differential Scanning Calorimetry (DSC) study

The prepared phytosome of norbixin was characterized by Shimadzu DSC-60 Differential scanning calorimeter (Shimadzu DSC-60, Japan) using aluminum pans. [29]

Determination of % yield

Assurance of % yield of phytosomes of norbixin was calculated by the accompanying equation:

$$(\%)\ \text{Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Solubility study

The solubility study for the phytosome of the drug was determined in different solvents. [30-31]

Determination of entrapment efficiency and drug loading

The entrapment efficiency and drug loading of phytosomes of norbixin were detected by the centrifugation method (RemiElektroTechnik Ltd, Vasai, India). The entrapment efficiency and drug loading were figured by the accompanying formula:[32-35]

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{Amount of free drug} \times 100}{\text{Total amount of drug}}$$

$$\text{Drug loading (\%)} = \frac{\text{Weight of the entrapped drug} \times 100}{\text{Weight of the Formulation}}$$

Determination of particle size and zeta potential

The average diameter and surface charge property of phytosomes of norbixin were estimated by dynamic light scattering (DLS) using particle size analyzer (Zetasizer 2000, Malvern Instruments Ltd., UK).[36-38]

Scanning Electron microscope (SEM) study

Scanning electron microscopy (Hitachi Ltd., S-3400N type II model, Tokyo, Japan) was utilized to decide the surface morphology of the optimized phytosome of a drug. Dry samples of norbixin were separately set on an electron magnifying instrument metal stub and covered with gold in a particle sputter. Digital pictures of the formulation were taken by irregular examining of the stub at various magnifications.[39-41]

***In-vitro* drug release**

Based on the literature survey phosphate buffer pH 7.4 (900 ml) was utilized as a dissolution medium for 24 hours and maintained at 37 ± 0.5°C. The dissolution study was completed utilizing the dissolution apparatus (Electrolab TDT-08L, Mumbai) by the USP II paddle method at 50 rpm. The cotton paper pack was utilized to complete the *in-vitro* drug release. The cotton paper packs containing phytosomes of the drug were dipped into a jar containing medium and it was shut with cover to avoid vanishing of the dissolution medium. At predetermined time intervals, aliquots were withdrawn from the discharge medium and supplemented with a similar measure of phosphate buffer. The samples were assayed at respective wavelengths by UV spectrophotometer (Shimadzu-1800, Japan). The experiment was repeated three times for both the drugs and the values recorded as mean ± standard deviation (SD).[40]

Stability Study

In vitro stability study is a standout amongst the most basic variables of preparation and in ensuring the safety and adequacy of the product. Optimized phytosome and phytosome loaded complex were packed individually in glass vials with nitrogen purging and sealed by rubber stoppers and crimped using aluminum seals. The samples were divided into three sets for the stability study and stored at:

- In refrigerator (5 ± 3℃) for 3 months
- In humidity control chambers
  - 25 ± 2℃/60 ± 5 % RH for 3 months
  - 40 ± 2℃/75 ± 5% RH for 3 months

Samples were withdrawn on 0, 1, 2 and 3 months and evaluated for changes in physical appearance, % entrapment efficiency, % drug-loading and average particle size as per ICH Q1A (R2) guidelines.

RESULTS AND DISCUSSION

Pre-formulation Studies:

Organoleptic properties:

The organoleptic properties of norbixin were characterized by color, odor and appearance and the results were shown in Table 3.

Table No. 3: Organoleptic properties of Norbixin

Drug	Color	Odor	Melting point
Norbixin	Yellowish to orange	Bitter	196°C

FT-IR spectroscopy:

FT-IR spectrum of drug samples showed all the characteristic peaks as reported in the literature indicating the presence of functional groups of Norbixin.

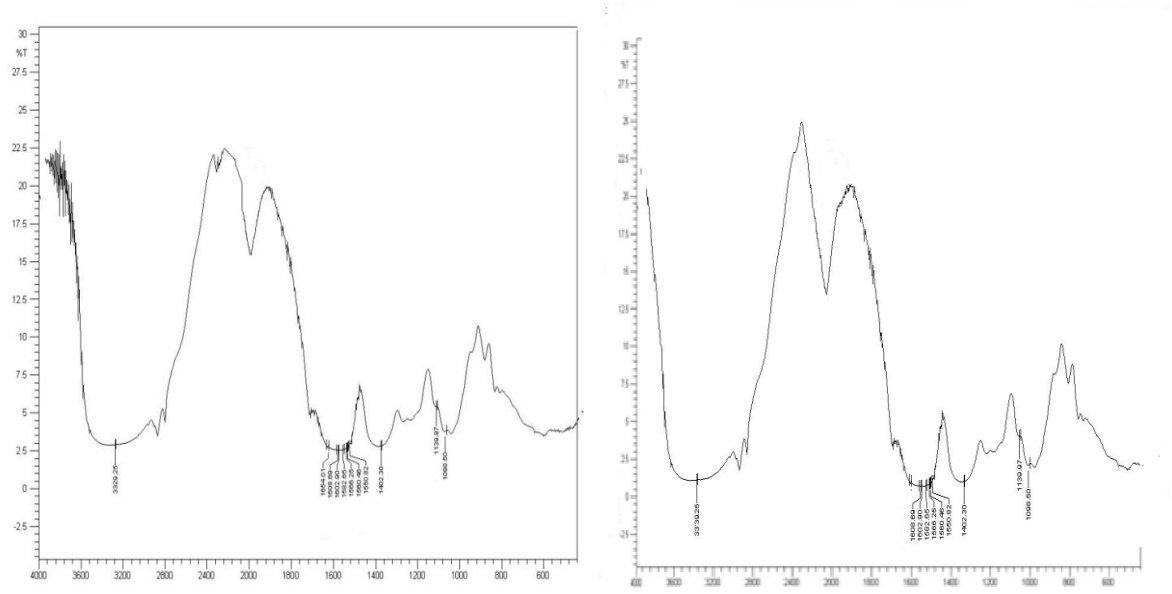

Figure No. 1: FTIR spectra of Norbixin and physical mixture

The FT-IR spectrum of norbixin and its physical mixture with soya lecithin was determined. The functional group with corresponding peaks of norbixin and in its physical mixture of norbixin& soya lecithin was found to be correlative (Figure 1). The prominent peaks of norbixin at 3338.25 cm^{-1} due to O-H stretching while peaks at 1608.69 cm^{-1} correspond to the Carbonyl group and strong absorption peaks were observed at 1620.90 and 1582.65 cm^{-1} corresponds to C=C aromatic groups were found in the FT – IR spectra of the physical mixture. As evident from the results, there was no interaction between norbixin and selected soya lecithin as prominent peaks were present in the physical mixture. Hence, norbixin and selected phospholipids were compatible with each other. Therefore, from the FT-IR study, it can be concluded that norbixin and selected phospholipids are compatible with each other.

Differential scanning calorimetry studies

Differential scanning calorimetry studies of norbixin and its physical mixture with soya lecithin were determined using DSC 60. The DSC thermogram of norbixin showed a sharp melting endotherm at a temperature of 211.69°C as presented in Figure 2.

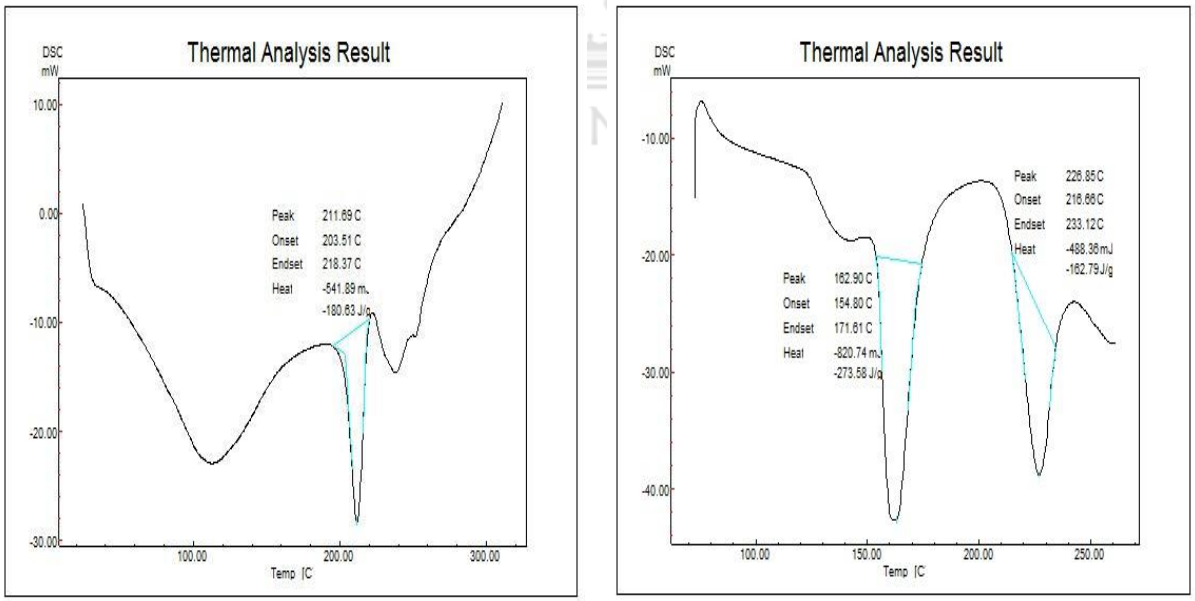

Figure No. 2: DSC Thermogram of Norbixin and physical mixture

DSC study shows that the characteristic peaks of norbixin were present in the physical mixture and hence no interactions between norbixin and selected phospholipid were seen.

Determination of solubility

The phytosome consist of drug and phospholipid. The selection of the right solvent for the preparation of the phytosome is mainly based on the solubility and compatibility of the drug with phospholipid in the respective solvents. The solubility of norbixin was determined in different solvents such as acidic pH 1.2 HCl, phosphate buffer pH 6.8 & pH 7.4, organic solvents acetone, acetonitrile (ACN), dimethyl sulfoxide (DMSO), dichloromethane (DCM), ethanol (EtOH), methanol (MeOH) and distilled water (DW). The solubility analysis of norbixin in different solvents is presented in Table 4. Except for distilled water, all other solvents showed good solubility profile of Norbixin.

Table No. 4: Solubility analysis of Norbixin

Sr. No.	Solvents	Concentration (mg/mL)
1.	pH 7.4	4.05 ± 0.08
2.	Acetonitrile	0.73 ± 0.05
3.	Methanol	9.08 ± 0.17
4.	Ethanol	7.53 ± 0.02
5.	DCM	2.1 ± 0.07
6.	Water	0.23 ± 0.05
7.	DMSO	1.43 ± 0.19
8.	Ethyl acetate	2.52 ± 0.10

Mean ± SD, n = 3

UV Spectroscopy studies

The wavelength of maximum absorption (λmax) of norbixininin phosphate buffer pH 7.4 and methanol are shown in Table 5.

Table No. 5: Maximum absorbance wavelength (λmax) of norbixin in

Solvents	Wavelength of maximum absorption (λmax) (nm)	
	Observed	Reported
Phosphate buffer pH 7.4	264	270
Methanol	283	285

The calibration curve of norbixin was prepared in phosphate buffer pH 7.4 and methanol is depicted in Table 6&7 and Figures 3 & 4, respectively. The regression coefficient (R2) of norbixin was found to be 0.9948 in phosphate buffer pH 7.4 and 0.9977 in methanol, respectively. The results indicate a linear relationship between concentration and absorbance in the range of 30-210 µg/ml & 20-140 µg/ml of norbixin in phosphate buffer pH 7.4 & in methanol, respectively).

Table No. 6: Calibration curve data of Norbixin in pH 7.4 Phosphate buffer

Concentration of (µg/mL)	Absorbance at 264 nm
30	0.1767 ± 0.03
60	0.2386 ± 0.04
90	0.3095 ± 0.01
120	0.3689 ± 0.09
150	0.4473 ± 0.05
180	0.5383 ± 0.03
210	0.6209 ± 0.08

Mean ± SD, n = 3

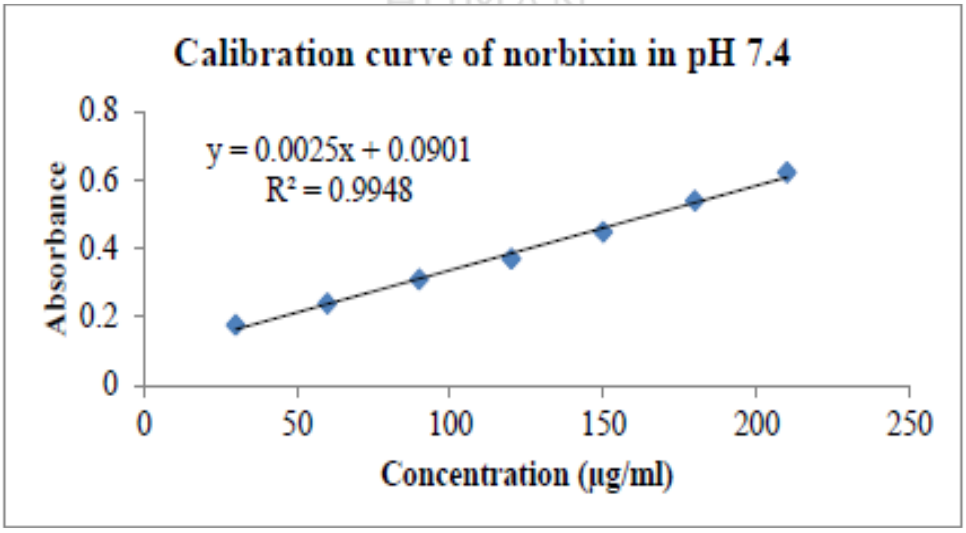

Figure No. 3: Calibration curve of norbixin in pH 7.4 Phosphate buffer

Table No. 7: Calibration curve data of Norbixin in methanol

Concentration (µg/ml)	Absorbance at 283 nm
20	0.1981± 0.06
40	0.2618 ± 0.04
60	0.3282 ± 0.01
80	0.3938 ± 0.02
100	0.4635 ± 0.05
120	0.5313 ± 0.03
140	0.6201 ± 0.07

Mean ± SD, n = 3

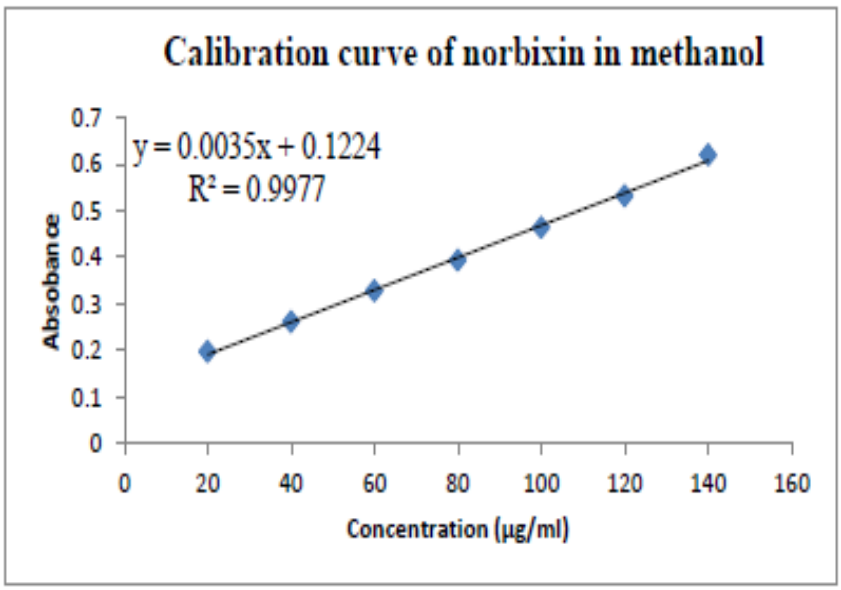

Figure No. 4: Calibration curve of Norbixin in methanol

Formulation and Development:

Selection of plant drug

The plant Phyto-constituents carotenoid drug norbixin was selected as an antibacterial drug for the preparation of phytosome.

Selection of phospholipids

Soya lecithin was selected as a phospholipid for the preparation of phytosome based on compatibility and interaction with the drug.

Selection of method

Preparation and Evaluation of preliminary batches for selection of working method

The preliminary batch of the phytosome of norbixin was prepared by different methods as mentioned in Table 1. The quantitative results indicate that the phytosome of norbixin obtained from various methods shown in Table 8. It was found that the anti-solvent precipitation method showed 83.96±0.02 % yield, 565.19±0.48 nm particle size, 87.05±1.20 % entrapment efficiency and 15.66±0.30 % drug loading as compared to 83.88±1.01 % yield, 663.06±0.05 nm particle size, 71.28±0.03 % entrapment efficiency, 14.12±0.70 % drug loading by solvent evaporation, 76.75±0.05 % yield, 779.89±0.31 nm particle size, 84.62±0.01 % entrapment efficiency, 10.88±0.21 % drug loading by rotary evaporation technique, respectively.

Table No. 8: Evaluation of Preliminary Batches of NorbixinPhytosome

Sr. No.	Methods	Evaluation Parameters			
		Yield (%)	Particle size (nm)	Entrapment efficiency (%)	Drug loading (%)
1.	AP	83.96±0.02	565.19±0.48	87.05±1.20	15.66±0.30
2.	SE	83.88±1.01	663.06±0.05	71.28±0.03	14.12±0.70
3.	RE	76.75±0.05	779.89±0.31	84.62±0.01	10.88±0.21

Mean ± SD, n = 3

The phytosome of norbixin by anti-solvent precipitation method showed better % yield, particle size, % entrapment efficiency and % drug loading as compared to other methods. The microscopic view of the phytosome of norbixin indicated the presence of sphere-shaped vesicles. Thus, the anti-solvent precipitation method was selected to prepare the final batches of the phytosome of norbixin.

Preparation of final batches of Phytosome

The different molar ratio of the phytosome of norbixin was prepared by the anti-solvent precipitation method, mentioned in Table 9.

Table No. 9: Final Batches of NorbixinPhytosome

Sl. No.	Phytosomes	Molar ratios	
		Norbixin	Soya lecithin
1.	NOP1	0	1
2.	NOP2	1	1
3.	NOP3	1	2
4.	NOP4	2	1
5.	NOP5	2	2

 # NOP1 (Blank phytosome)

Evaluation of final batches of Phytosome:

All the prepared phytosome of norbixin were characterized by FT-IR, DSC and evaluated for % yield, entrapment efficiency, drug loading, particle size, zeta potential and *in vitro* drug release, etc.

Physical Appearance

The physical appearance of the phytosomes of norbixin was found to be yellowish to orange color powder and bitter odor.

Percentage Yield

The evaluation results of the phytosome (NOP1 – NOP5) of norbixin shown in Table 10. NOP1 and NOP3 showed the lowest 37.31±0.04 % and the highest 85.35±0.02% yield, respectively.

Entrapment Efficiency and Drug Loading

The entrapment efficiency and drug loading of phytosome of norbixin vary from 60.12±0.07 to 84.36±0.42 % and 00.92±0.06 to 11.80±0.10 %, respectively as shown in Table 10. It was

found that the phytosome of norbixin (NOP4) showed the highest 84.36±0.42 % entrapment efficiency and 08.05±0.03 % drug loading due to the proper bounding of norbixin with the polar head of soya lecithin as compare to the others.

Particle Size and Zeta Potential

The particle size varies from 193.67±0.04 nm to 490.19±0.10 nm as shown in Table 10 and Figure 5 (blank) and Figure 6. It was found that the phytosome of norbixin (NOP4) showed the lowest 431.21±0.90 nm particle size due to the availability of numbers of norbixin molecule and combined interaction with soya lecithin as compare to the others, indicating uniformity in the particle size distribution. The zeta potential of the phytosome of norbixin (NOP4) was found to be -13.11 mV. These results suggest that the selected ratio of soya lecithin favored the formation of a phytosome, resulting in the formation of a uniformly distributed nanosizedphytosome.

Table No. 10: Evaluation of Final Batches of Phytosome of Norbixin

Sr. No.	Phytosome Formulations	Evaluation Parameters			
		Yield (%)	Entrapment efficiency (%)	Drug loading (%)	Particle size (nm)
1	NOP1	37.31±0.04	00.00±0.00	00.00±0.00	193.67±0.04
2	NOP2	46.69±0.09	60.12±0.07	00.92±0.06	490.19±0.10
3	NOP3	85.35±0.02	82.60±0.04	11.80±0.10	436.01±0.07
4	NOP4	73.24±0.06	84.36±0.42	08.05±0.03	431.21±0.90
5	NOP5	67.52±0.01	79.27±0.01	06.01±0.05	437.33±0.02

Mean ± SD, n = 3

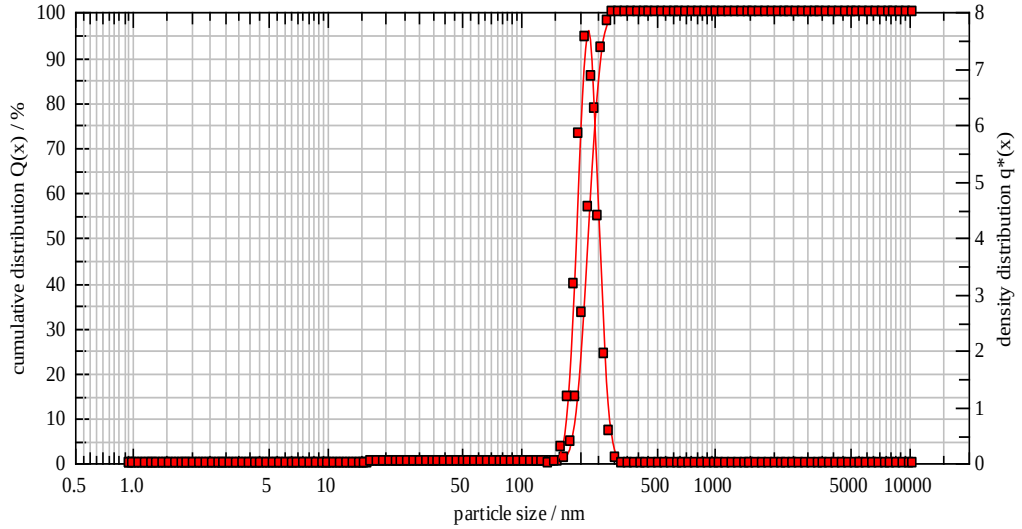

Figure No. 5: Particle size of blank phytosome of norbixin (NOP1)

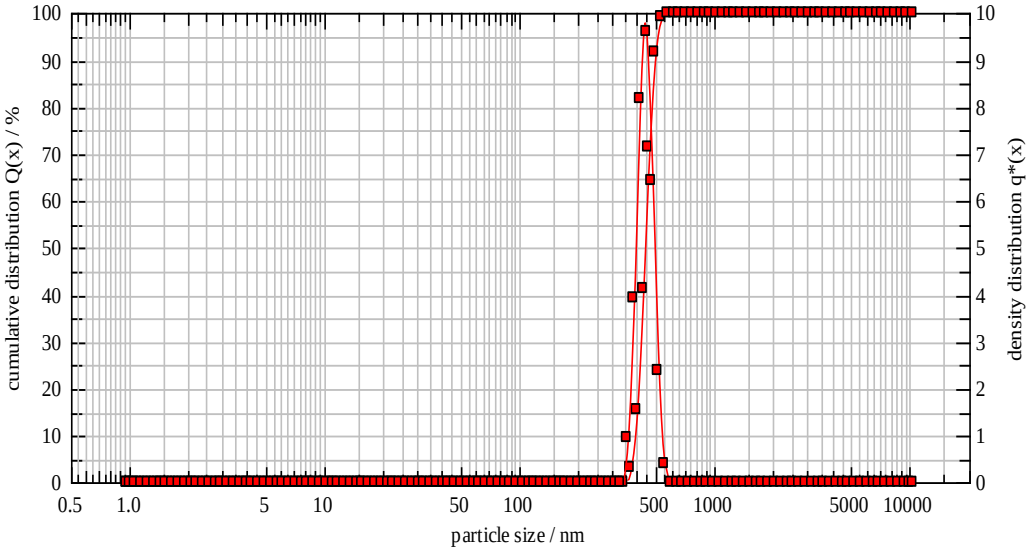

Figure No. 6: Particle size of Optimized Norbixinphytosome (NOP4)

FT-IR study

The optimized phytosome of norbixin NOP4 was characterized by the FT-IR spectrum as shown in Figure 7. The FT-IR spectrum of optimized phytosome NOP4 revealed the presence of a peak at 3333.50cm^{-1} due to O-H stretching while peaks at 1608.50 cm^{-1} correspond to the Carbonyl group. Strong absorption peaks were observed at 1602.30 and 1560.46 cm^{-1} corresponds to C=C aromatic groups. In the FT-IR spectrum of the optimized phytosome NOP4, the major peak position of functional groups of norbixin and soya lecithin doesn't affect or changed.

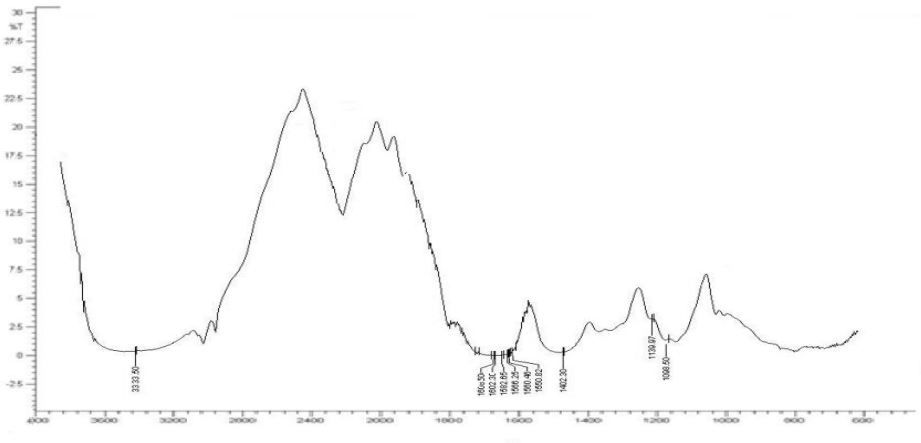

Figure No. 7: IR spectrum of Optimized Norbixinphytosome (NOP4)

Differential Scanning Calorimetry (DSC) study

DSC thermogram compatibility study of optimized phytosome NOP4 is shown in Figure 16. The DSC thermogram of norbixin and optimized phytosome NOP4 showed a sharp melting endotherm at temperature 195.28°C. The almost same melting was also observed for Norbixin indicating the absence of norbixin and soya lecithin interactions. Thus, it was concluded that the norbixin and soya lecithin did not interact with each other and optimized phytosome NOP4 was compatible.

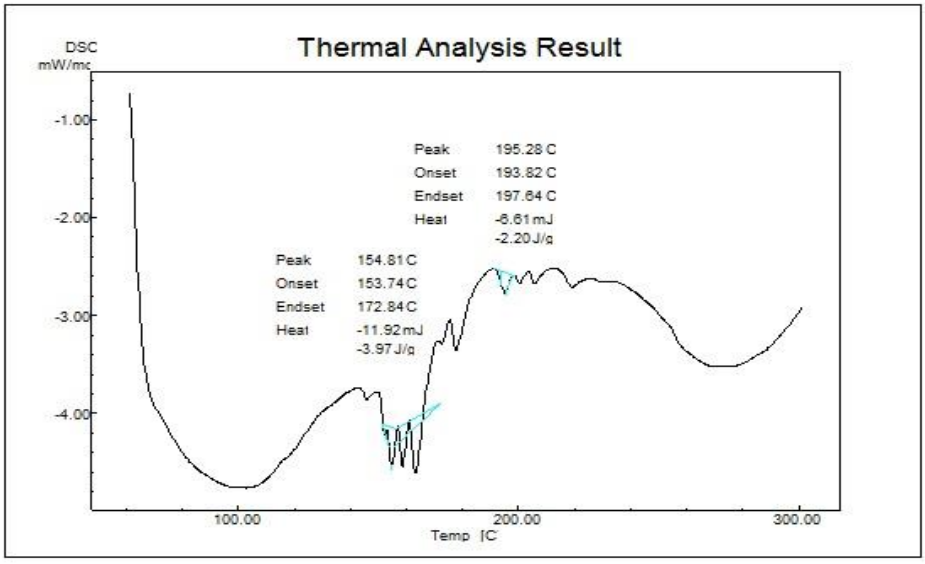

Figure No. 8: DSC thermogram of Optimized Norbixinphytosome (NOP4)

Solubility Study

The solubility comparison of norbixin and optimized phytosome NOP4 was observed in 0.1 N HCl (pH 1.2), pH 6.8 & pH 7.4 phosphate buffer, acetone, acetonitrile, methanol, ethanol, dichloromethane, distilled water, chloroform, and dimethyl sulfoxide are presented in Table 11. From the solubility comparison results, it was found that the phytosome of norbixin NOP4 was showing better solubility profile (Figure 9) in all solvents as compared to the norbixin due to the wettability and dispersion properties of soya lecithin.

Table No. 11: Solubility Comparison of Norbixin with Norbixinphytosome (NOP4)

Sr. No.	Solvents	Solubility Concentration (mg/mL)	
		Norbixin	NOP4
1.	pH 7.4	4.05 ± 0.08	5.21± 0.16
2.	Acetonitrile	0.73 ± 0.05	1.2 ± 0.04
3.	Methanol	9.08 ± 0.17	9.91± 0.23
4.	Ethanol	7.53 ± 0.02	8.07± 0.06
5.	DCM	2.1 ± 0.07	2.4± 0.90
6.	Water	0.23 ± 0.05	1.47± 0.00
7.	DMSO	1.43 ± 0.19	2.35± 0.11
8.	Ethyl acetate	2.52 ± 0.10	3.84± 0.02

Mean ± SD, n = 3

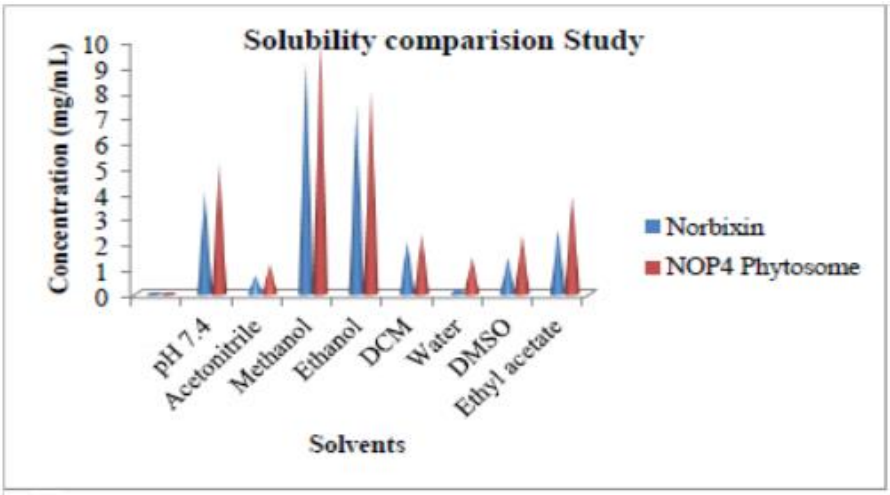

Figure No. 9: Solubility Comparison of Norbixin with Norbixinphytosome (NOP4)

Scanning Electron Microscope (SEM)

The surface morphology, shape and structure of the optimized phytosome of norbixin (NOP4) at various magnifications are shown in Figure 10, by scanning electron microscope (SEM). It was observed that the norbixin particles are associated with the polar head of soya lecithin that is forming phytosome NOP4 with irregular particle shapes, spherical and crystalline structures.

***In-vitro* drug release**

The *in vitro* cumulative drug release of phytosome of norbixin (NOP1-NOP5) given in Table 12. It showed that the highest 86.03±0.06 % cumulative drug release of NOP4 at the end of 24 h. NOP2, NOP3, and NOP5 showed 78.29±0.05, 82.23±0.01 and 79.01±0.02, respectively at the end of 24 hours.

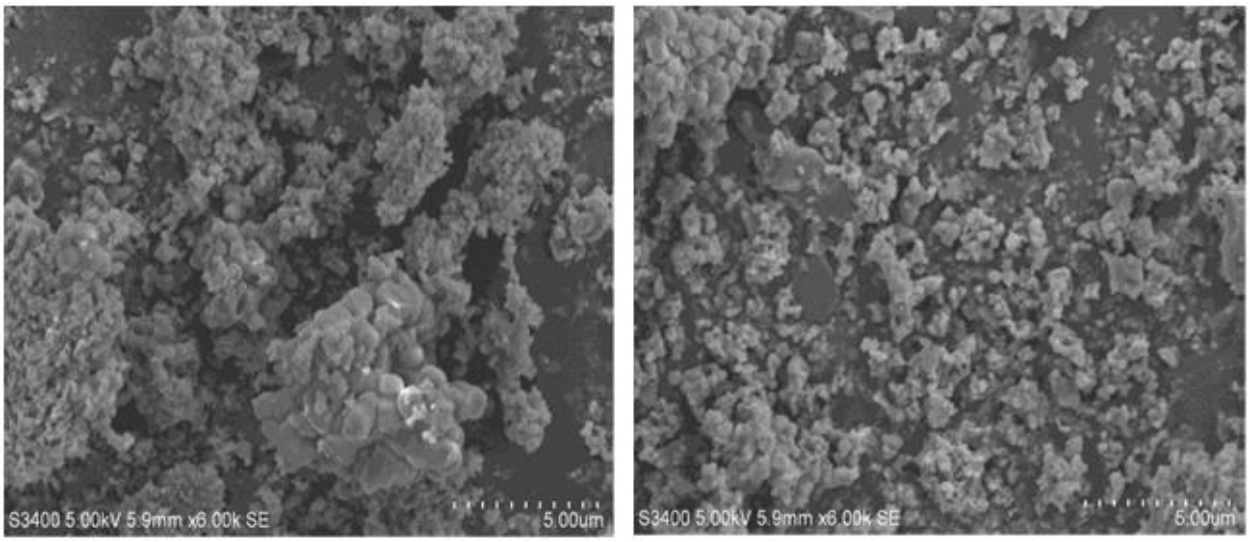

Figure No. 10: Surface morphology of optimized phytosome of norbixin (NOP4)

Table No. 12: Cumulative percentage drug release of phytosome of Norbixin

Time (h)	NOP1	NOP2	NOP3	NOP4	NOP5
0.5	0.00±0.00	03.40±0.10	03.81±0.04	05.20±0.21	01.92±0.05
1	0.00±0.00	13.01±0.05	15.06±0.01	14.97±0.03	12.74±0.25
2	0.00±0.00	21.40±0.02	22.35±0.26	23.35±0.01	19.09±0.12
3	0.00±0.00	34.55±0.06	32.09±0.05	34.09±0.14	32.66±0.60
4	0.00±0.00	46.07±0.35	47.01±0.01	50.22±0.31	41.02±0.04
5	0.00±0.00	54.50±0.23	59.17±0.62	64.38±0.07	52.67±0.02
6	0.00±0.00	67.02±0.03	72.66±0.03	76.07±0.90	65.12±0.45
8	0.00±0.00	74.04±0.08	79.45±0.08	82.95±0.21	71.03±0.61
12	0.00±0.00	80.63±0.70	84.70±0.29	87.21±0.09	79.94±0.08
24	0.00±0.00	78.29±0.05	82.23±0.01	86.03±0.06	79.01±0.02

Mean ± SD, n = 3

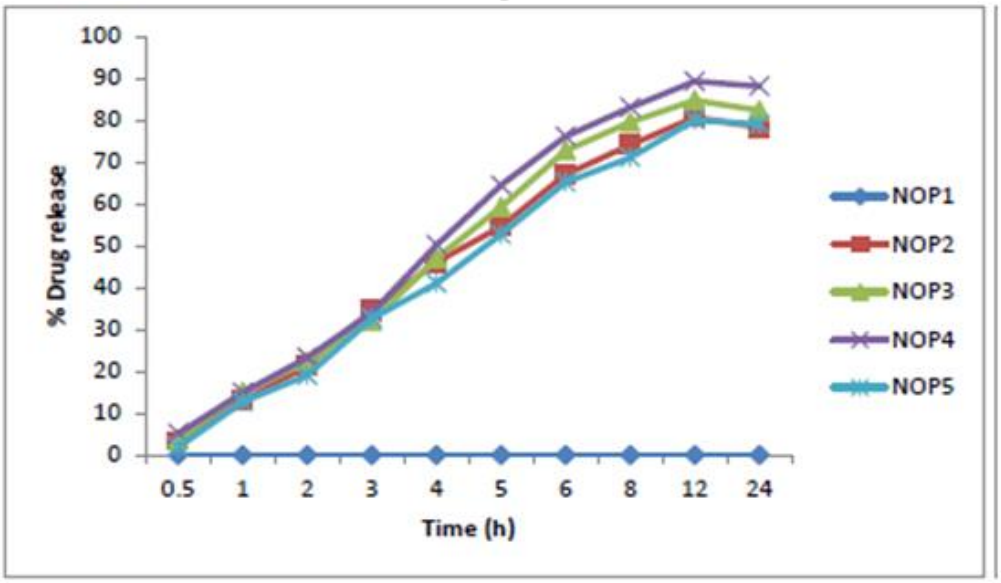

Figure No. 11: *In-vitro* cumulative drug release of phytosome of Norbixin

Stability studies

The results of the stability study of the optimized phytosome of Norbixin after 0 month, 1 month, 2 months and 3 months at different storage conditions are shown in Table 13 and are illustrated in Figure 12. At predetermined intervals (0 month, 1 month, 2 months and 3 months), there was an insignificant increase in mean PS (particle size) of optimized

phytosome of Norbixin that did not much affect % EE (Entrapment Efficiency) & % DL (Drug Loading) during the study period, indicating good stability of the optimized phytosomes. These results confirmed the compatibility of Norbixin with the polar groups of soya lecithin.

Table No. 13: Stability study data of phytosome of Norbixin

Time storage (month)	At 5±3˚C			At 25 ± 2˚C/60 ± 5 % RH			At 40 ± 2˚C/75 ± 5 % RH		
	% EE	%DL	PS (nm)	% EE	%DL	PS (nm)	% EE	%DL	PS (nm)
0	84.36± 0.42	08.05± 0.03	431.21 ±0.90	84.36± 0.42	08.05± 0.03	431.21 ±3.90	84.36± 0.42	08.05± 0.03	431.21 ±3.90
1	84.12± 0.24	07.79± 0.34	419.23 ±2.20	85.36± 0.33	08.27± 0.56	442.34 ±4.90	83.21± 0.11	07.99± 0.12	451.32 ±3.70
2	85.45± 0.54	07.75± 0.23	451.29 ±2.60	85.78± 0.47	08.24± 0.13	451.71 ±4.46	87.34± 0.48	08.26± 0.27	481.89 ±4.82
3	85.75± 0.85	07.65± 0.89	462.27 ±3.65	85.63± 0.51	08.56± 0.58	452.67 ±4.25	88.69± 0.86	08.34± 0.23	481.26 ±2.34

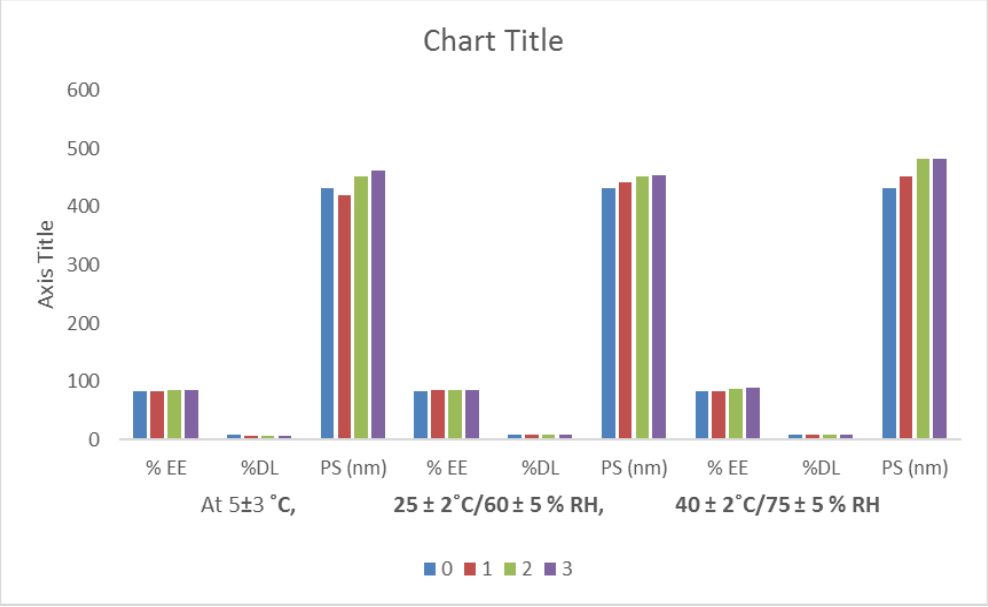

Figure No. 12: Stability study of phytosome of Norbixin

CONCLUSION

The present research work was directed towards the formulation of phytosome of plant drug, which would increase bioavailability and solubility of phytoconstituents through various mechanisms, improve the stability and also showed sustained drug release for 24 h.

Plant phytoconstituents, phospholipids soya lecithin, and their molar ratio and selection of method play a vital role in the formulation of phytosome. Based on trial batches, an anti-solvent precipitation technique was selected for the formulation of phytosome. Through anti-solvent precipitation technique, phytosomes in a different molar ratio such as 0:1, 1:1, 1:2, 2:1 and 2:2 were prepared for further studies.

Infra-red (IR) studies and Differential Scanning Calorimetry (DSC) revealed that there was no interaction between the phytoconstituents and phospholipids. The entrapment efficiency and drug loading rate of prepared phytosome of drug confirmed the effective loading of drug and also sustained delivery of drug at the specific target site. The particle size, zeta potential assured the nanoparticle size of prepared phytosome and confirmed the formation of phytosome within range. In vitro drug release showed the release rate of phytoconstituents through the phytosome formulation.

Based on evaluation parameters 2:1 molar ratio of phytosome formulation of the drug was selected for further evaluation like Scanning electronic microscopes that confirm that the actual shape and size of the prepared phytosome of a drug. Thus from the above observations, it can be concluded that the phytosome could be helpful for the treatment of various disorders and also to deliver the other plant phytoconstituents.

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