Mucuna Pruriens Seeds Extract Loaded Phytosomes for The Effective Treatment of Parkinson's Disease

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KEYWORDS

Phytosome, *Mucuna pruriens*, factorial design, Parkinson's disease.

ABSTRACT

One phytoconstituent derived from Mucuna pruriens (legumes) is levodopa. This medication's oral usage is limited by its high first-pass metabolism and low absorption. The goal of the present research was to develop a optimized Phytosomal drug delivery system with Mucuna pruriens seeds extract for better delivery. Various techniques, including solvent evaporation, salting out anti-solvent precipitation, direct egg yolk, and egg lipids methods, were used to create phytosomal formulations. Scanning electron microscopy, particle size, x-ray diffraction, and other techniques were used to characterize phytosomes. The most effective Phytosomes were those made via the antisolvent precipitation approach. In this investigation, a 3^2-randomized complete factorial design was employed. Batch F4 had an entrapment efficiency of 70%, a particle size of 15 (µg) and 60% CDR. For improving Parkinson's disease treatment, the optimized phytosomal formulation delivered via the nasal route would be the ideal option.

1. BACKGROUND

Drugs of origin from nature are significant and vital components of contemporary medicine. as they are occurring in nature, herbal remedies have fewer adverse consequences, which is why many traditional medical practitioners and scientists utilize them to treat conditions including diabetes, cancer, rheumatoid arthritis, inflammation, and several more (Haidan Yuan et al 2016; Henry Ivanz A. Boy et al 2018). These products are made to provide specific medical advantages using different ways to deliver drugs (DDS). According to reports, these unique drugs delivery methods provide remarkable benefits over traditional formulations of plant extracts or active ingredients. Improvements in solubility, bioavailability, toxicity, pharmacological activity, stability, tissue distribution, prolonged administration, and protection against physical/chemical deterioration are some of the factors that may (Sarangi et al 2018).

1.1 Phytosome for herbal drug delivery;

The reaction between phosphatidylcholine and phospholipids results in lipid-compatible molecular complexes known as phytosome, which are complexes of phospholipids and naturally occurring potent phytochemicals that are bound in their structures (Arsul V.A. et al 2014; Mahmood Barani, et al 2021). It mostly consists of the lipid-bound, bioactive phytoconstituents of the herb. Polar or water-soluble substances constitute most organisms' physiologically active components. However, water-soluble phytoconstituents (such as flavonoids, tannins, glycosidic aglycones, etc.) have poor absorption because of their large molecular size, which hinders passive diffusion, or because they are poorly soluble in lipids, which greatly limits their ability to cross lipid-rich biological membranes, resulting poor bioavailability (Joseph A. Kareparamban et al 2012; Jagruti Patel et al 2009, Bhupen Kalita1 et al 2014, Mahmood Barani et al 2021).

1.2 **Optimization:**

Statistical optimization enables a pharmaceutical scientist to define a formulation with optimum characteristics. A large amount of data can be generated from a limited no of experiments, which facilitate an in-depth understanding of the formulation and its manufacturing process. Statistical optimization can also provide solution to large scale manufacturing problems, which occasionally arise. Importantly statistical optimization experimentation and analysis provide strong assurance to regulatory agencies regarding superior product quality; statistical optimization allows formulator to study a wide range of independent and dependent variables. Independent variables include formulation issue such as granulating solvent/ lubricant/ polymer concentration.etc. Dependent variables i.e. response that can be measured, include tablet dissolution/ disintegration/ hardness/ friability etc.

There are many other advantages to use of statistical method; it is strongly favored by regulatory agencies as it.

- It justifies the choice of ranges and finds a robust (optimum) region.
- It gives researcher the ability to study interaction between factors, in contrast merely studying one factor at a time does not allow the researcher to study interactions and is not scalable to production
- The statistical design method often provides a more economic use of resources, especially when many factors exist and provide a greater chance of finding conditions.

Finally, predictions can be made about future experiments

Optimization techniques

There are several types of statistical design for pharmaceutical formulation:

1. Factorial Designs: (both full and fractional factorials),
2. Sequential Simplex Techniques,
3. Response Surface Methodology,
4. D-Optimal Techniques and
5. L-Optimal Techniques.

Optimization using Factorial design (Dhaneshwar Vishwakarma et al 2022)

Factorial designs are used in the experimental where the effects of different factors, or conditions, on experimental result are to be elucidated. Factorial designs are a design of choice for simultaneous determination of affects several factors and their interactions.

Factors – A factors is an assigned variable such as concentration, temperature, and formulation ingredient or drug treatment. The choice of factors to be included in an experimental dependent on experimental objectives and is predetermined by the experimenter. A factor can be qualitative or quantitative. Qualitative factors are assigned names rather than numbers.

Level- The levels of factor are the values or designation assigned to the factor.
Effect- The effect of a factor is the change in the response caused by varying the level of the factor.
Interaction- Interaction may be in the thought of as a lack of "additivity of factor effects".
In factorial designs, level of factors are independently varied, each factors at two or more levels. The effects that can be attributed to their factors and their interactions are assessed with maximum efficacy in factorial designs. Also, factorial design allows for estimation of effects of each factors and interaction, uncompounded by other experimental factors (Mr. Siddhant Shelke et al 2021)

1.3. Parkinson's disease and its treatment:

Parkinson's disease is a brain disorder that causes unintended or uncontrollable movements, such as shaking, stiffness, and difficulty with balance and coordination. Symptoms usually begin gradually and worsen over time. As the disease progresses, people may have difficulty walking and talking. They may also have mental and behavioral changes, sleep problems, depression, memory difficulties, and fatigue.
Mucuna pruriens is a species of bean that grows in the tropics. It is very rich in natural L-Dopa. Mucuna seed extract has been an effective treatment of Parkinson's disease (PD) in many patients (Chayarit Vilairat et al 2023). Mucuna seeds are known to produce the unusual nonprotein amino acid 3-(3,4 dihydroxyl phenyl)-l-alanine (l-Dopa), a potent neurotransmitter precursor that is, at least in part, believed to be responsible for the toxicity of Mucuna seeds (Cilia et al. 2017). One of the causes of neurodegeneration is a disturbed balance between reactive species of oxygen and cellular activity showing an antioxidation effect (Bingjing Zheng et al 2020). It includes Alzheimer's disease (AD) and Parkinson's disease (PD). In Parkinson's disease, there is a loss of dopaminergic neurons in the part of the brain known as substantia nigra with the formation of Lewy bodies.

2. Need of present investigation and plan of the work

Macuna pruriens seeds have been reported to contain about 8-9% L-Dopa which is known to be active for the treatment of neurodegenerative disease particularly by loss of dopaminergic neurons. Therefore, a systematic probe is necessary to evaluate the synergistic effect of the active moiety present in the herbs.
In the present investigation, we have attempted to prepare the whole extract at a suitable pH ranging from 3-4 in the aqueous solvent system while the organic extracts are attempted in a series of solvents called n-Hexane in various proportions. Thus, one fraction being polar and another fraction nonpolar was a serious challenge for providing a suitable interface for its release and absorption. On the other side for neurodegenerative diseases, the site of action is located somewhere in the brain perhaps beyond Blood Brain Barrier (BBB) (Emily G. et al 2022). Similarly, nasal sites of administration L-Dopa extract can be considered.
To resolve the above complexity suitable nanocarrier system was designed for L-Dopa as the active moiety has been derived from plant material. Phytosome seems to be the appropriate option as a carrier for drug moieties. After having optimized and designed phytosome for the said active moieties the site of administration connected to the brain/nearest to the brain can be opted to be nasal mucosa.

3. EXPERIMENTAL

3.1 Authentication of *Plant*

The plant specimen used for the research work was collected from the forest of Kusgaon Bk. Lonavala, Pune, Maharashtra, India. The plant specimen was authenticated by the Botany Department at Agharkar Research Institute, Pune, India.

Pharmacognostic Characterization

Pharmacognostic studies of the plant were conducted by the standard prescribed method, which includes macroscopic, microscopic, and other characteristic properties.

3.2 Preparation of the Extract

L-Dopa extract

Different methods and solvent systems were used for the extraction of L-Dopa from Mucuna Pruriens seed powder. The pH of the solvent blend played a significant role.

a) Seed powder was defatted with acetone and then suspended in water: ethanol (1:1) for 3 days (Chayarit Vilairat et al 2023) at room temperature. Then maceration technique was used for extraction. The extract was passed through a suitable filter medium.

b) A significant amount of seed powder was mixed with a stipulated volume of acidified water and followed by ultrasonication at 250 rpm at room temperature. The extract was passed through a suitable filter medium (Narisa Kamkaen et al 2022). The yield obtained was around 5-6 % w/v.

3.3 Preliminary Phytochemical analysis of the extract of Levodopa:

The extracts were concentrated and subjected to phytochemical screening using standard procedures. Alkaloids, glycosides, flavonoids, tannins, proteins, carbohydrates, amino acids, and steroids are the preliminary phytoconstituents that were analyzed.

3.4 Standardization of Extract: L-Dopa

3.4.1 Thin Layer Chromatography TLC (Ratna Wulandari et al 2018)

The presence of L-DOPA in the test samples was confirmed by comparing the TLC pattern of testing seeds and the standard L-DOPA using an optimized solvent system; butanol: acetic acid: water (4:1:1). After elution with appropriate solvent, the TLC plates were air dried for 10 min, sprayed with a 0.1% solution of ninhydrin in methanol, and dried at 105 °C for 5 min. The *Rf* values and brownish-pink spots revealed the presence of L-Dopa

3.4.2 High-Performance Liquid Chromatography (HPLC) (Mrs. Carmen Tesoro et al 2022; Bruno Fonseca-Santos et al 2023),

HPLC conditions:

Column: Lichro CART Purosphere STAR, 4.6 x 250 mm, Rp 18e, 5µm

Mobile Phase:

Solvent A: consisted of a 30 mM phosphate buffer pH 2.50 (20 mM sodium dihydrogen phosphate dihydrate and 10 mM disodium hydrogen phosphate dihydrate). The pH of the solvent was adjusted with orthophosphoric acid.

Solvent B: was composed of a mixture of water that before mixing had been adjusted to pH 2.50 with orthophosphoric acid and acetonitrile (50:50, v/v). Gradient Elution: HPLC analyses were carried out by applying the gradient conditions

3.4.3 High-performance thin layer chromatography (HPTLC) (Archana P. Raina et al 2011; Sharad Kharat et al 2017)

The HPTLC plates were developed in a saturated twin trough chamber using a mobile solvent phase of n-butanol: glacial acetic acid: water (4:1:1, v/v) for 30 min up to 8 cm and then air dried. After chromatographic development, the peak areas of the individual bands were measured at 280 nm UV using a Camag TLC Scanner 3 integrated with WinCATS software at a slit width of 5 × 0.45 mm. The analysis was done in duplicate and the average was taken for L-dopa estimation. A stock solution of L-dopa standard (0.1 µg/µL) was used in different concentrations (100 ng/spot - 1000 ng/spot) for preparing a calibration graph of peak area versus concentration

3.4.4 Identification of Levodopa in the extracted sample by Fourier Transformed Infrared Spectroscopy (FTIR)

To adapt the conventional KBr disc sampling technique for quantitative determination, the thickness of the KBr disc must be kept constant (to obtain the same path length) for all the analyzed samples. The desired drug quantity was obtained from the 2.0 g% (w/w) drug/KBr mixtures and ground in agate mortar for 2 min, completed to 0.15 g with KBr, then ground again and pressed under 15,000 lbs by the hydraulic pressure system in the die-press for 3

min. The FTIR of the sample was compared with the FTIR of the standard compound.

3.5 Preparation of Phytosome (Gaikwad Abhijeet R et al 2021)

The phytosome were prepared for L-dopa extract by following methods

a) Solvent evaporation method (Sanjay Saha et al 2013, Vishal Gaurav et al 2021)

Different molar concentrations of extract and Phosphatidylcholine (1:0.5, 1:1, and 1:2) were taken in the beaker. 50 ml methanol was added. Then the mixture was taken in round bottom flask and evaporated in rotary vacuum at 60^0 C. prepared phospholipids complex washed with N- Hexane with continuous stirring. The Extract–phospholipids complex was precipitated and the precipitate was filtered and dried under vacuum to remove traces of solvents. The resultant phospholipids complex was placed in an amber-colored bottle stored at room temperature.

b) Salting out anti-solvent precipitation method (Vishal Gaurav et al 2021)

Different molar concentrations of extract and Phosphatidylcholine (1:0.5, 1:1, and 1:2) were taken in the beaker with 20 ml methanol refluxed at 60^0 C temperatures for 02 Hrs on a magnetic stirrer at 100 rpm. The solution was later concentrated and anti-solvent like n-hexane was added. The resultant phospholipids complex was placed in an amber-coloured bottle stored at room temperature.

c) Direct egg yolk method

The egg yolk was separated from the egg. The aqueous extract was heated and 50 ml of which was added to 20 ml of egg yolk with homogenization at 250 rpm for not less than 20 min. The Extract–phospholipids complex was precipitated and the precipitate was filtered and dried.

d) Egg Lipid method:

20 ml of egg yolk was added into 50 ml of ethanol and 30 ml of ethyl ether. The mixture was stirred continuously. Then 10 ml of extract was added into it with homogenization for 20 min. The Extract–phospholipids complex was precipitated and the precipitate was filtered and dried.

3.6 Optimization of the method used for the preparation of phytosome

Phytosomes thus prepared for L-dopa were characterized as follows:

a) Scanning Electron Microscopy (SEM)

The surface morphology of the Phytosome was investigated by scanning electron microscopy. The study provided a better understanding of the morphological characteristics of the Phytosome (Sundaresan Nandhini et al 2021). The morphology of the phytosome was determined using scanning electron microscopy (Hitachi S-3700N). SEM gives a three-dimensional image of the globules. One drop of phytosome suspension was mounted on a clear glass stub. It was then air-dried and gold-coated using sodium aurothiomalate to visualize under a scanning electron microscope. The studies were carried out at Agarkar Research Institute, Pune.

b) **Entrapment efficiency** (Annamma Anthrayose et al 2018; Deivasigamani Senthil Kumar et al 2021) The entrapment efficiency of phytosome was determined by centrifuging 2 mL of the phytosome formulation at 1500 rpm for 30 min at room temperature. The supernatant was taken carefully using a pipette. Pure supernatant was then dissolved in ethanol to disrupt the vesicles and appropriate dilution was made and measured using a UV spectrophotometer.

c) Selection of method and optimization of Design of Phytosome

Based on the results obtained from Scanning Electron Microscopy and Entrapment Efficiency, the salting out anti-solvent precipitation method was found to be a better method than other tried techniques for the preparation and design of Phytosome. The process parameters like temperature and rotating speed were optimized for better results.

d) Optimization of parameters of the method selected (salting out anti-solvent precipitation method)

The optimum temperature required to form Phytosome was found to be 60^0C

The optimum Rotating Speed required to form a Phytosome was found to be 500 rpm.

3.7 Characterization of Phytosome (prepared by Salting out anti-solvent precipitation

method): the various batches of phytosome prepared for L-Dopa extract and were subjected to characterization as follows:

a) Surface morphology

The morphology of the phytosome was determined using scanning electron microscopy (Hitachi S-3700N). SEM gives a three-dimensional image of the globules. One drop of phytosome suspension was mounted on a clear glass stub. It was then air-dried and gold-coated using sodium aurothiomalate to visualize under a scanning electron microscope.

b) Zeta potential (Sachin K S et al. 2019)

Zeta potential was determined using a zeta sizer (HORIBA SZ-100). Measurements were performed for the samples prepared. Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in a dispersion system.

For the measurement, 1ml of the sample was diluted to 10ml with water, 5ml of this diluted sample was transferred to a cuvette, and the zeta potential was measured

c) Particle Size Determination

The mean size of the Phytosomal suspension was determined by photon correlation spectroscopy. The experiments were carried out using a 4.5mW laser diode operating at 670nm as a light source. Size measurements were carried out at a scattering angle of 90^0C.To obtain the mean diameter and polydispersity index of colloidal suspensions, a third-order cumulative fitting correlation function was performed by a Malvern PCS sub-micron particle analyzer. The samples were suitably diluted with filtered water-methanol mixtures at the same ratio used for phytosome preparations and those were placed in a quartz cuvette to avoid the multi-scattering phenomenon.

d) XRD (X ray Diffraction Techniques)

Samples of the prepared phytosomes were examined using Shimadzu XRD -6000 (X-Ray Diffract meter SERIAL NO. Q30344700643CZ, Tokyo, JAPAN). The measurements were carried out using Cu as an anode material and operated at a voltage of 25 KV with a current of 40 mA. The samples were analyzed in the 2 Theta angle ranges of 4 to 50 degrees and a scanning speed of 1.2degree/min.2

e) Drug Entrapment Efficiency and % Drug Loading

The vesicles were separated in a high-speed cooling centrifuge at 10000 rpm for 90 minutes. The sediment and supernatant liquids were separated, amount of drug in the sediment was determined by lysing the vesicles using methanol. It was then diluted appropriately and estimated using a UV visible spectrophotometer at 624 nm for L-Dopa From this, the entrapment efficiency was determined by the following equation, EE% = (Total drug) - (free drug) X 100 /Total drug.

Drug loading was determined by % Drug Loading = weight of Entrapped drug X 100 /weight of formulation. Based on the results obtained for L-dopa extract phytosomal formulation batch LEF3 (extract: Phosphatidylcholine: cholesterol in the ratio of 1:2:0.25) was further explored for optimization studies.

3.8 Optimization of formulation: After having characterized the phytosome of Macuna pruriens extract was further optimized for finalizing the formulation

A 3^2-randomized full factorial design was used in the study. In the design 2 factors identified were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations shown in the table. Percentage of Phosphatidylcholine (X1) and Percentage of Cholesterol (X2) were selected as independent variables. Entrapment efficiency (Y1), and in-vitro drug release (Y2), were selected as dependent variables. The dependent variables were selected based on the fact that the release rate would be directly affected due to an increase in the bond strength (Extract and Lipid) with an increase in the concentration of both excipients and with an increase in the concentration of Phosphatidylcholine and Cholesterol there would be more availability of sites for bonding with lipid and increase in the stability

due to cholesterol that will directly affect Entrapment efficiency. The more the entrapment efficiency more will be the economical method. And if the release rate is slowed down that will reduce the dosing frequency. Formulation (Batch F5) containing 2 %w/v of Phosphatidylcholine and 0.5% w/v of Cholesterol was selected as the optimized formulation. The optimized formulations were further ensured by in vitro release and entrapment efficiency.

Table 1: Batch code

Batch code	Variable levels in coded form	
	X_1	X_2
F1	+1	0
F2	-1	-1
F3	0	-1
F4	0	+1
F5	+1	+1
F6	+1	-1
F7	-1	0
F8	-1	+1
F9	0	0

Transformation of code levels in actual units			
Variable levels	**Low**	**Medium**	**High**
Percentage of Phosphatidylcholine (X_1)	1	1.5	2
Percentage of Cholesterol (**X2)**	0.25	0.375	0.50

a) In-vitro drug release studies of optimized batches:

The release tests were performed according to the USP II paddle method at a speed of 50 rpm using 500 ml of SNEF as a dissolution medium at 35°C ± 0.5°C. Aliquots of 1 ml were withdrawn at time intervals of 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min and each aliquot were replaced by 1 ml of fresh SNEF. The samples were measured spectrophotometrically as mentioned earlier.

b) Entrapment Efficiency of optimized batches:

The entrapment efficiency of the Phytosome was determined by centrifugation. The vesicles were separated in a high-speed cooling centrifuge at 10000 rpm for 90 minutes. The sediment and supernatant liquids were separated, amount of drug in the sediment was determined by lysing the vesicles using methanol. It was then diluted appropriately and estimated using a UV visible spectrophotometer. From this, the entrapment efficiency was determined by the following equation, EE% = (Total drug) - (free drug) X 100 /Total drug

Based on the results obtained for in vitro drug release and entrapment efficiency during the optimization study batch PLEF5 (Phytosomal levodopa extract batch code F5 with 2% Phosphotidylcholine and 0.50% cholesterol) can be further transferred to suitable dosage form for treatment of Parkinson's Disease.

5. Result

5.1 Study of plant and extract

Plant authentication and Pharmacognostic evaluation results confirmed the identity of plant. Biological name of plants was confirmed as ***Mucuna*** *pruriens* (Fabaceae)

5.1.1 Pharmacognostic Characterization

Organoleptic Evaluation

Table. 2 Organoleptic Evaluation (Seeds of Macuna pruriens)

Sr. No	Macroscopic Characters	Seeds
1.	Shape	ovate, oblong, flattened
2.	Size	Length -0.8–1.29 cm,
3.	Surface	hard, smooth, glossy
4.	Colour	brown to black
5.	Weight	Upto 0.5 gm
Sr. No	**Macroscopic Characters**	**Powder**
1	Colour	Creamish green
2	Texture	Course
3	Odor	Odorless
4	Taste	Starchy- Slightly bitter

5.1.2. Physicochemical Evaluation

Table 3 Physicochemical constants of Macuna pruriens seeds

Sr.No.	Parameters	Value % w/w
1	Total Ash	4.31
2	Acid insoluble ash	2.25
3	Water soluble ash	1.37
4	Water soluble extractives	18.25
5	Alcohol soluble extractives	11.84
6	Loss on drying	6.38

5.1.3 Phytochemical screening:

Phytochemical screening confirmed the presence of phenolic compound in Macuna pruriens extract, which is an antioxidant considered as active moiety available in the plant material. However, the moieties have varying chemical structure reported as L-dopa

Table 4 Preliminary Phytochemical analysis of the extract of L-Dopa

Sr. No.	Test	Presence (+)/ Absence (-)
1	Glycosides	+
2	Saponins	-
3	Oils and fats	-
4	Alkaloids	+
5	Steroids	+
6	Flavonoids	-
7	Proteins and free amino acids	+
8	Tannins and phenols	+

5.2 Standardization of plant extract

5.2.1 Thin Layer Chromatography (TLC)

Rf value of Standard and Extract was found to be identical. Thus, the compound extracted is considered as L-Dopa

Fig.No. 1 Thin Layer Chromatography of Macuna pruriens extracts

A= Extract 1 B=EXTRACT 2 C=STD Levodopa

5.2.2. High-performance liquid chromatography (HPLC)

The HPLC technique was used to determine the quantity of drug in the extract. The area of peak was use to determine the same by using following formula:

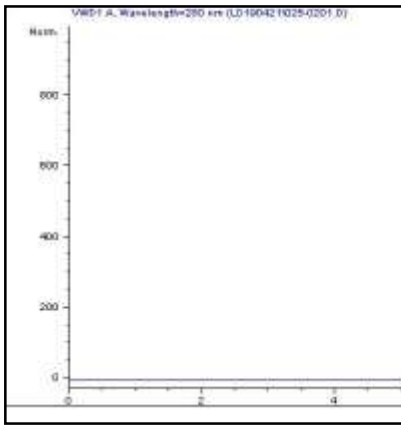
Concentration of levodopa in % = Area of sample/area of standard x concentration of Standard

For L1 sample- 353.577/3404.497x0.5=0.50% (Method 1 for extraction)

For L2 Sample -3660.380/3404.497x0.5=5.37% (Method 2 for extraction)

Based on the amount of Levodopa in the extract the second method was further selected for extraction

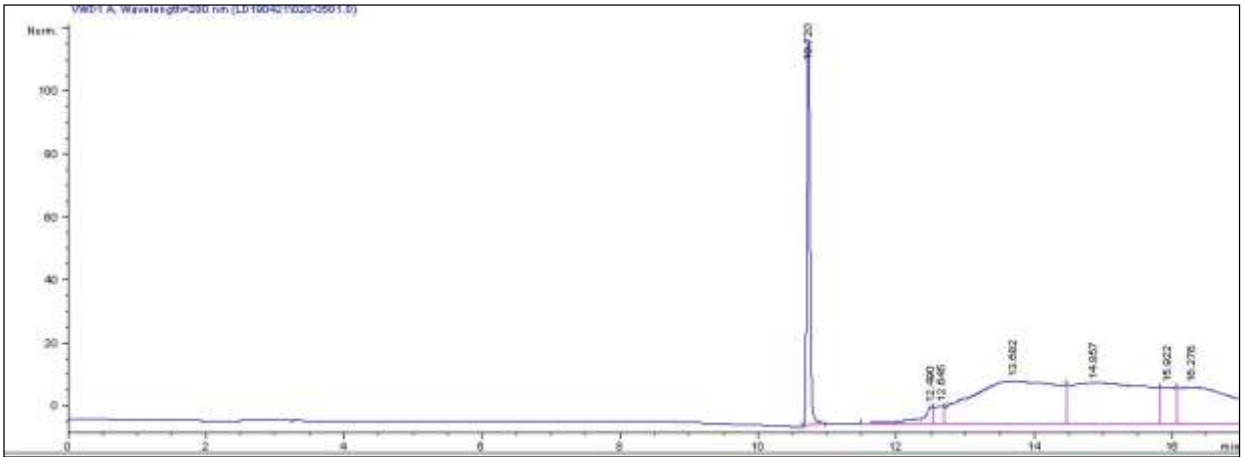
Fig.No. 2 Estimation of Levodopa in the extract samples by HPLC analysis of Macuna pruriens Extract 1



A B C

Fig.No. 3 Estimation of Levodopa in the extract samples by HPLC analysis of

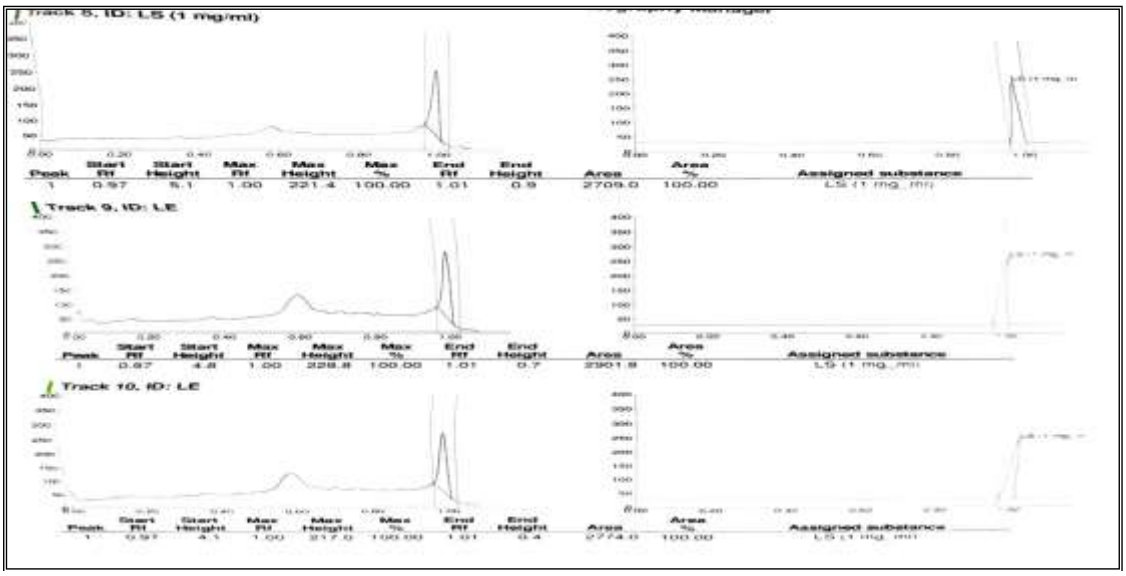
Macuna pruriens Extract 2



5.2.3 High-performance thin layer chromatography (HPTLC)

To identify presence of drug in the extract of Macuna pruriens HPTLC method was found suitable where comparison of chromatogram of standard with sample was done. Comparison of standard L-Dopa with Extract at peak apex and peak base was done, which confirmed that method was selective.

Fig. No. 4 Estimation of Levodopa from the extract samples by HPTLC



5.2.4 Fourier Transformed Infrared Spectroscopy FTIR

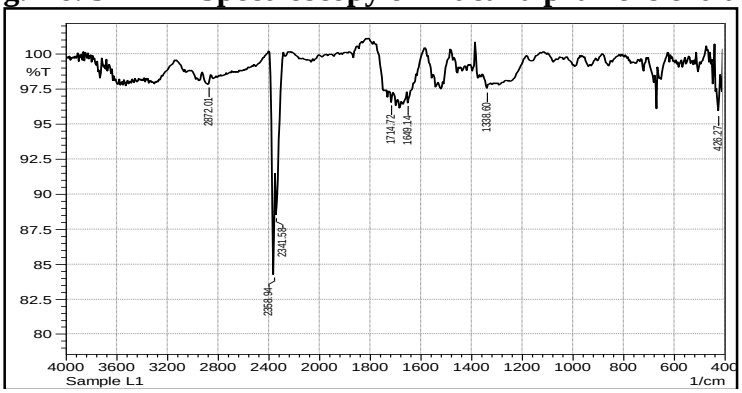
To ensure the functioned group and their detection FTIR study of extract was done that gives following results. Based on the functional group present it was confirmed that L-dopa was present in the given samples of extract.

Table 5 Macuna pruriens extracts FTIR Results

Sr.No.	Vibrational Frequencies	Present Functional group
1	3643	Aromatic OH Group
2	2357	Ammonium Group
3	1714	C=O Stretching, COOH

4	1556	Ring def. coupled OH bend
5	1236	Ring breathing
6	1211	Coupled X-H bending

Fig. No. 5 FTIR Spectroscopy of Macuna pruriens extract



5.3 Formulation and Evaluation of Phytosomal formulation

Four different methods were tried to form phytosome. And method selection was done based on the results obtained for SEM and Entrapment efficiency as follows:

5.3.1. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy confirmed the formation of vesicles (Phytosomes) with methods one (Solvent evaporation method) and two (Salting out anti solvent precipitation method) only.

Fig No. 6 Method 01 Solvent evaporation method (SEM)

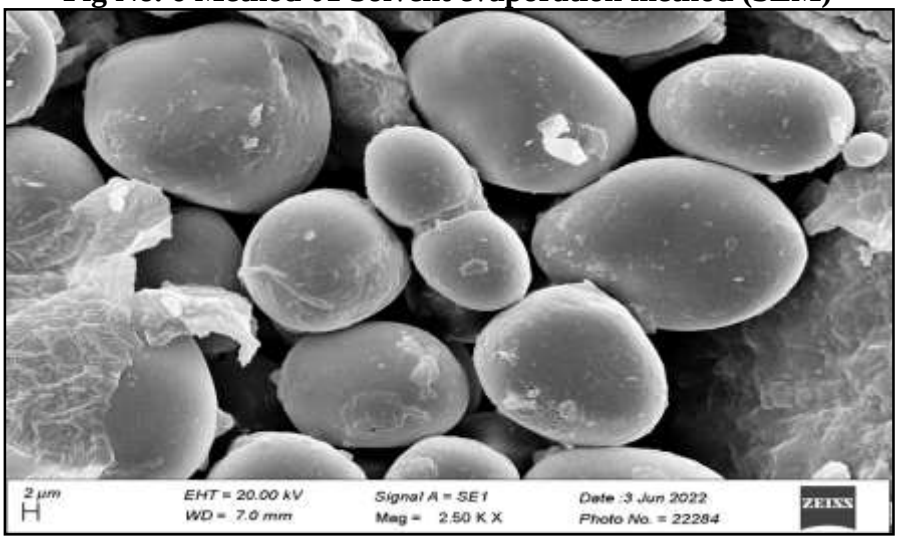


Fig. No. 7 Method 02 Salting out anti solvent precipitation method (SEM)

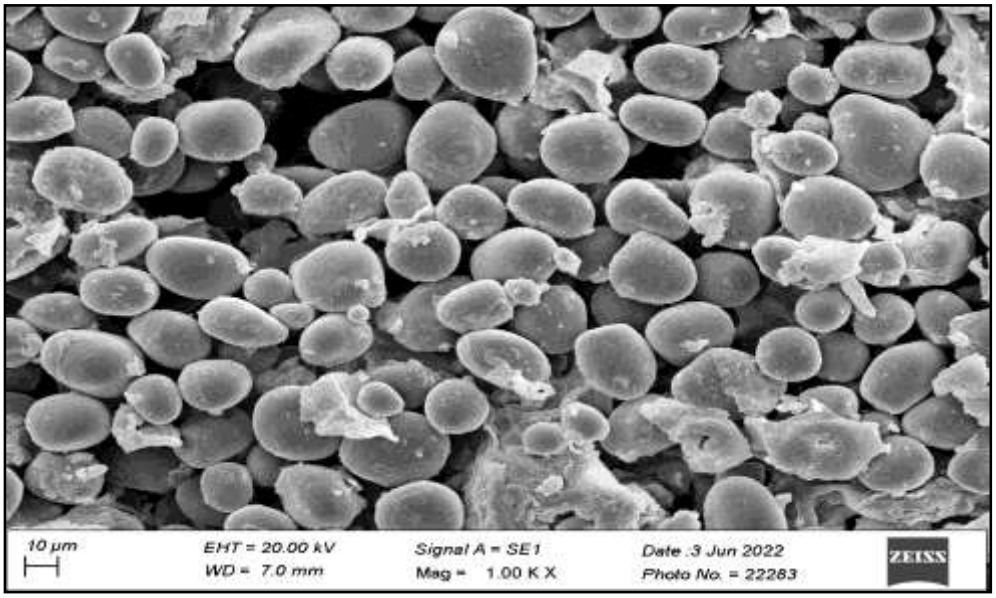


Fig No. 8 Method 03 Direct egg yolk method (SEM)

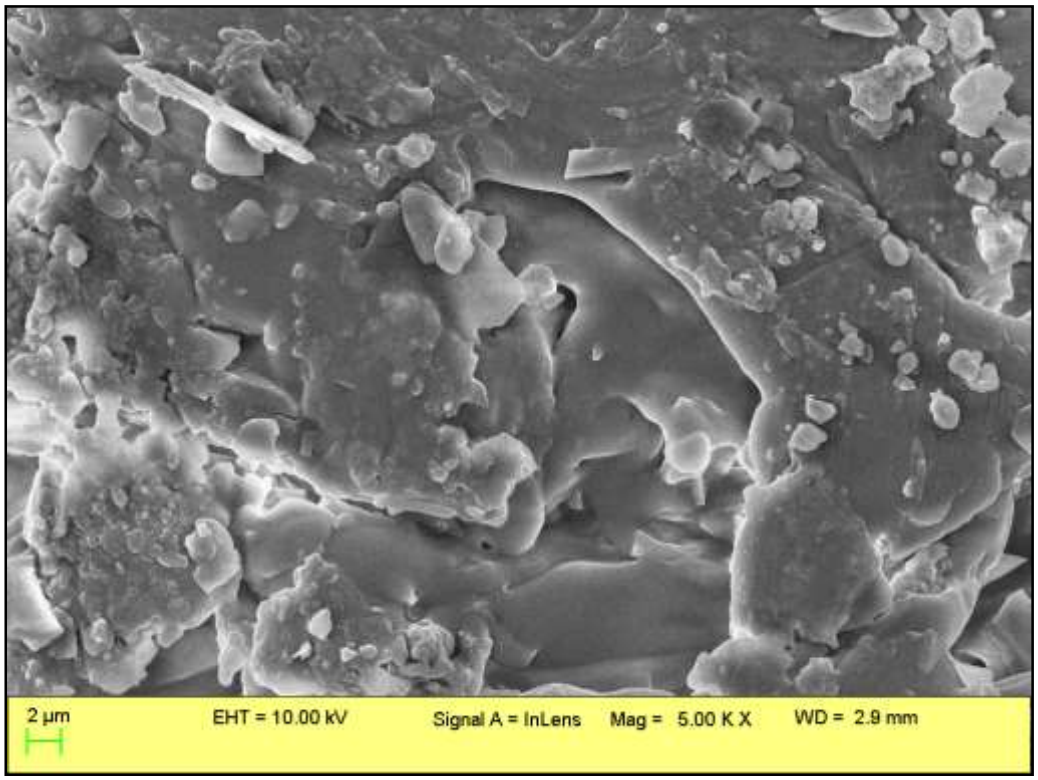
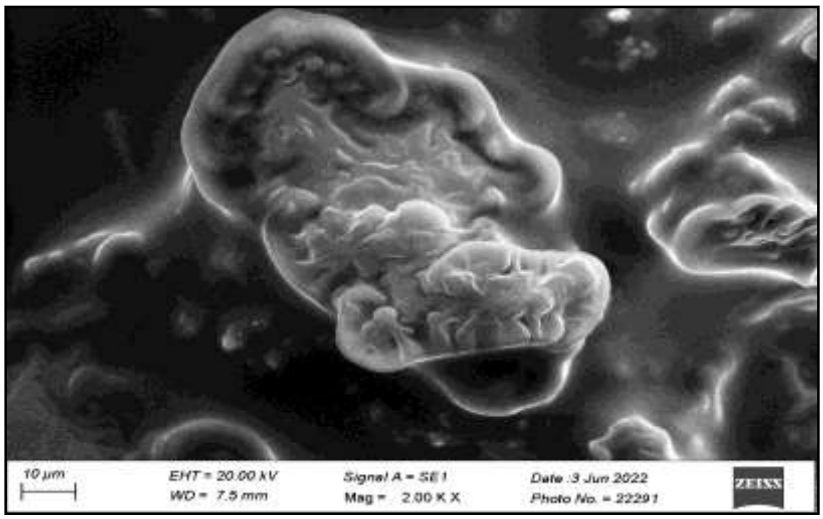


Fig.No.9 Method 04 Egg Lipid method (SEM)



5.3.2 Entrapment efficacy and Drug loading results

The entrapment efficiency was found better in the Phytosome prepared by using salting out and antisolvent precipitation technique **68.30±0.7%** and Drug loading was found to be **12.25±0.25%** this is higher than obtained by solvent evaporation technique.

Based on this salting out and antisolvent precipitation technique was further explored for the preparation of L-Dopa Extract Phytosome. HPTLC study of drug, Extract and Phytosome confirmed that extract get entrapped well in the Phytosome.

Phytosome prepared by using salting out and antisolvent precipitation technique were evaluated for various parameters and following results were obtained

Fig No. 10 HPTLC study of Standard Drug and Extract (Levodopa and Macuna pruriens)

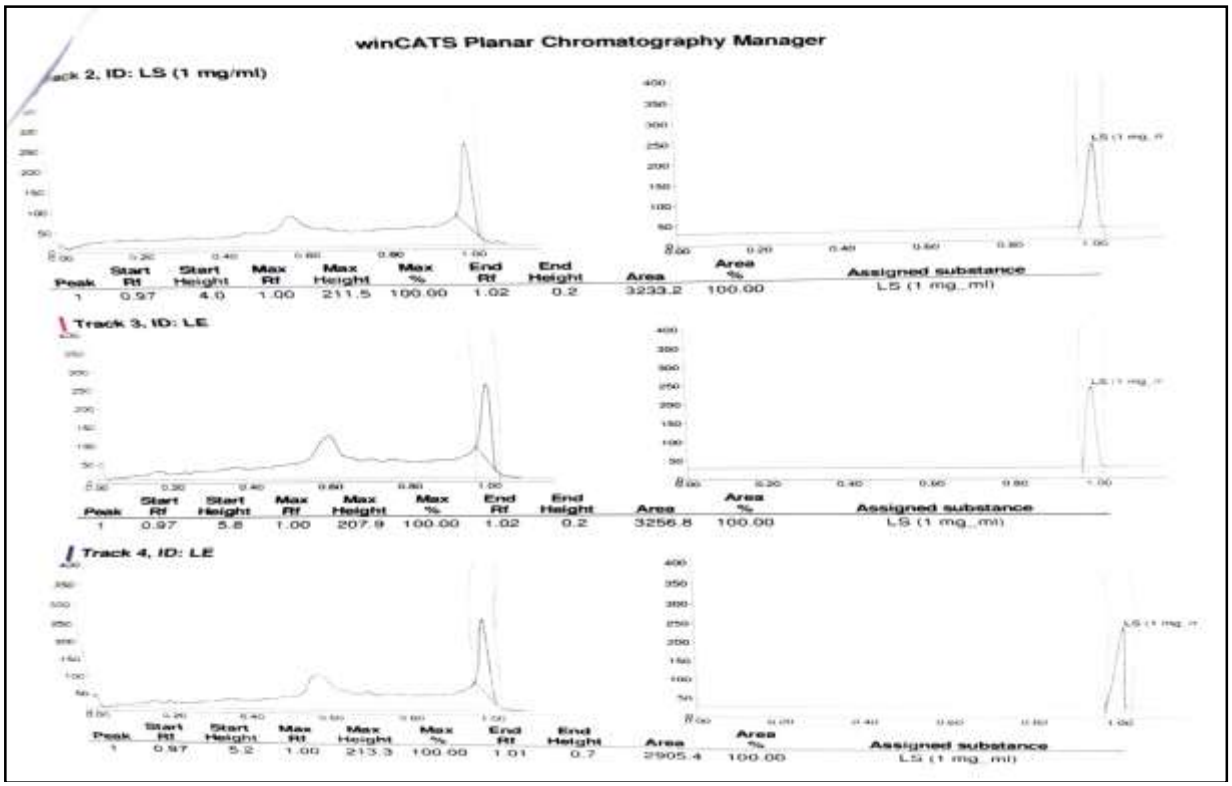
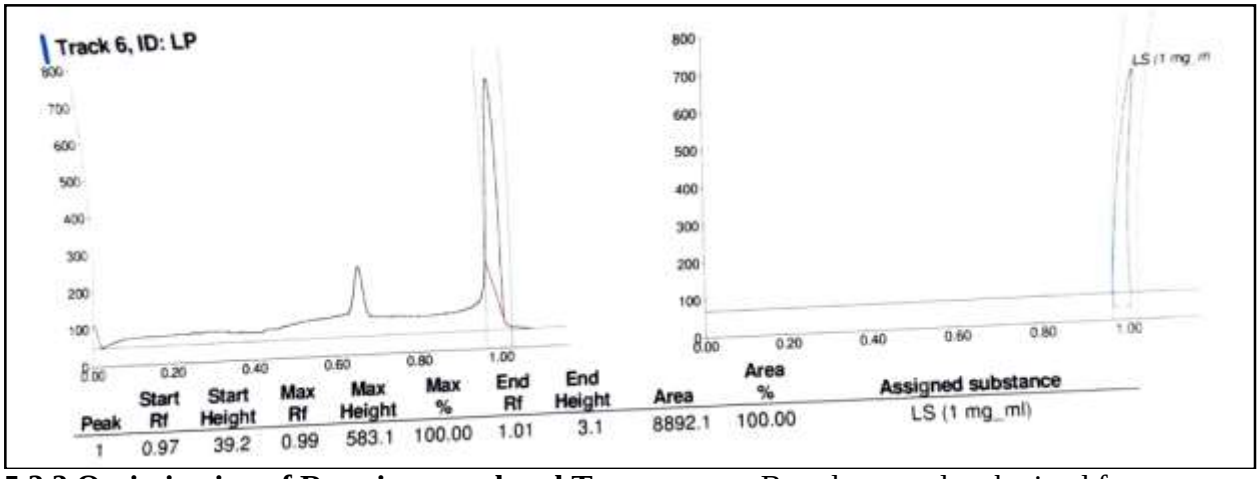


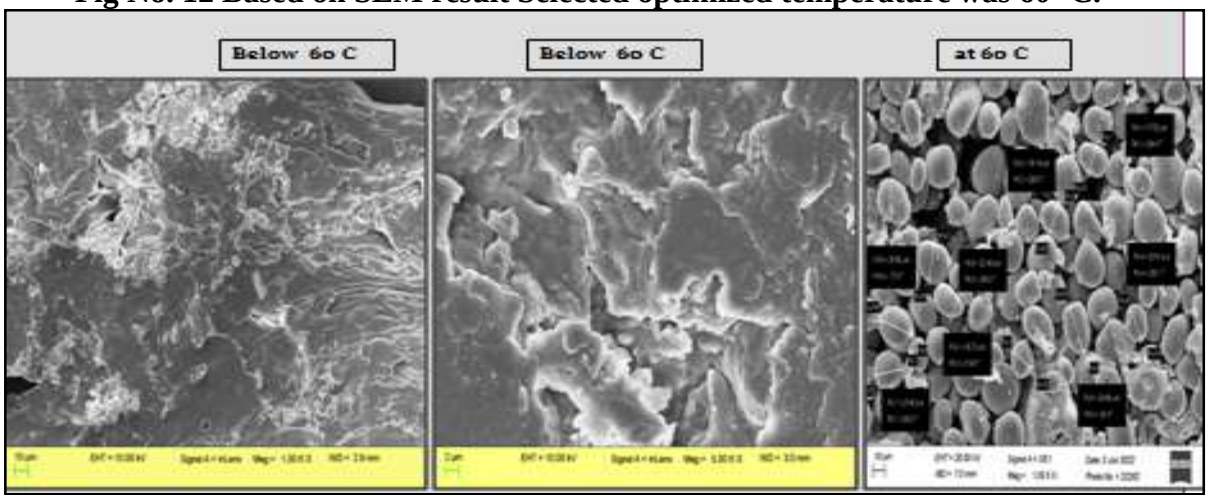
Fig No. 11 HPTLC study of Phytosomal extracts (Macuna pruriens)



5.3.3 Optimization of Rotating speed and Temperature Based on results obtained for Scanning Electron Microscopy (SEM) temperature of 60^0C.and Magnetic stirrer rotating speed of 500 rpm was selected further for the preparation of phytosome.

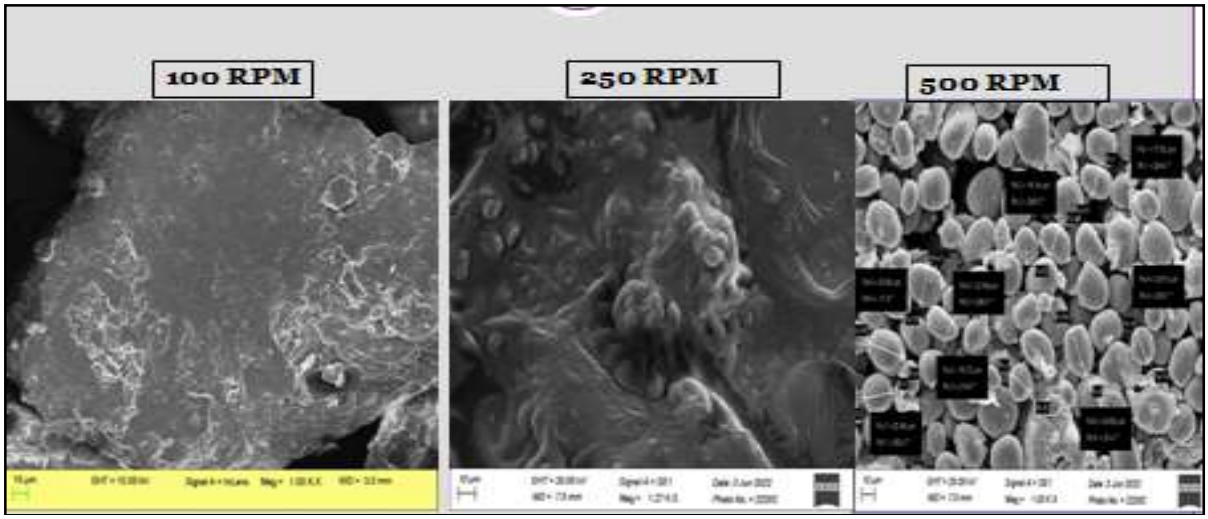
Optimization of Temperature

Fig No. 12 Based on SEM result Selected optimized temperature was 60^0 C.



Optimization of Rotating Speed

Fig.No. 13 Based on SEM result Selected optimized rotating was 500 RPM



5.3.4. Particle size determination:

Formulation F3 Showed best result with minimum average particle size of 12 micrometre.

Fig. No. 14 Batch F1 Particle Size Distribution

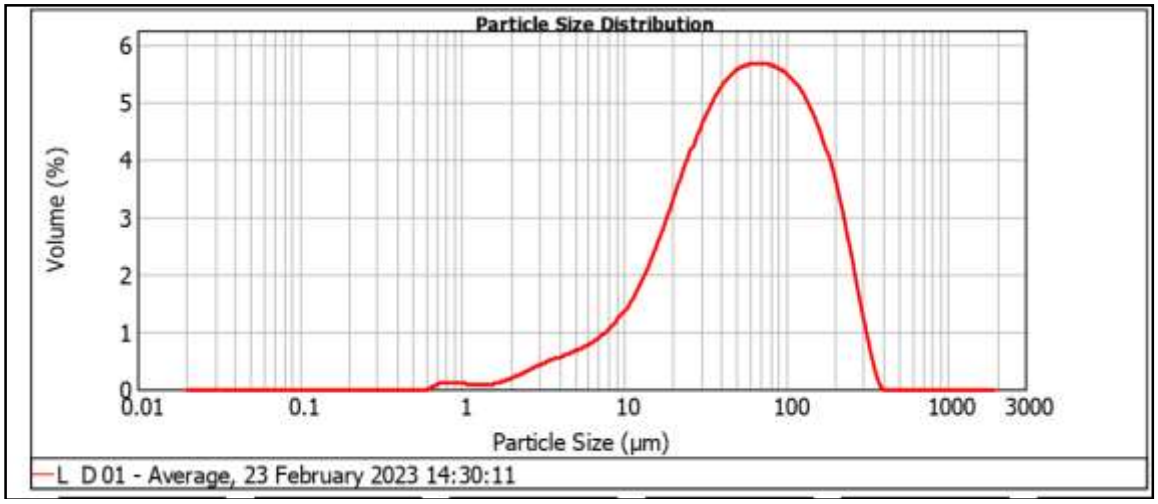


Fig.No. 15 Batch F2 Particle Size Distribution

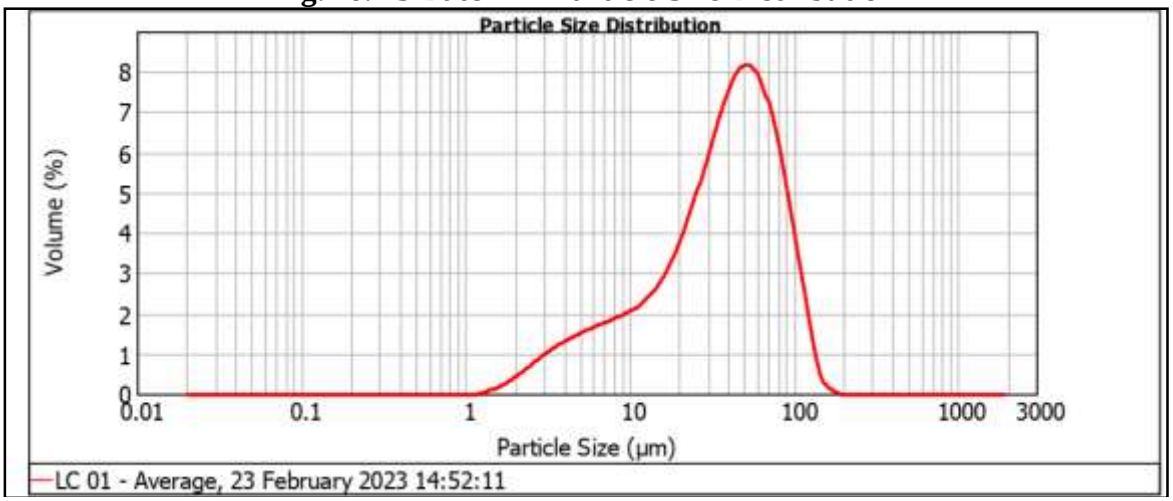
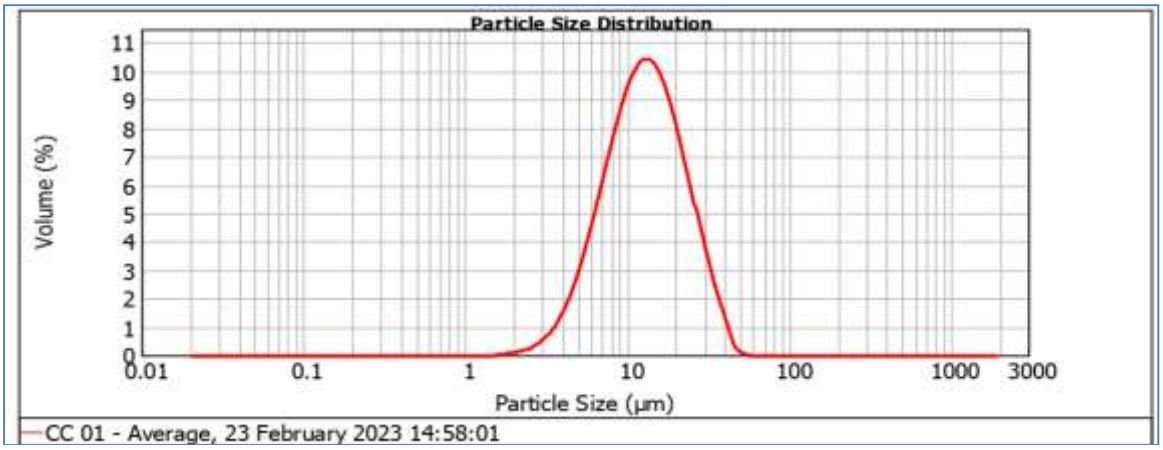


Fig.No. 16 Batch F3 Particle Size Distribution



Particle size determination of all three batches was done

5.3.5 Zeta Potential:

Zeta Potential of the batch F3 was found to be -55.1mv. It showed good stability for the Phytosomal formulations.

Fig.No. 17 Zeta Potential Batch F1

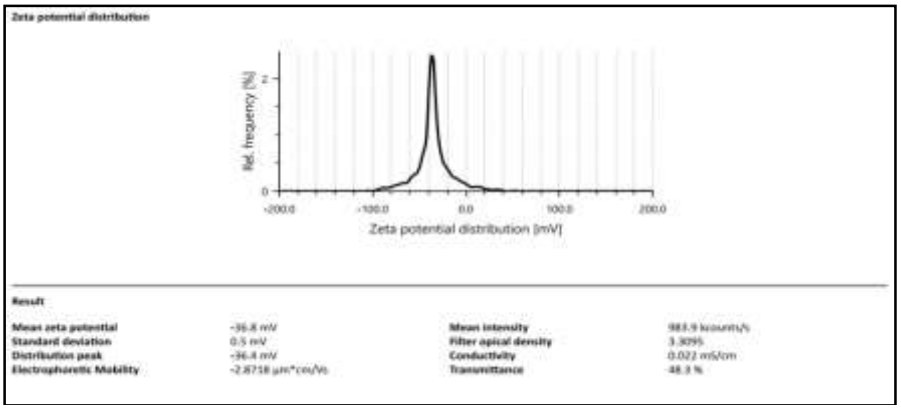


Fig.No. 18 Zeta Potential Batch F2

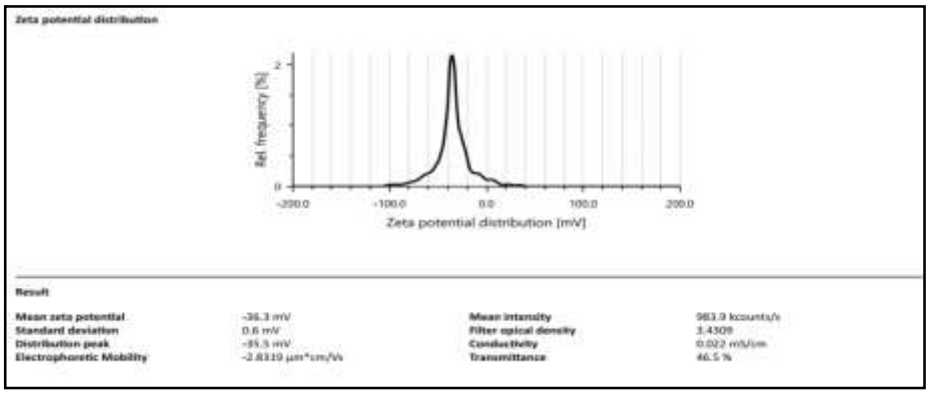
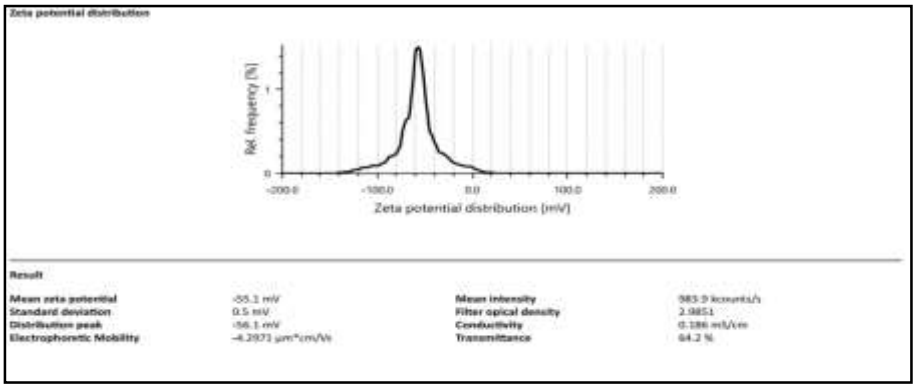


Fig.No. 19 Zeta Potential Batch F3



5.3.6 Powder X-ray diffractometry

In diffraction pattern the intense and sharp crystalline peaks at 2θ of 20 & 28 is observed. With Batch F3 crystallinity of 48.67% .It is possible that the extract is molecularly distributed in the phospholipid matrix and exists in an amorphous state.

Fig.No. 20 Batch F1 XRD

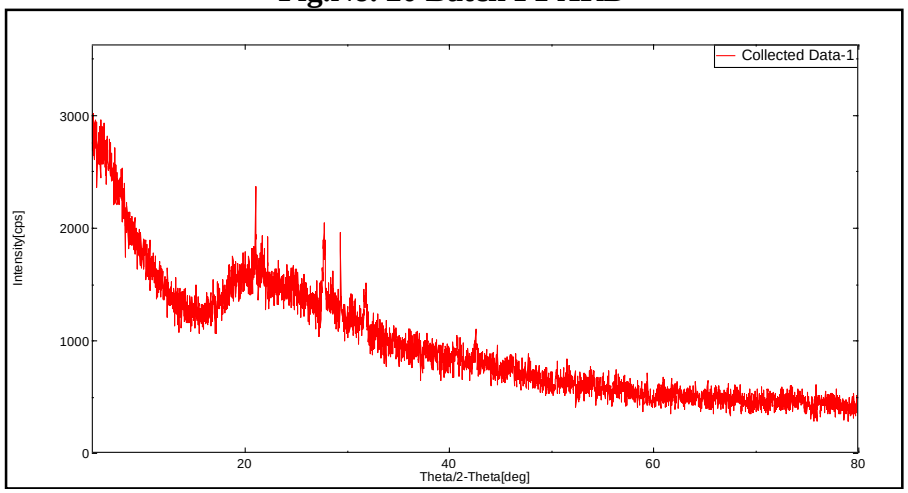


Fig.No. 21 Batch F2 XRD

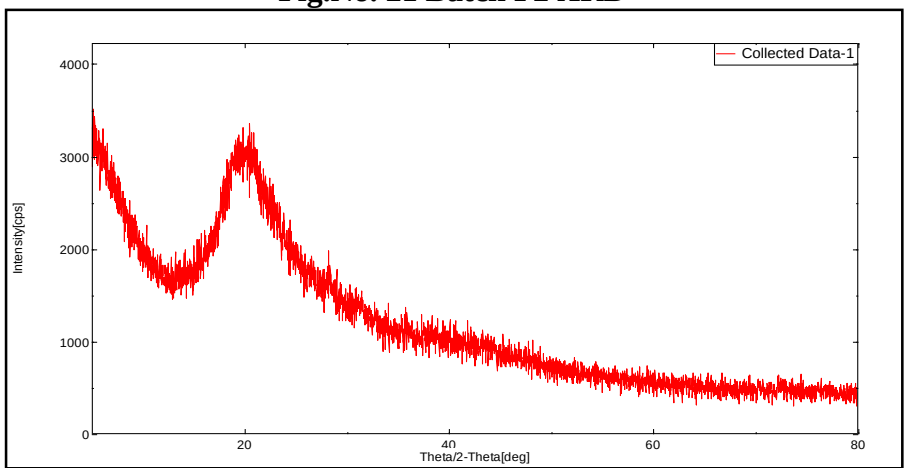
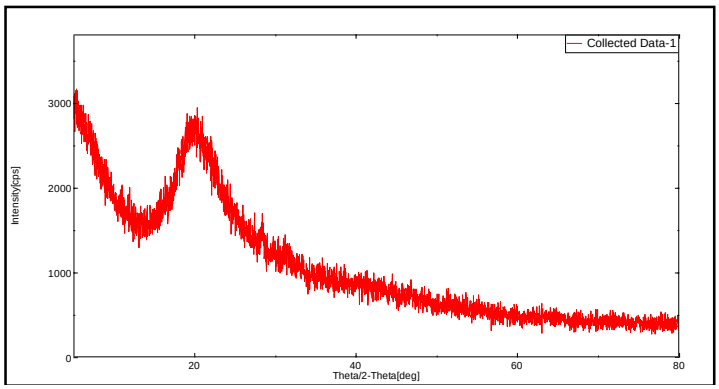


Fig.No. 22 Batch F3 XRD



Drug 5.3.7 Entrapment Efficiency

The maximum entrapment percentage of 67.23% was found for L-Dopa extract in the formulation with 2% (w/w) Lipid (Batch F3) . It was observed that Phytosome with higher lipid content gives better results.

Table 6 Batch Drug Entrapment Efficiency

Formulation	Entrapment efficiency (%)
F1	62.35±0.30
F2	65.80±0.12
F3	67.23±.0.41

5.4 Optimization study

Selected method was further optimized after phytosome were considered ready for formulation. 3D Plot showed that with increase in concentration of Phosphotidylcholine and Cholesterol there is decrease in the release of drug at 8 hours. It may be due to increase in the bond strength (Extract and Lipid) with increase in the concentration of both excipients. 3D Plot showed that with increase in concentration of Phosphotidylcholine and Cholesterol there is increase in the drug entrapment efficiency. This may be due to more availability of sites for bonding with lipid and increase in the stability due to cholesterol. Therefore formulation (Batch F5) containing 2 %w/v of Phosphatidylcholine and 0.5% w/v of Cholesterol were selected as optimized formulation.

Table 7 Different batches (F1-F9) with results (Drug Release in 8 Hr and % Entrapment Efficiency)

BATCH	% Phosphotidylcholine	% Cholesterol	% Drug Release in 8hr	% Entrapment Efficiency
F1	2	0.375	59±1.23	75±1.28
F2	1	0.25	80±2.62	50±2.63
F3	1.5	0.25	65±3.67	70±0.87
F4	1.5	0.5	60±0.29	68±0.52
F5	**2**	**0.5**	**58±1.45**	**80±1.25**
F6	2	0.25	58±2.3	78±3.14
F7	1	0.375	70±0.28	55±0.36
F8	1	0.5	60±3.14	68±0.87
F9	1.5	0.375	59±0.69	62±1.027

Fig. No. 23 in vitro drug release profile Batches F1-F9

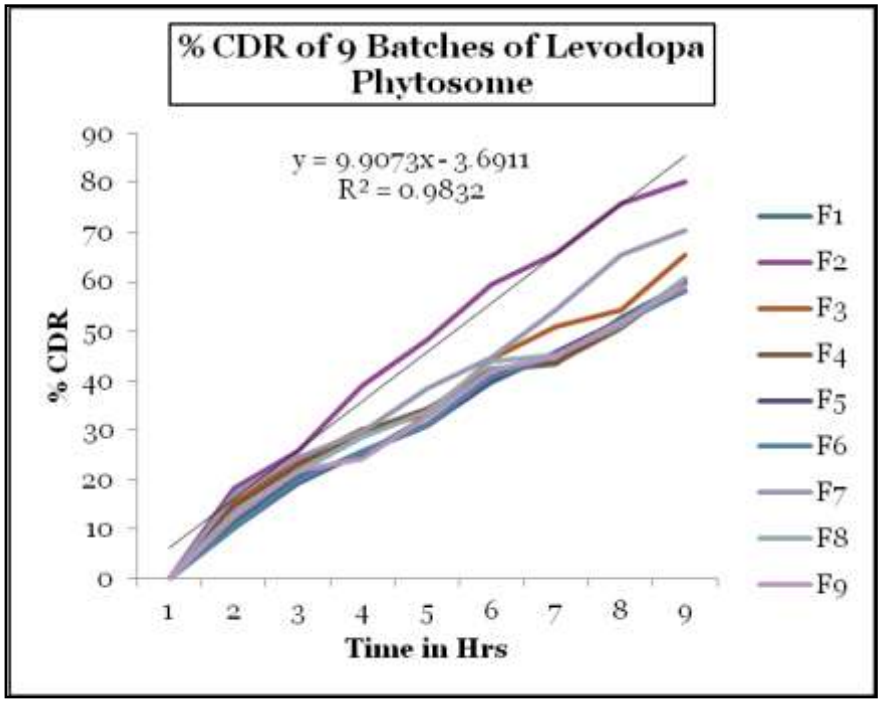


Fig. No. 24 Overlay plot % drug release in 8 Hrs

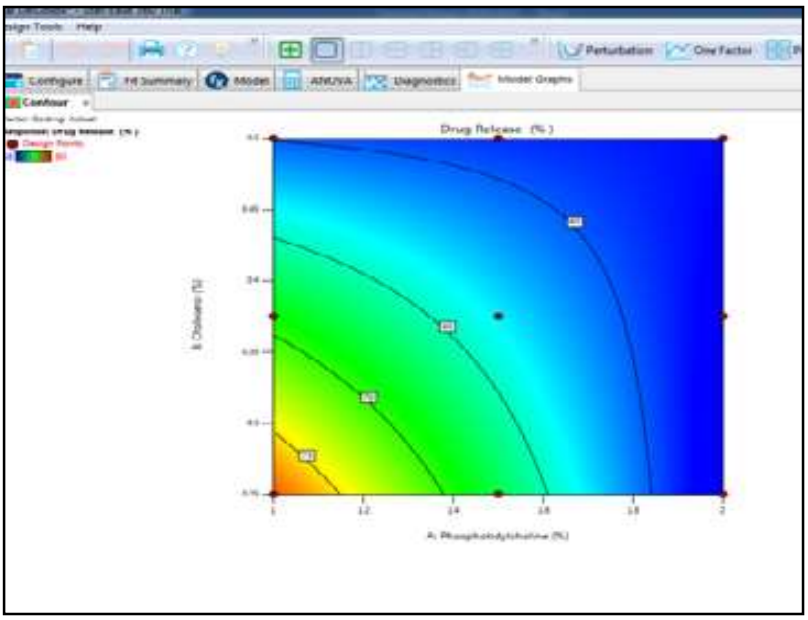
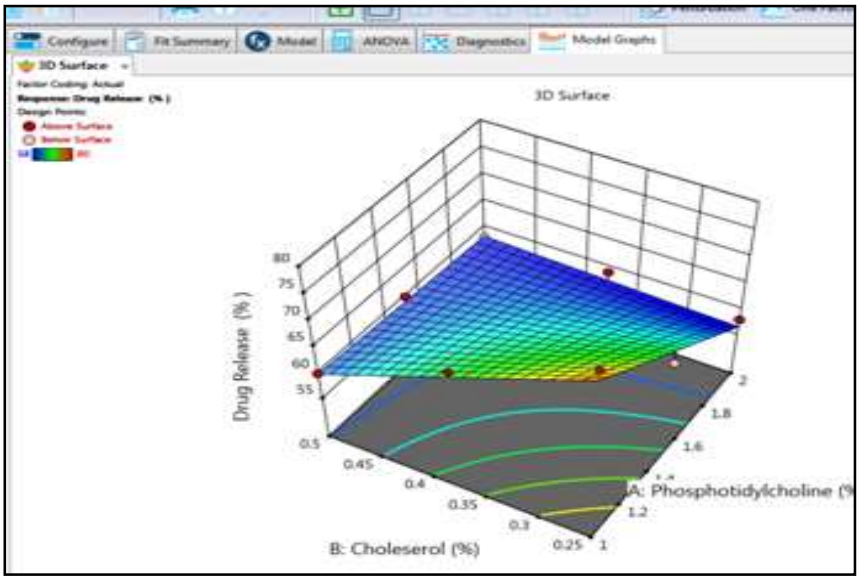


Fig. No. 25 3D Plot % Drug Release in 8 Hrs



As seen from **Fig.24 and 25** the surface response plot and 3D Plot revealed that a corresponding decrease in the drug release at 8 hours was observed with increase in concentration of Phosphatidylcholines and Cholesterol. This may be due to increase in the bond strength (Extract and Lipid) with increase in the concentration of both excipients.

Fig. No. 26 Overlay plot Entrapment Efficiency

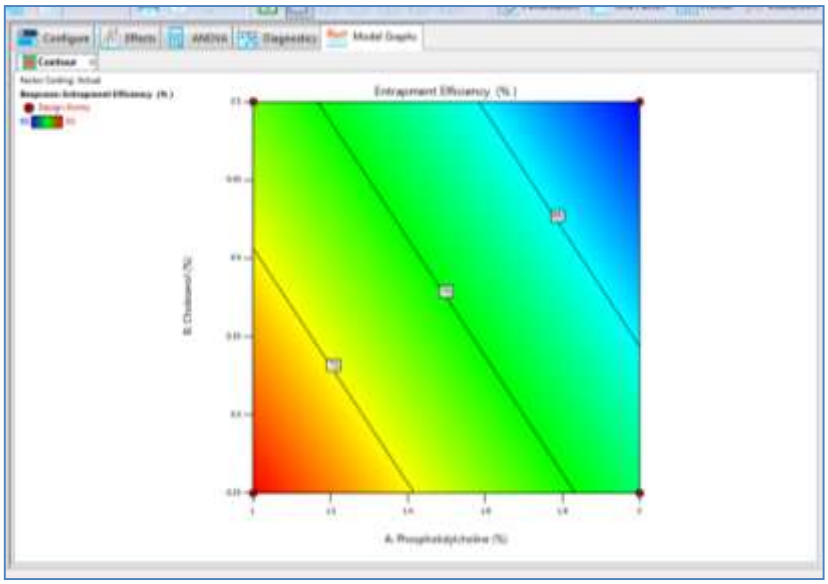
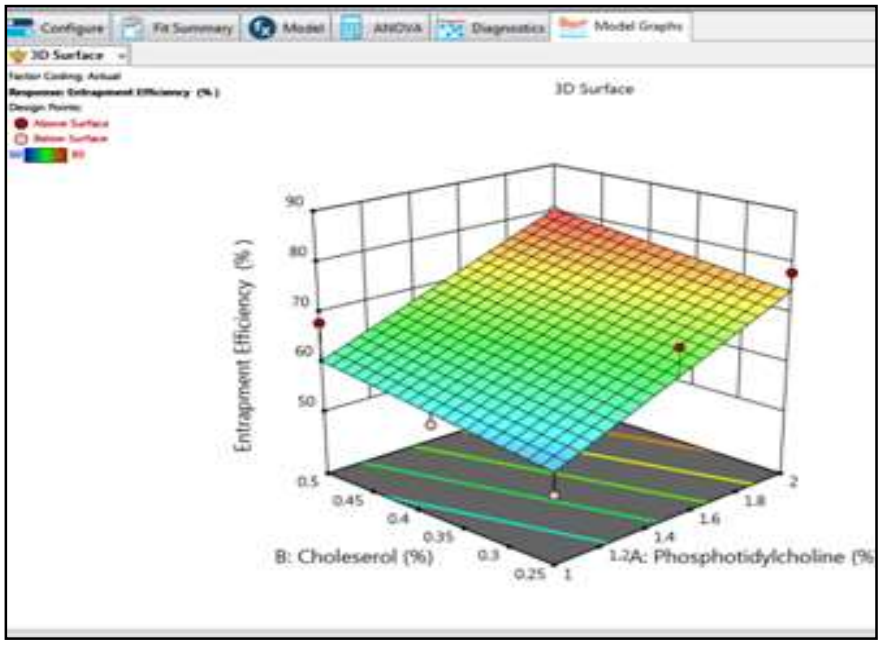


Fig. No. 27 3D Plot Entrapment Efficiency



As seen from **Fig.26 and 27** the surface response plot and 3D Plot revealed that a corresponding increase in the drug entrapment efficiency was observed with increase in concentration of Phosphotidylcholine and Cholesterol. This may be due to more availability of sites for bonding with lipid and increase in the stability due to cholesterol. Therefore formulation (Batch F5) containing 2 %w/v of Phosphotidylcholine and 0.5% w/v of Cholesterol was selected as optimized formulation.

Discussion:

The therapeutic efficacies of many indigenous plants for various diseases have been described by traditional herbal medicine practitioners. Natural products are a source of synthetic and traditional herbal medicine. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential properties. The Indiantraditional system of medicine described several drugs of plant, mineral, and animal origin Scientists who are trying to develop newer drugs from natural resources are looking toward the Ayurveda. Since time immemorial, man has used various parts of plants in the treatment and prevention of many ailments. Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts.

After the procurement of the plant parts it was subjected to authentication. The macroscopic characters were studied. The plants were then subjected to preliminary evaluation which included the determination of physical constants and preliminary phytochemical evaluation. *Macuna pruriens* is used as antiparkinson's drug. It is given in tablet or capsule However it has low permeability which leads to inadequate therapeutics effect. Recent work was done to develop vesicular system having enhanced penetration power. As a result novel vesicular system like phytosmes were developed.

The *Macuna pruriens* phytosmes was prepared by salting out methods by using Lecthin,Cholestrol,methonao and Distilled water.The prepared phytosmes were characterized for their Entrapment efficiency percent, Zeta potential, Particle size, Vesicle Morphology.

Percent Entrapment efficiency was higher in F3 formulation than that of other formulations. The particle size revealed that the F3 formulation gave particle size of 12 micron which gave that there should be little study done for the size of the particles. Zeta potential was more towards the negative charge for the F3 formulation.SEM analysis of the phytosme reveled that the F3 formulation gave a best result in the form of vesicular formation. Further Full Factorial Design: A 2^2 randomized full factorial design was used in this study. In that batch F4 showed 60 % CDR, particles size of 15 (µg) and entrapment efficiency of 70%. The formulation of this batch can be further utilized to be converted into suitable dosage form for better treatment of Parkinson's disease

LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORMS
%	Percentage
ºC	Degree Centigrade
µg/ml	Microgram per milliter
Mg	Milligram
Gm	grams
ml	Milliliter
pH	Hydrogen ion concentration
UV	Ultraviolet
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
HPTLC	high-performance thin layer chromatography
XRD	X Ray Diffraction
SEM	Scanning Electron Microscopy
L-DOPA	Levodopa
RH	Relative Humidity
rpm	Revolution per minute
min	Minutes
Hrs	Hours
etc	Etceteras
±	Plus, or minus
<	Less than
>	More than
v/s	Versus
λ	Lambda
CDR	Cumulative Drug Release
CADD	Cumulative Amount of Drug Diffuse
ICH	International Conference on Harmonisation
IP	Indian Pharmacopiea

Conc	Concentration
Abs	Absorbance
i.e	That is
FT-IR	Fourier Transform Infrared
R^2	Regression Coefficient
et.al	et alii,'and others′
ANOVA	Analysis of variance
e.g	For example
Fig.No.	Figure Number

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