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ABSTRACT

SOY PROTEIN – POLY (L – LACTIC ACID) BLEND NANOFIBERS FOR DRUG DELIVERY OF NARDOSTACHYS JATAMANSI

by
Swetha Tiwari

Electrospun nanofibers when nanofibers are impregnated with drugs play a significant role in drug delivery. Nanomedical approaches to drug delivery focus on developing nanoscale particles or molecules to improve the bioavailability of a drug.

Biodegradable nanofibers were developed using Poly (L – lactic acid) and Soy protein for the delivery of an ayurvedic drug, *Nardostachys jatamansi*. A weighed quantity of powdered drug – *Nardostachys Jatamansi* was subjected to Soxhlet extraction using water and ethanol in equal quantities. Various blends of the drug extract and soy protein solution are prepared and mixed in Poly (L-lactic acid). Nanofibers of Soy Protein – Poly (L-lactic acid) blend along with the drug *Nardostachys Jatamansi* developed, were morphologically analyzed using Scanning Electron Microscope (SEM) and also for the presence of the drug content with UV spectrometry.

This research has opened a new avenue for the drug delivery of *Nardostachys jatamansi* using nanofibers. However, there are more opportunities to be exploited from the electrospinning of PLLA and the corresponding drug-loaded nanofibers for drug delivery.

SOY PROTEIN – POLY (L – LACTIC ACID) BLEND NANOFIBERS FOR DRUG DELIVERY OF NARDOSTACHYS JATAMANSI

by
Swetha Tiwari

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SOY PROTEIN – POLY (L – LACTIC ACID) BLEND NANOFIBERS FOR DRUG DELIVERY OF NARDOSTACHYS JATAMANSI

by
Swetha Tiwari

Dr. Michael Jaffe, Thesis Advisor — Date
Research Professor of Biomedical Engineering, NJIT

Dr. Cheul H Cho, Committee Member — Date
Assistant Professor of Biomedical Engineering, NJIT

Dr. Treena L Arinzeh, Committee Member — Date
Professor of Biomedical Engineering, NJIT

BIOGRAPHICAL SKETCH

Author: Swetha Tiwari

Degree: Master of Science

Date: January 2012

Graduate and Undergraduate Education:

- Master of Science in Biomedical Engineering,
  New Jersey Institute of Technology, Newark, NJ, 2012

- Bachelor of Science in Biomedical Engineering,
  Jawaharlal Nehru Technological University, Hyderabad, India, 2009

Major: Biomedical Engineering

TVAMEVA MATA CHA PITA TVAMEVA,
TVAMEVA BANDHU CHA SAKHA TVAMEVA,
TVAMEVA VIDYA DRAVINAM TVAMEVA,
TVAMEVA SARVAM MAMA DEVA DEVA.

DEDICATED TO THE MOST IMPORTANT PEOPLE IN MY LIFE – 'MY PARENTS,' WHO MADE THIS AND EVERYTHING ELSE POSSIBLE AND WORTHWHILE!!!☺

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CHAPTER 1

INTRODUCTION

1.1 Overview

Nanofibers are finding a presence everywhere in human life. They have been identified for their various applications and one of the applications is use of nanofibers for drug delivery. As a drug delivery device, the carbon fiber hollow nanotubes are capable of carrying drugs into blood cells owing to their small size. Such fibers can also be used to carry medicines to internal tissues of the body. Anti – adhesion materials using cellulose nanofibers are already available for commercial use. The use of nanofibers in making bandages or sutures that eventually get dissolved in the body are of significance as it may be able to reduce infection rate and blood loss (1).

Nanofibers can be fabricated using techniques like phase separation, drawing, template synthesis etc., but electrospinning with better flexibility and simplicity of the process, has emerged as one of the most successful and versatile methods (2). Electrospun nanofibers are considered promising novel drug delivery systems due to their special characteristics and effective top – down fabricating process (3).

The polymeric drug delivery systems are known to have many advantages in comparision to the conventional dosage forms like improved therapeutic effect, reduced toxicity, convenience, etc. (4). Some drug delivery systems need to deliver the drug that would be therapeutically effective over a period of time and this can be efficiently achieved with the drug delivery systems produced by nanotechnology (5).

1.2 Objective of the present study

The main objective of this research was to envision innovation for the utilization of the use of biodegradable nanofibers for the drug delivery of *Nardostachys jatamansi*, an ayurvedic drug known for its prominent medicinal uses. This research is focused on developing blended nanofibers of a synthetic polymer, poly l-lactic acid; a protein, soy protein isolate and *Nardostachys jatamansi* and to analyze them for the presence of the drug. Nanofibers were developed by electrospinning solutions from the blends containing these three components. The initial stage of this study was to prepare and determine a superior blend that can be electrospun to develop nanofibers with no or limited depletion of the drug solution and the next stages were morphological and spectroscopic analyses of nanofibers. The morphological characterization was carried out using Scanning Electron Microscope (SEM). The drug content in the nanofiber was determined using UV spectroscopy analysis.

CHAPTER 2

BACKGROUND AND SIGNIFICANCE

2.1 Materials

The nanofibers were electrospun from a blend prepared by using three materials – a synthetic polymer, poly (L – lactic acid); a protein, soy protein isolate and a drug, *Nardostachys jatamansi*. Nanomedicine is a rapidly growing area exploring new possibilities for drug delivery and with the increasing demand of the use of eco – friendly compounds in various areas has instigated the use of soy protein in this research.

2.1.1 Poly (L – Lactic Acid)

Poly (L – lactic acid) is a biodegradable and biocompatible polymer. It's applications includes wound closure, prosthetic implants, controlled drug delivery systems and also in the production of three – dimensional scaffolds for tissue engineering. It is also used in textiles and the packaging industry. Pure polylactic acid (PLA) is a semi – crystalline polymer with a glass transition temperature in the range of 50 - 65°C and melting point of about 180°C (6).

Poly (L – lactic acid) is a product formed by the polymerization of L,L – lactide. Due to its thermoplastic nature, poly (L – lactic acid) can be processed into fibers and films.

The total degradation time of poly (L – lactic acid) of is few years and it is currently being researched for its applications in tissue engineering and various other biomedical areas (7).

Poly (L- lactic acid) has been investigated for its application in drug delivery. With its polymeric matrice, it can slowly release drugs incorporated in its interiors and is also among the best biodegradable polymers that get hydrolyzed without enzymes and also get metabolized by the body. Thus, it has the characteristics for a biocompatible and nontoxic biomaterial (8).

2.1.2 Soy Protein

Soy protein is a protein isolated from soybean, which is the only vegetable food with all eight amino acids and is considered one of the most complete foods. It is known for its use in health food products. It is the richest sources of arginine and glutamine. It is storage (9). Figure (2.1) demonstrates the cycle of soybean.

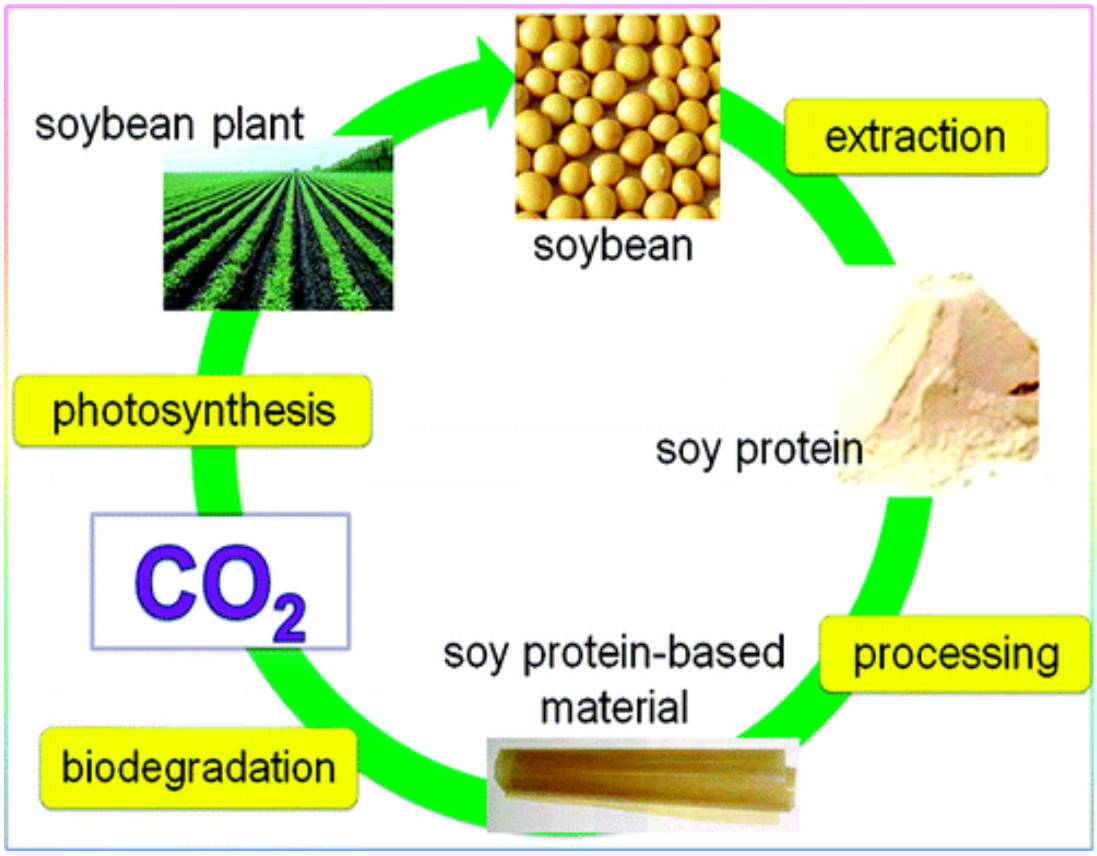

Figure 2.1 Diagrammatic representation of the cycle of Soy bean (10).

Soy protein has been researched for its use to develop fibers for the textile industry and also have been investigated for its use in the drug delivery along with other biodegradable polymers (9) (11).

Soy protein has many nutritional as well as health benefits and is highly recommended during pregnancy, cardiac diseases, diabetes and is also known for its property of reduction of cancer development (12).

Considering the use of soy protein during pregnancy and in cardiac patients, its use in drug delivery is desirable and should be considered.

2.1.3 *Nardostachys Jatamansi*

Nardostachys jatamansi is an ayurvedic herb found in India, Nepal, Bhutan, Tibet and China. The root of this plant shown in figure (2.2), is used for treatment of heart disease, high blood pressure and insomnia. The root and rhizome contains active components with carminative, sedative, antispasmodic and tranquilizing properties.

Nardostachys jatamansi belongs to the family Valerianceae. It is a small, perennial, herbaceous endangered species (13) and is known as Indian spikenard, but has various synonyms like *Fedia grandiflora* (Wall)., *Patrinia jatamansi* (Jones, D. Don), *Valeriana jatamansi* (Jones), *Valeriana jatamansi* (Wall), *Valeriana wallichii* (DC) (14).

Figure 2.2 Dried roots of *Nardostachys jatamansi* (15)

2.2.1.1 Phytochemistry. The roots of *Nardostachys jatamansi* consist of many chemical constituents that contribute to its medicinal properties. It consists of volatile and non – volatile constituents, with major part being sesquiterpenes (16).

The roots contain essential oil and are rich in sesquiterpenes and coumarins. The principle sesquiterpene is jatamansone or valeranone with others like nardostachone, dihydrojatamansin, jatamansinol, jatamansic acid, jatamansinone, jatamansinol, oroseolol, oroselone, seselin, valeranal, nardostachynin, nardosinone, spirojatamol, jatamol A and B, calarenol, seychellene, seychelane; a coumarin, jatamansin or xanthogalin (17).

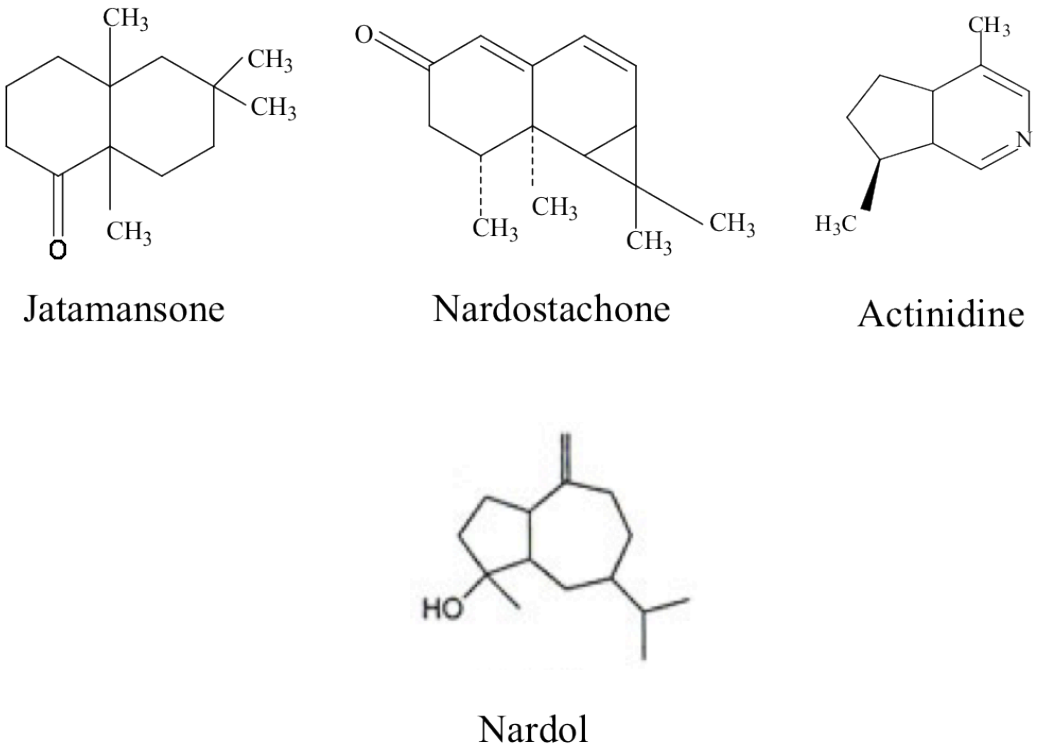

Figure 2.3 Chemical structures of few constituents of *Nardostachys jatamansi*. (17) (19).

The complex phytochemistry of the plant is being researched and new compounds are seen to be found like a sequiterpene acid, nardin; pyranocoumarin, 2', 2' – dimethyl – 3' – methoxy – 3', 4' – dihydropyranocoumarin and an alkaloid, Actinidine (17).

2.2.1.2 Pharmacological and Medicinal uses. *Nardostachys jatamansi* is known for its excellent therapeutic activity and is used for the treatment of various ailments in the Indian Ayurvedic system. It is also known for its hepatoprotective, cardioprotective, hypolipidimic and antifungal activities (16). It also has antioxidant and anticataleptic effects and is known to ameliorate and reversing the effects of induced Parkinsonism or catalepsy in rats. It is efficiently used as a medicine to improve learning and memory (19) (20).

2.2 Methods

2.2.1 Soxhlet Extraction

The Soxhlet apparatus was used for the extraction of the drug, *Nardostachys jatamansi* and the apparatus as described in the figure (2.4) mainly consists of the following (21)

i. Condenser tube

ii. Extraction tube holding thimble

iii. Distilling pot containing the solvent and a stirrer

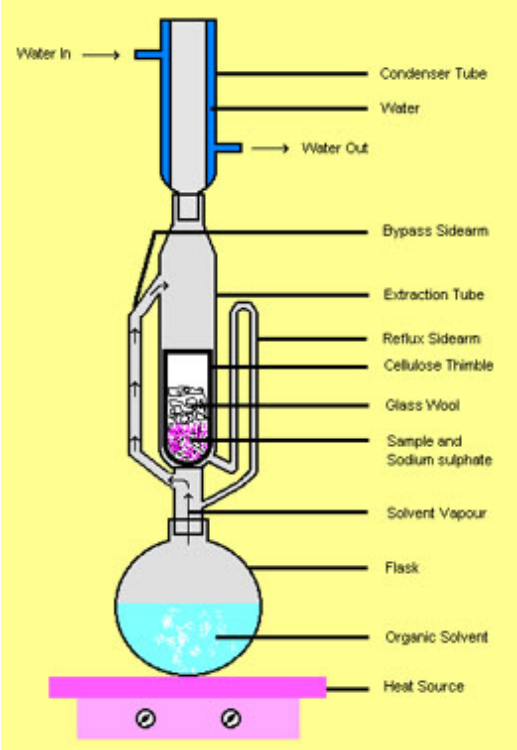

Figure 2.4 Soxhlet Apparatus (23).

Soxhlet extraction is a process where the solvent is heated to reflux and its vapors move up, gets condensed by the condenser tube and dripped into the thimble containing the material. As the solvent passes through the thimble, some amount of the material gets

dissolved in it. The solvent containing the extracted material is siphoned back into the distilling pot. This process is continued for several hours to few weeks. (22)

2.2.2 Electrospinning

Electrospinning is one of the simple and the most potential methods used widely for the preparation of nanofibers. It is an inexpensive process that can be scaled up by employing multiple spinnerets. Electrospinning process has been identified to be versatile which can process almost any soluble polymer into nanofibers. Conductive as well as dielectric polymer solutions have been successful with electrospinning technique (24).

2.2.2.1 Apparatus. A standard electrospinning apparatus consists of a syringe with needle or spinneret connected to a high – voltage direct current power supply, a metered syringe pump and a grounded collector (25).

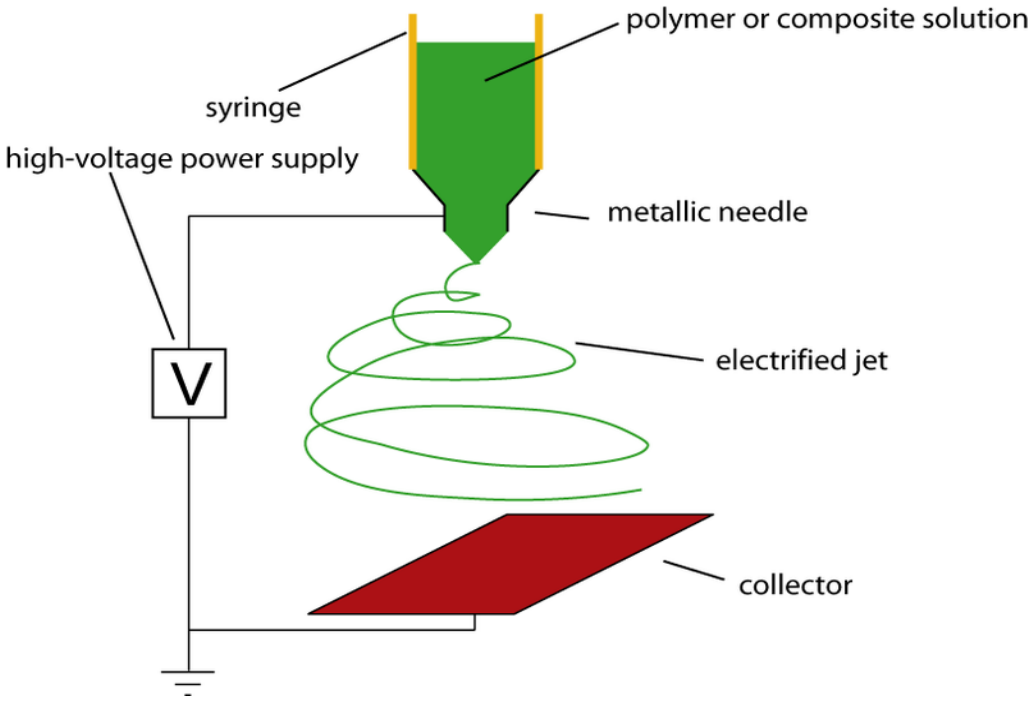

Figure 2.5 Schematic of electrospinning apparatus (25).

2.2.2.2 Principle and Operation. The process of electrospinning is based on the principle that the strong electrical forces in a charged polymer liquid overcome the weaker forces of surface tension (24). At sufficient supply of high voltage, the body of the liquid gets charged producing an electrostatic repulsion counteracting the surface tension. At this critical point, the droplet is stretched to form a stream of liquid resulting in the fibers. This point of eruption is known as 'Taylor cone' as shown in figure (2.6). A continuous stream would be possible with sufficient cohesion of the liquid (25).

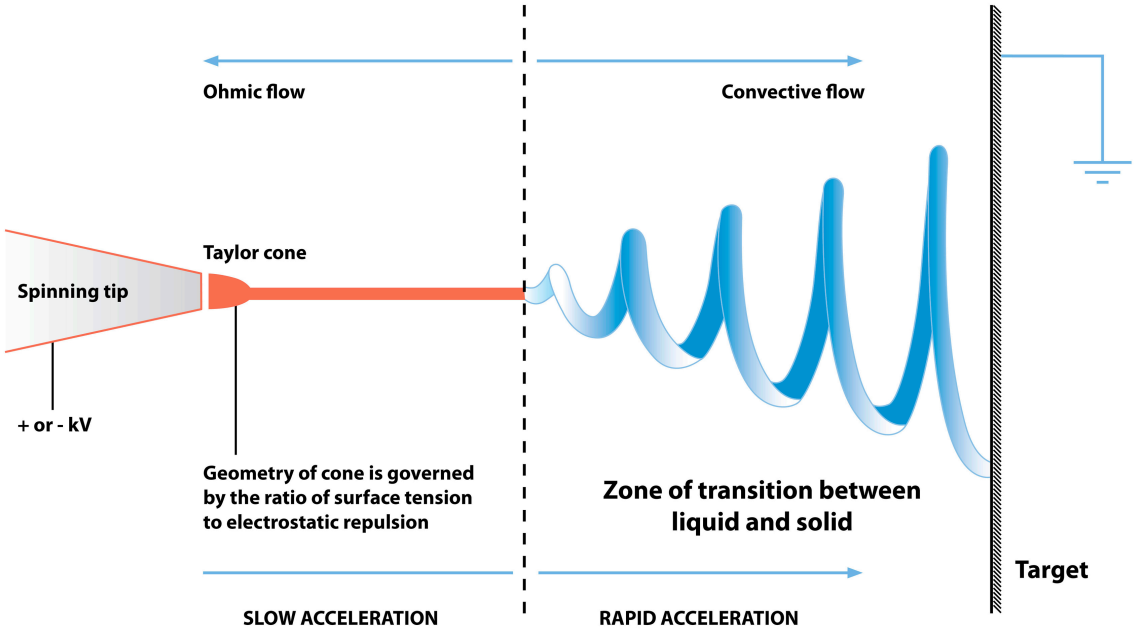

Figure 2.6 Fiber formation by electrospinning (25).

As the liquid travels from the syringe to the collector plate, the solvent is evaporated leaving behind a solid form of polymer fibers deposited on the collector.

2.2.2.3 Parameters. Electrospinning process could be influenced by the following parameters (25):

i. Polymer being electrospun
ii. Solution properties
iii. Electric potential
iv. Flow rate
v. Working distance between the spinneret and the collector
vi. Temperature and humidity
vii. Motion of the collector

2.2.3 Scanning Electron Microscope

Scanning Electron Microscope (SEM) is a type of electron microscope that used a high – energy beam of electrons to scan a sample. These electrons interact with the sample, resulting in production of signals that help in interpreting the sample's surface topography, composition and also other properties like electrical conductivity (26). The SEM with a large depth of field allows a large amount of the sample to be focused at one time. The samples can be examined at high magnification and SEM produces high-resolution images (27).

2.2.3.1 Instrumentation. As shown in figure (2.7), SEM consists of the following components (28):

i. Electron source or gun
ii. Electron lenses
iii. Sample stage

iv. Detectors

v. Display or data output devices

The electron beam is emitted by thermo-ionic emission from an electron gun mostly fitted with a tungsten filament cathode that acts as a source of electrons. It has two condenser lenses and pairs of deflection coils for scanning the electron beam. Electronic amplifiers of various types are used to amplify signals to be detected by specific detectors. The image is captured digitally and displayed on a computer monitor and saved as required (26).

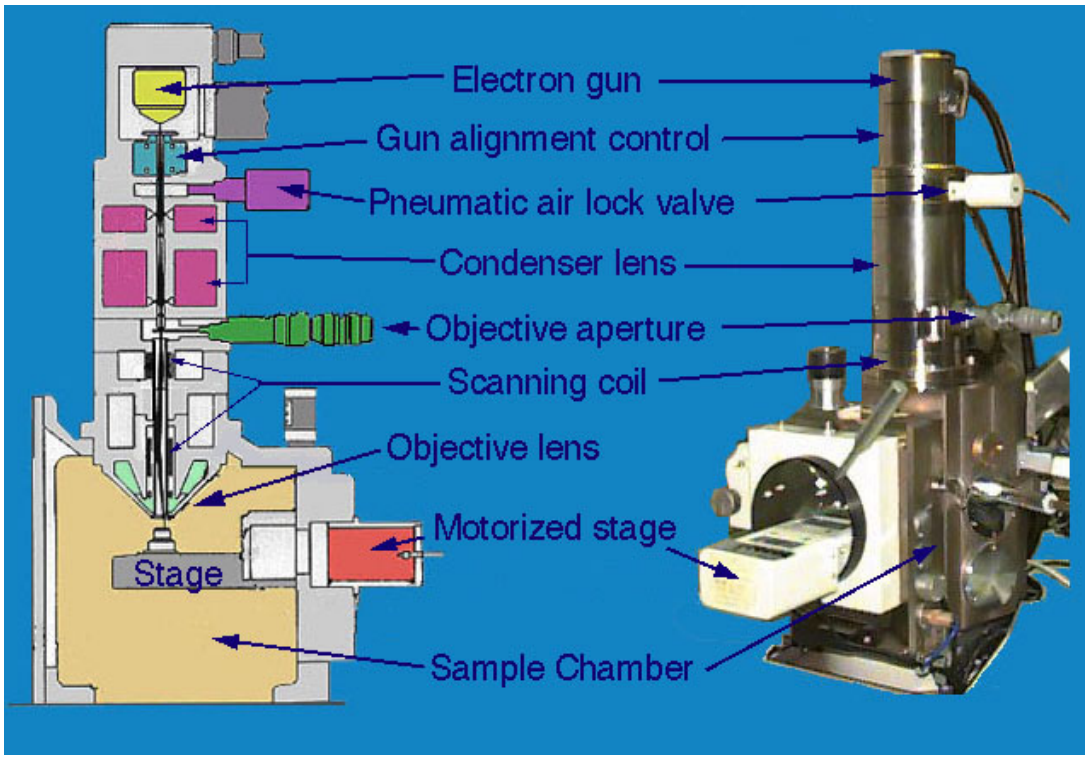

Figure 2.7 Instrumentation – Scanning Electron Microscope (29).

2.2.3.2 Principle. The high – energy incident electron beam is composed of accelerated electrons with significant amount of kinetic energy that gets dissipated during the electron – sample interactions to produce a variety of signals as shown in figure (2.8).

The signals comprises of secondary electrons, backscattered electrons, diffracted backscattered electrons, photons, visible light and heat. The secondary electrons produce SEM images and show the morphology and topography of the sample. Backscattered electrons illustrate rapid phase discrimination in multi – phase samples and diffracted backscattered electrons are used to determine crystal structures as well as orientations of light. Photons are characteristics of X – rays produced by the inelastic collisions of the incident electrons with the electrons in atoms of the sample (28).

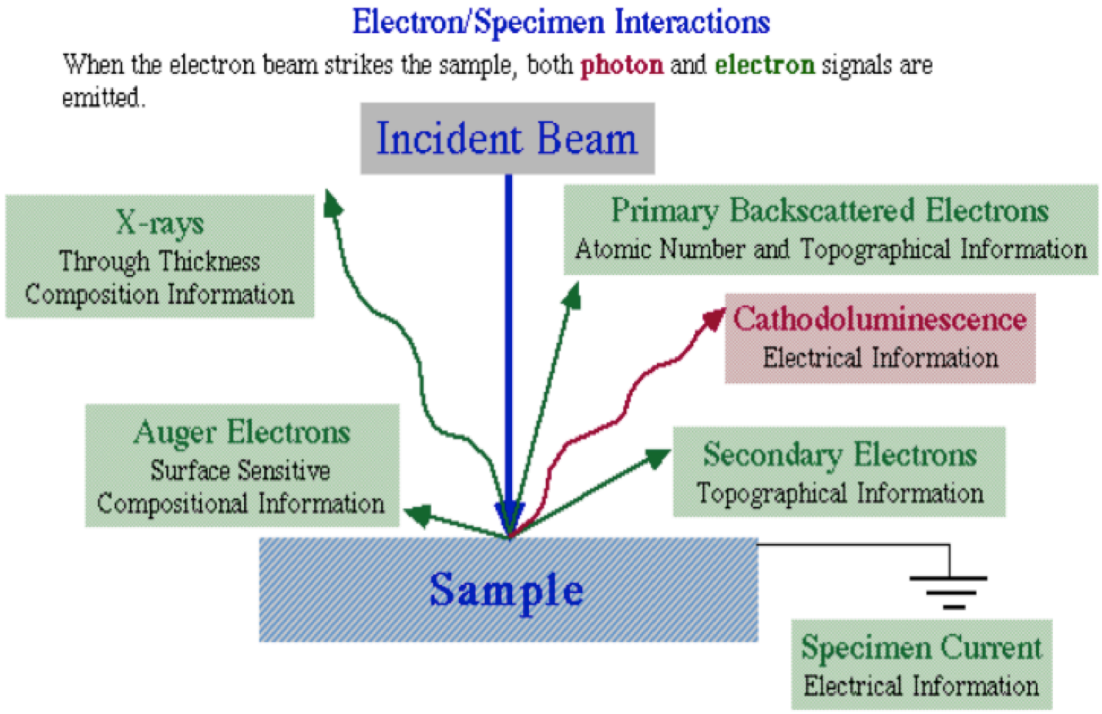

Figure 2.8 Schematic demonstration of scattering of electrons (29).

2.2.3.3 Preparation of Sample. The sample to be examined by SEM should be free from water, solvents or any other material that could get vaporized while in vacuum. Samples should be of appropriate size to be fitted in the sample chamber and firmly mounted on the stubs. Scanning using a regular SEM requires a sample to be conductive.

Non – conductive samples are coated with a thin layer of conductive layer using 'sputter coater' (26)(29)(30).

2.2.3.4 Working. The electron beam produced by the electron gun follows a vertical path through the microscope held within a vacuum. The beam passes through the lenses that focus the beam towards the sample and as it hits the sample, electrons and X – rays are emitted. These electrons and X – rays are detected by the various detectors and converted into signals that are sent to a screen giving a final image (30).

2.2.4 Ultraviolet (UV) Spectroscopy

Ultraviolet spectroscopy is an absorption in the Ultraviolet (UV) region. The light supplied from a source, is absorbed by the sample molecule in the form of radiation, which results in excitation of an electron to higher molecular orbital. This absorbance energy is plotted against the wavelength to yield a UV spectrum.

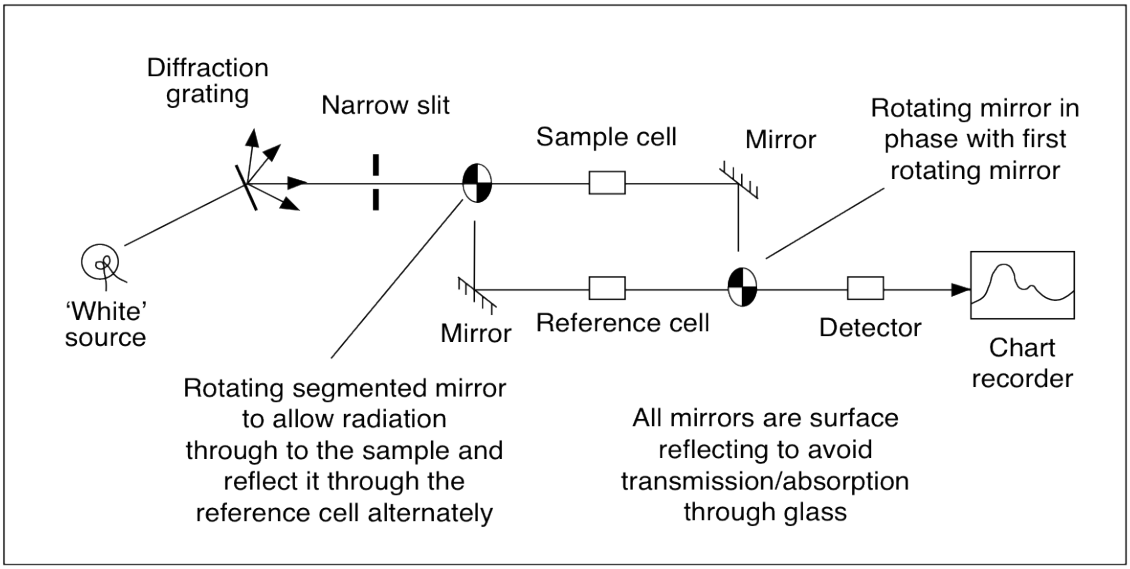

Figure 2.9 Diagrammatic representation of working of UV spectroscopy. (31)

The UV spectrophotometer consists of a sample holder with marked units for the blank and the samples. The composition of the sample cells should be considered appropriately as soda glass is known to absorb below 365 nm and also pyrex glass absorbs below 320 nm. So, the use of cells made of pure silica is recommended (31). The blank unit is fitted with a cuvette consisting of the solvent and is used to determine the baseline of the spectrum with the UV cutoff range of the solvent.

Table 2.1 UV cutoff of different solvent (32)

	UV Cutoff (nm)
Acetonitrile UV	190
Pentane	190
Water	190
Hexane UV	195
Cyclopentane	198
Cyclohexane	200
Heptane	200
Isopropyl Alcohol	205
Methanol	205
Ethyl Alcohol	210
2-Methoxyethanol	210
Methyl *t*-Butyl Ether	210
n-Propyl Alcohol	210
Trifluoroacetic Acid	210
Tetrahydrofuran UV	212
n-Butyl Alcohol	215
1,4-Dioxane	215
Ethyl Ether	215
Iso-Octane	215
n-Butyl Chloride	220
Glyme	220
Isobutyl Alcohol	220
Propylene Carbonate	220
Ethylene Dichloride	228
1,1,2-Trichlorotrifluoroethane	231
Dichloromethane	233
Chloroform	245
n-Butyl Acetate	254
Ethyl Acetate	256
Dimethyl Acetamide	268
N,*N*-Dimethylformamide	268
Dimethyl Sulfoxide	268
Toluene	284
N-Methylpyrrolidone	285
Chlorobenzene	287
o-Xylene	288
o-Dichlorobenzene	295
1,2,4-Trichlorobenzene	308
Methyl Ethyl Ketone	329
Acetone	330
Methyl Isoamyl Ketone	330
Methyl *n*-Propyl Ketone	331
Methyl Isobutyl Ketone	334

Not included:

Isopropyl Myristate
Petroleum Ether
Pyridine
Triethylamine

UV spectroscopy is used for quantitative determination of concentration using the Beer – Lambert law which states that the absorption is proportional to the number of absorbing molecules which is the concentration of absorbing molecules and the fraction of the radiation absorbed is independent of the intensity of the radiation (31) and is given by the equation -

$$A = \log_{10} (I_0 / I) = \varepsilon.c.l \qquad (2.1)$$

Where A is the measured absorbance, I_0 is the intensity of the incident light at a given wavelength, I is the transmitted intensity, l is the path length through the sample, ε is the extinction coefficient and c is the concentration of the sample (33).

The extinction coefficient, ε and the wavelength at which the maximum absorption occurs are sufficient to identify a compound, but are known to be influenced by the nature of the solvent and also by the degree of conjugation and substitution of the organic compounds. (31)

UV spectroscopy is commonly used for quantitative determination of various analytes like organic molecules, biological macromolecules, etc., as the concentration of the sample can be determined with known ε value.

CHAPTER 3

EXPERIMENTAL PROTOCOL

The dried roots of *Nardostachys jatamansi* were subjected to extraction and the extract obtained after evaporation of the solvent was used for further procedures. The initial step in the formation of the blend was to prepare different concentrations of poly (L – lactic acid solutions. The appropriate concentration was selected and different ratios of poly (L – lactic acid solution and soy were formed, electrospun to observe the convenience of electrospinning soy protein and poly (L-lactic acid) blend. The extract of *Nardostachys jatamansi* (drug extract) was mixed at 8% and 10% concentrations in soy protein solution. This solution was then mixed with poly (L-lactic acid) solution in different ratios and electrospun. The process of electrospinning was vigilantly observed and the final nanofiber mats were analyzed using Scanning Electron Microscopy (SEM) to determine the finest combination that would yield superior nanofiber mat. Further analysis of the finalized nanofiber mat was then analyzed for the presence of the drug component and the amount of the drug and to determine the possible time-release profile of the drug using UV spectroscopy.

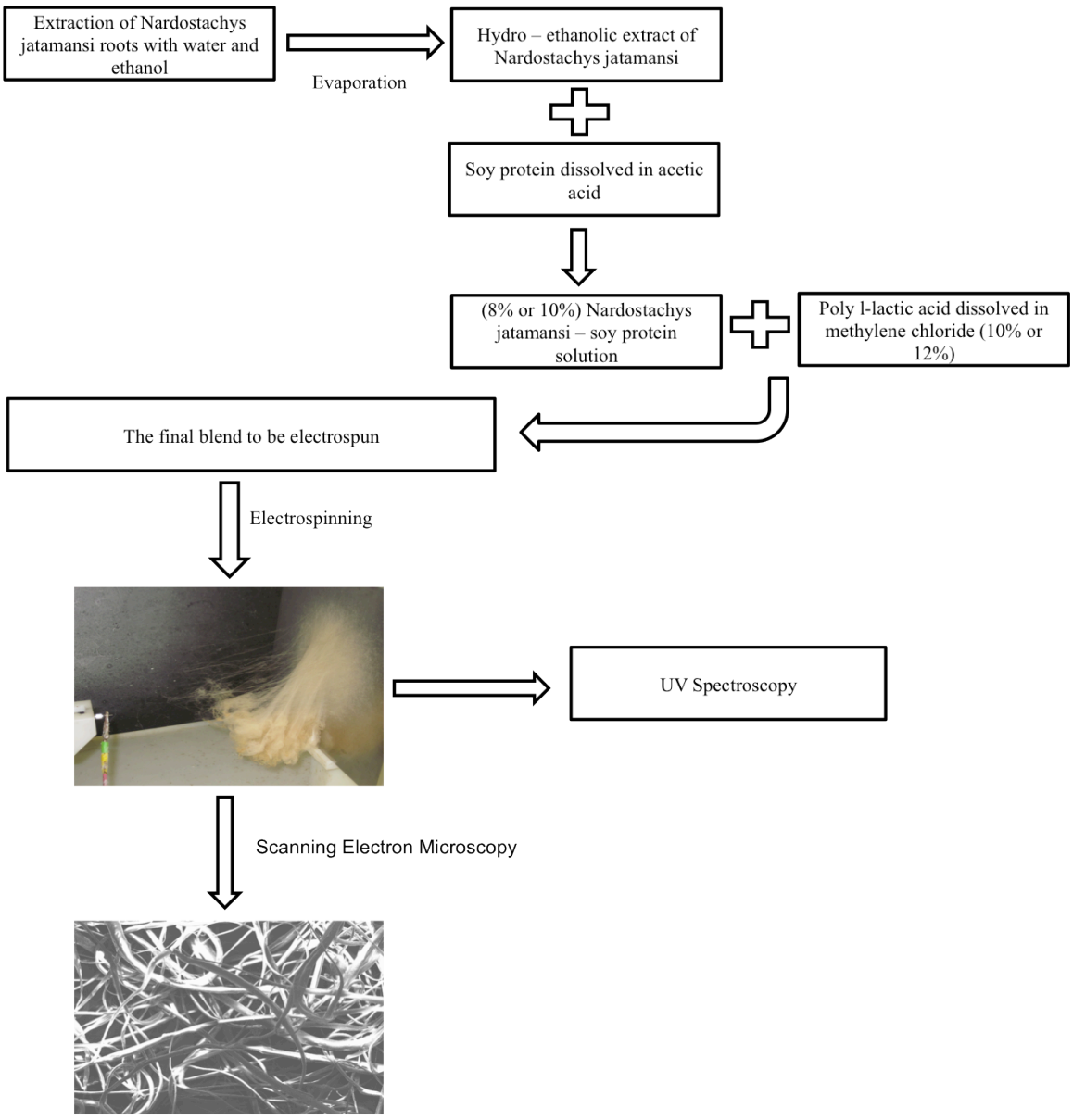

Figure 3.1 Schematic representation of the experimental protocol.

3.1 Hydro – Ethanolic Extraction of Nardostachys Jatamansi

The dried roots of *Nardostachys jatamansi* were treated with equal quantities of ethanol and distilled water using Soxhlet apparatus to undergo percolation process.

The dried roots of *Nardostachys jatamansi* were powdered and weighed quantity of the drug is placed in the thimble and loaded in the extractor tube. The extractor tube was placed on a round-bottomed flask containing a stirrer bar and equal quantities of absolute ethanol and distilled water. The condenser was placed above the extractor tube. This apparatus was placed on an electric heater and mounted on a magnetic stirrer. The extraction procedure was carried out for about a week.

The solvent was then evaporated using rotary evaporator and the drug extract was obtained. The percentage yield from the extract was about 15%.

3.2 Blend Preparation

The blend that was electrospun contained a mixture of poly (L-lactic acid) in methylene chloride and soy protein and the extract of *Nardostachys jatamansi* dissolved in acetic acid at varying ratios.

3.2.1 Poly (L – Lactic Acid) Solution

Weighed amount of poly (L-lactic acid) was dissolved in methylene chloride and 8%, 10% and 12% solutions were prepared.

3.2.2 Nardostachys Jatamansi – Soy Protein Solution

10% soy protein solution was prepared using acetic acid as solvent. Weighed amount of soy protein, 0.5M sodium sulfite, 1M Urea and few drops of sodium hydroxide were dissolved in acetic acid. It was stirred on a magnetic stirrer until a solution with uniform consistency was formed. The addition of the sodium hydroxide, sulfite and urea are known to decrease the viscosity of the soy protein solution and the sulfite added is known to break the disulfide bonds to denature the soy protein to ease the process of fiber formation at increased pH conditions (34) (35) (36). Acetic acid as solvent for soy protein has shown to form fibrous structure (37) and also the solubility of the extract of *Nardostachys jatamansi* was also considered.

Weighed amount of the hydro – ethanolic extract of *Nardostachys jatamansi* was dissolved in the 10% soy protein solution to prepare 8% and 10% *Nardostachys jatamansi* – soy protein solutions with the amount of drug being 8% and 10% respectively in the solution.

3.2.3 Final blend

The *Nardostachys jatamansi* – soy solution was dissolved in the poly (L – lactic acid) solution by varying concentrations and with various possible combinations. The solutions were stirred until a final blend of uniform consistency was obtained. The various combinations used during the experiment are illustrated in table 3.1. These blends were electrospun at constant parameters to yield the nanofiber mats.

Table 3.1 The different compositions used for electrospinning

Sl. No.	Final blend composition	*Nardostachys Jatamansi* (NJ) extract in 10% soy protein solution (%)	Poly (L – lactic acid) solution (%)	*Nardostachys jatamansi* in soy solution : Poly (L – lactic acid) solution (ratio)
1	8% NJ in soy – 10% PLLA	8	10	2 : 8
2	8% NJ in soy – 10% PLLA	8	10	3 : 7
3	8% NJ in soy – 10% PLLA	8	10	4 : 6
4	8% NJ in soy – 12% PLLA	8	12	3 : 7
5	8% NJ in soy – 12% PLLA	8	12	4 : 6
6	8% NJ in soy – 12% PLLA	8	12	5 : 5
7	10% NJ in soy	10	12	3 : 7

	– 12% PLLA			
8	10% NJ in soy – 12% PLLA	10	12	4 : 6

3.3 Electrospinning Procedure

The Electrospinning apparatus consisted of

i. A syringe containing the blend to be electrospun

ii. A high voltage supplier for constant supply of current

iii. A collector metal plate

iv. A metered syringe pump to maintain an uniform flow-rate of the solution

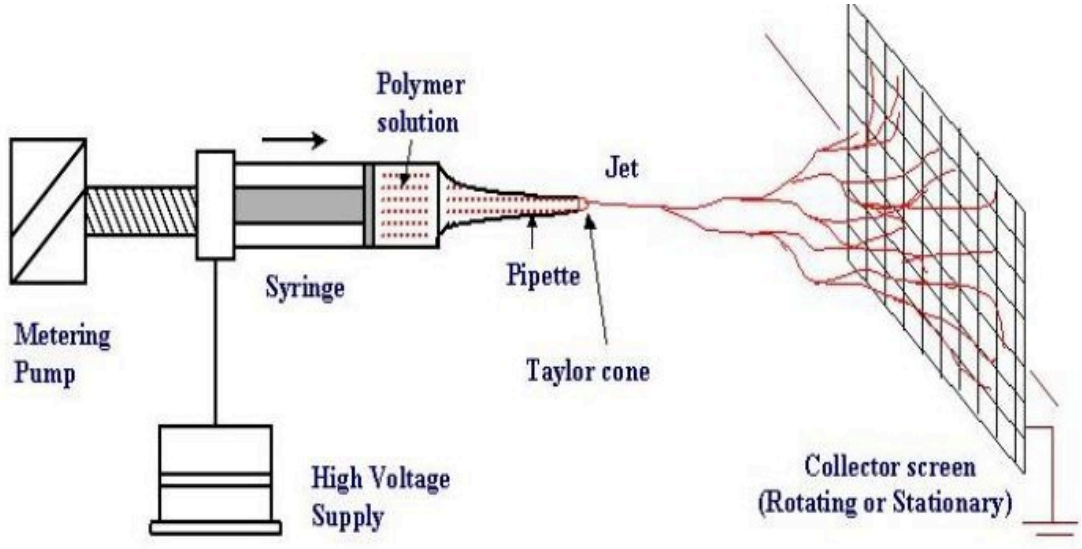

Figure 3.2 Schematic of the electrospinning set up (38).

The *Nardostachys jatamansi* – soy protein in poly (L – lactic acid) blend was placed in the syringe with a stainless steel syringe needle of gauge size 24. The needle and the collector plate were connected with the alligator clips that supplied the power supply of 25kV to the apparatus. The flow rate of the blend solution was maintained at

1ml/hr with the metered syringe pump. The working distance between the syringe needle and the collector plate was maintained at 25cm and the electrospinning was carried out at room temperature. The process was carried out for the complete volume of the blend solution in the syringe. This procedure was carried out to develop various mats with varying concentrations of poly (L – Lactic acid) and / or concentration of the drug, *Nardostachys jatamansi* extract. The electrospun fiber mat formed was stored at room temperature.

3.4 Scanning Electron Microscopy

The morphology and the size of the electrospun nanofibers were studied by scanning electron microscope (SEM).

The sample for the SEM was taken and mounted on a stub containing a carbon tape that helped for proper fixation of the sample. The stub with the sample was placed in a carbon coater and carbon thread was used to coat the samples with carbon. The stubs with the coated samples were placed in the sample holder of the SEM and were analyzed to get the SEM images at magnifications of 500X and 1000X. The SEM results helped in determination of the appropriate blend that would give a nanofiber mat with very less loss of the solution being electrospun and also with more better and distinct fibers.

3.5 UV Spectroscopy

The nanofiber mat that was determined to be superior based on the electrospinning observation and SEM results was used for the analysis of the presence of the drug using UV spectroscopy and the instrument used was Genesys 6 with VisionLite software. The wavelength of 285 nm which is considered for known maximum absorbance of jatamansone (13) (39), a major constituent of *Nardostachys jatamansi* was used for the analysis of the drug using UV spectroscopy.

The solvent used was ethanol due to a known solubility of *Nardostachys jatamansi* and also limited solubility of poly (L-lactic acid) and soy protein in it. The reference sample was prepared by dissolving the *Nardostachys jatamansi* extract in ethanol at known concentration. The nanofiber was weighed, dissolved in ethanol and stirred. The solution containing the nanofiber in ethanol was filtered using Whatman filter paper 1.

The extinction coefficient (ε) of the drug was calculated by using Beer – Lambert law. The absorbance (A) of the drug at three different wavelengths appropriate for *Nardostachys jatamansi* – 250 nm, 284 nm and 300 nm were recorded at known concentration (c) and the specific extinction coefficients were calculated. The path length (l) of the light was maintained constant at 1 cm.

$$A = \varepsilon c l \tag{3.1}$$

The extinction coefficient of the drug at specific wavelength was used to determine the yield percentage of the drug form the electrospun mat using the same Beer

– Lambert law and then calculating the percentage from the known concentration used to make the solution of the electrospun mat solution in ethanol.

The time – drug release profile was also determined at different time intervals – 2hr, 4hr, 8hr, 16hr and 24hr. Five different samples were prepared by dissolving equally weighed amount of the electrospun mat in ethanol and stirring the solution for the specified time. After the appropriate time the solution was filtered. The solutions were then analyzed by UV spectroscopy and the resultant absorbances were observed and the concentration of the drug released was determined at wavelength 284 nm which is the nearest to the wavelength characteristic of higher absorption for jatamansone, a major constituent of *Nardostachys jatamansi*.

CHAPTER 4

RESULTS

The electrospun nanofibers developed from the blend containing the *Nardostachys jatamansi* extract, Soy and Poly (L – lactic acid) were initially analyzed with Scanning Electron Microscope (SEM) to study their morphology, followed by UV Spectroscopy studies focused on the detection of the amount of the drug that could be extracted from the nanofibers.

Different nanofibers were electrospun by varying the amount of the *Nardostachys jatamansi* extract in Soy solution and/or the percent of Poly (L – lactic acid) and/or also by varying the ratio of the *Nardostachys jatamansi* extract in Soy to Poly (L – lactic acid). The various electrospun nanofibers were then analyzed and compared. The UV analysis was carried out using the nanofiber determined to be the superior from its SEM analysis.

4.1 SEM Analysis

4.1.1 Poly (L – Lactic Acid) Nanofibers

Poly (L – lactic acid) nanofibers electrospun from three different concentrations – 8%, 10% and 12% at uniform parameters, were analyzed by vigilantly observing the electrsospinning process and by scanning electron microscope to study the morphology of the fibers formed.

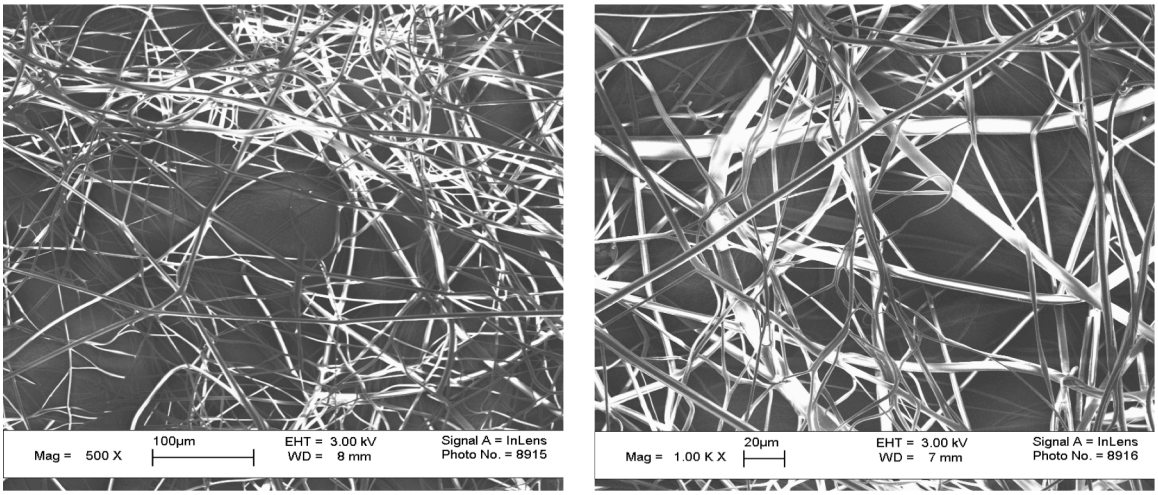

Figure 4.1 SEM analysis of 8% Poly (L – lactic acid) in methylene chloride.

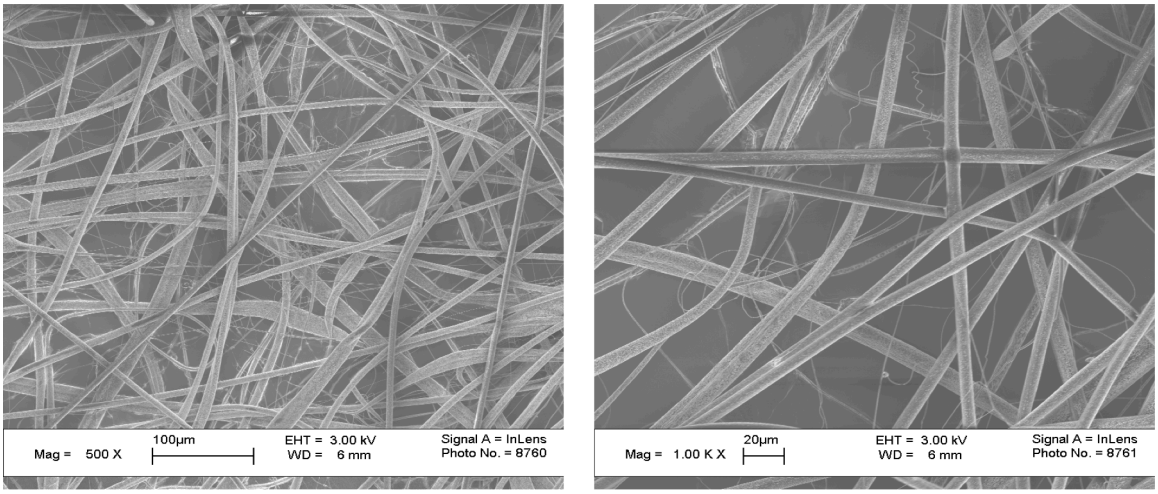

Figure 4.2 SEM analysis of 10% Poly (L – lactic acid) in methylene chloride.

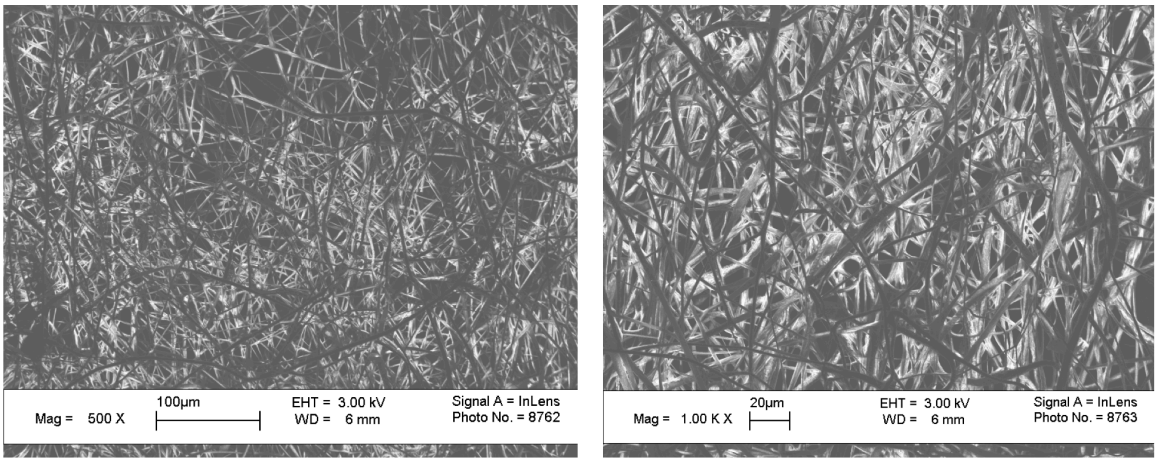

Figure 4.3 SEM analysis of 12% Poly (L – lactic acid) in methylene chloride.

Table 4.1 Observed results from the SEM analysis for PLLA

Sl. No.	Concentration of PLLA in Methylene Chloride	Observations		
		Electrospinning	SEM Analysis	
			Average diameter (µm)	S.D.
1.	8%	The nanofiber mat formed was prominent without globules.	2.71	1.34
2.	10%	The nanofiber mat formed was prominent, very distinct with better interspacing between the fibers and without globules.	3.84	2.64
3.	12%	The nanofiber mat formed was prominent without globules.	1.60	1.02

4.1.1 Soy protein - Poly (L – Lactic Acid) Nanofibers

The nanofiber mats formed from three different combination of 10% soy solution in PLLA solution were successfully electrospun. Table (4.2) depicts the results for the various combinations.

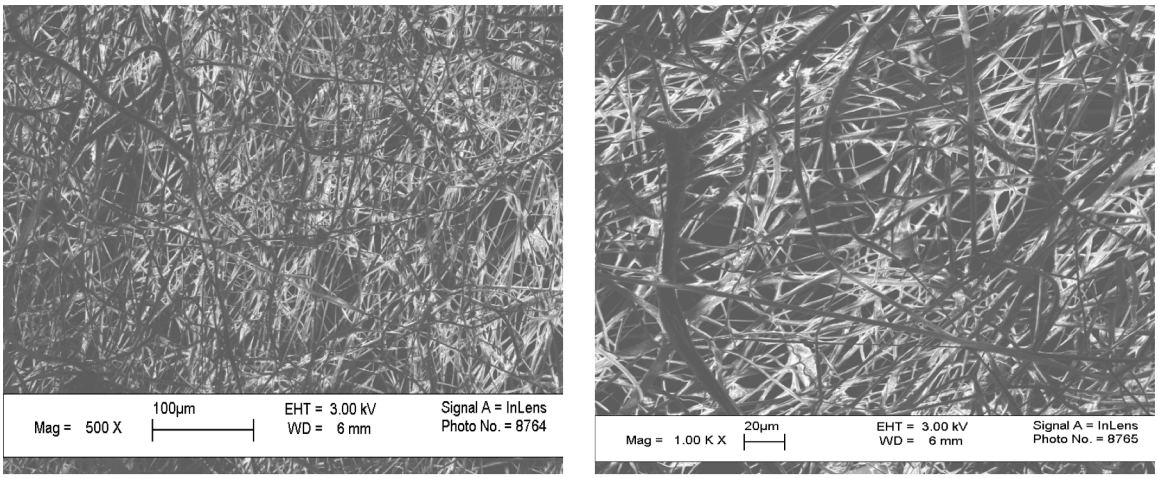

Figure 4.4 SEM analysis of (1:9) Soy protein solution in 10% Poly (L – lactic acid) solution.

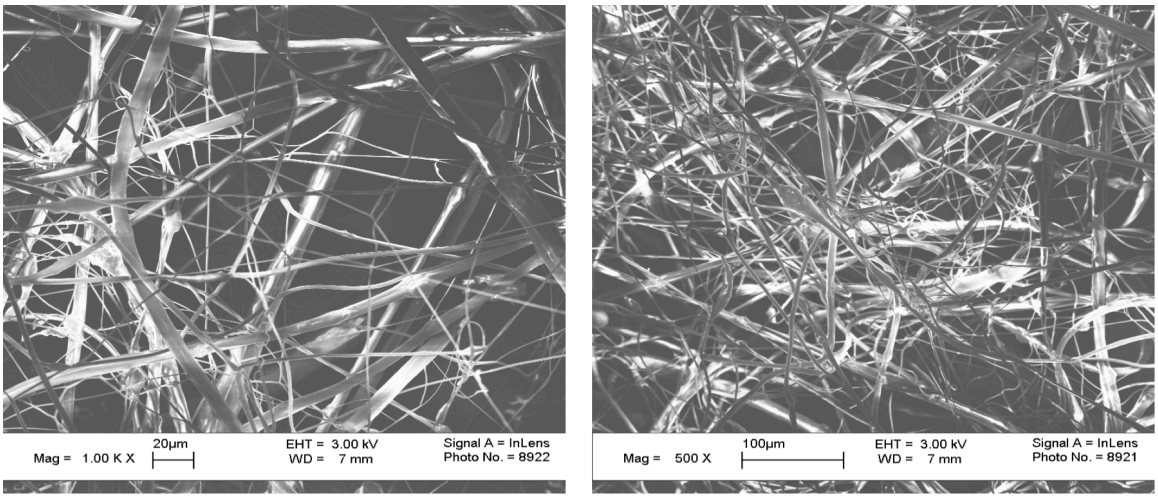

Figure 4.5 SEM analysis of (2:8) Soy protein solution in 10% Poly (L – lactic acid) solution.

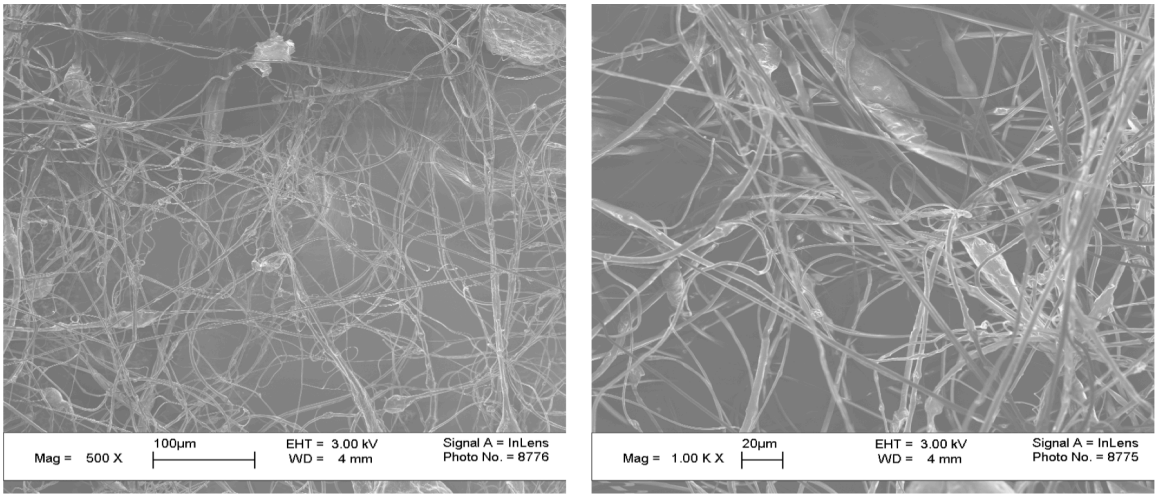

Figure 4.6 SEM analysis of (3:7) Soy protein solution in 10% Poly (L – lactic acid) solution.

Table 4.2 Observed results from the SEM analysis for Soy solution in PLLA

Sl. No.	Soy solution : PLLA	Observations	Average Diameter (µm)	S.D.
1.	1 : 9	Nanofiber mat was formed with distinct fibers.	1.21	0.55
2.	2 : 8	Nanofiber mat was formed with distinct fibers and few particles attached to the fibers.	1.67	1.02
3.	3 : 7	Nanofiber mat was formed with distinct fibers and few particles attached to the fibers.	1.45	1.53

4.1.1 *Nardostachys Jatamansi* – Soy protein in Poly (L – Lactic Acid) Nanofibers

The SEM results of the electrospun mats with 8% or 10% *Nardostachys jatamansi* solution in 10% or 12% PLLA, are as shown below and Table (4.3) depicts the results for the various combinations.

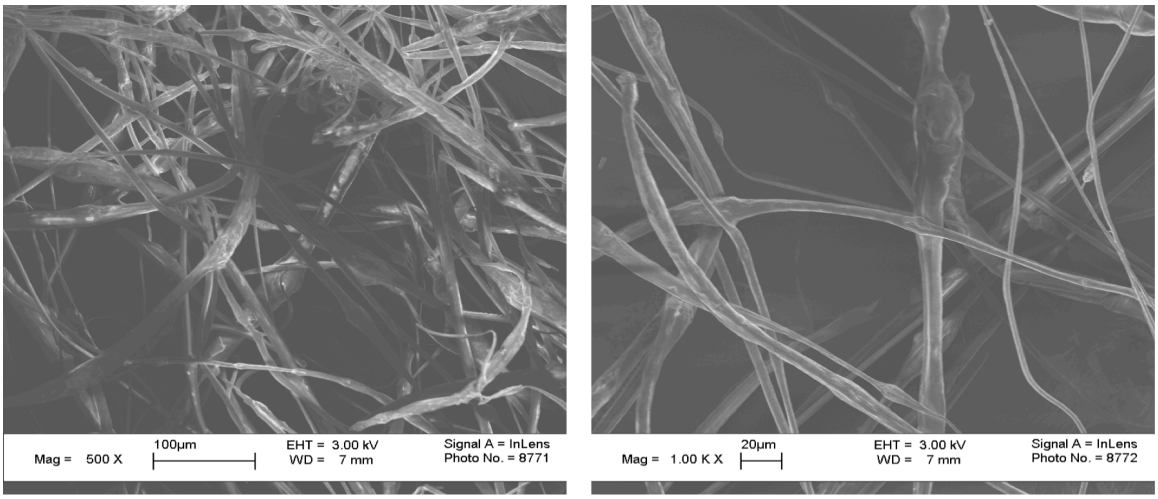

Figure 4.7 SEM analysis of (2:8) 8% *Nardostachys jatamansi* in Soy protein solution : 10% Poly (L – lactic acid) solution.

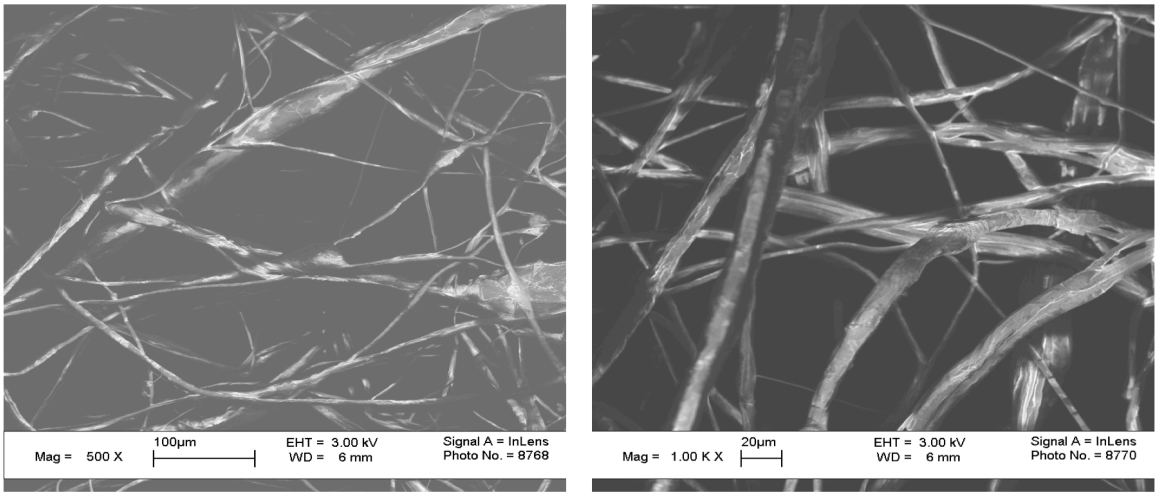

Figure 4.8 SEM analysis of (3:7) 8% *Nardostachys jatamansi* in Soy protein solution : 10% Poly (L – lactic acid) solution.

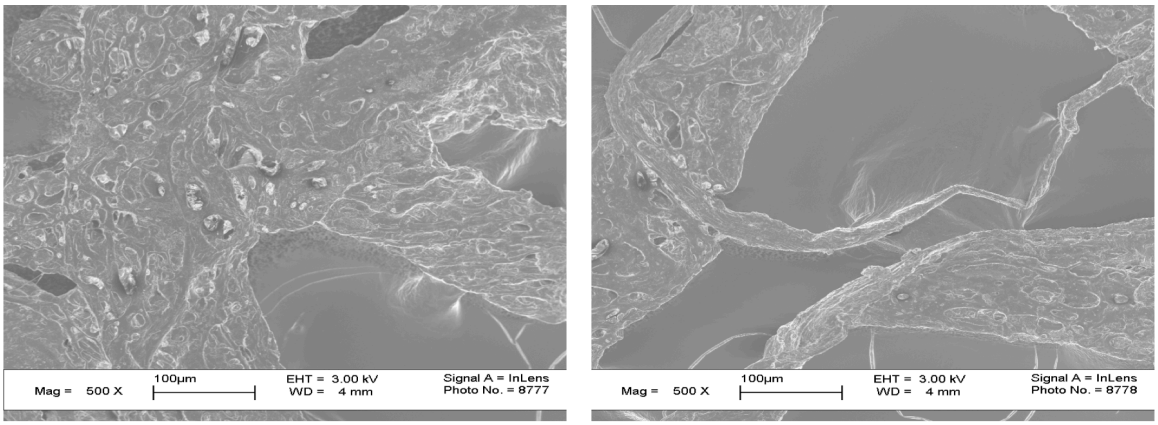

Figure 4.9 SEM analysis of (4:6) 8% *Nardostachys jatamansi* in Soy protein solution : 10% Poly (L – lactic acid) solution

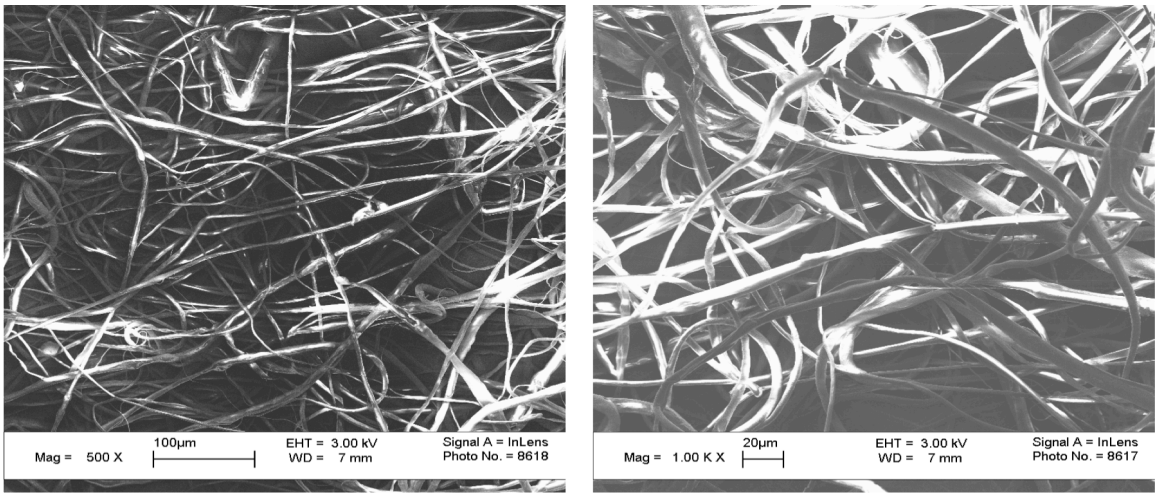

Figure 4.10 SEM analysis of (3:7) 8% *Nardostachys jatamansi* in Soy protein solution : 12% Poly (L – lactic acid) solution.

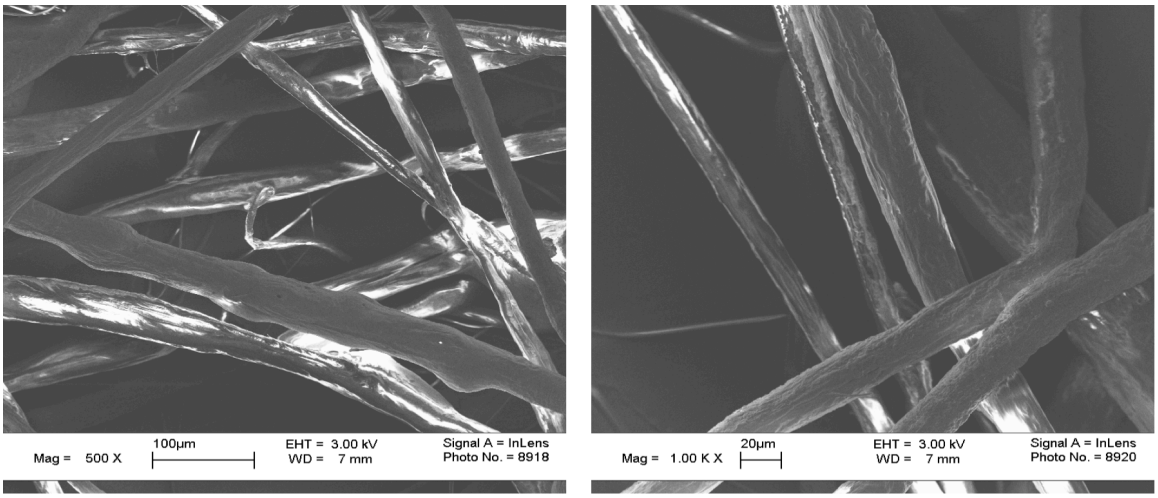

Figure 4.11 SEM analysis of (4:6) 8% *Nardostachys jatamansi* in Soy protein solution : 12% Poly (L – lactic acid) solution.

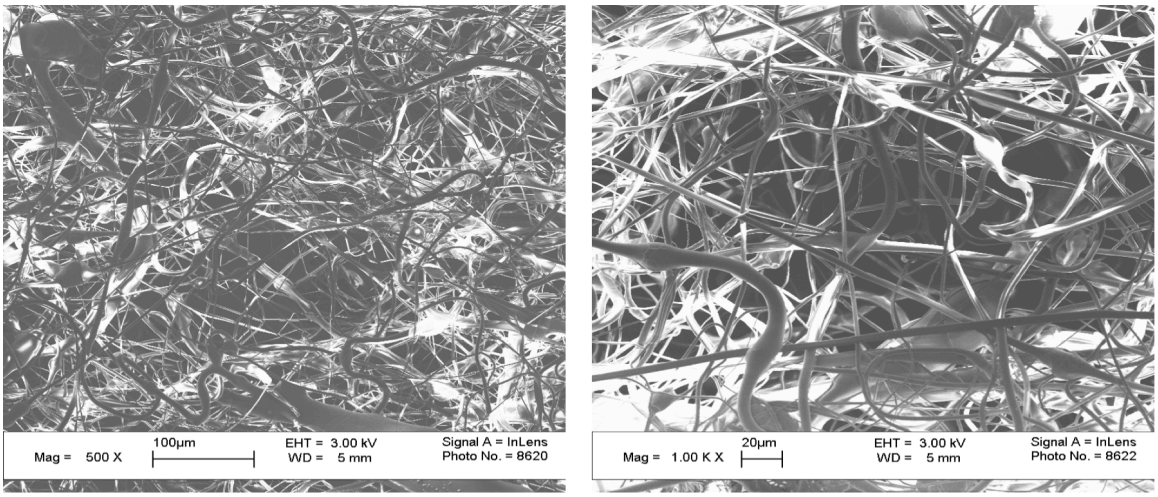

Figure 4.12 SEM analysis of (5:5) 8% *Nardostachys jatamansi* in Soy protein solution : 12% Poly (L – lactic acid) solution.

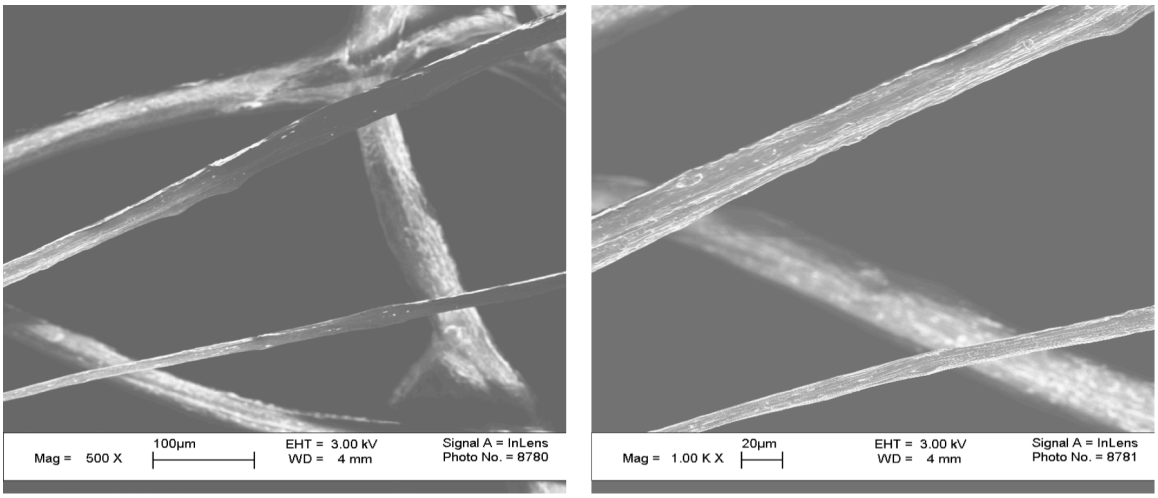

Figure 4.13 SEM analysis of (3:7) 10% *Nardostachys jatamansi* in Soy protein solution : 12% Poly (L – lactic acid) solution.

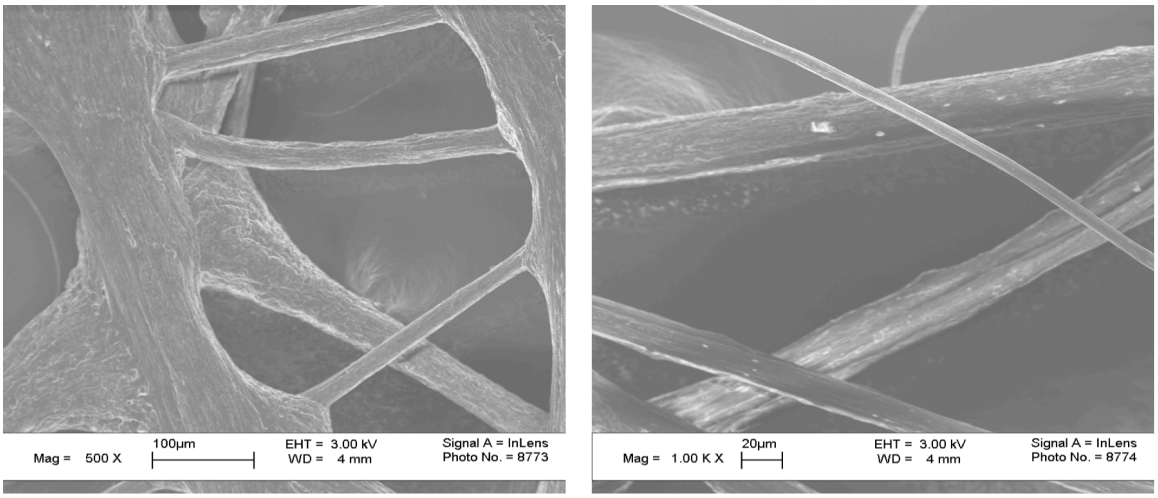

Figure 4.14 SEM analysis of (4:6) 10% *Nardostachys jatamansi* in Soy protein solution : 12% Poly (L – lactic acid) solution.

Table 4.3 Observed results from the SEM analysis for *Nardostachys Jatamansi* – Soy protein in Poly (L – Lactic Acid) Nanofibers

Sl. No.	Final blend	*Nardostachys jatamansi* in soy solution : Poly (L – lactic acid) solution (ratio)	Observations			
			Electrospinning	SEM analysis	Average Diameter (µm)	S.D.
1	8% NJ in soy : 10% PLLA	2 : 8	The flow of the solution was inconsistent but no loss of the solution due to dripping.	Very distinct fibers were visible and presence of very few globules was reported.	9.35	6.05
2	8% NJ in soy – 10% PLLA	3 : 7	The flow of the solution was inconsistent and loss of the solution due to dripping was observed.	The fibers were distinct with very few globules.	3.47	1.32
3	8% NJ in soy – 10% PLLA	4 : 6	The flow of the solution was very inconsistent and higher loss of the solution due to dripping was observed.	Fibers of very high diameter were seen and were not distinct	74.94	82.66
4	8% NJ in soy – 12% PLLA	3 : 7	No loss of solution.	Very distinct fibers with no globules.	3.47	2.65
5	8% NJ in soy – 12%	4 : 6	The flow of the solution was inconsistent.	Distinct fibers were	22.5	7.47

	PLLA			observed		
6	8% NJ in soy – 12% PLLA	5 : 5	The flow of the solution was inconsistent and loss of the solution due to dripping was observed.	Distinct fibers with limited globules.	3.06	1.23
7	10% NJ in soy – 12% PLLA	3 : 7	The flow of the solution was inconsistent and loss of the solution due to dripping was observed.	Distinct Fibers with high interspac e distance were observed.	17.34	8.47
8	10% NJ in soy – 12% PLLA	4 : 6	The flow of the solution was very inconsistent and higher loss of the solution.	Fibers of very high diameter and not highly distinct.	17.34	11.03

The SEM analysis showed that the blend containing 8% *Nardostachys jatamansi* in soy protein and 12% poly (L – lactic acid) in the ratio of 7:3 was the superior among the others and this nanofiber mat was used for the UV spectroscopy.

4.2 UV Spectroscopy

4.2.1 Determination of the drug content in the nanofiber

The absorbance of the drug extract in ethanol was observed at a wavelength range 250 nm to 550 nm and the extinction coefficient at 284 nm specific to the main constituent, jatamansone was determined with known concentration. The extinction coefficient was used to estimate the total content of the drug in the nanofiber by comparing the calculated amount of the concentration to the known concentration of the drug in nanofiber dissolved in ethanol.

Some assumptions were made during the determination of the amount of the drug content. The UV absorbance at 280 nm was considered assuming that it was specific for the major constituent, jatamansone and other chemical constituents did not absorb at the same wavelength.

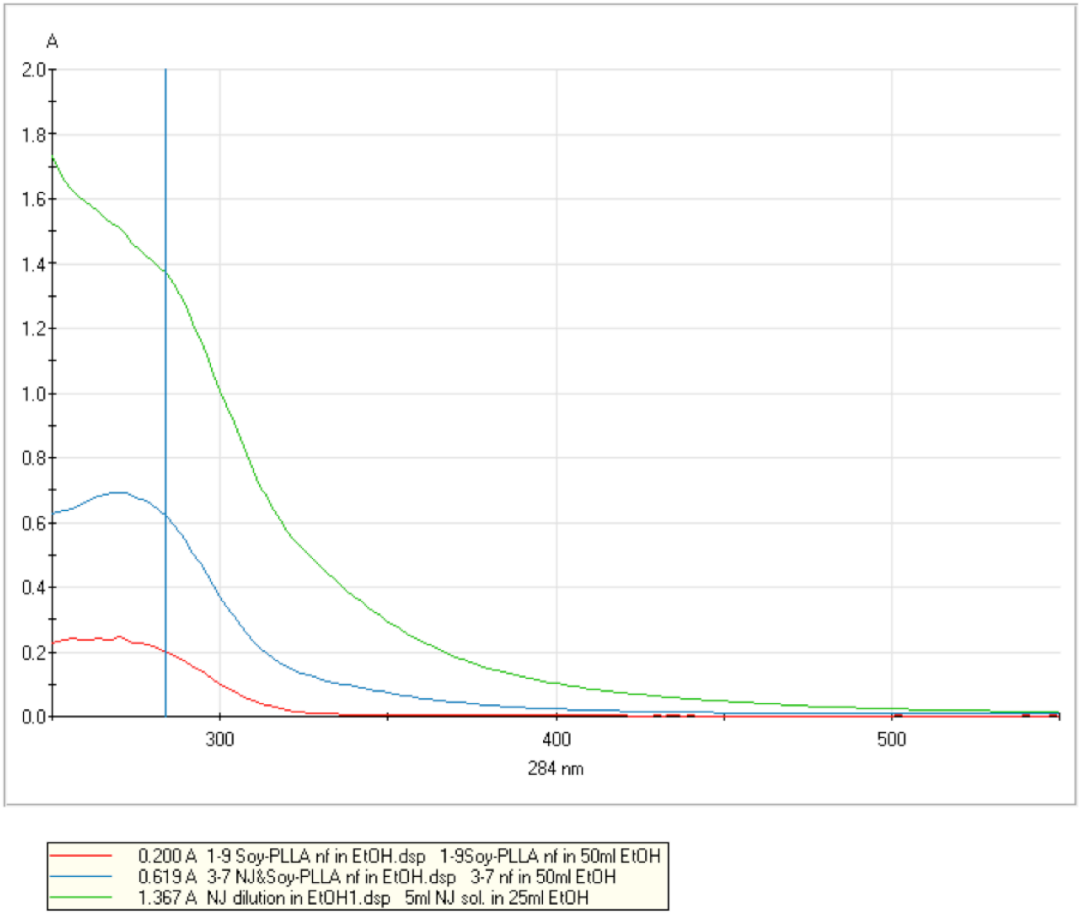

Figure 4.15 UV Absorbance at 284 nm.

As some absorbance was seen along a range of 250 nm to 300 nm, which could be due to other constituents present in the drug, the extinction coefficients were determined also at 250 nm and 300 nm.

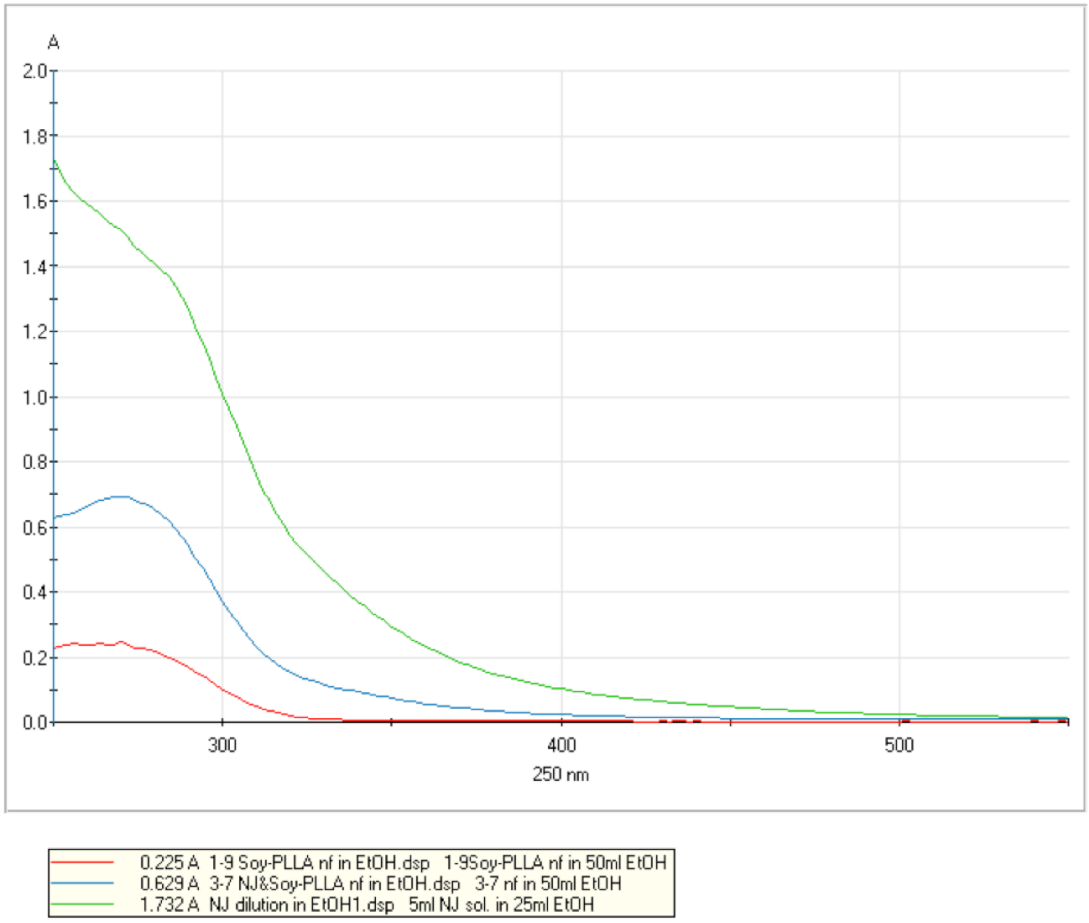

Figure 4.16 UV Absorbance at 250 nm.

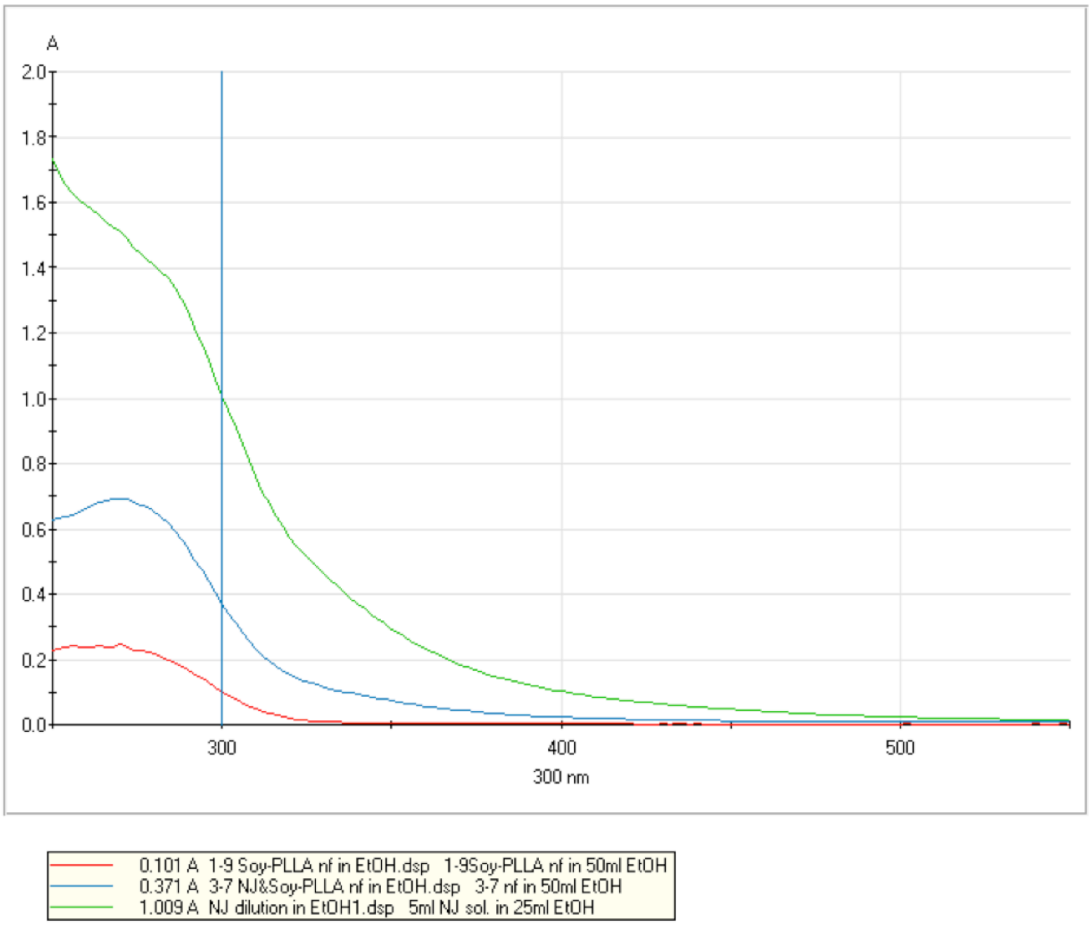

Figure 4.17 UV Absorbance at 300 nm.

Table 4.4 Calculated yield of drug (%)

Sl. No.	**Wavelength (nm)**	**ε value of NJ (ml/mg.cm)**	**Absorbance of nanofiber (A)**	**Known conc. of the drug in nanofiber (mg/ml)**	**Observed conc. of the drug in nanofiber (mg/ml)**	**Yield (%)**
1.	250	9.45	0.629	0.1140	0.0665	58.30
2.	284	7.46	0.619	0.1140	0.0829	72.71
3.	300	5.50	0.371	0.1140	0.0674	59.12

It can be observed that the yield of the major constituent, jatamansone has determined to be 72.71% indicating the presence of the drug in the nanofiber and also successful extraction of it using ethanol.

4.2.2 Time – release profile of the drug

The drug release from the nanofiber was determined at time periods – 2hr, 4hr, 8hr, 16hr and 24hr using UV spectroscopy.

The time – release profile of the drug at 284 nm with reference to the major constituent jatamansone was determined.

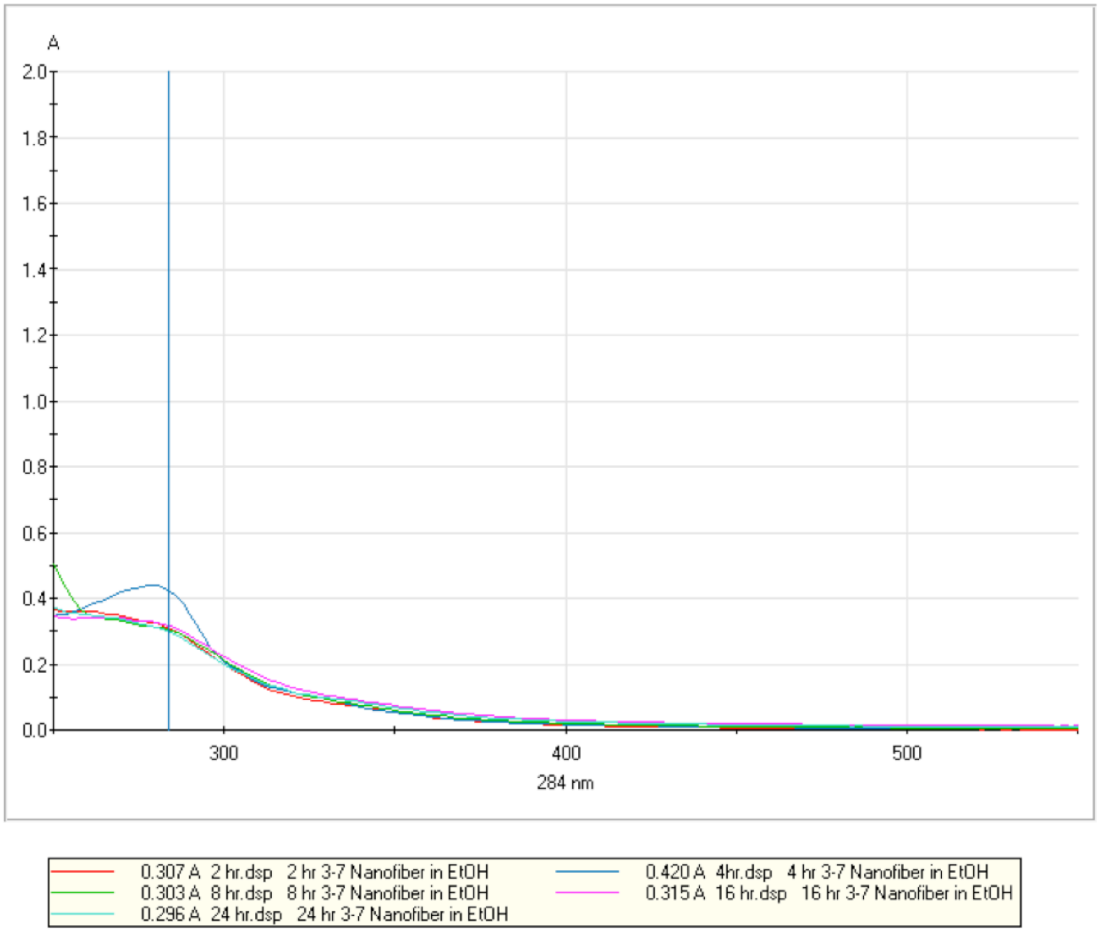

Figure 4.18 UV Absorbance at 284 nm at specific time intervals.

The absorbance observed could not well predict the release profile of the drug. It can be predicted that the unusual absorbance at for the drug sample at 4hr could possibly be an outlier due to the inconsistency of the drug content along the electrospun mat and so; the drug has completed its release within 2 hours.

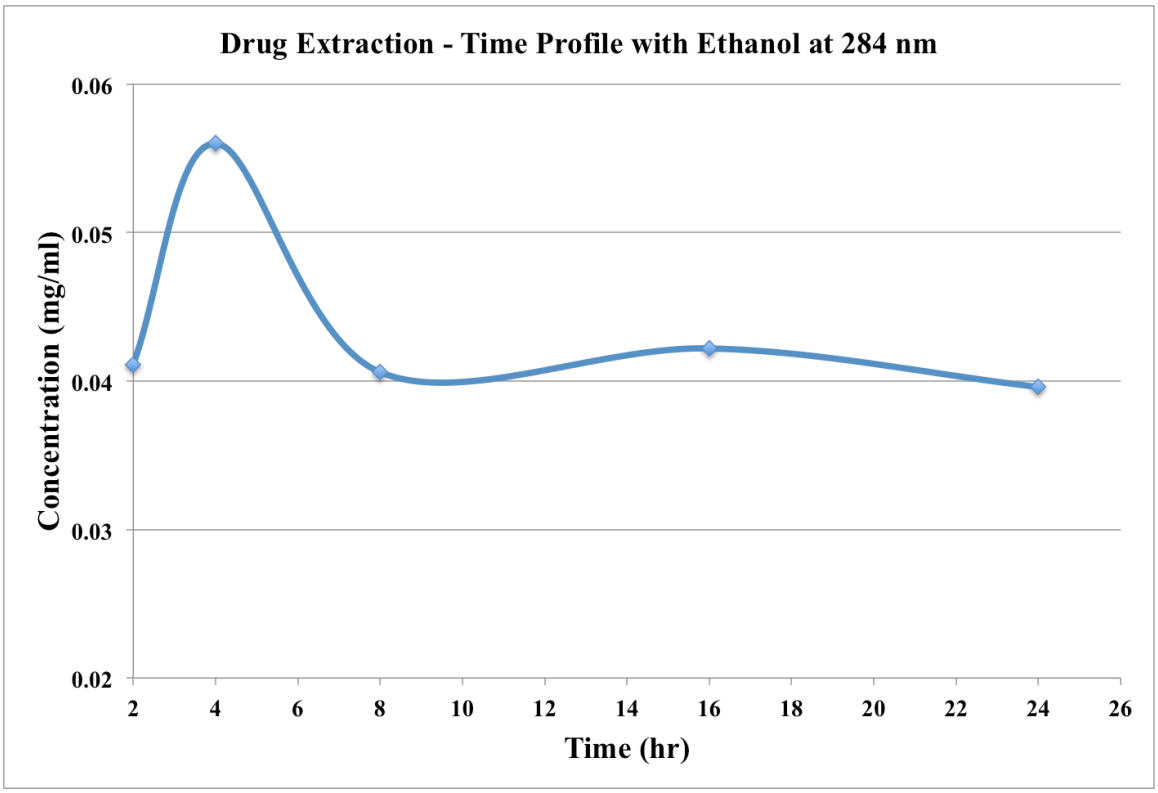

Figure 4.19 Concentration vs time using the calculated concentration of the drug at 284 nm.

Further calculations were done using a deconvolution method: Wagner – Nelson method to determine the elimination rate constant (ke) of the drug in the nanofiber and Area under the curve (AUC) using the mentioned calculations with initial (C_1) and final (C_2) concentrations.

$$ke = \text{Slope (Conc. : Time)}$$

$$AUC = \frac{(C_2 + C_1)}{2} \times (t_2 - t_1)$$

$$AUC_{0\text{-}t} = \sum_{i=0}^{t} (AUC)$$

$$AUC0\text{-}\infty = \frac{(C_t)}{ke} \times AUC_{0\text{-}t}$$

$$\text{Fraction of the drug absorbed (Fabs)} = \frac{(C_t + ke\ AUC_{0\text{-}t})}{ke\ AUC_{0\text{-}\infty}} \quad (4.1)$$

Table 4.5 Wagner – Nelson Method

Time (hr)	**C%**	**C** (mg/ml)	**AUC** (t1 to t2)	**AUC** (0-t)	**Fabs**
0	0	0	0.0411	0.0411	0.00023965
2	39.9	0.0411	0.0971	0.1382	0.40022274
4	54.4	0.056	0.1932	0.2903	0.54591039
8	39.5	0.0406	0.3312	0.5244	0.39761554
16	41.0	0.0422	0.3272	0.6584	0.41394596
24	38.5	0.0396	-0.4752	-0.148	0.38397667

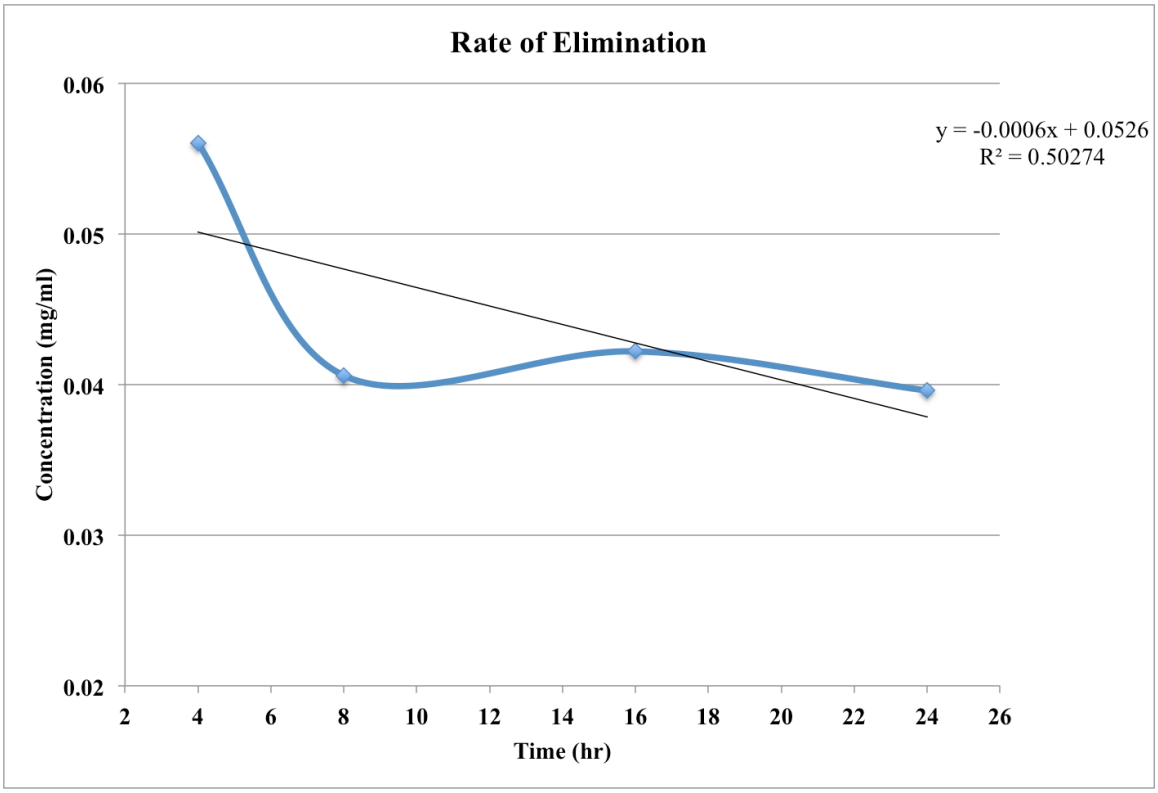

Figure 4.20 Concentration vs time to calculate the rate of elimination.

From the above calculations the elimination rate constant was calculated to be 0.0006/hr. This would be useful to calculate invivo profile if used with an ethanol based drug delivery system.

CHAPTER 5

DISCUSSION

The successful electrospinning of a blend containing *Nardostachys jatamansi* and also the determination of the drug content in the electrospun mat has set new avenues for future use of it along with biodegradable polymers for better and improved drug delivery. These electrospun mats could be investigated to determine an appropriate delivery system to improvise the treatment of central nervous system diseases like Parkinson's disease.

During the electrospinning process with the various concentrations of the drug in soy protein solution and their various combination ratios, it can be noticed that with increase in the concentration of the poly (L – lactic acid) solution from 10% to 12% has resulted in very discrete fibrous mat. The poly (L – lactic acid) is known for its use to produce nanofibers should be considered as the factor that is responsible in developing the nanofibers and providing the base for their production. Thus, if higher concentrations of the drug were desired to be electrospun then increasing the percentage of the poly (L – lactic acid) in methylene chloride and combining it at relevant ratio would yield distinct nanofibers.

The UV analysis was also performed using chloroform as solvent, but it was considered inappropriate as poly (L – lactic acid) also shows higher solubility with chloroform and this resulted in complete dissolving of the electrospun mat leaving behind possible soy protein. When analyzed, the results were found to be very noisy and thus could not be considered accurate. The UV absorption observed is characteristic of only the main component, jatamansone of the drug, which can be reported for its presence in the electrospun mat also and there are other constituents that should be analyzed,

resulting in demand for a design of process for investigation of this electrospun mat further. The choloroform solution of the drug showed distinct separation of compounds with Thin Layer Chromatography (TLC), which was not seen with the drug solution in ethanol. The drug, *Nardostachys jatamansi* is known for its complex chemical constituency and hence characterization of it would require many more methods like gas chromatography, NMR, HPLC, etc. However, there is no reference that could be referred for the characterization of the drug content and its constituents that are extracted into ethanol from the electrospun mat, making its characterization complicated.

CHAPTER 6

CONCLUSIONS

The experiments performed and the analysis of the results obtained concludes that:

- Electrospinning of the extract of *Nardostachys jatamansi* in soy protein along with poly (L – lactic acid) resulted in successful formation of electrsospun mat at appropriate concentration of the compounds.
- The scanning electron microscopy of the selected superior nanofiber mat showed no presence of globules indicating a continuous process for its formation.
- The presence of the drug was also successfully determined by using the UV spectroscopy that distinctly showed similar absorbance between the pure drug solution and the electrospun solution.
- The drug – release profile of the drug also helped in determining the predicted elimination rate of the drug that can be useful for future applications.

CHAPTER 7

FUTURE RESEARCH

The present research work has opened new avenues to be investigated.

- The same electrospun mat with the appropriate concentration should be investigated for the presence of various other chemical constituents of *Nardostachys jatamansi* with the use of different analytical methods.
- More research to determine an appropriate drug delivery system that would improve the release of the drug from fiber into the body should be highly considered.
- Similar research with varying concentrations of the drug and also the polymers to explore new possibilities should be carried out.
- The electrospun mat should also be investigated for its dissolution profile specific to the biological environment resulting in in vitro and in vivo studies that could improvise treatment of various diseases.

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