

Design and Development of Brahmi Extract Loaded Nanofibers for Cognitive Disorder and Its Optimization

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ABSTRACT

Cognitive disorders (CDs), also known as neurocognitive disorders (NCDs) are a category of mental health disorders that primarily affect cognitive abilities including learning, memory, perception, and problem solving. Global population with depression ranges mostly between 2% and 6% around the world today. Mental health disorders are also attributed to significant number of indirect deaths through suicide and self-harm. The aim of designing a drug delivery system is to enable drug release at a controlled rate over a desired period. nanofibers, with their large specific surface areas, can improve the solubility and dissolution rates of drugs, thereby resulting in fast release of poorly soluble active drugs. Drug release from nanofibers in terms of processing setup and modulate the release kinetics, to achieve site of action with increased plasma half life and resulting increasing residence time of drug in plasma, slowing down rapid renal clearance and helping extended presence of drug at the site of action and also minimize the toxicity of drug. Bacopa monniera an Ayurvedic drug which is mainly used for health practices. Polyvinylpyrrolidone as synthetic polymer was preferably used as a carrier for preparation of nanofibers. Nanofibers mainly prepared by using synthetic polymer because it does not have any toxic effect. Pre-formulation studies were carried out. Experimental design based on trial and error base. Total three batches were prepared using chitosan, cellulose acetate and Polyvinylpyrrolidone. FT-IR Spectroscopy study showed that there was no interaction between drug and polymer. Nanofibers were prepared by using electrospinning method. After preparation of nanofibers were subjected to various evolution parameters includes practical yield, in-vitro drug release, drug entrapment efficiency, SEM, XRD, Zeta Potential, FTIR. The formulations were optimized with respect to In-Vitro drug release and drug entrapment efficiency. Among the all formulations, the Brahmi Extract loaded PVP nanofibers batch F1 containing 300mg of brahmi extract and 600 mg polymer was optimized because this batch showed 97.57% drug release, 99.24% drug entrapment efficiency, -26.4mV Zeta potential of the optimized batch which is mainly used to check the stability of the nanofibers formulation. An optimized formulation *brahmi extract* loaded nanofibers batch F1 is well acceptable, pleasant, palatable and with better compliance.

Keywords : Cognitive disorders, brahmi extract loaded PVP nanofibers, Scanning electron microscopy, Zeta potential, In-Vitro drug release, drug entrapment efficiency practical yield, XRD, FTIR.

I. INTRODUCTION

Cognitive improvement typically acts as a toxicological and psychological tool.²⁰⁻²³ The medium of nootropic phytochemicals discovered in Bacopa

monnieri (BM), mainly triperpenoid saponins called bacosides, exhibit minimal observable shows minimal observable negative impacts at ordinary doses. BM shows anti-oxidant,²⁴ hepatoprotective,²⁵ and neuroprotective²⁶ activity. Emerging analysis

shows lots of mechanisms of action of brahmi extract —acetylcholinesterase inhibition, choline acetyltransferase activation, β -amyloid reduction, increased cerebral blood flow and monoamine potentiation. *Bacopa monnieri* (also known as brahmi, water hyssop, *Bacopa monniera*, and *Herpestis monniera*), is a creeping perennial with small oblong leaves and purple flowers, found in warm wetlands, and native to Australia and India. Commonly found as a weed in rice fields, BM grows throughout East Asia and the United States.²⁷

Phytochemical based antioxidants may have neuroprotective (preventing apoptosis) and neuroregenerative roles,²⁸ by decreasing or reversing cellular damage and by slowing of neuronal cell loss development. In nature, AOX are categorized as endogenous or exogenous. The endogenous group comprises of enzymes such as superoxidase demotes (Zinc, manganese, and copper), glutathione peroxide (selenium) and catalus and albumin, transferin, ceruloplasmin, metallothiynin and haptoglobin. AOX offer a promising strategy to control or slowing down progressive of neurodegenerative disorders such as Alzheimer's disease, parkinson's disease, huntington's disease, amyotrophic lateral sclerosis, ischaemic and haemorrhagic stroke.^{29, 30}

Several methods, such as the formation of nanoparticles, micelles and liposomes, have been recorded to improve curcumin bioavailability. Although these novel techniques are very promising, some constraints to their apps are still being practiced. The preparation technology and circumstances of curcumin nanoparticles are relatively complicated, so these particles are not appropriate for comprehensive manufacturing. Besides this, the biocompatibility of some of the polymers used in micelles and liposomes is still unknown and therefore this formulation cannot be implemented in clinical therapy. Furthermore, the structural stability and loading

capacities of these formulations are also regarded as limitations for their use in practice.³³

Electrospinning is a versatile and compatible technique to use a large prospective electrical field to create ongoing nanofibers from submicron diameters down to nanometer diameters. Nanofibers with diameters varying from 1-100 nanometers can be produced.^{34, 35} These methods can be readily used in the laboratory and can be cultivated in industrial processes³⁶. The asspun fibers are mostly gathered in the form of nonwoven nanofiber mat on electrode collectors. It is also feasible to achieve aligned nanofibers using supervised fiber deposition techniques³⁷. Optimal nanofibers can be formed by regulating solution, process and ambient parameters, as these parameters determine the features of electrospun fibers.³⁸ The engineering of nanofibers is an significant component of nanotechnology. For the first time more than four decades before electrostatics were created nanofibers. Nanofibers were developed more than four decades before electrostatics. Nanofibers have nanometer fibers in the range of diameters.³⁹ Nanofibers can be produced with distinct polymers and therefore nanofibers have distinct physical characteristics and application strengths. Over the past two decades, ultrafine structures including microspheres and nanostructures such as nanoparticles and nanospheres have been of interest in pharmaceutical science for the formulation of new drug delivery systems, Because of their controllable elevated surface area, surface-to-volume ratio, high porosity and elevated drug loading and controlled payload capacities.^{40,41} Natural polymers include collagen, cellulose, silk, fibroin, keratin, gelatin, and polysaccharides such as chitosan and alginate.⁴² Electrospinning is the most helpful method and can generate ultrafine diameter nanofibers and well-ordered surface morphologies.⁴³

Advantages of nanofibers⁴⁶

1. High surface area to volume ratio
2. Ease of fiber functionalization
3. Ease of material combination
4. Relatively low start up cost
5. Low learning curve for basic electrospinning
6. Ease of fiber deposition onto other substrates
7. Variety of nanofibrous structures have been constructed
8. Mass production capability demonstrated
9. Commercial applications:
 - a) Air filtration membrane
 - b) Face mask
 - c) Water filtration membrane
 - d) Tissue engineering
 - e) Drug delivery
 - f) Clothing
 - g) Wound dressing
 - h) Battery

OBJECTIVES

In this study, our goal was to prepare Brahmi Extract loaded nanofibers by electrospinning using polyvinylpyrrolidone (PVP). Furthermore, evaluation of various important factors, such as the applied voltage, feed rate, and the concentrations of the polymer and the drug, was used to determine the effects of these parameters on the different properties of the resulting nanofibers. In addition, in terms of drug-release characterization, the nanofibers are compared to the solid dispersion preparation obtained from the freeze-drying technique.

II. METHODS AND MATERIAL

Preparation of Electrospinning Solution

To prepare the initial solution, 30 and 35 g of PVP were dissolved in 100 mL of ethanol. The polymeric solutions were placed on a magnetic stirrer for 15

minutes at room temperature; subsequently, loratadine was added to this solution at ratios of 1: 2 and 1: 4 relative to the weight of the polymer and mixed until the loratadine was completely dissolved.

• Method for Preparation of Brahmi extract loaded PVP nanofibers

The Brahmi extract loaded nanofibers were prepared by electrospinning technique in ethanol. The PVP polymer was selected for the preparation of Nanofibers. Brahmi extract loaded chitosan nanofibers and cellulose acetate nanofibers were prepared. These batches were failed because there was no formation of nanofibers. The Brahmi extract loaded polyvinylpyrrolidone nanofibers was formed. PVP nanofibers Solution containing 300mg concentration of Brahmi extract with 600mg Polyvinylpyrrolidone(Batch F1), 300 mg concentration of Brahmi extract with 800mg Polyvinylpyrrolidone(Batch F2) and 300mg concentration of Brahmi extract with 500mg Polyvinylpyrrolidone(Batch F3) such as (1:2, 1:4 etc) were added 99%ethanol into PVP-Brahmi extract sample stir by using magnetic stirrer at 50°C for 90 min. Then these solutions were placed in ultrasonic bath for 20 min. After that solution added to 20 ml plastic syringe of 10mm diameter and syringe was placed in syringe pump and the was connected with high voltage supply. The flow rate were 0.8ml/hr, tip to collector distance 150mm, applied voltage 9 KV and needle diameter 0.7mm. After electrospinning process, the nanofibers were removed from aluminum foil and store in Desicator for further study.

• Formulation of Brahmi extracts loaded nanofibers based on trial and error base

Table 1.1 : Formulation table of Brahmi extract loaded nanofibers

Sr No	Batch code	Drug Conc. (mg)	Polymer concentration (mg)	Solvent Used (mL)	Flow rate (mL)	Needle Diameter (nm)	Voltage (Kv)	Drum speed (rpm)	Distance bet ⁿ tip and collector (mm)	Result
A. By Using Polyvinylpyrrolidone(PVP)										
1	F1	300	600	Ethanol + Dis. Water	1.5	10	9	500	150	formed
2	F2	300	800		0.9	10	10	500	140	
3	F3	300	500		2	10	12	500	150	

• **Characterization of *Brahmi extract* loaded nanofibers**

1. Practical Yield

The % practical yield of all prepared *Brahmi extract* loaded nanofibers were calculated. For determination of % practical yield following formula was used

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

2. Drug Entrapment efficiency⁷⁸

The entrapment efficiencies of prepared systems were determined by measuring the concentration of free drug was determined by adding 10 mg of nanofibers to 9.9 ml ethanol (95%) in order to dissolve the untrapped drug; the obtained suspension was centrifuged for 45 min at 2000 rpm. The Supernatant was separated and then filtered through filter paper (0.2µm). The filtrate was diluted using ethanol and measured spectrophotometrically at 286 nm. The entrapment efficiency was calculated using the following equation.

$$\text{Entrapment efficiency (\%)} = \frac{\text{Weight of total drug} - \text{weight of free drug} \times 100}{\text{Weight of total drug}}$$

3. In-vitro drug release study⁷⁷

The drug release of the 10 mg of *Brahmi extract* loaded PVP nanofibers were measured in order to determine the release profile of the drugs. Samples of *Brahmi extract* loaded nanofibers sheet were immersed in phosphate buffered saline solution ph 7.4 containing 0.5% sodium dodecyl sulphate at 37°C and samples of PVP-*Brahmi extract* were immersed in PBS only at 37°C. This release study was based on the continuous stirring method. The solutions were agitated using a shaker at a rate of 80 rev/min. the UV absorbance of the drug was measured at *Brahmi extract* 286 nm respectively. Measurements were taken at intervals at 4min, 8 min, 12min, 16min, 20min, 24min, 28min, 32min, 36min and 40 min. At each point, 4 ml of solution was taken from the vial and replaced with fresh in order to satisfy the perfect-sink conditions and keeping the volume of the solution constant. By using this procedure an in-vitro drug release of three batches were studied.

4. UV-Vis spectroscopy

The UV-Vis spectrum of *Brahmi extract* and the *Brahmi extract* drug loaded PVP nanofibers conjugates were measured with a UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan) to provide direct evidence of the attachment of *brahmi extract* to the PVP. The absorbance at 786 nm was

used to determine the concentration of **Brahmi extract** in Drug loaded PVP nanofibers using a visible spectrophotometer (Shimadzu, Kyoto, Japan).

5. Zeta potential determination:

A 10 mg aliquot of *Brahmi extract* loaded PVP nanofibers diluted in 0.5 ml of ethanol and 9.5 ml of ultra pure water. After the shaking of this solution properly wait until the proper mixing of the solution. Then clear solution was taken from the mixture and by using the zetasizer zeta potential was determined of optimized batch F1. From the optimized batch sample zeta potential of the *Brahmi extract* drug loaded PVP nanofibers were determined this diluted sample was transferred to a cuvette and the zeta potential was measured in triplicate using a Delsa™ Nano zetasizer.

6. FT IR Spectroscopy

To determine and interpret functional groups in *brahmi extract* loaded PVP nanofibers. FTIR of procured sample of drug loaded PVP nanofibers was studied by directly taking the drug loaded nanofibers on the ATR disk and proceed for the next step to monitoring data which shows the peaks on particular wavelengths. After taking of graphs peaks was found and taken the results for it. The analysis of IR peaks was done by JASCO 4600, Japan spectrometer. From the results data were interpreted with the standard values.

7. Scanning electron microscopy

SEM test is carried out for the change in morphology of *Brahmi extract* loaded PVP nanofibers images were performed on a JSM 6360 images clearly illustrated in different figures, show that the nanofibers synthesized in this study display widely different morphologies according to the values and the concentrations of variable parameters. The SEM image taken at 20 kV with the magnification 15 KX,

5KX. These samples however display different mutual orientations.

8. X- Ray diffraction Spectra

The particle size and nature of the *Brahmi extract* loaded PVP nanofibers was determined using X-Ray diffraction. XRD is a rapid analytical technique primarily used for crystalline phase identification and then provide information on unit cell dimensions. Sample was scanned in the angular range of 100 to 600 in instrument. The drug loaded nanofibers was placed into instrument and X-ray passed through sample and then results were noted.

9. Short term stability study

The purpose of stability testing is to provide evidence on how the quality of *Brahmi extract* loaded PVP nanofibers with time under influence of a variety of environmental factors such as temperature and humidity. Optimized batch F1 selected for short term stability study. Stability studies were carried out for the optimized formulation by storing the formulation at temperature $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$ and $65\%\pm 5\%$ relative humidity in stability chamber. The samples were withdrawn periodically after 30 days. Entrapment efficiency and *in-vitro* drug release was estimated for three months to find any change in the entrapment efficiency drug release kinetics of the prepared *Brahmi extract* loaded PVP nanofibers.

III. RESULTS AND DISCUSSION OF BRAHMI EXTRACT LOADED PVP NANOFIBERS

The prepared batches of Brahmi extract loaded nanofibers were characterized by percentage practical yield, particle size, entrapment efficiency and In-vitro drug release study. From the obtained results, specific batch was selected as optimized batch.

1. Percentage practical yield

The results of percent practical yield studies are shown in Table 9.8. The Nanofibers are prepared by

solvent electrospinning method. From the different ratio of drug, and Polyvinylpyrrolidone, ratio 1:2 shows maximum practical yield. It was found that electrospinning method gives the practical yield in the range of 62-35%. The maximum yield was found 61.70% in *Brahmi extract* loaded PVP nanofibers batch F1.

Table.1.2 : Practical yield of *Brahmi extract* loaded PVP nanofibers

Sr. no	Batch code	% Yield
1	F1	61.70%
2	F2	38.11%
3	F3	34.95%

2. Determination of entrapment efficiency of *Brahmi extract* loaded PVP nanofibers

The entrapment efficiency of prepared nanofibers was carried out by ultra centrifugation method. The drug: PVP (Polyvinylpyrrolidone) ratio 1:2 found to be maximum drug entrapped. The batch F1 shows maximum drug content capacity. The drug entrapment efficiency was found to be 91.60%. This result is summarized in Table no 9.9.

Table 1.3 : % Entrapment efficiency of *Brahmi extract* loaded PVP nanofibers

Sr.No	Batch Code	%Drug Entrapment Efficiency
1	F1	99.44
2	F2	98.03
3	F3	97.76

3. In-Vitro drug release study of *Brahmi extract* loaded PVP nanofibers

Table no 9.13 shows the comparison between drug release rates of all the batches. From the observation

it is clearly seen that the drug release rate of batch F1 is highest by graphical representation in comparison with remaining 2 batches F2 and F3.

Table 1.4 % Drug release of *Brahmi extract* drug loaded PVP nanofibers of all the batches

Sr. No	Time (min)	F1 (%)	F2 (%)	F3 (%)
1	0	0	0	0
2	4	24.50	16.27	18.12
3	8	30.89	20.38	22.03
4	12	47.95	46.75	31.75
5	16	54.78	46.95	33.77
6	20	57.25	54.37	38.10
7	24	62.19	55.60	41.60
8	28	67.55	60.34	45.51
9	32	70.02	63.63	48.81
10	36	76.40	63.22	52.51
11	40	97.52	63.22	52.92

IV. RESULTS AND DISCUSSION

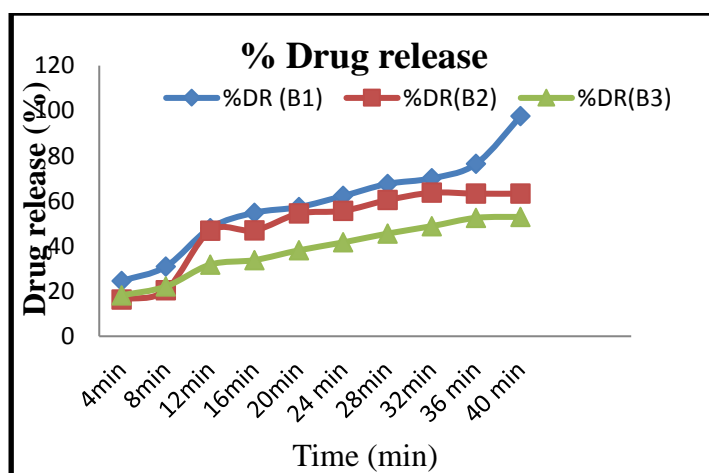


Figure 1.1 : Comparative Graphical Presentation of *in-vitro* % drug release for *Brahmi extract* loaded PVP nanofibers

4. FTIR of pure *Brahmi extract* loaded nanofibers

The IR spectra of pure drug *Brahmi extract* loaded PVP nanofibers shows sharp peak at 1741.41 cm⁻¹ which shows presence of C=O stretch of ester group. 1650.77 cm⁻¹ shows presence of medium N-H primary amine. There is presence of C-N stretch of aliphatic amine at 1027.5 cm⁻¹. C-O stretch of alcohol

is presented at 1157.08 cm⁻¹. There is sharp peak at 2923.58 cm⁻¹ which shows the alkene (=CH bend). Figure 9.8 shows FTIR spectra of *Brahmi Extract* in the range of 4000 cm⁻¹ to 400 cm⁻¹ and Table 9.11 shows the data interpretation of *Brahmi extract* loaded PVP nanofibers.

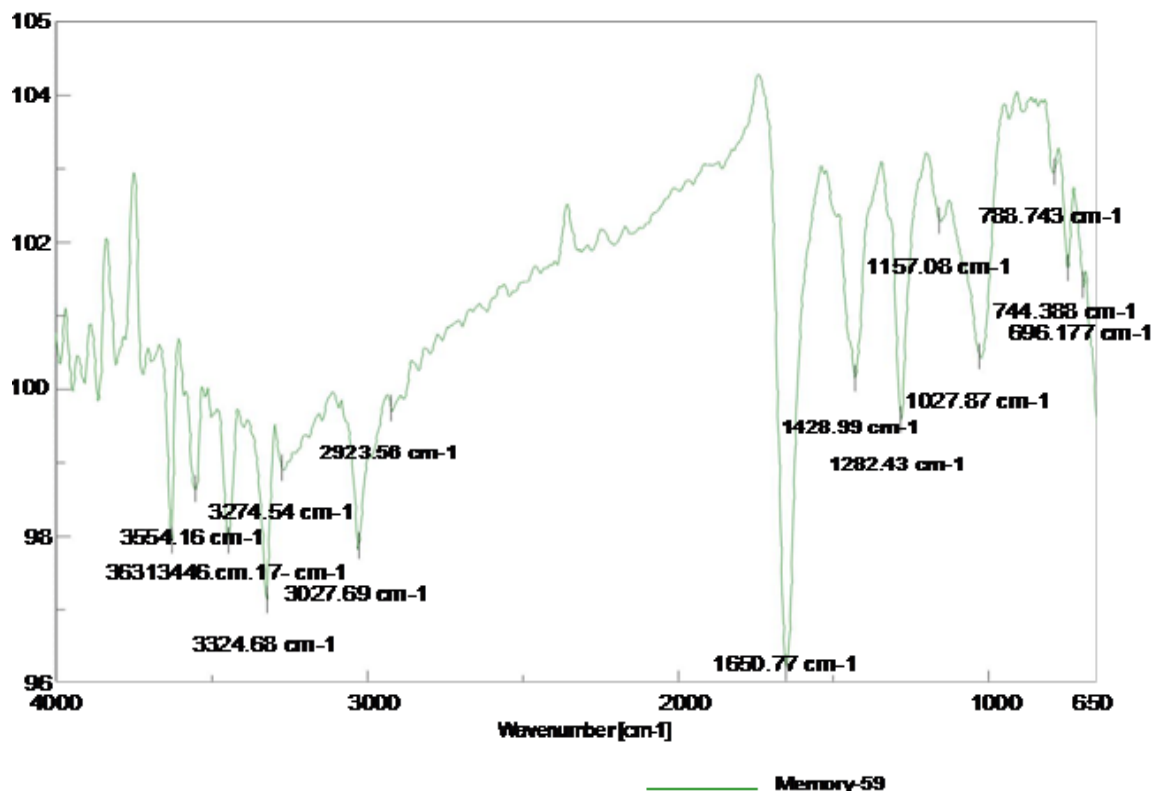


Figure 1.2 : IR Spectra of *Brahmi extract* loaded Polyvinylpyrrolidone nanofibers

Table 1.5 : IR data interpretation of *Brahmi extract* loaded PVP nanofibers

Sr. No	Wave number (cm ⁻¹)	Vibration	Functional Group
1	696.177	Bending	C-H monosubstituted
2	744.388	Bending	C-H 1,4 disubstituted
3	788.744	Stretching	C=C alkene
4	1027.87	Stretching	C-N secondary amide
5	1157.08	Stretching	C-O primary alcohol
6	1282.79	Stretching	C-O primary alcohol

7	1650.77	Bending	C=O secondary amide
8	2923.68	Stretching	C-H alkene
9	3027.69	Stretching	O-H alcohol
10	3274.54	Stretching	O-H alcohol
11	3446.0	Stretching	O-H primary alcohol
12	3554.16	Stretching	O-H alcohol
13	3631.17	Stretching	O-H alcohol

5. Zeta Potential

Optimized nanofibers were analyzed to determine their zeta potential values. The zeta potential value of nanofibers ranging between 25mV to -35mV shows good stability of fibers. It was observed that for optimized formulation of *Brahmi extract* loaded nanofibers batch F1 zeta potential value was found to be -26.7 mV indicating good stability of the formulation. Figure 9.9 shows zeta potential of optimized batch.

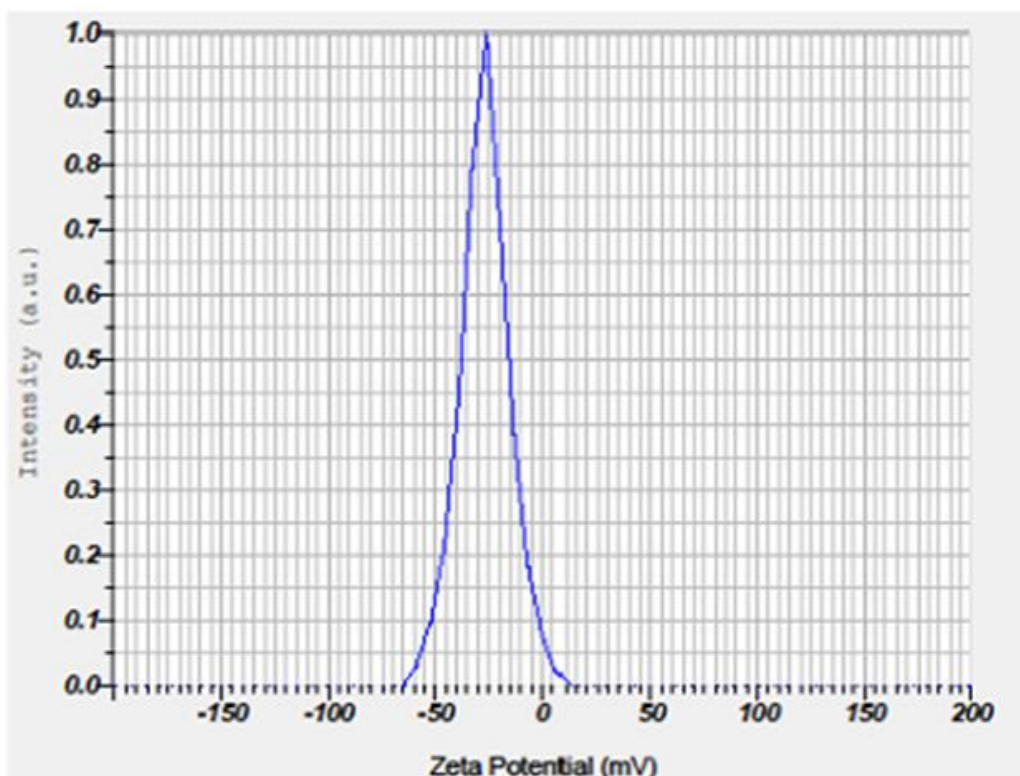
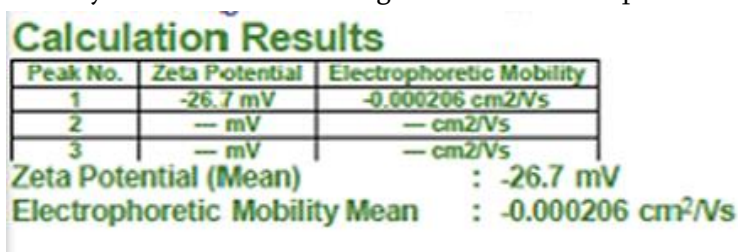


Figure 1.3 : zeta potential of *Brahmi extract* loaded nanofibers

6. UV visible Spectroscopy

The UV absorbance was taken on Shimadzu UV spectrophotometer and the absorbance of before and after the drug loading reaction was taken as the process going through drug loading mechanism. The absorbance after drug loading was found to be 0.350 nm.

7. Scanning Electron Microscopy

The surface morphology of Brahmi Extract Loaded PVP Nanofibers as shown in Figure 9.12 (a), and 9.13(b) indicates the presence of fiber shape of complex. Result of surface characterization reveals that uniform fiber shape of Brahmi Extract Loaded PVP nanofibers with 450nm at 15KX shows optimum uniform size.

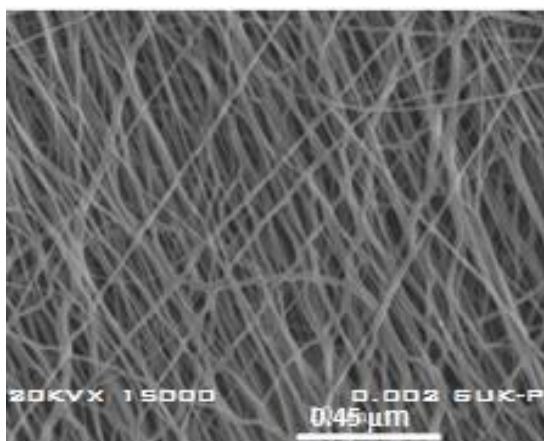


Figure 1.4 a : Morphology of *Brahmi extract* loaded nanofiber

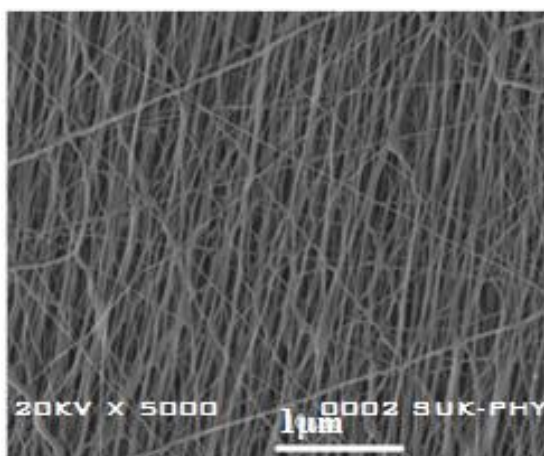


Figure 1.4 b : Morphology of *Brahmi Extract* loaded nanofibers

8. X- Ray diffraction

Figure 9.10 shows XRD spectra of prepared Nanofibers. Dried sample was analyzed under XRD instrument the result was shown that given sample contains crystalline material in the sample. A sharper peak indicates that more crystalline structure present in the sample.

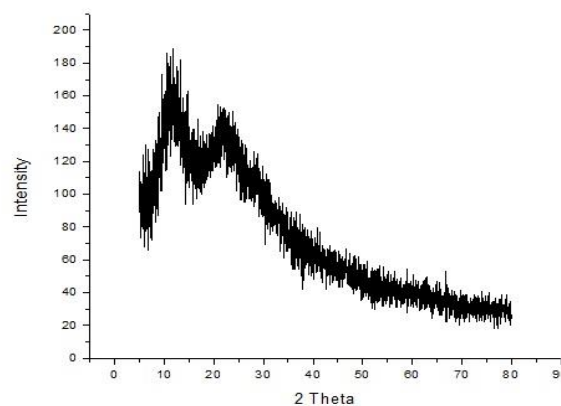


Figure 1.5 : XRD Pattern of *Brahmi extract* of prepared nanofibers

9. Short Term Stability studies of optimized formulation

Stability studies were conducted on *Brahmi Extract* loaded nanofibers optimized formulation. The sample was withdrawn periodically after 30 days. Stability studies conducted for 90 days. After performing the stability study it was observed that at intermediate stability condition there was no change in the entrapment efficiency as well as drug release from prepared *Brahmi extract* loaded PVP nanofibers. From this study it is concluded that the prepared *Brahmi extract* loaded nanofibers formulation were stable during storage. Observed data of stability studies of optimized formulation shown in Table 1.6

Table 1.6 : Stability study of optimized *Brahmi extract* loaded PVP nanofibers batch F1

Formulation	Test	Immediately after preparation	30th Day 25°C±2°C/ 65%±5%	60thDay 25°C±2°C/ 65%±5%	90th Day 25°C±2°C/ 65%±5%
Batch 9	Entrapment efficiency	99.44%	99.10%	99.01%	98.95%
	<i>In vitro</i> drug release	97.52%	97.35%	97.13%	97.01%

V. SUMMARY AND CONCLUSION

Based on preformulation studies for various tests such as organoleptic properties, UV and FTIR spectroscopic study, drugs and excipients were characterized. The study of compatibility with drugs and excipients was conducted. Drugs and excipients were discovered to be consistent with the outcomes to each other. There was not formation of any new compound.

The nanofibers batches F1 to F3 were ready with the use trial and error method. Brahmi extract Electrospinning technique was used to prepare charged nanofibers. In all the lots of formulations F1 to F3, drug concentration was kept constant and polymer concentration was varied. From obtained results of all nanofibers batches it is drug: polymer concentration is required.

The prepared nanofibers batches (F1 to F3) were characterized for different parameters like % practical yield, entrapment efficiency, and determination of zeta potential, Electron microscopy scanning, X-ray diffraction research and nanofibers Prepared drug release in vitro research. From the various drug ratio: polymer ratio, 1:2 ratio indicates maximum effectiveness of drug trapping and release of drug in vitro.

The entrapment efficiency of Ultracentrifugation technique was used to prepare for the nanofibers. The effectiveness of drug trapping was discovered to be 99.40% and 97.54% of drug release in vitro. From this the brahmi extract loaded PVP nanofibers batch F1 batch. The optimized batch F1 further evaluated for determination of, spectroscopic analysis of X-ray diffraction.

For the good stability of prepared nanofibers formulation, Nanofibers ' zeta potential should range from 25mv to -35mv. To determine their zeta potential values, optimized nanofibers were evaluated. It has been noted for optimized formulation brahmi extract loaded PVP nanofibers batch F1 has zeta potential value – 26.4 mV An indication of excellent formulation stability. Using scanning electron microscopy, Drug lipid and polymer complex (nanofibers) surface morphology was conducted. The result demonstrates that fiber shape. was found in the in vitro release of drugs research depends upon optimum drug: polymer ratio and particle size of prepared nanofibers.

The formulation Brahmi extracts loaded nanofibers batch F1 containing the optimum drug: polymer ratio 1: 2, shows the maximum drug release from nanofibers batch F1 within 40 min. As we increase the concentration of polymer there was decreases the

drug entrapment of drug in polymeric nanofibers and also decreases the drug release.

From all results, formulation Brahmi extract loaded PVP nanofibers batch F1 containing 1:2 drug: polymer ratio was optimized formulation having maximum drug release 97.52% within period of 40 min. with maximum drug entrapment efficiency that is 99.44% and zeta potential -26.4mV showing good stability of formulation. The nature of prepared nanofibers was performed with X-ray diffraction spectroscopy. less diffused peaks in the X-Ray diffraction spectrum indicates that pure drug Brahmi extract material. Sharper peaks of diffraction show more crystallize the drug. An optimized formulation of Brahmi extract loaded PVP nanofibers batch F1 was well acceptable and palatable with better absorption and stability.

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