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Synthesis, characterization and parametric study for diameter of nano fiber of Chitosan, AzadirachtaIndica leaves extract composite

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Abstract

In the present examination, AzadirachtaIndica Leaf Powder and chitosan-based nanofibers are orchestrated utilizing electro-turning process. Acquired nanofibers are portrayed by FTIR, SEM and XRD considers. Chitosan can be acquired normally and are utilized in various fields, for example, biomedical, natural, tissue designing, body embed and so forth. AzadirachtaIndica leaves powder to have their fluctuated antimicrobial application. Both AzadirachtaIndica leaves and chitosan polymers were difficult to electro-spun, to create nanofibers yet we figure out how to do as such by utilizing extraordinary solvents like 90% acidic corrosive. It has found to diminish the surface strain making electrospinning practical. Parametric Study for distance across of Nanofibers is finished utilizing ANOVA table for every one of the three factors. Ends are produced using various plots like region chart, shape plot and so forth. These outcomes can be utilized in future for electro spinning of Nano fibers of explicit distance across

Keywords: *Electrospinning, Nanofiber, parametric study, Chitosan, AzadirachtaIndica, characterization*

I. METHODOLOGY

Nanofibers

Nanofiber has known for varied characteristics and distinctiveness in their properties such as surface area to volume ratio. Ithas very large and high porosity with very minute pore sizes that is why the nanofibers shows high potential materials for countless applications such asprotective clothing, wound dressings, bone tissue engineering, drug delivery etc. It is also used in the formation of filter for water purification and for sensing applicationsetc [1].

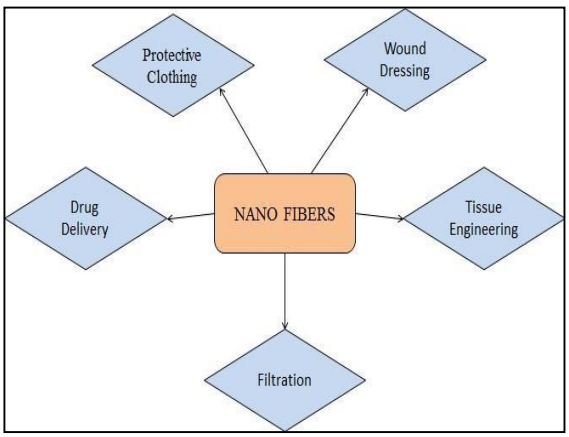

Figure 1: Applications of electrospunnanofibers in the biomedical field

Electro-spinning

Electrospinning is a technique for synthesis of nanofibers using electric field. Theelectrostatic force is used to "produce fine fibers from polymeric solution.Nanofibers Made out of this

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electrospinninghave a nano distance across (inside the request for nanometers to micrometers). The electrospinningstrategy can combination nanofibers that have more noteworthy floor territory than that received from everyday turning process [2]. Electrospinning has giving a nanofiber which might be having higher porosity, excessive percentage of floor to quantity and specifically nanofibers are adaptable and can be formed into special styles and sizes [3].Electrospinning method makes use of high electric powered fieldwhich is applied to the bead of an answer or dissolve turning out from the top of a narrow. It calls for a Direct Current voltage supply within the scope of a few numerous kV in an effort to deliver electrostaticforces.Strong electrical lousy powers created by using this voltage supply beat the greater prone powers of floor pressure within the fluid bead to deliver electrospinningnanofibers. The electrostatic powers influencing the fluid reason the kingdom of the bead to alternate from adjusted meniscus to the Taylor cone, a wonder noticed due to the electrostatic aversions between comparative expenses in the fluid [4].When fluid arrives at the simple factor, the electric discipline turns into beats the floor stress of the fluid prompting the association launched out from the top of the Taylor cone.As the fly is going thru the air there may be a vanishing of the solvents which favors the development of the nanofibers.The ventilated chambers are applied to preserve a strategic distance from even negative discharges of sure polymers or dissolvable fumes duringthe spinning[5].- Electro turning method can be union nanofibers by using consolidating remedial professional within the fibers,this expands the appropriateness in medicate conveyance framework. The vast claims to fame of nanofibers explore within the biomedical area. Bioactive nature of the medication atoms" may be retainedby electro-turning strategy.

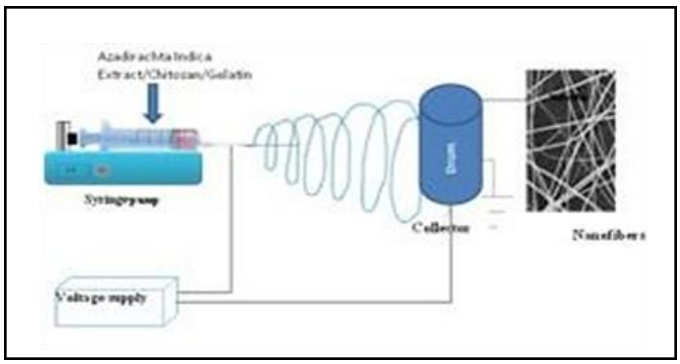
The arrival of meds at the harm can be doable increment and symptom may be decreased via utilizing electro-spun filaments regionally, which move approximately as a medicine transporter [6].

Restricted treatment by way of the medication is accelerated by way of Electro-spun strands. The debasement of the polymer filaments shows the arrival of the medication and can be appropriately overseen in tranquilize conveyance applicationselectro-spun strands

These filaments manage the medication and spare the drugs from the chemical substances and bacterial development. Right now, beneficial operators stay unaltered and secured until required on the website online of interest [7].

Tissue building is a board area requiring the joint and enormous data on medicine, science, designing and material technological know-how fields. Tissue culture is wherein we're utilizing the mixture of cells, substances, restorative experts and special biochemicals use and it offers recovery of tissues that have been demolished via illnesses, wounds or inherent defects[8].

The stringy section of ECM offers operational help and gadget-pushed uprightness to tissues which in turns bolster the form, recuperation, movement, and separation made of protein filaments, for instance, collagen, elastin, keratin, laminins, fibronectin and vitronectin. In this manner, ECM strands are possibly the most widespread section that's expected to take care during fake tissue regeneration[[9,10].



Schematic diagram of the Electro-spun nanofibers

Chitosan

Chitosan is a polymer obtained from chitin and which are now having large interest in the field of research and development [11]. Organic polymer chitosan has the spectrum of properties which are

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not only non-toxic, biocompatible, eco- friendly, bio-functional, and water loving. It is used in the field of various field of pharmaceutics, wastewater treatment, cosmetics industry agriculture, food science, and textiles industry as well as antimicrobial and antiviral material. In acidic conditions chitosan have very good activity.

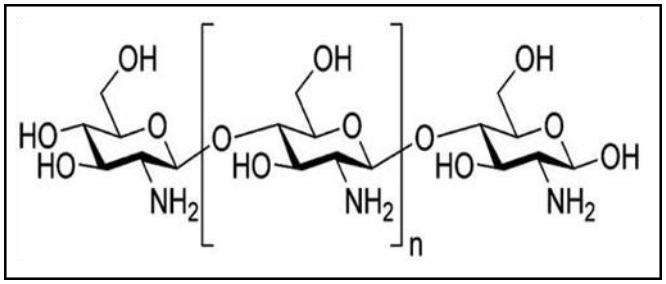

Figure 2: Structure of Chitosan

Gelatin is similar like collagen have naturally obtained easily as biopolymer. Gelatin is biocompatibility and biodegradability. It is abundantly found in animal tissue such as skin, muscle, and bone.

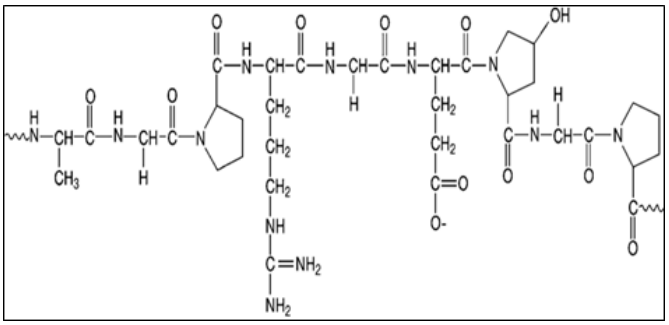

Figure 3: Structure of gelatin

AzadirachtaIndica

The Neem tree (AzadirachtaIndica) from ages (more than 2000 years) known in India and neighboring countries The AzadirachtaIndica always remains green, which generally cangrow up to the height of 15–20m and a trunk thickness of 1.5–3.5m.The plant is known for its fast-growing nature [28]. The significance of AzadirachtaIndica has been identified by the US National Academy of Sciences, resulting in a 1992 report entitled "AzadirachtaIndica – a tree which can solve

global problems". The AzadirachtaIndicatree survives any types of polluted environment such as cities having high pollution Index and village with low pollution [29]. Some of the most vigilant researchers are saying that AzadirachtaIndica deserves to be called a "wonder plant" because of its antifungal, antidiabetic, antibacterial, antiviral, contraceptive property.The main chemical component, present in various segments of the AzadirachtaIndica tree, consist of a few compounds belongs to the "triterpene", or more specifically "limonoid" group which is anatural product. The main components present in AzadirachtaIndica are azadirachtin, salannin, meliantriol, nimbinandnimbidin.

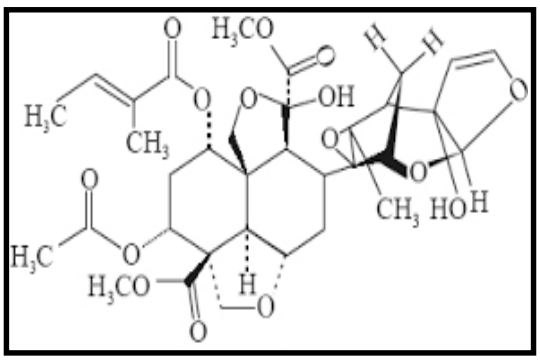

Figure: 4 Structure of AzadirachtaIndica

II. EXPERIMENT

Chitosan, Glacial Acetic Acid, Gelatin were precured of Sigma-Aldrich. Characterization of the nanofibers is done using FTIR, XRD, and SEM. FTIR was used to determine the functional group of AzadirachtaIndica Extract

XRD Chitosan/Gelatinnanofiber was used to determine the structure of nanofibers. The Surface

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morphology of nanofibers was easily studied by means of SEM Image processing software which was used to measure the diameter of the nanofiber captured in the SEM micrograph.

AzadirachtaIndica extract solution was prepared by heating 10gm of leaves of AzadirachtaIndica in 100 ml of water. Heat the solution until the volume of the solution reduces to half. Filtered the final heated solution via filter paper. Final solution after filtration appears brown in color.

Nanofibers solution was prepared by taking 10ml of AzadirachtaIndica extract and 5 ml of 50% diluted Glacial Acetic Acid. Add 0.3g of Chitosan slowly into the above solution. Once the chitosan is completely dissolved then add 0.3g Gelatin. Keep the solution for 1 hour on the magnetic stirrer. The solution was obtained by blending Chitosan,

AzadirachtaIndica Extract, Glacial Acetic Acid, and gelatin solution. The prepared AzadirachtaIndica Extract/Chitosan/Gelatin solution was then filled into 12-mL plastic syringe. 0.4-mm needle was used at a high voltage of 15 kV, with a tip-at a distance of 14 cm, was applied to the solution and fiber collected on the plane collector

Table-1Chemical composition of AzadirachtaIndica

Table No.1	Compound	Amount	Compound	Amount
1	Moisture	59.4 %	Niacin Vitamin	1.40 mg/100g
2	Proteins	7.1 %	C Carotene	218 mg/100g
3	Fat	1.0 %	Calorific value	1998
4	Fiber	6.2 %	Glutamic acid	microgram/100g
5	Carbohydrates	22.9 %	Tyrosine	1290 kcal/kg
6	Minerals	3.4 %	Aspartic acid	73.30 mg/100g
7	Calcium	510	Alanine	31.50 mg/100g
8	Phosphorous	mg/100g	Proline	15.50 mg/100g
9	Iron	80 mg/100g	Glutamine	6.40 mg/100g
10	Thiamine	17 mg/100g		4.00 mg/100g
		0.04		1.0 g/100g
		mg/100g		

III. CHARACTERIZATION: FT- IR SPECTROSCOPY

TABLE NO.2	WAVE NUMBER (CM-1)		
S. NO.	**NLP (BEFORE)**	**NLP**	**BOND STRETCHING**
1	3284.88 IN CASE OF NLP	3269.45 IN CASE OF NLP	-O-H IN ALCOHOLS, ACID, PHENOLS AND –N-H IN AMINES AND AMIDES.
2	2924.18,2854.74 IN CASE OFNLP,	2918.40, 2852.81 IN CASE OF NLP	-C-H IN –CH3 AND CH2.
3	1722.49 IN CASE OF NLP	1658.84 IN CASE OF NLP	-C=O IN CARBONYL, CARBOXYL GROUPAND LACTONES.

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4	1649.19, 1612.54,1543.10, 1442.80 IN CASE OF NLP	1543.10 IN CASE OF NLP	-C=C- IN AROMATIC RINGS, -C=O IN HIGHLY CONJUGATED.
5	1319.35, 1039.67 IN CASE OF NLP	1317.43 IN CASE OF PLP	-C-O IN ALCOHOLS, PHENOLS,
6	879.57, 761.91, 638.46 IN CASE OF NLP AND ALP 789.62, 686.62, 630.74 IN CASE OF PLP	651.96 IN CASE OF PLP 883.43, 615.31 IN CASE OF ALP	-C-C- DEFORMATIONS AND OUT OF PLANE - C-H DEFORMATION IN AROMATICRINGS.

Table No. 2: Bands assigned to the surface functional

The FT- IR spectroscopy method was used to obtain the informationabout the types of the functional groups [30, 31]. The principle of IR spectroscopy is based on the fact that when the molecules vibrates at with specific frequencies associated with internal vibrations of groups of atoms. These frequencies occur in the IR region which is a part of the electromagnetic spectrum, i.e. ~ 200 to ~4000cm -1. A Fourier Transform Infrared (FT- IR)helpful in knowing the type of functional present

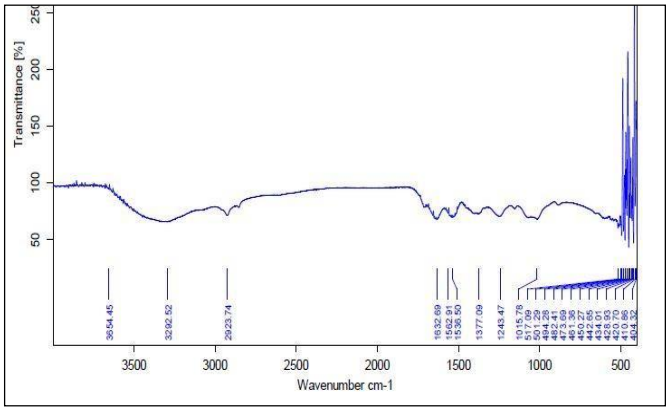

Figure-6: FTIR spectra of Nanofibers

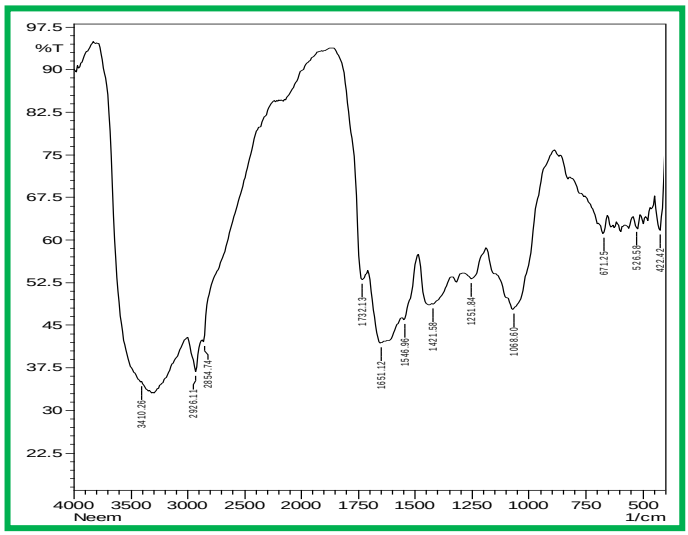

Fig.5 FTIR spectra of AzadirachtaIndica

The spectra change before and after formation of nanofibers can be visible in FTIR

IV. RESULTS AND DISCUSSIONS:

" BothAzadirachtaIndica leaves and chitosan polymers were found to be prepared extract and manage to produce nanofibers using special solvents 50% acetic acid which found to reduce the surface tension making electrospinning feasible. Electrospinning is shown potential applications in biomedical fields like tissue engineering scaffolds and wound healing capabilities."

FTIR spectra of NANOFIBERS

1) SEM Study of SurfaceTopography

Scanning Electron Microscopy (SEM) is a very important method to study the surface topography of a material in a solid state. SEM imaging gives better

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results than optical microscopy because of enhanced resolution and depth.

AzadirachtaIndica. Leaf Powder as shown in figure 7., shows quite rough texture of surface of the particles with quite big vides throughout the surface. This indicates that particles are having some porosity.

Indica Leaves Powder

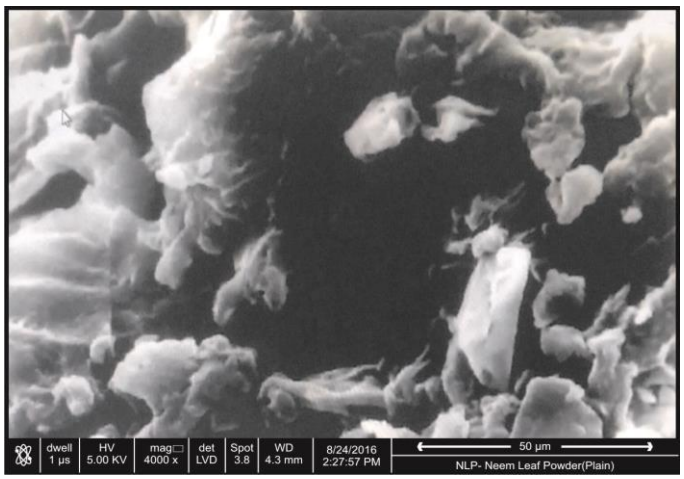


Figure 7: SEM image of the surface Azadirachta

SEM results of Nano fibers show the formation of the nanofiberswiththeAzadirachtaIndica Extract/Chitosan/Gelatin solution. The fibers are clearly visible in the image. The diameters of the nanofiberswerecalculated

The beads are more at this scale

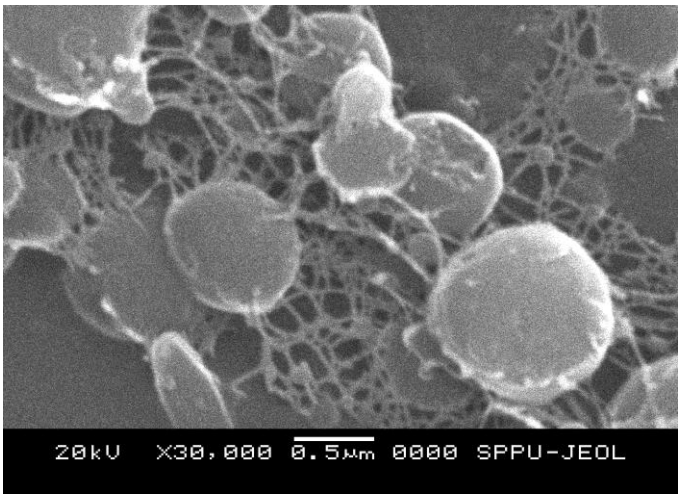


Figure-8 The beads and fibers are morevisible

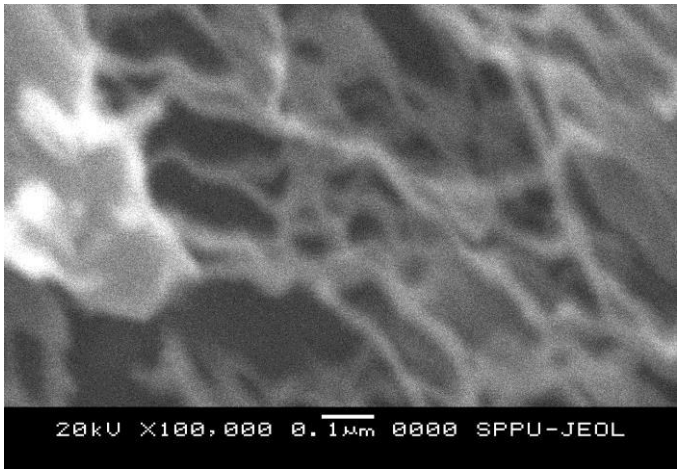
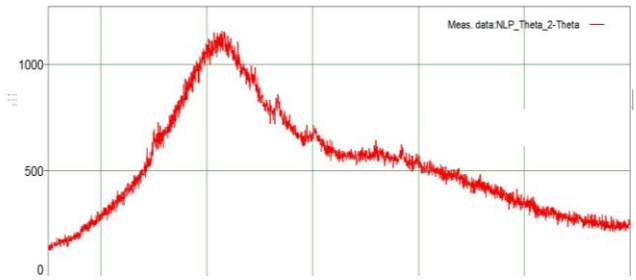


Figure-9 The beads and fibers are morevisible

to 80°, with a goniometric specification radius of 150 mm and Soller slit ±2.5° (divergence angle).

2)XRD

"Powder X-ray diffraction (PXRD) patterns were recorded using benchtop X-ray diffractometer (Model: RigakuMiniflex 600). The operating target voltage was 30 kV and the current were 15 MA. The radiations of Cu Kα were generated using X-ray generator and the β radiations were suppressed using Ni filter. The sample was powdered and scanned for 2θ ranges from 5° to 80°, with goniometer specification radius of 150 mm and roller slit ±2.5° (divergence angle)."



V. PARAMETRIC STUDY FOR DIAMETER OF NANOFIBER

Thefollowing factors are being selected for the experiment:

1. Distance(cm)
2. Flow Rate(ml/hr)
3. Voltage(kv)

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Number of Observation:

The experiment is designed .in the table No.3, there were three independent variables are varied at two levels i.e. low and high during experimentation. Hence the minimum numbersofobservation are 23 i.e.8.

Table No.3	Parameters	Nomenclature	Low (-1)	High(+1)
1	Distance	Cm	10	13
2	Flow Rate	Ml/hr	0.1	0.3
3	Voltage	KV	15	20

For the design of the experiment, the data is collected in Table No.4 through the experiments. In the synthesis of nanofiber by electrospinning method we have taken at two levels, one is higher and other is lower. The higher level abbreviated as '+1' and the lower abbreviated '-1' .The data table referred here combined as per the higher and lower levels.

VI. EXPERIMENTATION AND DATA COLLECTION

TableNo.4	Distance (cm)	Flow Rate(Ml/hr)	Voltage (Kv)
1.	-1	-1	-1
2.	1	-1	+1
3.	-1	+1	-1
4.	-1	+1	+1
5.	+1	-1	-1
6.	+1	-1	+1
7.	+1	+1	-1
8.	+1	+1	+1

Experimental Results [32]

Experimental Results are given in table No5

Table No.5	Experiment No.	Distance (cm) A	Flow Rate(Ml/hr) B	Voltage (Kv) C	Diameter (nm) X
1.	1.	-1(10)	-1(0.1)	-1(15)	40
2.	2.	-1(10)	-1(0.1)	+1(20)	35
3.	3.	-1(10)	+1(0.3)	-1(15)	70
4.	4.	-1(10)	+1(0.3)	+1(20)	55
5.	5.	+1(13)	-1(0.1)	-1(15)	35
6.	6.	+1(13)	-1(0.1)	+1(20)	32
7.	7.	+1(13)	+1(0.3)	-1(15)	35
8.	8.	+1(13)	+1(0.3)	+1(20)	32

VII. ANALYSIS OF EXPERIMENTS [33]

Mean square or variance (MS or V)

Variance is calculated for all the factors as well as interactions Analysis of Variance has been calculated in table No 6 for factors A, B, C.

Table No.6		Analysis of Variance			
Source	DF	Adj SS	Adj MS	F-Value	P-Value
A	1	544.5	544.5	6.02	0.07
B	1	312.5	312.5	3.45	0.137
C	1	84.5	84.5	0.93	0.389
Error	4	362	90.5		
Total	7	1303.5			

Analysis of Variance has been calculated in table No 7 for factors AB.

Table No.7	Analysis of Variance				
Source	DF	Adj SS	Adj MS	F-Value	P-Value
AB	3	1169.5	389.83	11.64	0.019
Error	4	134	33.5		
Total	7	1303.5			

Analysis of Variance has been calculated in table No 8 for factorsBC

Table No.8		Analysis of Variance			
Source	DF	Adj SS	Adj MS	F-Value	P-Value
BC	3	409.5	136.5	0.61	0.643
Error	4	894	223.5		
Total	7	1303.5			

Analysis of Variance has been calculated in table No 9 for factorsAC

Table No.9	Analysis of Variance				
Source	DF	Adj SS	Adj MS	F-Value	P-Value
AC	3	653.5	217.8	1.34	0.38
Error	4	650	162.5		
Total	7	1303.5			

MS = Sum of square / degree of freedom

Our experiments were a strong correlation between various operating conditions and diameters of

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nanofibers, we found that increasing the voltage(C) and distance (A) led to reduce the diameter of the nanofibers. However, increased flow rate resulted in thicker fibers. In our experiments, the diameters of the nanofibers varied from 32 nm to 70 nm.

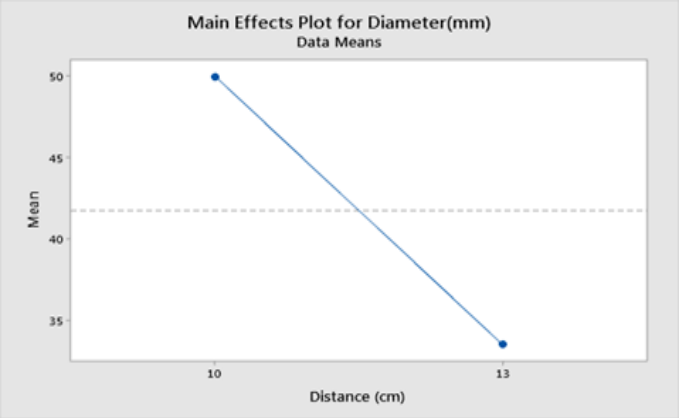

Figure-10 The main effects plot for diameter vs. distance

The main effects plot for diameter vs. distance shows that for distance of 10cm and 13cm mean diameter is 50 mm and 33.5mm respectively.

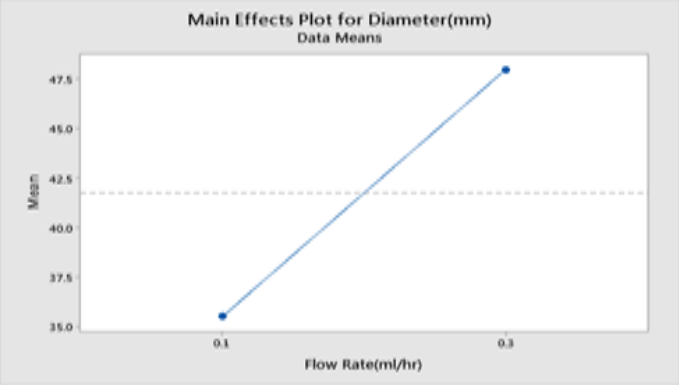

Figure-11 The main effects plot for diameter vs. flow rate

The main effects plot for diameter vs. flow rate shows that for flow rate of 0.1 ml/hr and 0.3ml/hr the mean diameter is 35.5 mm and 48mm respectively.

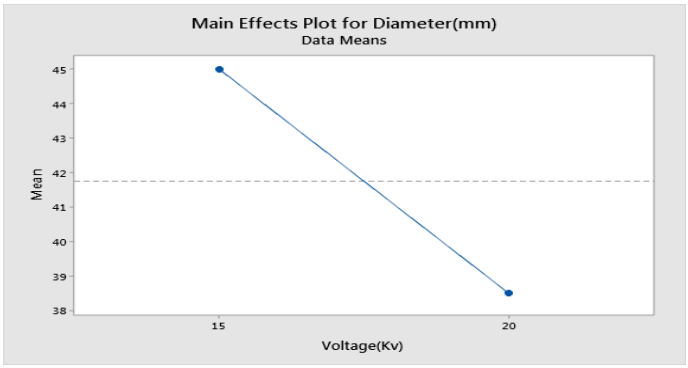

Figure-12 The main effects plot for diameter vs. voltage

The main effects plot for diameter vs. voltage shows that for voltage of 15Kv and 20Kv, the mean diameter is 45 mm and38.5 mm respectively.

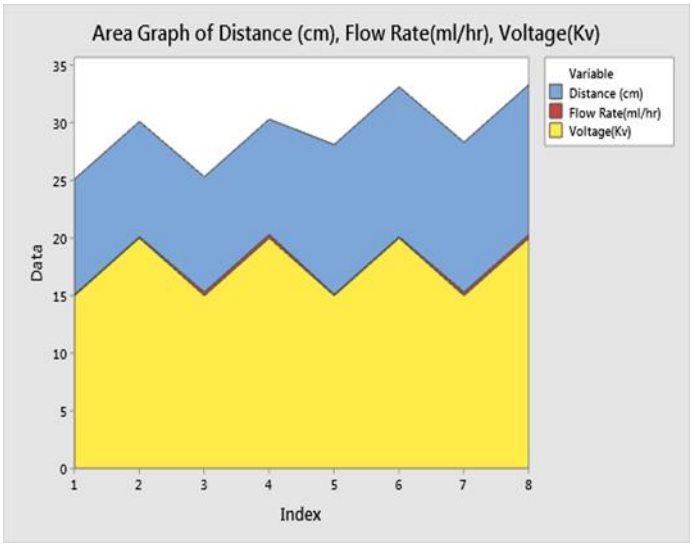

Figure-13 Area graph diameter across three variables

"The following area graph shows diameter across three variables distance, flow rate and voltage. Voltage contributes the most to the diameter from variables. Flow rate contribute least to the diameter.The highest values of diameter are in the upper left corner of the plot, which corresponds with low values of both voltage and distance.The lowest values of diameter are in the lower right corner of the plot, which corresponds with higher values of both Voltage and distance."

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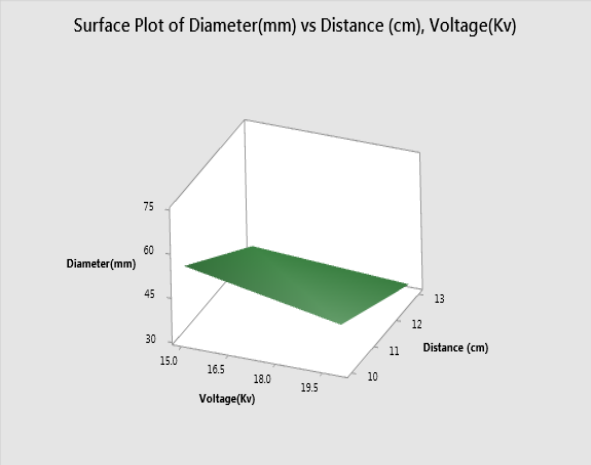

Figure-14 Surface plot of diameter (mm) vs. Distance (cm), Voltage (KV)

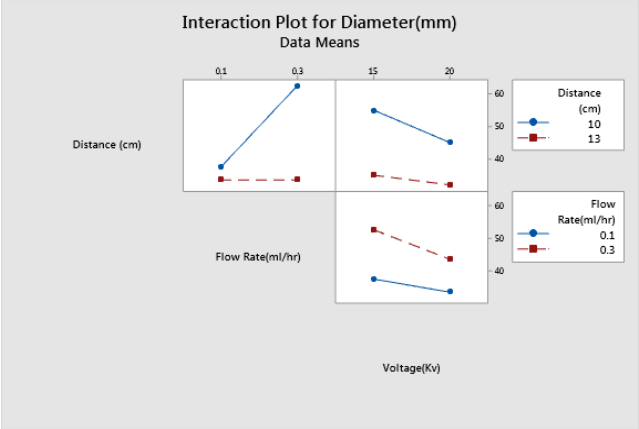

Figure-15 Interaction plot

In this connection plot regarded over heredoes no longer the parallellines.The first cooperation impact indicates that the connection between movement rate and width relies upon the estimation of separation. It indicates that for circulation rate zero.1ml/hr and 0.3ml/hr separation of 10cm is related with most expanded imply diameter.The second connection impact indicates that the connection among voltage and breadth is based upon the really worth ofdistance. It suggests that for voltage of 15 Kv and 20Kv, separation of 10cm is related with maximum noteworthy mean diameter.The 1/3 collaboration impact indicates that the relationship amongst voltage and distance across is predicated upon the estimation of flowrate. It suggests that for voltage of 15 Kv and 20Kv, circulate pace of zero.3ml/hr is associated with most expanded meandiameter.

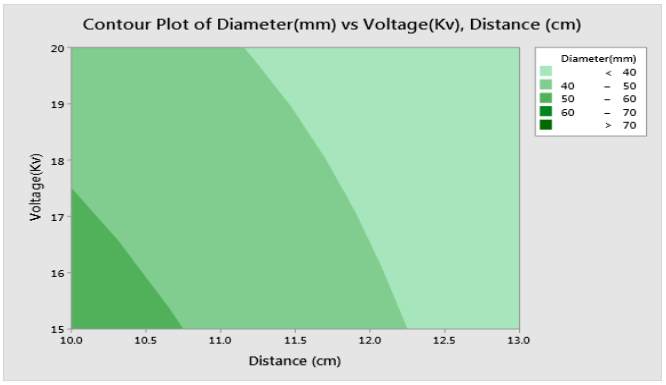

Figure-16 Contour plot

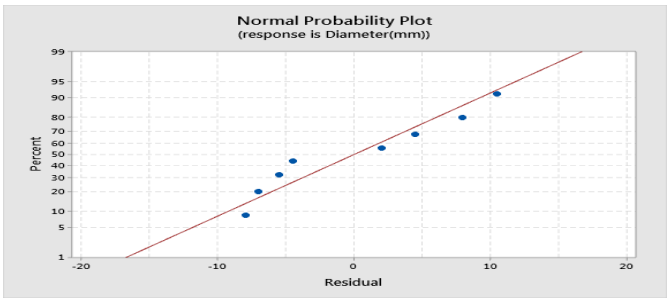

Figure-17 Normal Probability Plot

This contour plot shows the relationship between the distance and voltage settings used to get the response of diameter. Darker regions indicate higher diameters.

VIII. CONCLUSIONS

The AzadirachtaIndica Extract/Chitosan/Gelatinnanofiber wassuccessfullysynthesisbyelectrospinningmethod.There resultsof FTIR, XRD, and SEM display that AzadirachtaIndica Extract/Chitosan/Gelatinnanofiber is framed. SEM picture indicates the electro-spun came about in nanoscale distance throughout of the fiber. The combination of nanofibers can be successfully executed via way of electro-turning method at a voltage 15 to 20 kV, with a tip-authority separation of 10 to 13 cm. We located that increasing the voltage(C) and separation (A) caused lower the space across of the nanofibers. It became determined that the electrospinning of the ordinary filaments

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werevery difficult thinking about that they will be for the maximum detail include high atomic loads, the form consist of the solid spine.These tendencies make the association gooey that is tough to electro-spum.Thisobstaclewastheformationofsmall dabs with the strands. Utilization of focused acidic corrosive as a dissolvable (ninety%) which was observed to decrease floor stress or combo of solvents a Nanofibers from AzadirachtaIndica Extract/Chitosan/Gelatin will be framed successfully utilizing solvents. Parametric Study for width of Nanofibers is finished using ANOVA table for every one of the three factors. Ends are produced the use of various plots like region chart, form plot and so on. These effects can be applied in future for electrospinning of nanofibers of specificdiameter.

Zone plot display that voltage contributes the most to the distance throughout from factors. Stream price contribute least to the dimensions. Surface plot show that maximum noteworthy estimations of breadth are inside the higher left corner of the plot, which relates with low estimations of every voltage and separation. This form plot suggests the relationship between the separation and voltage settings used to get the reaction of breadth. Darker districts monitor better breadths above 70mm.The principle influences plot and verbal exchange plots are plotted to get the advise width at numerous ranges of things.

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