Synthesis of Biofunctionalized Nanofibers (Curcumin, Gelatin and Formic Acid) using Electrospinning Process and Optimization of Parameters for Diameter of Nanofibers

Sachin S. Chavan, Pramod V. Londhe, Avinash M. Pawar, S.M. Shendokar

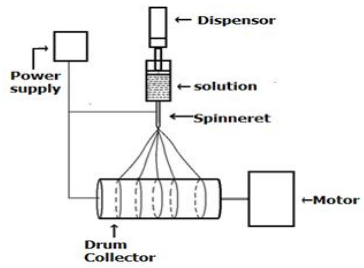
***Abstract*: Electrospinning is a easy and versatile method to synthesize the Nanofibers of different Polymers and compounds. The main merits of this process are we can get continuous and ultra thin fibers. Due to this we can use this process for mass production. This process overcome so many limitations of other processes.**

Hence in this present work manufacturing of fibers and optimization of process parameters has been carried out. There are near about 16 process parameter of electrospining process. From this four parameters are selected (distance between spinneret and drum collector, voltage, flow rate and viscosity). Then characterization of these manufactured nanofibers has been done by using SEM. Then applying the technique of DOE and ANOVA the effect of these parameters on the diameter of nanofibers has been predicted.

***Keywords* : About four key words or phrases in alphabetical order, separated by commas.**

I. INTRODUCTION

Electro spinning may be regarded as noncontact drawing process, where in a dragging force is generated by a potential difference applied between spinneret and collector plate. The spinneret dispenses solution which can be polymer, melt or composite solution. This solution droplet, when it is at the tip of the spinneret, is under the action of surface tension and potential difference applied. When potential difference applied exceeds, surface tension, the droplet starts elongating. At a semi-angle of 45^0, called as Taylor Cone, droplet start converting into a fiber, which further under the action of "Bending Instability" and whipping elongates exponentially into "Nanofibers".Electro spinning provides a simple and versatile method for generating ultrathin fibers from a rich variety of materials that includes polymers, composites and ceramics. It is also recognized as an efficient technique for the fabrication of polymer nanofibers, various polymer have been successfully electrospun into ultrafine fibers. Also electrospinning defined as feasible process for the fabrication of continuous fibers with diameters ranging from general micrometre's down to a few nanometers.[42-46]



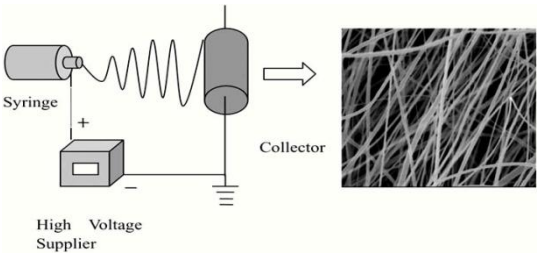


Fig No 01:Schematic of electrospinning Setup[15]Fig No 02:- Setup of electrospinning[16]

Electrospinning is a simple process which can produce polymer fibers ranging from micrometer to nanometer from melt or polymer solution using an electric field.When an electric potential is applied to melt or solution of polymer the changed polymer solution forms a cone shaped droplet at the tip of the nozzle [17, 18, and 19]. When an electrostatic force is sufficient enough to overcome the surface tension of the solution droplet, the tip of droplet elongates towards a collection plate which is in the form of a grounded metal target resulting in a formation of a jet. This charged jet undergoes whipping (stretching) mode, called instability region where it splits into multiple fine fibers and travels

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to the target. The solvent evaporates while the dry ultrafine fibers are deposited on the collection plate as illustrated in figure-1 [20-21][47-51].

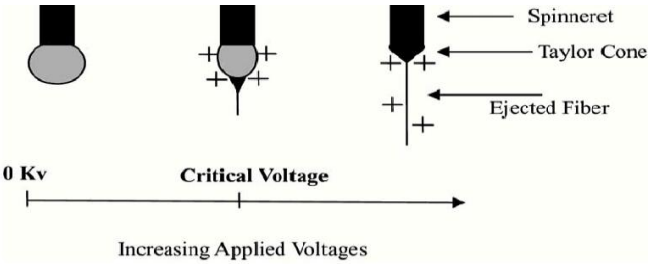


Fig No 03:- Scheme Illustration of a Taylor cone formation with increase in applied voltages[16]

II. Literature Survey

Literature survey: applications of biofunctionalised nanofibers in biological field

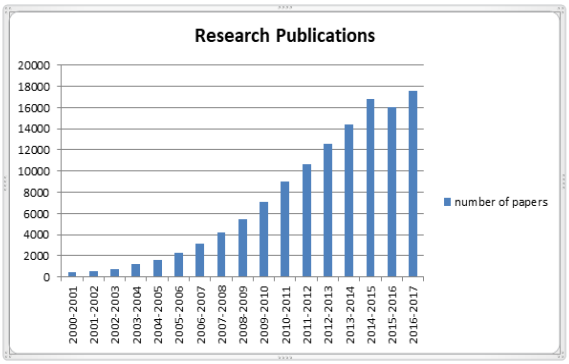


Fig No 04:- Research Publications(Graph)

J. Venugopal et.al. [24]Suggested recent advancements in the electro spinning method enable the production of ultra-fine solid and continuous fibers with diameters ranging from a few nanometres to a few hundred nanometres with controlled surface and internal molecular structures. Due to the facility of functionalizing, nanofiber surface can be functionalized to improve biochemical characteristics of nanofibers. This can be incorporated in the field of biomedicine for various applications such as multifunctional membranes, biomedical structural elements, scaffolds used in tissue engineering, wound dressing, drug delivery, and artificial organs. They also state that, due to the biocompatibility and biodegradability of the nanofibers, they can be useful in the replacement of unhealthy organs and tissues in the humans. They can also be used in small electronic devices, biosensors, super absorbents, automobile parts and armor components. In this paper, they also discussed about the patent by Smith et al. for production of skin mask fabricated by electrospinning nanofibers directly onto the surface of the skin for the protection of wounds.

Yanzhong Zhang,et.al.[25] Present review of recent advancements made in the nanotechnology regarding use of polymer nanofibers for biomedical applications. It compares different processing methods for creating polymer nanofibers/fibrous structure. Processing method, such as electrospinning, melt blowing, phase separation, self-assembly, template synthesis. It emphasizes on latest studies that used various processing methods mainly electrospinning.

Younanxia,et.al.[26], P.V. Londhe,,et.al[46] Have focus on use of Electrospun Functionalized Nanofiber for biomedical research and also they have reviewed some recent development to electrospinning technique they have introduced a typical set up and mechanism and used polymer and respective solvent. The solute plays a critical role in controlling the physical properties of polymer solution introducing the surface tension, electrical conductivity and viscosity. They have also suggested about How to controlling the composition and structure to the manufacturing of uniform nanofibers like as a polymer blend which offers the potential to prepare functional nanofibers for use in a variety of applications like as to enhance mechanical strength and duration of use. Manufacturing of porous nanofiber can be functionalised by introducing phase separation between two polymers. During the electrospinning of polymer blend followed by the selective removal of one component through thermal degradation solvent extrusion. Variety of secondary structure such as core sheath and micro tubes with single and multiple channels are manufactured. They have also suggested about the controlling assembly by which we can able to manufacture the fibers aligned to uniaxial array deposited across the gap through the use of an electrostatic force. Mainly they predict that nonwoven mats of electrospun nanofibers can serve as ideal scaffold for tissue engineering because they can mimic the cellular matrix. They have also given the information about Encapsulation of bioactive materials which is used in various applications.

Seema Agarwal, et.al.[27]Discuss about how electro spinning technique is used for getting nanofibers having a large surface area and superior mechanical properties which are used in many fields such as optical, electrical, sensors, composite. They also discuss the nanofibers applications in wound dressing, tissue engineering, targeted drug delivery, etc. They also discuss how3 nanofiber material can be used for regenerating new extracellular matrix which destroyed by any disease, congenital defects without stimulating any immune response. Here they use PVA/$AgNO_3$ fiber when this nanofiber is given heat or UV radiation, it can be reduced to Ag ions from PVA/$AgNO_3$ fibers into the Ag nanoparticles which can

be used for wound and burn treatment, because Ag nanoparticles are antimicrobial agents. Jonathan G. Merrell,et.al.[28] Had published a paper under title Curcumin Loaded Poly (CaproLactone) Nanofibres Diabetic wound dressing with antioxidant and anti-inflammatory Properties. In this they had highlighted the use and application of Curcumin loaded nanofibers. They also predicted that the diabetic wound dressing with antioxidant and anti-inflammatory properties of these nanofibers can be possible. Due to Curcumin is a naturally occurring Poly Phenolic component with a broad range of biological functions including anti-cancer, antioxidant and anti-inflammatory activities. In this study they had mainly investigated the feasibility and potential of PCL (polycaprolactone) nanofibers is a delivery vehicle for Curcumin for wound healing applications. By optimizing the electrospinning parameters bead free Curcumin loaded PCL nanofibers are developed. The antioxidant activity of Curcumin loaded nanofibers was demonstrating using an oxygen radical absobancecapacity (ORAC) and by the ability of the fibers to maintain the viability of HFF-1 cells under condition of oxidative stress. The fibers showed sustained release of Curcumin for 72 h and could be made to delivers a dose much lower than the repotedcytetoxic concentration while remaining will be bioactive Human fareskin fibroblast cells (HFF-1) showed more than 70% viability on Curcumin loaded nanofibers. Another advantage of Curcumin loaded nanofibers is to reduce inflammatory induction. The in vivo wound healing capacity of the Curcumin loaded nanofibers was demonstrated by an increased rate of wound closure. So this is predicted that the Curcumin loaded PLC nanofibers matrix is bioactive and has potential as a wound greasing with antioxidant and anti-inflammatory properties.

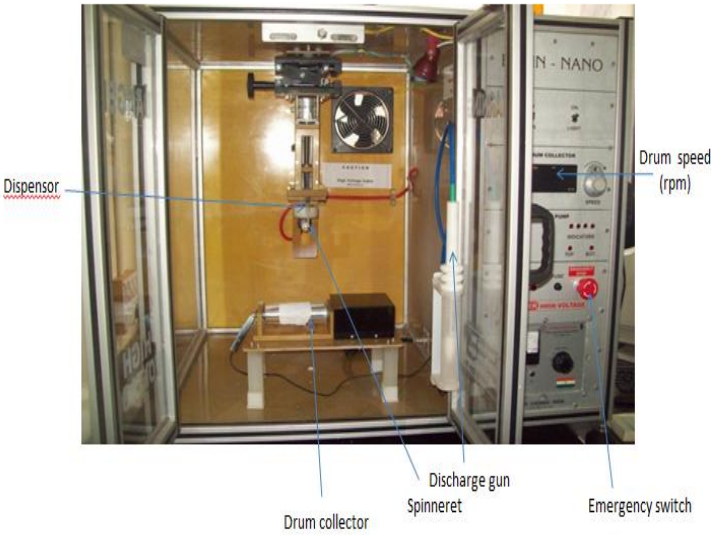
GholipourKanani,et.al.[29]Both have briefly covered the historical records of tissue engineering. The high surface area and micro porous structure of the nanofibers starts the signalling pathway and attract the fibroblasts to the upper dermatological layer of the skin excreting extracellular matrix components like collagen and cytokines. Tissue engineered scaffolds have unique properties and can be used as alternative to tissue plantation. It also has several applications in drug delivery, vascular tissue engineering, bone tissue engineering, heart tissue engineering, particular cartilage tissue engineering.

R. Rathinamoorthy, et.al. [30] Mentioned that natural fibers afford a bioactive matrix for design of more biocompatible and intelligent materials owing to remarkable molecular structure. A different type of wound dressing which could accelerate wound healing. Where wound dressing materials are classified as absorbent and non-absorbent depending on the type of the fibres' used, and also as passive products, interactive products and bioactive products based on its nature of action. Various polysaccharide fibers infused with alginate, chitin and chitosan have been observed for their biological properties and experimented on for various applications.

III. Design Of Experiments And ANOVA

In electro spinning by controlling some factors we can reduce the value of diameter of nanofiber.[22]. The factors are flow rate, voltage, distance between spinneret and drum and viscocity

ELECTROSPINNING SETUP (BVUCOEP, COMPOSITE LAB)



The following materials system are being identified for the experiment

- Gelatine
- Chitosan
- Curcumin
- Formic acid

The following factors are being selected for the experiment [22]:

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

Number of Observation:

The 2k factorial design is used. There are three independent variables are varied at two levels low and high [22] during experimentation. Hence the minimum number of observation are 2^4 i.e.16.

Parameters Nomenclature Low(-1) High(+1)

Distance Cm 10-15
Flow Rate Ml/hr 0.1-0.15
Voltage Kv 15-20
ViscocityCp 65-70

Table 1:- DOE with parameters and levels

Parameters	Nomenclature	Low(-1)	High(+1)
Distance	Cm	10	15
Flow Rate	Ml/hr	0.1	0.15
Voltage	Kv	15	20
Viscosity	CP	65	70

For design of experiment the data is collected the experimental level have some level i.e. the factors selected should be given some value. For example in the experiment of the electrospinning taken at two levels one is higher and other is lower. The high level abbreviated as '+1' and the lower abbreviated '-1' in the data refer table the runs combined as per the higher and lower levels as shown in table. We can easily do the 23=8 combinations. The experimental results were analyzed for getting significant parameters, interaction by using F-test of ANOVA.

IV. EXPERIMENTATION AND DATA COLLECTION

Table 2:-DOE in coded form

Sr. No	Experiment No.	Distance (cm) A	Flow Rate (ml/hr) B	Voltage (kv) C	Viscosity (cp) D
1	1	(-)1	(-)1	(-)1	(-)1
2	2	(+)1	(-)1	(-)1	(-)1
3	3	(-)1	(+)1	(-)1	(-)1
4	4	(+)1	(+)1	(-)1	(-)1
5	5	(-)1	(-)1	(+)1	(-)1
6	6	(+)1	(-)1	(+)1	(-)1
7	7	(-)1	(+)1	(+)1	(-)1
8	8	(+)1	(+)1	(+)1	(-)1
9	9	(-)1	(-)1	(-)1	(+)1
10	10	(+)1	(-)1	(-)1	(+)1
11	11	(-)1	(+)1	(-)1	(+)1
12	12	(+)1	(+)1	(-)1	(+)1
13	13	(-)1	(-)1	(+)1	(+)1
14	14	(+)1	(-)1	(+)1	(+)1
15	15	(-)1	(+)1	(+)1	(+)1
16	16	(+)1	(+)1	(+)1	(+)1

Setup for Measurement Of Diameter Of Nanofiber (IIG MUMBAI)

Diameter of Nanofiber: Diameter is measured in the form of nanometer (nm) value and SEM setupis used for the measurement of diameter of nanofiber.

Experimental Results

The results obtained so are shown in the table:

Table 3:-Analysis of variance

Sr. No	EXPERI-MENT NO.	DISTANCE (cm) A	FLOW RATE (ml/hr) B	VOLTAGE (kv) C	VISCOSITY (cp) D	RESULT DIAMETER (nm)
1	1	(-)10	(-)0.1	(-)10	(-)65	175
2	2	(+)15	(-)0.1	(-)10	(-)65	140
3	3	(-)10	(+)0.15	(-)10	(-)65	185
4	4	(+)15	(+)0.15	(-)10	(-)65	453
5	5	(-)10	(-)0.1	(+)15	(-)65	124.09
6	6	(+)15	(-)0.1	(+)15	(-)65	55
7	7	(-)10	(+)0.15	(+)15	(-)65	392
8	8	(+)15	(+)0.15	(+)15	(-)65	342
9	9	(-)10	(-)0.1	(-)10	(+)70	155
10	10	(+)15	(-)0.1	(-)10	(+)70	161
11	11	(-)10	(+)0.15	(-)10	(+)70	144.5
12	12	(+)15	(+)0.15	(-)10	(+)70	133
13	13	(-)10	(-)0.1	(+)15	(+)70	117
14	14	(+)15	(-)0.1	(+)15	(+)70	86
15	15	(-)10	(+)0.15	(+)15	(+)70	121
16	16	(+)15	(+)0.15	(+)15	(+)70	119
TOTAL ∑X = 2902.59						

V. ANALYSIS OF EXPERIMENTS

Mean square or variance (MS or V)

Sum of squares when divided degrees of freedom given mean square or variance. Variance is calculated for all the factors as well as interactions and following ANOVA table is formed.

MS = Sum of square / degree of freedom

Table 4:-Variance calculation

S.NO	FACTOR	SUMT OF T SQUARE	DEGREEST OFT FREEDOM	VARIANCE T OFT MEANT SQUARE
1	A	355.335	1	355.335
2	B	48005.9	1	48005.9
3	C	2266.0	1	2266.0
4	D	43013.72	1	43013.72
5	AB	6955.14	1	6955.14
6	AC	9005.5	1	9005.5
7	AD	1451.8	1	1451.8

8	BC	5906.23	1	5906.23
9	BD	48335.1	1	48335.1
10	CD	764.31	1	764.31
11	ABC	3522.72	1	3522.72
12	BCD	1529	1	1529
13	ACD	6584.9	1	6584.9
14	ABCD	6823.17	1	6823.17

Highest value = 48335.1

Following sources can be pooled together:

A+C+AB+AC+CD+ACD+BCD+ABC+ABCD

=355.335+2266+6955.14+9005.5+1451.8+5906.23+764.31

+3522.72+6584.9+

3522.72+6823.17

=45164.105

$MS_{error} = SS_{error} / V_{error}$

= 45164.105/11

= 4105.

These pooled figures are removed from their place in ANOVA table and recorded as error factor at the bottom of ANOVA table.

Various tables with Pooled Error

Table 5:-Table with pooled error

S.NO	Factor	Sum of Square	Degrees of Freedom	Variance of Mean Square
1	B	48005.9	1	48005.9
2	D	43013.72	1	43013.72
3	BD	48335.1	1	48335.1
4	Pooled Error	45164.105	11	4105.8

Calculation for F value [23]

Our main aim in this method of analysis is to see if the signal created by the factor is stronger than the background noise (error).the F test is used to compare two variances.

FO = SSA/Vfactor ÷ SSE/Verror =MSfactor/MSerror

Including this F factor, we can form final ANOVA table as below.

Table 6:-Final ANOVA table

S.NO	Factor	Sum of Square	Degrees of Freedom	Variance of mean Square	F0
1	B	48005.9	1	48005.9	11.70
2	D	43013.72	1	43013.72	10.47
3	BD	48335.1	1	48335.1	11.77
4	POOLED ERROR	45164.105	11	4105.8	

Now in calculation of F ratio:

Degrees of freedom for numerator= 1

Degrees of freedom for denominator= 11

Therefore consulting F-Distribution table, for 95% level of confidence we find that F value is $F_{0.05,1,11}$= 7.71i.e. F_{limit} Since all the F –values in the table are greater than the limiting value of F-ratio

VI. REGRESSION ANALYSIS

Model equation:

$$Y= (\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_K X_K + £) /N$$

β_0 =2902.59/16 = 181.41

Effect of factor B = $\overline{Y_{B+}} - \overline{Y_{B-}}$

= 109.5

Effect of factor D = $\overline{Y_{D+}} - \overline{Y_{D-}}$

= -103.70

Effect of factor BD = $\overline{Y_{BD+}} - \overline{Y_{BD-}}$

= -109.935

Effect of factor A = $\overline{Y_{A+}} - \overline{Y_{A-}}$

= 9.42

Effect of factor C = $\overline{Y_{C+}} - \overline{Y_{C-}}$

= -23.80

Effect of factor AB = $\overline{Y_{AB+}} - \overline{Y_{AB-}}$

= 41.69

Effect of factor AC = $\overline{Y_{AC+}} - \overline{Y_{AC-}}$

= -47.45

Effect of factor AD = $\overline{Y_{AD+}} - \overline{Y_{AD-}}$

= -19.02

Effect of factor BC = $\overline{Y_{BC+}} - \overline{Y_{BC-}}$

= +38.40

Table 7:-Effect estimation (% Contribution)

Remark	Factor/Interaction	Effect Estimate	SumOf Square	% Contribution
Model	B	109.5	48005.9	26.01
Model	D	-103.70	43013.72	23.31
Model	BD	-109.935	48335.1	26.19
Error	A	9.42	355.335	0.19
Error	C	-23.80	2266	1.23
Error	AB	41.69	6955.14	3.77

Error	**AC**	**-47.45**	**9005.5**	**0.03**
Error	**AD**	**-19.02**	**1451.8**	**0.78**
Error	**BC**	**38.40**	**5906.23**	**0.05**
Error	**CD**	**-26.13**	**764.31**	**0.4140**
Error	**ACD**	**40.5**	**6584.9**	**10.0**
Error	**BCD**	**-19.5**	**1529**	**0.82**
Error	**ABC**	**-29.75**	**3522.72**	**1.90**
Error	**ABCD**	**41.3**	**6823.17**	**3.70**

TOTAL = 184518.825

% Contribution for B= 48005.9/184518.825 X =26.01

B_1 = 1/2 X (effect estimate for factor B) = 54.75

B_2= 1/2 X (effect estimate for factor D) = -51.85

B_3 = 1/2 X (effect estimate for factor BD)= -54.96

VII. RESULTS AND DISCUSSION

Scanning electron microscope results (images), IIG Mumbai

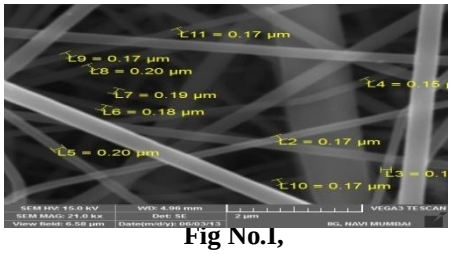


Fig No.I,
D-175 nm, Fr-0.1ml/hr,
V-10kv, Ds-10cm, Vs-65 Cp

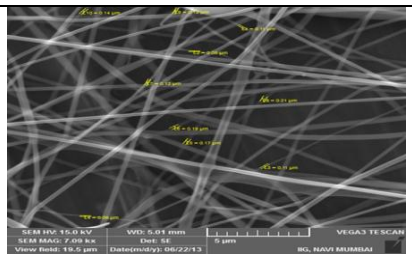


Fig No.II,
D-140 nm, Fr-0.1ml/hr,
V-10kv, Ds-15cm, Vs-65 Cp

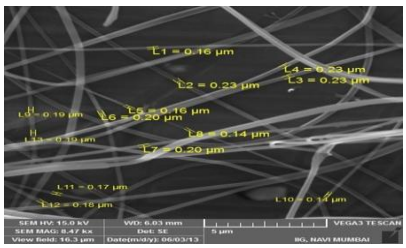


Fig No.III,
D-185 nm, Fr-0.15ml/hr,
V-10kv, Ds-10cm, Vs-65 Cp

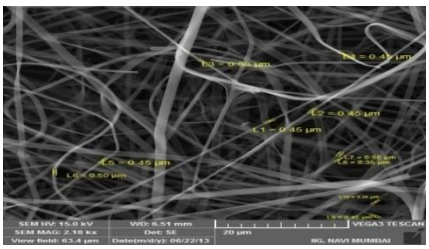


Fig No.IV,
Ds-453 nm, Fr-0.15ml/hr,
V-10kv, Ds-15cm, Vs-65 Cp

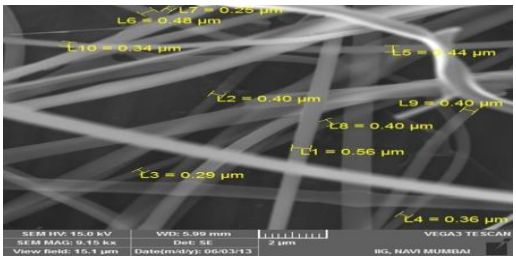


Fig No.VII,
D-392 nm, Fr-0.15ml/hr,
V-15kv, Ds-10cm, Vs-65 Cp

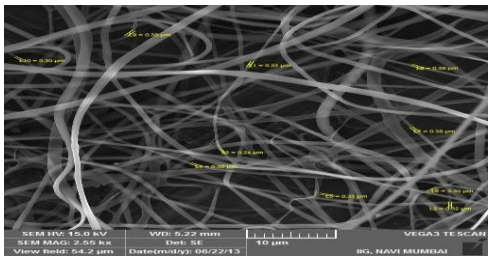


Fig No.VIII,
D-342 nm, Fr-0.1ml/hr,
V-15kv, Ds-15cm, Vs-65 Cp

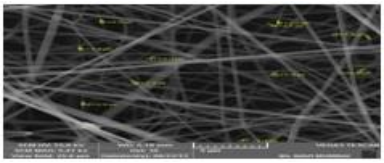


Fig No.IX,
D-155 nm, Fr-0.1ml/hr,

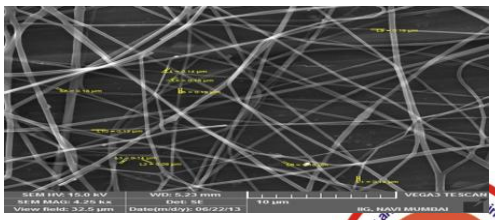
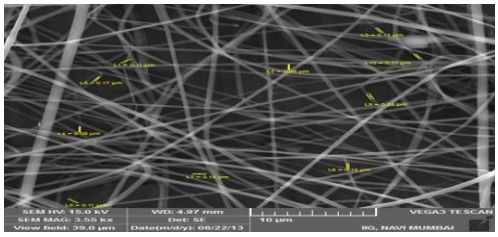
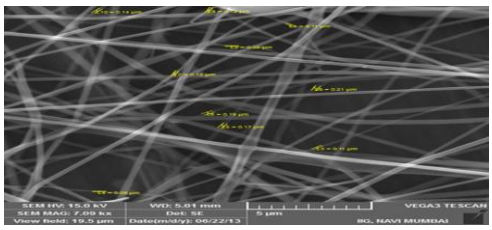
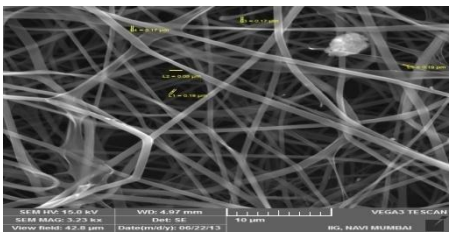
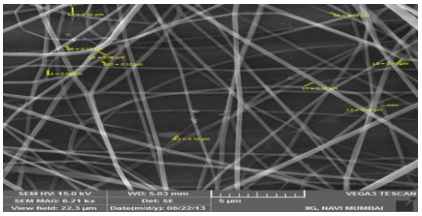
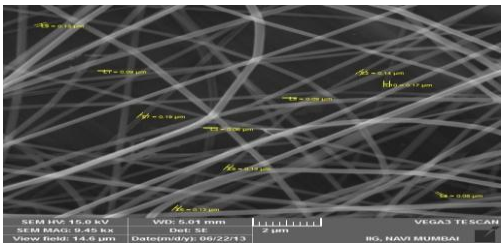
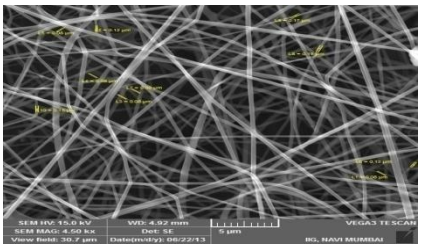
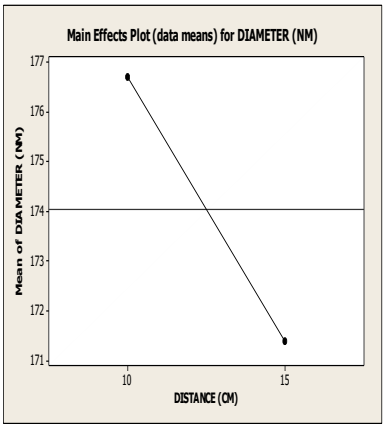


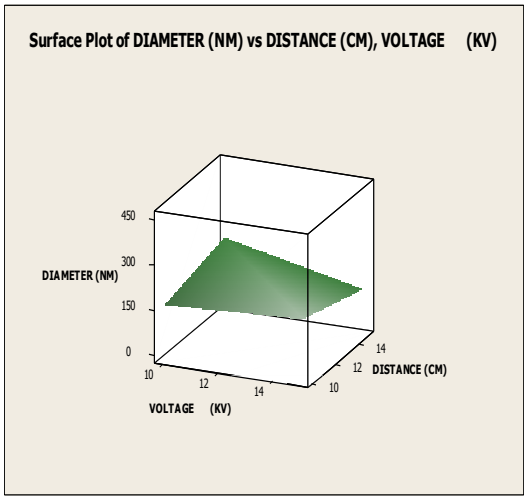
Fig No.X,

V-10kv, Ds-10cm, Vs-70 Cp	**D-161 nm, Fr-0.1ml/hr, V-10kv, Ds-15cm, Vs-70 Cp**
 **Fig No.XI, D-144 nm, Fr-0.15ml/hr, V-10kv, Ds-15cm, Vs-70 Cp**	 **Fig No.XII, D-133 nm, Fr-0.15ml/hr, V-10kv, Ds-15cm, Vs-70 Cp**

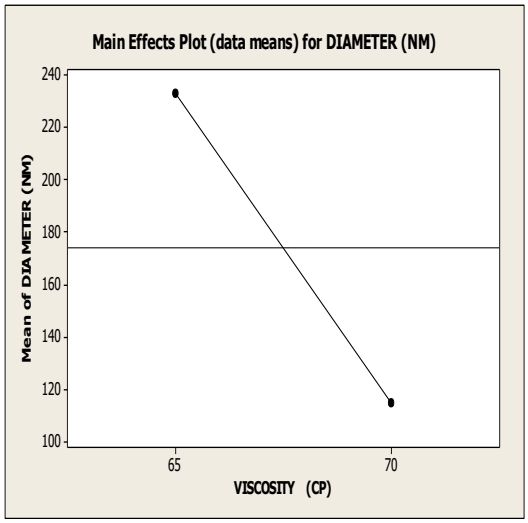
 **Fig No.XIII, D-117 nm, Fr-0.1ml/hr, V-15kv, Ds-10cm, Vs-70 Cp**	 **Fig No.XIV, D-286 nm, Fr-0.1ml/hr, V-15kv, Ds-15cm, Vs-70 Cp**
 **Fig No.XV, D-121 nm, Fr-0.15ml/hr, V-15kv, Ds-10cm, Vs-70 Cp**	 **Fig No.XVI, D-119 nm, Fr-0.15ml/hr, V-15kv, Ds-15cm, Vs-70 Cp**

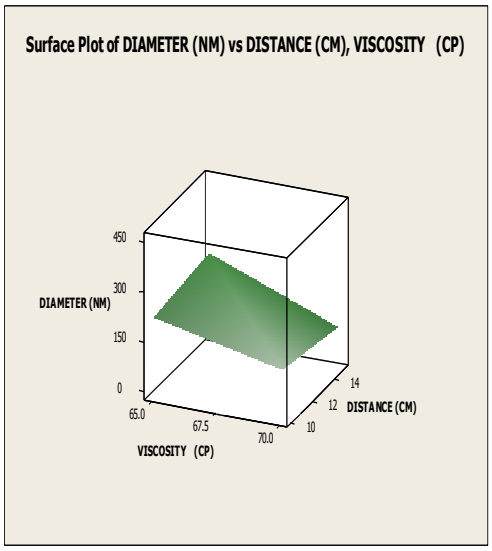
VIII. GRAPHICAL REPRESENTATION



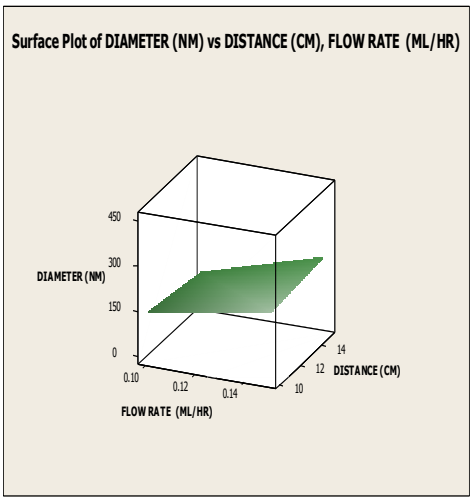


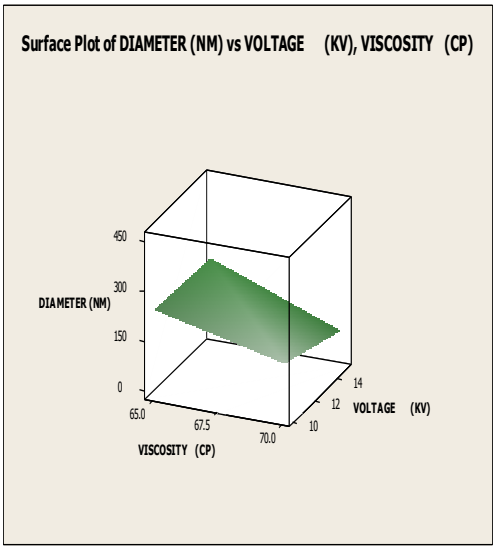
Graph 03Graph 04





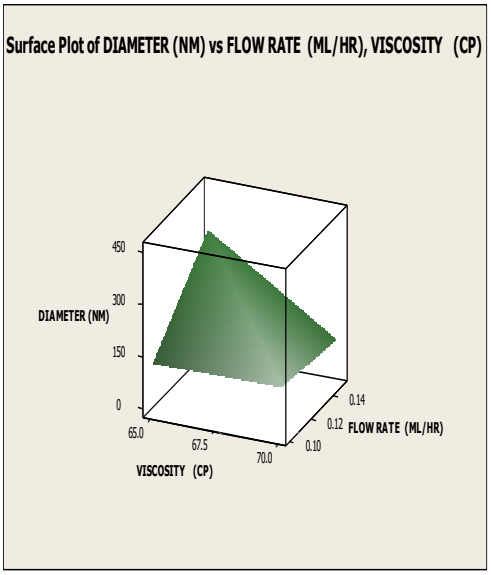
Graph 01Graph 02

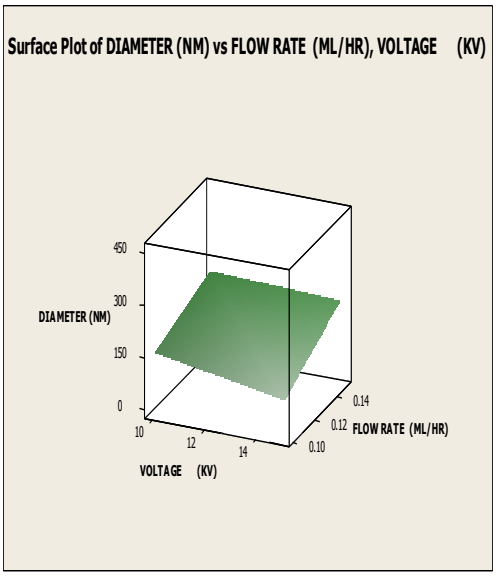




Graph 05

Graph 06





Graph 07

Graph 08

IX. CONCLUSION

In this research work, only some input parameters like as voltage, distance, flow rate And Viscosity areconsidered for the SEM and analysis of electro spinning has been done.

Following factors can be concluded.

1. Nano fiber of mixture of Curcumin, gelatine and formic acid can be manufactured.
2. These nano fibers having diameter less than 453 nano meter.
3. Distance affectsthe diameter (nm) BY 0.19% which is of no significance.
4. Voltage affectsthe diameter (nm) by 1.23% which is of no significance.
5. Flow rate considerably affectsthe diameter (nm) by 26.01%.
6. Viscosity considerably affectsthe diameter (nm) by 23.31%.
7. When flow rate and viscosity, taken together their effect is of prime importance on the diameter (nm) by 26.19%.
8. 1.D Structure nanofibre has been manufactured.
9. DOE with full factor has been carried out.

X. FUTURE SCOPE

1. Synthesis of Chitosan Nanofibres
2. Characteristics and testing of biofunctionalized nanofibers
3. Fibres to be tested for Antimicrobial activity
4. Applications to be checked for wound healing, tissue engineering, dental field

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