EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 7
358-370
Year: 2022

PREPARATION, PREFORMULATION & EVALUATION OF IMIQUIMOD LOADED NANOFIBER PATCH

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Article Received on 04/05/2022 **Article Revised on 25/05/2022** **Article Accepted on 16/06/2022**

ABSTRACT
Nanocarriers have been investigated for various biomedical applications for over a decade. In general, the use of nano-sized particles offers several advantages over other drug delivery systems.[1] In the electrospinning process a high voltage is used to create an electrically charged stream of polymer solution or melt. A high voltage electrode is linked with the polymer solution.[11] The solution is then spun through a capillary. Due to high voltage electric field between the tip of capillary and a grounded collector, Taylor cone is formed at the tip of capillary producing sub- micron in diameter fibers.[12] Fibers solidify as the polymer solvent evapourates and create an interlinked fiber layer on the surface of the collector[13] A number of synthetic, semisynthetic and natural polymers have been applied for production of Electrospun nanofibers.[19] Compared with natural sources, synthetic polymers have great flexibility in synthesis and modification. Meanwhile, natural polymers exhibit better biocompatibility and safety.[20] Natural polymers have also some unique properties such as antibacterial characteristics.[21] There are various types of natural polymers including polysaccharides, proteins, DNA and their derivatives which have been used in electrospinning process.[22] Synthetic polymers and copolymers such as poly (lactic acid) (PLA), poly (lactic-co- glycolic acid) (PLGA), poly(ε- caprolactone) (PCL), poly(vinyl pyrrolidone) (PVP), and poly(ethyleneoxide) (PEO) have been used to produce for tissue engineering and drug delivery.[23] In the field of drug delivery, different parameters of polymers such as molecular weight, polymer composition and ratio of amorphous to crystalline segments of the polymer could affect drug release from nanofibers.[24]

KEYWORDS: Nanofibre, Preformulation, FTIR, Transdermal Patch.

INTRODUCTION
Nanocarriers have been investigated for various biomedical applications for over a decade. In general, the use of nano-sized particles offers several advantages over other drug delivery systems.[1] They are used to

- Enhance the solubility of highly hydrophobic drugs.[2]
- Provide sustained and controlled release of encapsulated drugs.[3]
- Increase the stability of therapeutic agents by chemical or physical means.[4]
- Deliver higher concentrations of drugs to target areas due to an *Enhanced Permeation and Retention* (EPR) effect.[5]
- Provide targeted treatments when modified with cell-specific ligands.[6]
- Drug-loaded nanofibers often accumulate in hair follicles and thereby facilitate the penetration of drug molecules through the superficial layers of the SC, followed by drug release into the deeper layers of the skin.[7]

Applications of Nanofibers in Therapeutics Delivery

- **Anticancer Agents**

Electrospun nanofibers have been widely used to ameliorate the solubility of anticancer drugs. In this system, the drug-loaded nanofibers can circumscribe and crystallize the anticancer agents within the fiber, resulting in improvement of their dissolution rate in the biological system.[8]

- **Antibiotics**

Discovered and developed a true antibiotic named penicillin, antibiotics have been the most prescribed and used pharmaceutical agents to treat various bacterial infections.[9] Despite all the favourable characteristics of antibiotics, their appropriate delivery routes, toxicological profile, poor water solubility, in this scenario, electro spun nanofibers are considered as an alternative for antibiotic delivery because the large surface area and tuneable pore size offer maximum antibiotic loading

capacity and encapsulation efficiency.[10]

Electrospinning

In the electrospinning process a high voltage is used to create an electrically charged stream of polymer solution or melt. A high voltage electrode is linked with the polymer solution.[11] The solution is then spun through a capillary. Due to high voltage electric field between the tip of capillary and a grounded collector, Taylor cone is formed at the tip of capillary producing sub- micron in diameter fibers.[12] Fibers solidify as the polymer solvent evapourates and create an interlinked fiber layer on the surface of the collector.[13]

The process principle involves subjecting a polymer solution or melt held at its own surface tension at the end of a capillary to an electric field.[14] As the intensity of the electric field is increased, the hemispherical surface of the solution at the tip of the capillary elongates and forms a conical shape known as the Taylor cone.[15] The electric field reaches a critical value where the repulsive electric force overcomes the surface tension force.[16] At this critical value, a charged jet of the solution is ejected from the tip of the Taylor cone.[17] The solvent evapourates as the jet travels in air. In the case of the melt, the discharged jet solidifies when it travels in the air. The charged polymer fiber is randomly deposited on a collector.[18]

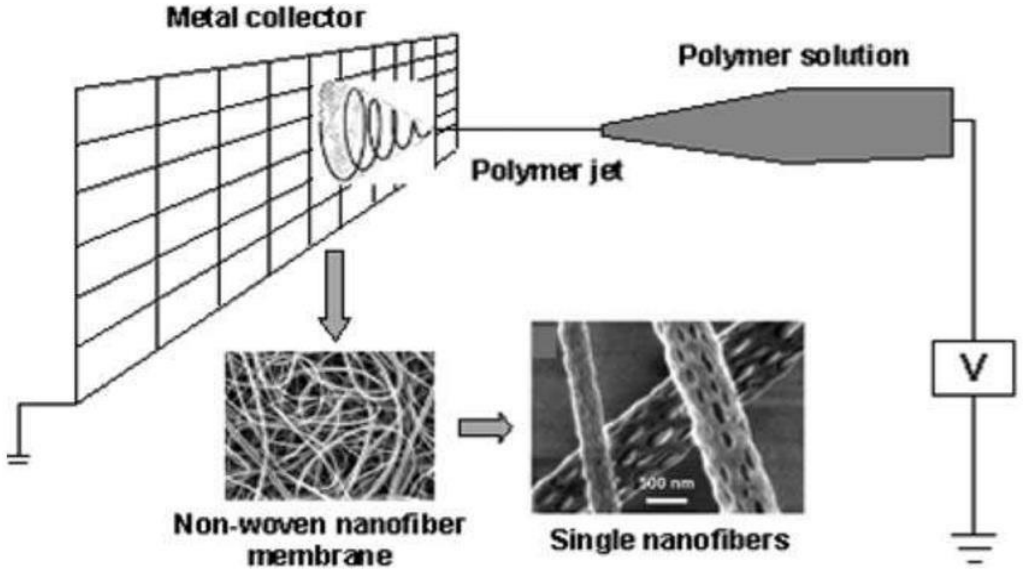

Figure 1: Schematic Diagram for the Electrospinning Process.

Polymers used in electrospinning

A number of synthetic, semisynthetic and natural polymers have been applied for production of Electrospun nanofibers.[19] Compared with natural sources, synthetic polymers have great flexibility in synthesis and modification. Meanwhile, natural polymers exhibit better biocompatibility and safety.[20] Natural polymers have also some unique properties such as antibacterial characteristics.[21] There are various types of natural polymers including polysaccharides, proteins, DNA and their derivatives which have been used in electrospinning process.[22] Synthetic polymers and copolymers such as poly (lactic acid) (PLA), poly (lactic-co- glycolic acid) (PLGA), poly(ε- caprolactone) (PCL), poly(vinyl pyrrolidone) (PVP), and poly(ethyleneoxide) (PEO) have been used to produce for tissue engineering and drug delivery.[23] In the field of drug delivery, different parameters of polymers such as molecular weight, polymer composition and ratio of amorphous to crystalline segments of the polymer could affect drug release from nanofibers.[24] The polymer composition is an important factor which is effective in drug loading and release from nanofibers.[25] Hydrophilic and amphiphilic copolymers could increase drug loading and inhibit burst drug release.[26] It was also shown that high crystallinity of polymer decreases drug release rate because of limitation of crystalline regions for water uptake compared to amorphous regions.[27]

Depending on the polymer carrier used, drug release from Electrospun nanofibers can be in a rapid, immediate, delayed, or extended manner.[28] Therefore, selection of the polymer is a crucial factor to achieve a desirable drug release profile.[29] Various polymers and their related solvents used for production of Electrospun nanofibers aimed for drug delivery.[29]

MATERIALS AND METHODS

Pre-formulation studies

It is the study of the physical and chemical properties of the drug prior compounding process. These studies focus on those physicochemical properties of the drug that could affect its performance and development of an efficacious dosage form.[31]

Organoleptic Characteristics

The drug sample was characterized for the physical characterization like appearance, color and odour. Color: Small quantity of drug was taken in butter paper and viewed in well illuminated place. Odour: Very less

quantity of drug was smelled to get the odour.

Melting point

For determination of melting point USP method was followed. Melting point of drugs was determined by capillary fusion method. The amount of the drug were placed in a thin walled capillary tube 10-15 mm long, about 1 mm inside diameter, and closed at one end. The capillary, which contains the sample, was suspended to heat the samples slowly and evenly and thermometer placed to check the temperature. The temperature range over where the sample is observed to melt is taken as the melting point of the drug.

UV absorption maxima of Imiquimod

UV-visible spectrophotometer is generally used for structural information of various drugs to obtain specific information on the chromophoric part of the molecules in solution when exposed to light in the visible/ultraviolet region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption. The UV spectrum is generally recorded as a plot of absorbance versus wavelength.

Double beam UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) was used to know the λmax of drug. A 20µg/ml solution of Imiquimod was scanned in the range of 200-400 nm.

Estimation of Imiquimod by UV-visible spectrophotometer Preparation of Stock Solution

Standard stock solution of Imiquimod was prepared by dissolving 10mg of Imiquimod in 10ml of 0.1N HCL which gives 1000µg/ml. 10ml of this stock solution was taken and was diluted up to 100ml by using 0.1N HCL to produce a concentration of 100µg/ml solution.

Preparation of Working Solution

The standard stock solution of Imiquimod (100µg/ml) was prepared in 0.1N HCL. This solution was diluted with 0.1N HCL, to obtain various dilutions from 2-20µg/ml. Absorbance of these solutions was recorded at 314nm against 0.1N HCL as blank using UV-visible spectrophotometer and standard curve was plotted against concentration. From the calibration curve intercept, slope, straight line equation and correlation coefficient were obtained.

Solubility Studies

For quantitative solubility study, excess amount of drug (Imiquimod) was taken in thoroughly cleaned culture tubes containing 5ml of different solvents and Culture tubes were tightly closed. These Culture tubes were shaking on water bath shaker at 25°C for 24 h at room temperature. After 24 h each sample was centrifuged 15,000 rpm and supernatant was withdrawal. After that supernatant was filtered and filtrates was suitably diluted and determined spectrophotometrically.

Partition Coefficient of Drug

Partition coefficient (oil/water) provides a means of characterizing the lipophilic/hydrophilic nature of the drug. Drugs having values of P much greater than 1 are classified as lipophilic, where those with values much less than 1 are indicative of a hydrophilic drug. The partition coefficient is commonly determined using an oil phase of n-octanol and water. In the case n- octanol and water.

$P_{o/w} = C_{n\text{-octanol}}/C_{water}$

The partition coefficient ($P_{O/W}$) therefore is the quotient of two concentrations of drug in n- octanol ($C_{n\text{-octanol}}$) and water (C_{water}) respectively and is usually given in the form of its logarithm to base 10 (log P).

Shake flask method was used for the determination of partition coefficient of Imiquimod. This is a classical and most reliable method of log P determination. The partition coefficient of Imiquimod was carried out in n-octanol/distilled water. The two phases were shaken together initially to ensure mutual saturation.

An accurately weighed quantity (100mg) of the drug (Imiquimod) dissolved in 10 ml of two solvents (n-octanol: Water) together (1:1) and placed in flask for 24 h at room temperature. After 24 h, the two layers were separated and centrifuge for 15 min's at 15,000 rpm. The absorbance was taken in UV spectrophotometer at the respective λ max after appropriate dilution.

FTIR of Imiquimod and Excipients

FT-IR Spectroscopy was used for structure analysis. The potassium bromide (KBr) disc technique was employed. Since the KBr has no absorption in the fundamental region of IR spectrum, only the spectrum of sample is obtained.

The KBr disc was prepared using 1 mg of Imiquimod /excipients plus drug in 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and Imiquimod mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm^{-1} region.

Drug-excipients Compatibility Study by FTIR

The compatibility of drug with excipients was ascertained by FT-IR. FTIR was used as toolto detect any physical and chemical interaction between drug and excipients. Drug and various excipients were mixed thoroughly in ratio of 1:1. Samples were scanned by FTIR under the range of 400-4000 cm^{-1}. The spectra of pure drug and drug with excipients were compared to check any incompatibility and physical changes.

Preparation and optimization of nanofibers based transdermal patch using electrospinning

Chitosan solution (1-3% w/v) was prepared by dispersing chitosan in 3% (v/v) acetic acid solution with mixture of pure drug Imiquimod (equivalent to dose) and PVA

solution (8-10% w/v) was prepared by dissolving in water at room temperature. Solutions were stirred on a magnetic stirrer overnight. Then these solutions were mixed and stirred to obtain a homogenous blend solution. The polymeric solution with mixture of drug was used for the preparation of nanofiber patch. Electrospinning was performed with laboratory electrospinning equipment (Model ES1a; Electrospinz, Kanpur). The assembly consists of a stainless steel spinning platform and grounded electrode that is connected to the metallic sheet/plate that can be covered with aluminium foil sheet to collect nanofibers. The process parameters feed rate (0.1–0.7 ml/min), electric voltage (15–22 kV) and tip to collector distance (12-15 cm) were investigated as mentioned in Table 7 & 8. The electrospinning process was carried out at ambient conditions. Briefly, the spinning solution was placed in a 3 mL plastic syringe. The feed rate of solution was adjusted by a syringe pump. The needle was connected with a positive electrode of the high voltage power supply, capable to generate 10 kv-30 kv. Grounded electrode was connected to collector which was covered with aluminium sheet for deposition and collection of nanofibers. The nanofibers formed were allowed to dry at room temperature for overnight in vacuum dissector. Nanofiber mats were carefully peeled out from sheets and stored at room temperature in hermetic plastic bags until further use.

Table 1: Composition of trial batches formulations for optimization.

Ratio (Chitosan:PVA)	Chitosan solution (%) w/v	PVA (%)w/v	Electrospinning condition		
			Potential (KV)	Flow rate (ml/hr)	TCD (cm)
5:5	1-3	8-10	15-22	0.3	12-15

Table 2: Various types of trail used for optimization of nanofiber film.

Formulation code	Ratio (Chitosan:PVA)	Chitosan solution (%) w/v	PVA (%)w/v	Electrospinning condition		
				Potential (KV)	Flow rate (ml/hr)	TCD (cm)
F1	4:6	1	10	15	0.1	15
F2	4:6	1	10	20	0.1	15
F3	4:6	1	10	15	0.2	15
F4	4:6	1	10	20	0.2	15
F5	4:6	1	10	15	0.3	15
F6	4:6	1	10	20	0.3	15
F7	4:6	1	10	15	0.5	15
F8	4:6	1	10	20	0.5	15
F9	5:5	1	10	15	0.1	15
F10	5:5	1	10	20	0.1	15
F11	5:5	1	10	15	0.2	15
F12	5:5	1	10	20	0.2	15
F13	5:5	1	10	15	0.3	15
F14	5:5	1	10	20	0.3	15
F15	5:5	1	10	15	0.5	15
F16	5:5	1	10	20	0.5	15
F17	3:7	1	10	15	0.1	15
F18	3:7	1	10	20	0.1	15
F19	3:7	1	10	15	0.2	15
F20	3:7	1	10	20	0.2	15
F21	3:7	1	10	15	0.3	15
F22	3:7	1	10	20	0.3	15
F23	3:7	1	10	15	0.5	15
F24	3:7	1	10	20	0.5	15
F25	3:7	1	10	20	0.6	15
F26	3:7	1	10	20	0.7	15

Evaluation of nanofiber formulation

1. Optical microscopy

In the process of electrospinning, nanofibers of each batch were collected on a glass side which was fixed with double faced adhesive on a collector. The nanofibers appearance and quality observed by optical microscopy.

2. Mechanical folding endurance

Number of times a film can be folded without breaking or visibly cracking it is defined as the folding endurance. The method was used to determine the folding endurance and flexibility. Ends of Electrospun nanofiber or films (2×2 cm^2) were held with forceps and folded to 180° for a maximum of 100 times or until it breaks.

3. Surface pH

Nanofiber patches were kept in contact with 0.5 ml of double distilled water for 1 h in glass tubes and were allowed to swell. A combined glass electrode was brought near the surface of patch and pH readings were taken after allowing an equilibration period of 1 min.

4. Drug content determination

Pieces of (2×2 cm^2) size were cut from each type of formulation and put in 10 ml of 0.1N HCL solution. The contents were magnetically stirred for 2 h. The solution was then filtered through Whatman filter paper (0.45 µ) and diluted suitably with 0.1N HCL. The solution was then analyzed for its absorbance at 289 nm using placebo patch as blank. From the absorbance values, the drug content was determined.

5. Drug loading Capacity (%)

The weighed Electrospun nanofiber film formulation samples were dissolved with 10ml of 0.1N HCL. Then, the mixture was shaken at 200 rpm for 24 h. Each sample solution was passed through the 0.45 µm syringe filter and then 1 ml of filtrate was analyzed by UV method.

Drug loading capacity (%) = Amount of drug content in nanofiber film / Total drug fed in nanofiber × 100

6. FTIR Spectra of formulation

The structure of the nanosized fibers was examined using Fourier transform infrared (FTIR) Avatar 380 spectrometer, in the region of 400–4000 cm−1 with the resolution of 4 cm−1.

7. Scanning electron microscopy

The morphology of the Electrospun nanofibers was observed using scanning electron microscope. The SEM samples were coated by gold sputtering in an argon atmosphere before analysis. Sample of nanofiber was placed on glass stub and vacuum dried. After the stub containing the sample was placed in the scanning electron microscope chamber and coated with gold palladium and observed microscopically at accelerating voltage of 10 Kv.

RESULTS AND DISCUSSIONS

1. Result of pre formulation study of drug

Organoleptic properties

Organoleptic properties of drug Imiquimod found to be as per I.P. monograph. The Organoleptic properties of Imiquimod were found to the given table 9.

Table 3: Organoleptic Properties of Imiquimod.

Description	Crystalline
Taste	Tasteless
Odor	Odorless
Colour	White to off white crystalline powder

2. Melting Point

Table 4: Melting Point of Imiquimod.

Drug	Specification	Observation
Imiquimod	292-294 °C	293.667±1.528°C

Discussion: The melting point of Imiquimod was found to be in range 293.667±1.528°C which is of the pure drug. Hence drug sample was free from any type of impurities.[113]

3. UV Spectroscopy

Determination of absorption maxima

Absorption maxima of Imiquimod were found to be at 314 nm similar to literature as shown in Figure 2.

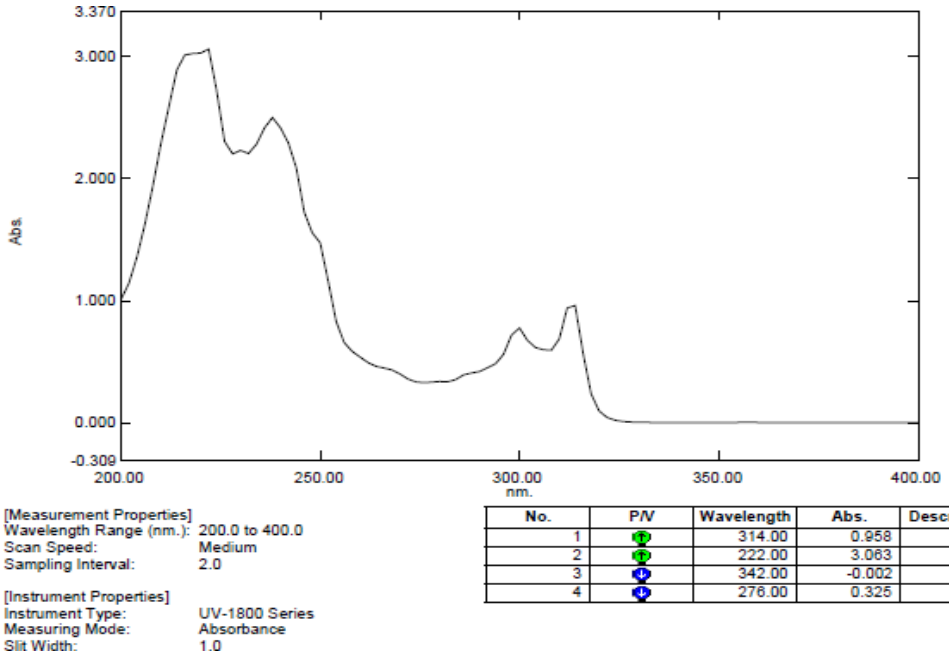

Figure 2: UV Spectrum of Imiquimod.

Preparation of standard curve of Imiquimod

Table 5: Calibration curve of Imiquimod

Sr.no.	Concentration µg/ml	Absorbance (Mean ± SD)
1	2	0.097±0.002
2	4	0.193±0.001
3	6	0.296±0.001
4	8	0.386±0.002
5	10	0.488±0.001
6	12	0.584±0.002
7	14	0.674±0.001
8	16	0.767±0.002
9	18	0.862±0.002
10	20	0.958±0.001

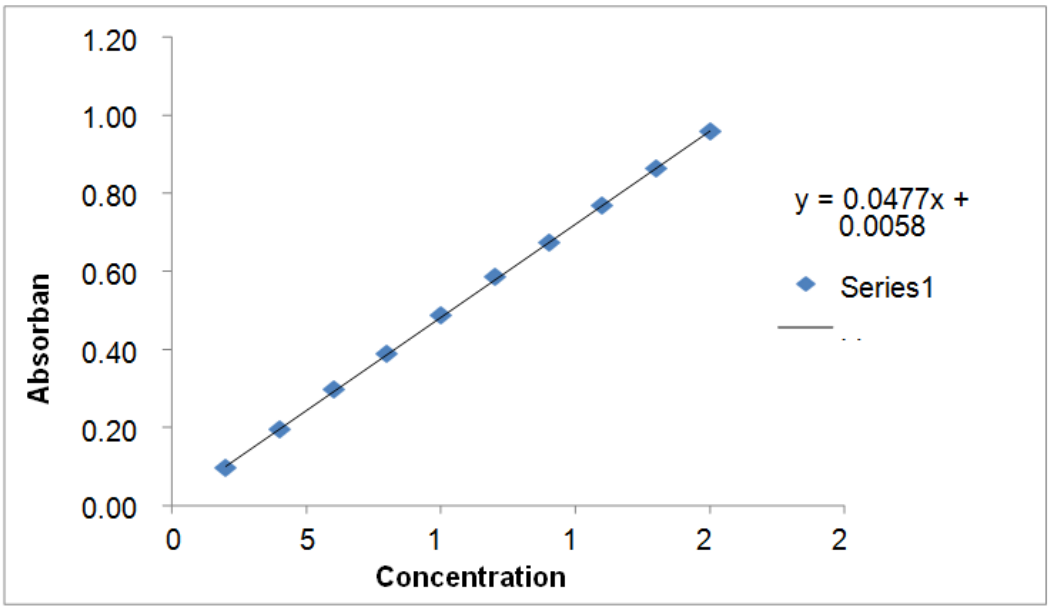

Figure 3: Graph of standard calibration curve of Imiquimod.

Table 6: Result of regression analysis of UV method for estimation of Imiquimod.

Statistical parameters	Results
λ max	314 nm
Regression equation: y=mx+C	Y=0.047x+0.005
Slope (m)	0.047
Intercept (C)	0.005
Correlation coefficient (r^2)	0.999

DISCUSSION

The calibration curve for Imiquimod was obtained by using the 2 to 20 µg/ml solution of Imiquimod. The absorbance was measured at 314nm. The calibration curve of Imiquimod as shows in graph indicated the regression equation Y = 0.047x+0.005 and R^2 value 0.999, which shows good linearity as shown in Figure 4.

Solubility studies

Solubility of drug in various solvents, were carried out in order to screen for the components to be used for formulation development. Analysis of the drug was carried out on UV Spectrophotometer at 314 nm.

Table 7: Solubility studies of Imiquimod for different solvents.

S.No.	Solvent Name	Solubility mg/ml (Mean±SD)	Solubility
1	Water	0.0003±0.0001	Insoluble
2	Phosphate Buffer pH 7.4	0.0005±0.0001	Insoluble
3	Phosphate Buffer pH 6.8	0.001±0.0003	Insoluble
4	Acetone	0.055±0.001	Insoluble
5	Chloroform	0.279±0.004	Very Slightly Soluble

6	Ethanol	0.741±0.004	Very Slightly Soluble
7	Methanol	0.977±0.003	Very Slightly Soluble
8	0.1N HCL	7.000±0.043	Slightly Soluble

* Each value is average of three independent determinations

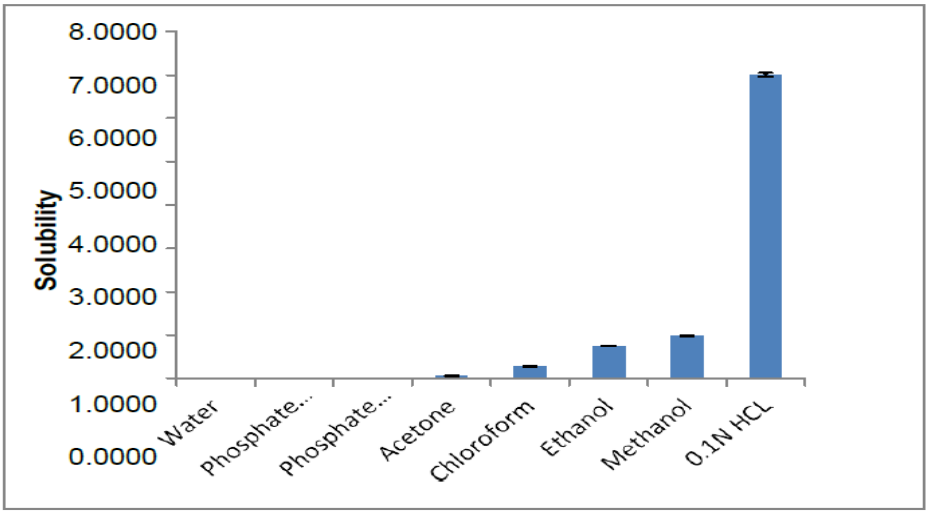

Figure 4: Solubility study of drug in different solvents.

Discussion: From the above data, it was clearly seen that Imiquimod is highly insoluble in aqueous media; its solubility increases slightly at acidic pH because it is a weak base. The solubility in different vehicles (Methanol, Chloroform, Ethanol, Acetone): which was found to be lower than 1 mg/ml.

Partition coefficient determination

Partition coefficient of the Imiquimod was determined using n-octanol and water. Log P greater than one indicates that the drug is lipophilic in nature, whereas those with partition coefficients less than one are indicative of a hydrophilic drug. This indicated the lipophilicity and purity of drug.

Table 8: Partition coefficient determination of Imiquimod.

Partition coefficient of drug	Solvent system	Log P Values Specification	Log P Values Observed
Imiquimod	n-octanol:water	2.7	2.663± 0.029

Discussion: The partition coefficient of Imiquimod in n-Octanol: Water was found to be 2.663± 0.029 this indicates that the drug is lipophilic in nature.

FTIR of Imiquimod and Excipients

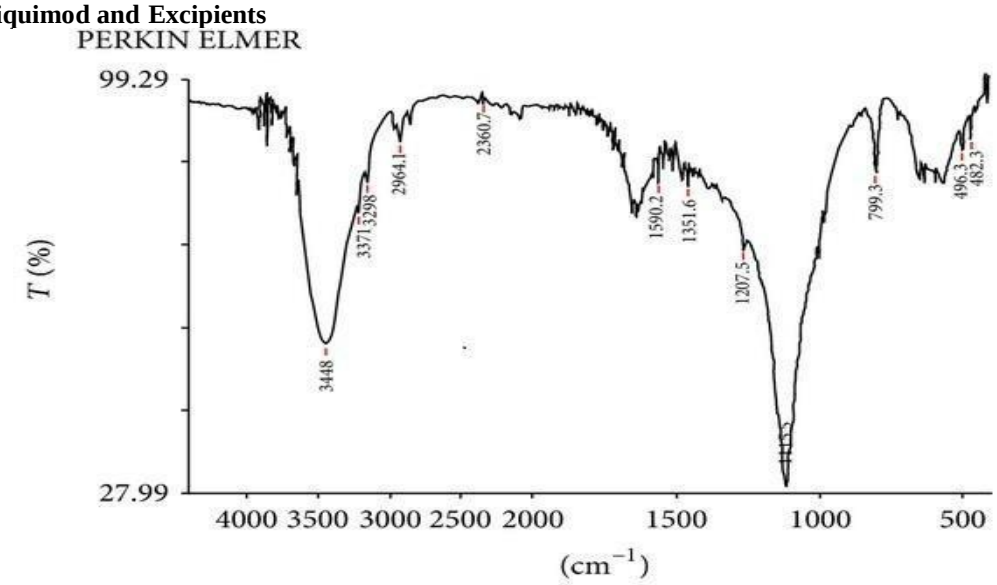

Figure 5: FTIR spectrum of Imiquimod.

The FTIR spectra of Imiquimod were shown in the **Figure 5**. The principal IR absorption peaks of Imiquimod at 2964.1 cm^{-1} (C-H stretch), 3448 cm^{-1} (OH, stretch, COOH), 1590.2 cm^{-1} (CO stretch CONH), 1351.6 cm^{-1} (C N stretching, aromatic), 1770.71 cm^{-1} (C O, stretch, COOH), 799.3 cm^{-1} (C H, bending), 1207.5 cm^{-1} (C C, stretch), were all observed in the spectra of Imiquimod. These observed principal peaks. This observation confirmed the purity and authenticity of the Imiquimod.

- **FTIR spectrum of Chitosan**

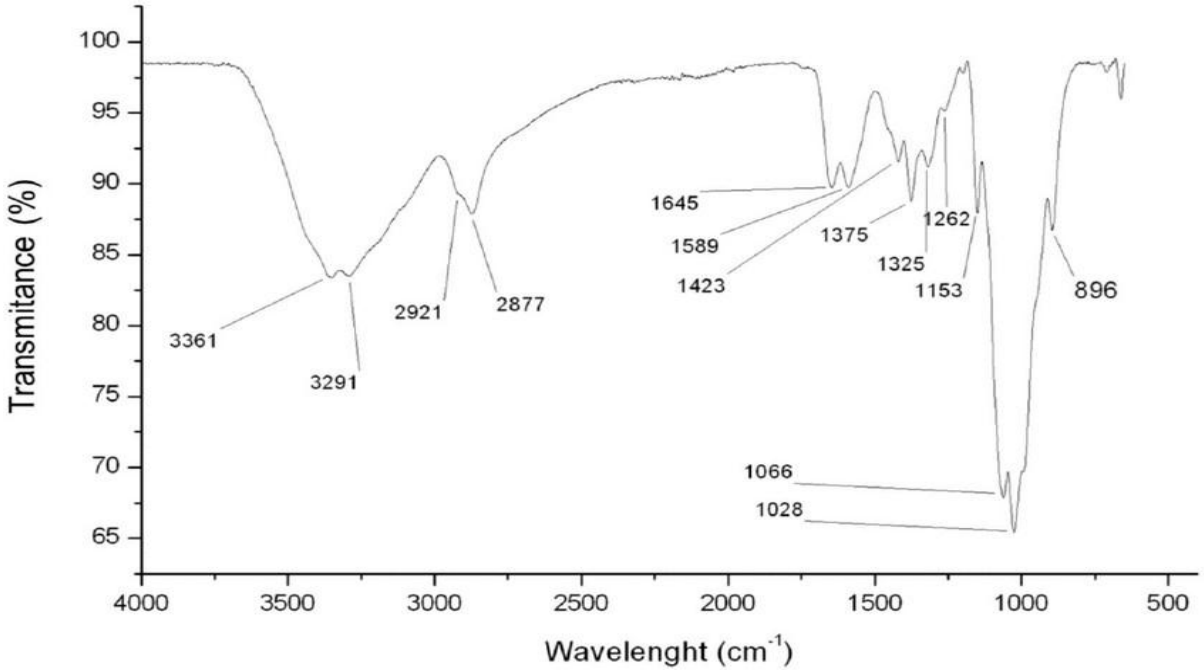

Figure 6: FTIR spectrum of Chitosan.

The FTIR spectra of Chitosan were shown in the **Figure 6**. The principal IR absorption peaks of Chitosan at 2921 and 2877 cm^{-1} (C–H stretching band of long fatty acid chain), 1645 cm^{-1} (Carbonyl stretching band in the fatty acid ester), 1262 cm^{-1} (P=O stretching band), 1066 cm^{-1} (P–O–C stretching band) and 896 cm^{-1} (N+(CH3)3 stretching) were all observed in the spectra of Chitosan These observed principal peaks. This observation confirmed the purity and authenticity of the Chitosan.

- **FTIR spectrum of Polyvinyl alcohol (PVA)**

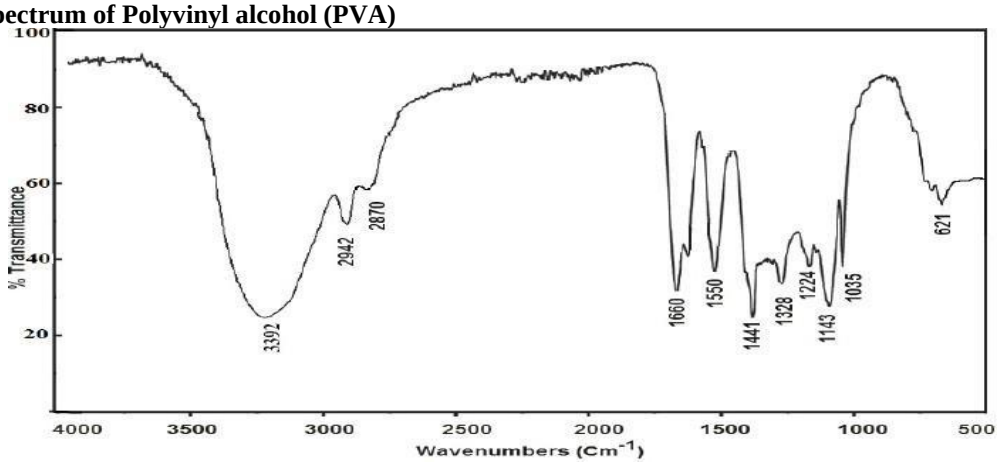

Figure 7: FTIR spectrum of Polyvinyl alcohol

The FTIR spectra of Polyvinyl alcohol were shown in the **Figure 7**. The principal IR absorption peaks of Polyvinyl alcohol at 2942 and 2870 cm^{-1} (C–H stretching band of long fatty acid chain), 1660 cm^{-1} (Carbonyl stretching band in the fatty acid ester), 1224 cm^{-1} (P=Ostretching band), 1143 cm^{-1} (P–O–C stretching band) and 1035 cm^{-1} (N+(CH3)3 stretching) were all observed in the spectra of Polyvinyl alcohol. These observed principal peaks. This observation confirmed the purity and authenticity of the Polyvinyl alcohol.

- **FTIR of Pure drug and physical mixtures (Chitosan and Polyvinyl alcohol):**

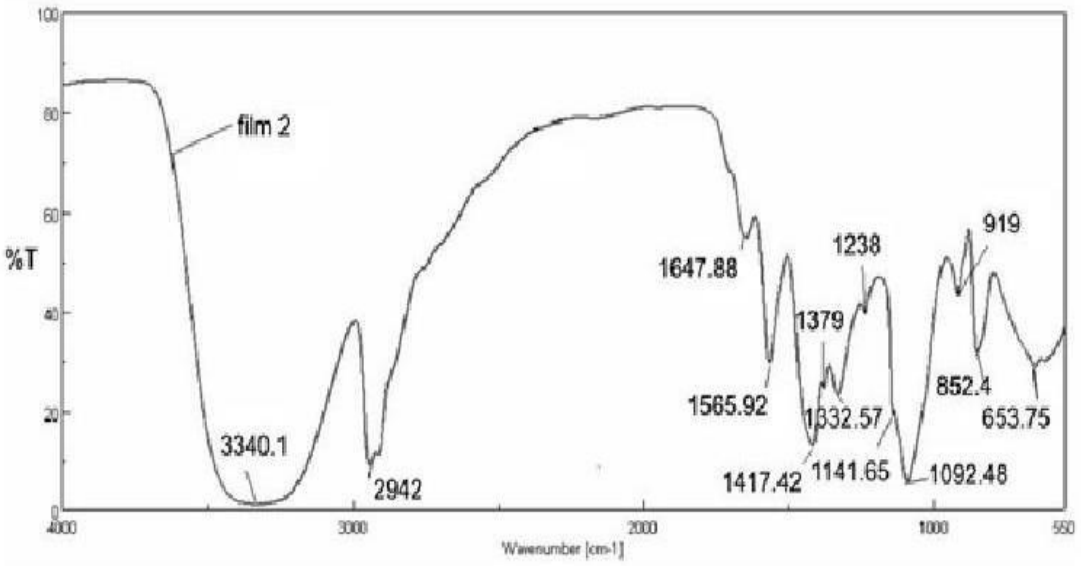

Figure 8: FTIR spectra of Imiquimod, Chitosan and Polyvinyl alcohol.

FTIR of Pure drug and physical mixture studies **(Figure 8)** were carried out to eliminate the possibility of interaction between drug and excipients. All the spectrum peaks revealed that corresponding peaks of drugs are present in the above spectra along with excipients peaks. Hence no interaction was observed in this mixture.

Preparation & Optimization of Nanofiber Patch

The morphology and diameter of Electrospun nanofibers are dependent on the various parameters such as solution concentration, applied voltage, and elecrospinning distance and so on. Optimization of nanofiber patches was determined by using different concentration of chitosan (1-3% w/v) and PVA solution (8-10% w/v) at different process parameters; feed rate (0.1–0.7 ml/min), electric voltage (15–22 kV) and tip to collector distance (12-15 cm).

Evaluation & characterisation of nanofiber Patch

Optical Microscopic Analysis

RESULT: The optical microscopic revealed that F20, F22, F24 & F25 nanofiber were formed with uniform nonwoven texture and no bead was appeared but in rest of nanofiber image indicted that nanofiber were not uniform and highly thick with bead (Table 15).

Folding endurance studies

Folding endurance of the films is an important mechanical property that characterizes the ability of films to withstand the stresses applied in the packaging, storage and handling. It has been suggested that the film with folding endurance of 40 or more could be considered as flexible. The nanofiber mat had passed the folding endurance test limit more than 100 time and no visible cracks were observed (naked eye). The nanofiber mat was more flexible as compared to casted film during folding process. It might be due to the non-woven inter porous network of nanofiber mat. However, further improvement in the mechanical properties of both films may be required to avoid the physical damage during packing and patient handling.

Surface pH

Table 9: pH values of formulations (F20-F25)

Sr. No.	Formulation Code	pH (Mean ± S.D)
1	F20	7.25±0.04
2	F22	7.16±0.04
3	F24	7.29±0.01
4	F25	7.22±0.03

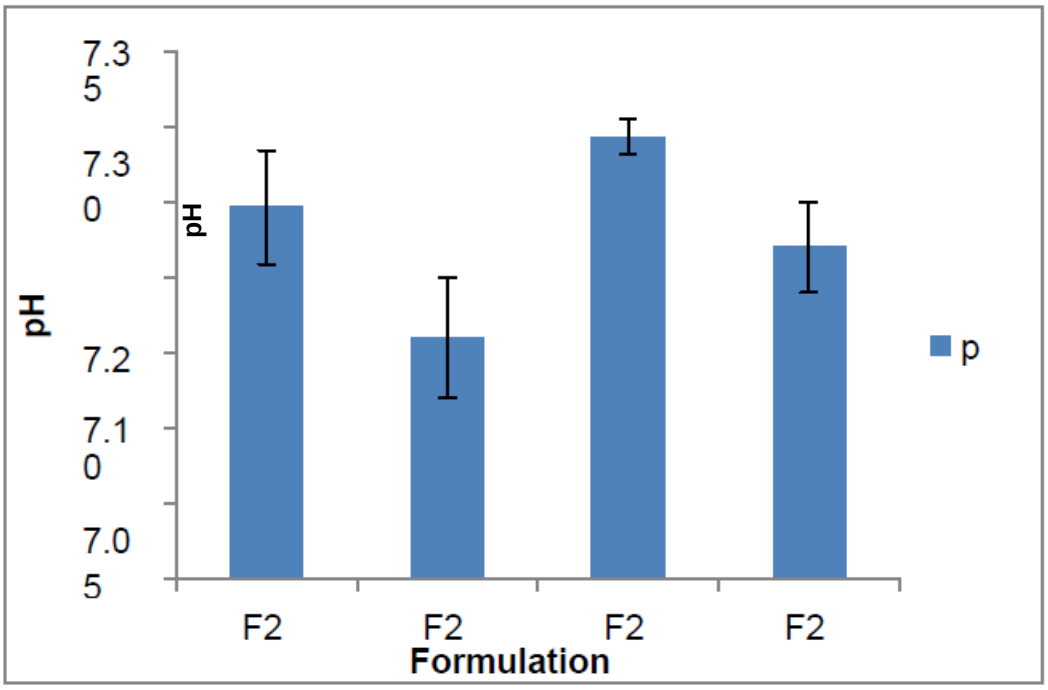

Figure 9: pH values of Nanofiber formulations.

Discussion: From the Table 16, it was found that pH of formulations were found to be in a range 7.16±0.04 to 7.29±0.01.

Drug Content (%)

Table 10: Percentage Drug Content of nanofiber patch.

Formulation Code	Drug Content (%)
F20	94.320±0.379
F22	96.927±0.569
F24	98.043±0.692
F25	93.235±0.478

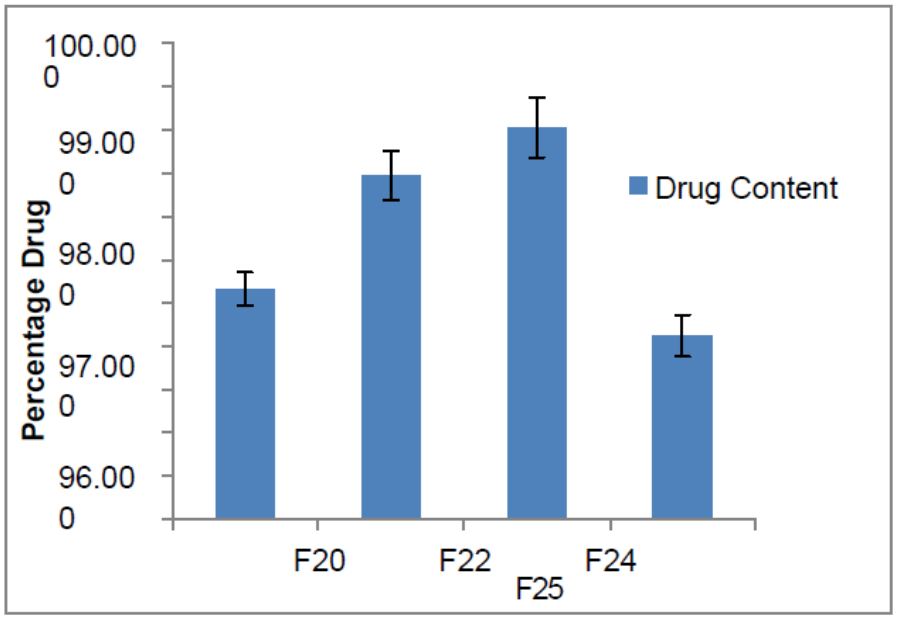

Figure 10: Percentage Drug Content of Nanofiber Patch.

Discussion: The results show that Percentage drug content of all formulation was found to be in a range 93.235±0.478 to 98.043±0.692.

Drug loading Capacity (%)

Table 11: Percentage Drug Loading capacity of nanofiber patch.

Formulation Code	Drug Loading Capacity (%)
F20	8.274±0.071
F22	8.892±0.045
F24	10.159±0.069
F25	9.246±0.069

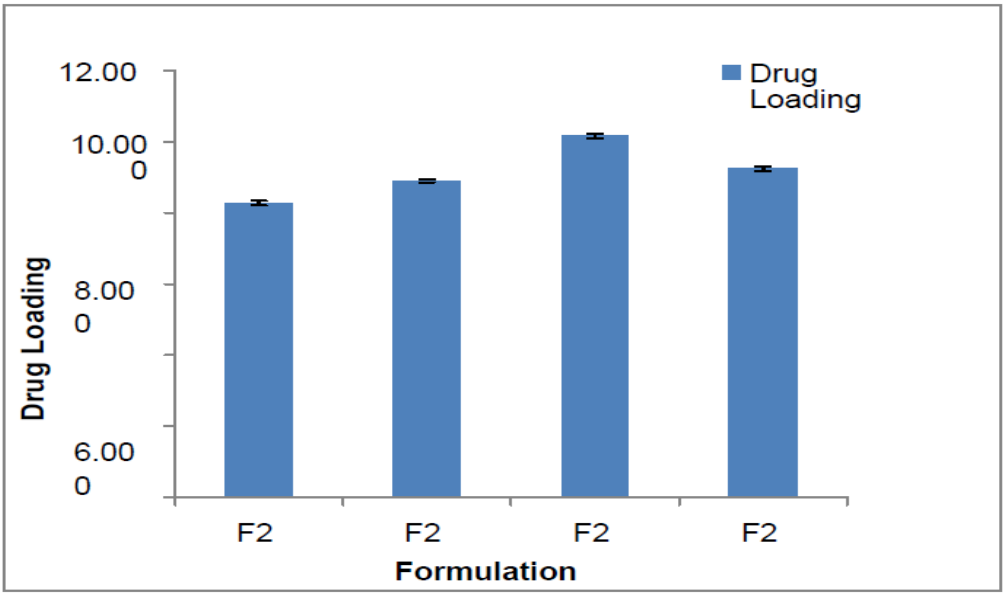

Figure 11: Percentage Drug Loading capacity of Nanofiber Patch formulations.

Discussion: From Figure 12 & Table No. 22, it was found that drug loading capacity of nanofiber in the range from 8.274±0.071 to 10.159±0.069. Drug availability in F24 the formulation was satisfactory. The linkage structure of fiber allowed the drug to associate within the fiber. F24 showed good loading capacity of drug and smooth nanofiber was appeared in SEM image. So, F24 was carried out for further studies.

On the basis of result of above parameters Formulation F24 was selected for further in-vitro drug release study.

FTIR analysis of nanofiber film (F24)

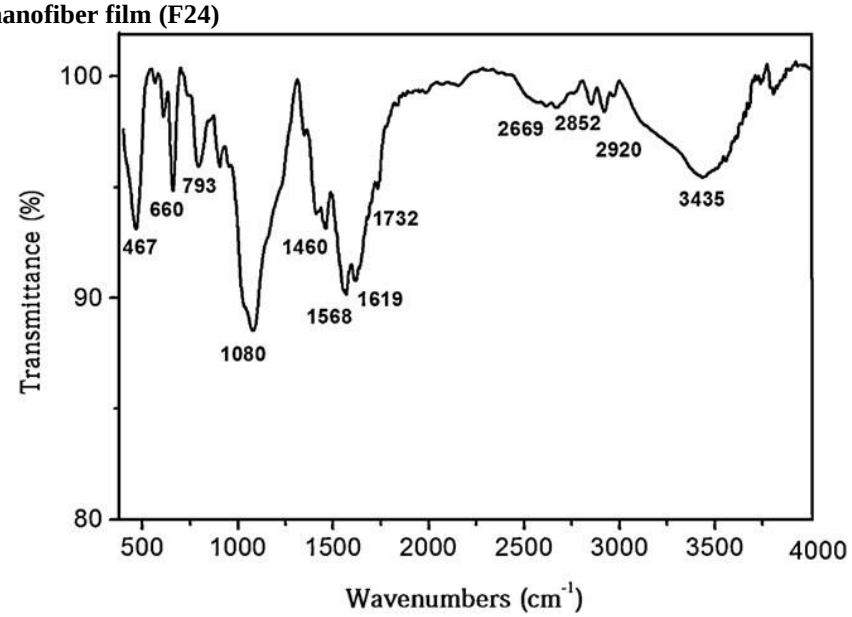

Figure 12: FTIR analysis of nanofiber film (F24)

As seen from Figure 13, FTIR spectrum of Nanofiber displayed some peaks of Imiquimod with reduced intensity. FTIR spectrum of pure drug Imiquimod showed the characteristic peaks at wavenumber of 3435 cm^{-1}, 2920 cm^{-1}, 793 cm^{-1}, representing to the aromatic C-H stretch, aliphatic C-H stretch, carboxylic acid, respectively.

Discussion: There was no alteration in the characteristic peaks of drug, chitosan and PVA suggesting that there was no interaction between the drugs and polymer.

Morphology characterization by scanning electron microscopy (SEM) analysis

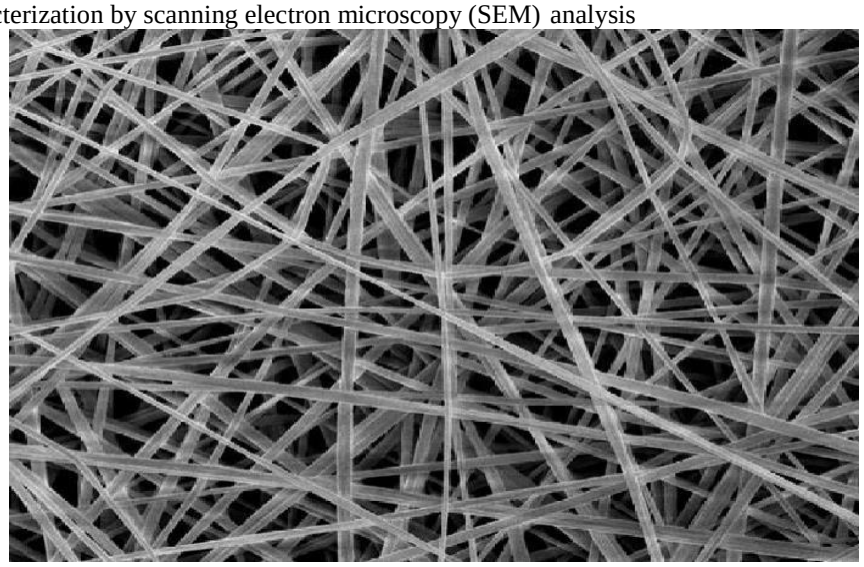

Figure 13: SEM micrograph of Electrospun nanofiber mat of F24.

Result: SEM was used for the examination of morphology of Electrospun nanofiber patch. The SEM micrographs revealed that F24 nanofiber patch were formed with uniform nanofiber (Figure 14). There was no phase separation or drug crystals observed in PVA Electrospun nanofiber mats, possibly suggesting that the drug is homogenously dispersed in PVA.

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