Design and Development of Drug Eluting Stent Graft for the Treatment of Coronary Diseases

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A thesis submitted in partial fulfillment of the requirement for the degree of Masters of Science

In

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Thesis Supervisor: **Dr. Murtaza Najabat Ali**

Thesis Supervisor's Signature: _____

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DEDICATION

I dedicate my thesis to my parents and family who have always been my nearest and have been so close to me that I found them with me whenever I needed. It is their unconditional love that motivates me to set higher targets.

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List of Abbreviations

PVA	Polyvinyl alcohol
P. Aeruginosa	Pseudomonas Aeruginosa
E. coli	Escherchia coli
MRSA	Multiple resistant staphylococcus aureus
PBS	Phosphast buffer solution
%AA	%Antioxidant activity
AA	Ascorbic acid
SEM	Scanning electron microscopy
FTIR	Fourier transformed infrared spectroscopy
XRD	X-ray diffraction
DPPH	1, 1-diphenyl-2-picrylhydrazyl
C-O	Carbonyl stretching
CH-	Alkyl stretching
A1	0.65% curcumin concentration
A2	1.09% curcumin concentration
A3	2.19% curcumin concentration
A^1	Absorbance of reference and sample extract
A^0	Absorbance of control reaction
VSMCs	Vascular smooth muscle cells
DSC	Digital scanning calorimetry

CD	Cyclodextrin
HNT	Halloysite nanotubes
THF	Tetrahydrofuran
PDLLA	Poly (D,L-lactide)
PU	Polyurethane
PCU	Poly carbonate urethane
MWCNT	Multiwalled carbon nanotube
PAN	Polyacrylonitrile
Hap	Hydroxyapatite
Pul	Pullulan
API	Amaranth protein isolate
DES	Drug eluting stent
FDA	Food and Drug Administration
PET	Poly (ethylene terephthalate)
PCU	Poly carbonate urethane
PLGA	Poly (D, L-lactide-co-glycoide)
ACP	Amorphous calcium phosphate
PCI	Percutaneous coronary intervention
ARE	Antioxidant response time

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Abstract

Novel curcumin loaded polyvinyl alcohol (PVA) nanofibrous mats were fabricated using single nozzle electrospinning technique. Curcumin, an antioxidant and anti-microbial drug was incorporated in PVA which is a synthetic, nontoxic and biodegradable polymer. Combination of the two components was used to get an added benefit for the prevention of late in-stent restenosis. Fixed ratios of curcumin and PVA were used for the production of polymeric films. Antioxidant activity of the polymeric films was evaluated and films with highest concentration of the curcumin showed highest antioxidant activity. Antimicrobial activity was tested against E. coli, P. aeruginosa and MRSA and the composition with lowest curcumin content (0.65%) showed highest activity of all compositions. In vitro drug release and degradation profiles were also evaluated. The degradation and drug release results suggested the composition with 0.65% curcumin to be the best for use in the treatment of coronary artery disease along with the use of DES. The study concluded the electrospun mats to have potential use in the treatment of post-stent complications i.e. delayed endothelialization and late in-stent restenosis.

Key words: Curcumin, PVA, antioxidant, drug release, in vitro studies, nanofibrous mats, electrospun mats

1. Introduction

Coronary artery disease or the ischemic heart disease is caused due to the hardening of the arteries occur due to low density lipid deposition on the endothelium of the vessel which cause narrowing of the arteries, hence narrowing the pathway for blood flow. The patients may represent with the symptoms of stable angina or they might not show any symptom but later the patients suffer from acute coronary syndrome due to the rupture of the plaque and thrombus formation (O'Gara et al. 2009). It was found to be the leading cause of death of all the diseases in the world. About 54 million people experience angina pectoris worldwide. World health organization report (2008) indicated that 12.2% people died of the disease. Those who survived angina pectoris at first died of a cardiac arrest within a year. To treat the disease metallic stents and bypass grafts have been used so far (Bhatia, S. K., 2010).

Drug eluting stents (DES) have been brought in use for the treatment of cardiovascular diseases. Sirolimus eluting stent was the first Food and Drug Administration (FDA) approved DES, also known as Cypher. Xience, Endeavour and Taxus are the other FDA approved DESs which are coated with everolimus, paclitaxel and zotarolimus, respectively (Sheiban et al., 2008). The two primary drugs used for coronary artery disease treatment are sirolimus and paclitaxel. In-stent restenosis has been one of the major concerns after stent implantation. Both the drugs are capable of inhibiting recurrence of the stenosis through various mechanisms (Ma, X., Oyamada, S., Gao, 2011; Iijima, R., Ikari, Y., Miyazawa, A., 2004; Schofer et al., 2003).

Stent graft, an endoprosthesis, is usually composed of a polymeric non-biodegradable sheath for the treatment of endovascular aortic aneurysms, coronary artery aneurysms, etc. Stent grafts are sheaths that provide the blood a single conduit to flow through the blood vessels (Zhang, P., Li, Q. F., & Zhang, H., 2012). They reduce the pressure of the blood onto the arterial walls, hence, keeping it from rupturing which is lethal in most of the cases. But the problem remained to recur even after the treatment (Desai, M., Eaton-Evans, J., Hillery, C, 2010).

Endovascular aortic repair (EVAR) has been performed using stent-grafts. Bioabsorbable stent graft has been produced consisting of two parts; the stent itself and a permanent graft. The grafts are made up of flexible but non-biodegradable materials such as polyethylene terephthalate (PET), polycarbonate urethane (PCU) and polyurethane (Burnside et al., 2003).

Polyvinyl alcohol is a synthetic polymer that has been extensively used in various applications. Owing to its non-toxicity, biocompatiblility, biodegradablility and hydrophilicity, good physical and chemical properties, it has been arduously studied in biomedical applications like contact lenses, wound dressings, etc (Shao, C., Kim, H. Y, 2003; Walker, Young, Hunt, & Henderson, 2007; Yang, Lee, Lin, Yang, & Chen, 2007; Yang, E., Qin, X., & Wang, S., 2008.; Jia, Y. T., Gong, J., Gu, X. H., 2007).

Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a natural, yellow colored, bioactive, polyphenol, extracted from the rhizomes of Curcuma Longa which belongs to Zingiberaceae family. Owing to its potential medicinal properties, it has been commonly used in South Asians. It is a low molecular weight and a lipophilic agent. Curcumin has been widely used due to its potent pharmacological properties like antioxidant, anti-inflammatory, anti-cancer and anti-microbial activities (Fallah et al., 2016; Dhurai, B., 2013; Shehzad, A., 2013; Perumal, G., 2017; .Saeed, S. M., Mirzadeh, H., 2017; Ravikumar et al., 2017; Mouthuy et al., 2017).

The current work focuses on the antioxidant and anti-bacterial activity of the electrospun nanofibrous mats of PVA and curcumin for the treatment of post-stent complications; late stent thrombosis and re-endothelialization. In-vitro analysis of electrospun films has been performed. In- vitro degradation and drug release tests were performed as well. Characterization tests; SEM, FTIR and XRD were performed for PVA-curcumin electrospun films.

2. Literature Review

Neointimal growth after stent placement has been a major setback for decades. Drug eluting stents have been introduced to treat the problem. Sirolimus and paclitaxel loaded poly (D, L-lactide-co-glycolide)/ amorphous calcium phosphate, PLGA/ACP biodegradable drug eluting stents were created to take care of the in-stent restenosis. The effect of both the drugs was retained. The study revealed that the combination of the two drugs can be brought in use for coronary arterial diseases (Ma, X., Oyamada, S., Gao, F., 2011).

Drug eluting stents (DES) of first and second generation, used in coronary arteries, present with late and very late stent thrombus formation. Anti-platelet therapy has been used in combination with DES to prevent these problems. Follow up is necessary for ensured well being of the patient (Takayama, T., Hiro, T., & Hirayama, A., 2011).

The major setback with Percutaneous coronary intervention (PCI) is the neointimal formation that cause the arterial blockage. Drug eluting stents have been proven to have minimized this problem to a greater extent. Bringing an improvement in DES technology can enhance its effectiveness (Htay, T., & Liu, M. W., 2005).

Due to the failure of balloon angioplasty and bare metal stents (BMS) to cater the problem of in-stent restenosis, Drug eluting stents have been introduced. Risk of late and very late stent thrombosis has been drastically reduced with the development of the second generation DES. Biodegradable and bioresorbable polymeric stents and scaffolds, respectively, have been created. Delayed endothelilalization have been treated with the biodegradable drug eluting stents containing antiproliferative drugs (Martin, K., & Mehran, R., 2018). However, complete elimination of the in-stent thrombosis has not been achieved up till now. Also the polymeric by products were found to cause inflammation (Bhatia, V., Bhatia, R., & Dhindsa, M., 2004).

Curcumin, a hydrophobic and biologically active polyphenolic compound (Perumal, G., 2017; Dhurai, B., 2013), is a yellow colored, non-toxic, turmeric extract which has been used as an anti-inflammatory for the treatment of diseases like obesity and neurodegenerative diseases, metabolic diseases and pancreatitis, arthritis and irritable bowel syndrome, etc (Shehzad, A.,

Rehman, G., & Lee, Y. S., 2013; Shehzad, A., Lee, J., & Lee, Y. S., 2013; Shehzad, A., Wahid, F., & Lee, Y. S., 2010).

Cardiovascular diseases are also reported to be treated with curcumin. Curcumin causes reduction of cell proliferation of inflammatory cells by acting on various molecular targets. Curcumin enhances the expression of p21 by inducing HO1 expression that occurs due to the activation of Nrf2-dependent antioxidant response element (ARE) which suppresses the vascular endothelial cell proliferation and vascular smooth muscle cell proliferation. Curcumin is an inhibitor of p300 which helps in fighting cardiomyoctes hypertrophy (Shehzad et al, 2013)

Curcumin (diferuloylmethane) was found to be effective for the inhibition of vascular smooth muscle cell (VSMC) proliferation. It also caused cell cycle arrest and cellular apoptosis in the VSMCs. Protein kinase C and protein tyrosine kinase and various mRNAs expression inhibition was found responsible for the apoptosis and antiproliferation of the cells (Chen et al., 1998).

Curcumin has also been used for the treatment of a variety of cancers e.g. digestive and urological cancers, reproductive cancers, breast and bone cancers and hematological cancers, etc. It was found to be chemo-preventive; it binds to various kinases, transcription and growth factors, etc, directly or indirectly and causes the modulation of the cell cycle. It also enhances and decreases the expression of proapoptotic and antiapoptotic proteins respectively (Shehzad et al., 2010 & 2017).

Antibacterial property and antioxidant are few of the many therapeutic properties of curcumin. Polycaprolactone/gelatin/ curcumin electrospun nanofibers were fabricated and tested for anti-microbial activity. Curcumin was proven to have a strong anti-bacterial effect against *methicilin-resistant staphylococcus aureus (MRSA)* and *extended spectrum b lactamase (ESBL)* (Fallah, et al., 2016).

Curcumin loaded PLA/chitosan nanofibers were fabricated. Antioxidant activity of the fibrous mats was conducted. % Antioxidant activity (AA) was observed till 40 minutes after incubation. The %AA increased with respect to time. Curcumin was proven to have a potent antioxidant activity. In vivo cytotoxicity test results proved it to be non-toxic and showed improved healing rate which suggests it to be useful in wound management (Dhurai, B. et al., 2013).

Complete or partly hydrolyzed polyvinyl acetate results in the formation of polyvinyl alcohol, a synthetic polymer. The chemical, physical, film forming and mechanical behavior of PVA is dependent on the degree of hydrolysis and the extent of polymerization. Higher amount of polymerization and hydrolysis makes PVA less hydrophilic. Owing to its hydrophilicity, biodegradability and elevated chemical resistance, PVA has been used as a commercial as well as an industrial product. PVA is an FDA approved material. Due to its non-toxicity, biocompatibility and bioadhesive properties, it has also been used as a biomaterial in biomedical applications (Baker, M. I., Walsh, S. P., Schwartz, Z., & Boyan, B. D., 2012).

Nanofibrous mats of PVA/silica gel were fabricated and their characterization tests (scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), Thermogravimetric analysis (TGA) were performed. An increase in silica content caused an increase in bead formation in fibers and higher degree of crystallization in the mats and caused a decrease in hydrophillicity of PVA (Shao, C. et al., 2003).

PVA (a polyhydroxy polymer) and PVA/maleic anhydride (MA) were investigated for solubility, morphological appearance and thermal behavior through water durability test, SEM analysis and DSC, respectively. DSC and SEM indicated enhanced crystallization and a greater fiber diameter for PVA/MA fibers as compared with PVA mats (Yang, E., Qin, X., & Wang, S., 2008).

Nanofibers of PVA/chitosan (CS) were fabricated with varying concentration of CS. The membranes were investigated for DSC, XRD, SEM and FTIR. The results concluded the membranes to have an increase in fiber diameter and fiber uniformity with stronger bonding among the PVA and CS (Jia, Y. T., Gong, J., 2007).

Wang and his coworkers investigated the thermal behavior and catalytic activity of lipase enzyme loaded polyvinyl alcohol (PVA) as-spun mats in 2008. Both the activities showed homogenous dispersion of the enzyme in the polymeric fibers. The addition of lipase in hydrophilic polymers, immobilized the enzyme so stability of the enzyme was enhanced which made its effect more potent.

Sun et al. conducted a study in 2013 in which electrospun nanofibers of polyvinyl alcohol (PVA)/curcumin and PVA/cyclodextrin (CD) were fabricated. Crystalline curcumin was found accumulated in clusters in the PVA fibers while CD complexes were more evenly disseminated

in PVA. Thermal stability of the drug was enhanced while the chemical structure remained intact. A decrease in fiber diameter was observed with an increase in curcumin content. The results confirmed both the nanofibrous complexes to put forward greater stability and solubility to the drug. The developed mats were found to be potentially capable for use in the fields of wound healing and drug delivery system.

Various concentrations of polyvinyl alcohol (PVA) of different molecular weights were electrospun to investigate the effect of molecular weight on the fiber morphology of the mats. The fiber diameter varied with respect to varying molecular weights. Lower concentrations and low molecular weight PVA showed round and thin fibers while higher concentrations of PVA and high molecular weight PVA resulted in thick fibers (Koski et al, 2004).

PVA has also been used for orthopedic and cartilage implants. It has been proven to be a potentially useful material for use in tissue replacement implants (Baker, M. I., Walsh, S. P., Schwartz, Z., & Boyan, B. D., 2012). PVA has also been used along with halloysite nanotubes (HNT) in clinical operations like cartilage replacement or cartilage transplantation. PVA/HNT composites were found to have a potential use in targeted drug delivery systems, contact lenses and tissue engineering (Gaaz, T. et al., 2015).

PVA composites and blends were used to prepare hydrogels and were investigated for their mechanical behavior. Various mechanical testing measures were developed. Stiffness of the material was evaluated while using PVA/chitosan and PVA/sorbitol hydrogels. Inclusion of chitosan in PVA did not improve the stiffness of the gel while sorbitol inclusion lowered the stiffness of the hydogel. PVA only showed lesser elastic behavior than PVA/sorbitol gels. Cotton fibers gave more strength to the gels (Afghan et al., 2016).

Polymeric Aloe vera and PVA films were fabricated using solvent casting technique. Aloe vera showed up to be an excellent antimicrobial agent against *Escerchia coli (E.coli)* and *Psuedomonas aeruginosa (P.aerugenosa).* In vitro degradation, drug release and anit-fungal activities were also performed. The study concluded PVA/aloe vera films to be effective for the treatment of surgical infections (Ghafoor et al., 2016).

Mechanical properties of polyvinyl alcohol were determined while using PVA of four various molecular weights. Contact angle, Young's Modulus and tensile strength were evaluated. PVA

with the highest molecular weight had the maximum tensile strength and modulus of all the other PVAs and was found to be most hydrophilic of all (Ngadiman et al., 2015).

Various aqueous solutions of PVA with different concentrations were prepared for the investigation of the effect on fiber diameter, number of fibers per area and morphology of the mats. The PVA aqueous solutions were electrospun using a range of voltage, distance and concentrations of PVA. All the characteristics were mainly affected by polymeric concentration. Beads were observed in the fibers with an increase in polymeric concentration. Higher voltage and increasing distance decreased the effect of beading (Phachamud, T., Manisara, P., 2011).

In electrospinning, a polymeric solution is filled in a syringe or a capillary tube. The solution is induced to be charged with a high voltage supply. The voltage applied helps break the surface tension of the solution and let it reach to the metal collector surface of the machine. The increase in voltage causes the semi circular jet into a conical shape also known as the Taylor cone. Hence, a fibrous mat is fabricated with the fiber diameter ranging from nanometers to microns. Surface tension, conductivity and viscosity of the solution, voltage range, temperature and humidity have a strong impact on the morphological characteristics, mechanical, chemical and physical properties of the electrospun mats. Various water soluble polymeric solutions were prepared for electrospinning. The study accomplished that lesser diameter of the fibers provides mats with a greater length to diameter ratio and increased surface area to volume ratio (Doshi, J., & Reneker, D. H., 1995).

Electrospinning process has turned out to be favorable technique in drug delivery and tissue engineering applications. Co-axial electrospinning was found to be useful for applications that require sustained drug release. Fiber morphology and diameter varies with a change in collector size, distance between tip and the collector, applied voltage and the geometry of the needle (Rogina et al, 2014; Tan, S. H., Inai, R., Kotaki, M., & Ramakrishna, S., 2005).

Ceramic along with its composite based electrospun mats were also reviewed. The study focused on the morphological appearance of the mats, alignment and encapsulation of the carbon nanotubes (Chronakis et al., 2005).

Multi-jet electrospinning machine has also been used for an amplified output. Poly (ethylene oxide) solutions were formed using a number of nozzles. Deviation in the direction of jets was

observed which was minimized by deploying another electrode other than the primary electrode. The polarity of the metallic collector and the charged solutions was changed and electrospinning was performed which resulted in high-quality nanofibers (Varesano et al., 2009).

Poly (D, L-lactide) (PDLLA) of various molecular weights in different concentration was dissolved in tetrahydrofuran (THF) and was electrospun. A validation test was performed to relate the quantitative and qualitative results of processing parameters and morphological appearance and confirmed the results in conformity with the calculated values (Cui et al., 2007).

Electrospinning technique was brought in use to produce novel nanofibers of polyurethane (PU) only and PU/multiwalled carbon nanotube (MWCNT). Surface morphology of the films was evaluated and the results showed the fibers of 20-40 nm diameters (Kimmer, D., Slobodian, P., Petráš, D., 2009).

Collagen based tissue engineering scaffolds were formed using electrospinning machine. Collagen containing electrospun mats can accelerate cell growth and cellular penetration into the engineered matrix. The properties of the fabricated mat were different from the original tissues (Matthews, J. A. et al., 2002).

Conductive fillers and additives were incorporated in polyacrylonitrile (PAN). PAN alone and in combination with salt and carbon nanotubes (CNTs) was used to fabricate mats. All the compositions were optimized by varying different parameters of electrospinning machine. Elevation in the level of conductive material leads to the formation of large fibers of diameter in microns (Heikkilä, P., & Harlin, A., 2009).

Cobalt acetate tetrahydrate containing PVA nanofibrous mats were fabricated. These mats were calcined at 700°C which lead to the formation of cobalt (II, III) oxide nanofibers. The band gap energy of the mats was determined using Ultra violet visible spectroscopy (Barakat, 2008).

Nylon-6 and nylon-12 were electrsopun and analyzed for conformational changes. The study implied that electrospinning induces a high stress on the fibers, hence, changes in chemical structure of nylon was changed (Stephens, J. S., Chase, D. B., & Rabolt, J. F., 2004).

Curcumin loaded amaranth protein isolate (API) and carbohydrate polymer pullulan (Pul) were fabricated through electrospinning process. The fibers containing curcumin possessed greater antioxidant activity in comparison with the free curcumin (Blanco-Padilla, A,, 2015).

Nanofibrous mats were prepared using poly (ethylene terephthalate) (PET). Morphological changes were observed with variation in polymer concentration. Fibers were uniform and no bead formation was observed at higher polymeric concentration and vice versa (Veleirinho et al, 2008).

Hydroxyapatite (Hap) nanoparticles were incorporated in polyvinyl alcohol (PVA) and their mats were created through elecrtsospinning. Morphological and crystallographic analysis was evaluated which suggested that the HAp had nanopores and it was well embedded in the fibers. They had good thermal properties owing to their strong OH interaction with PVA (Kim et al., 2008).

3. Materials and Methods

3.1 Materials

Curcumin (95% total curcuminoid content) was purchased from Alfa Aesar. Polyvinyl alcohol (PVA) molecular weights 72,000 Da and 100,000 Da were purchased from MP Biomedicals, LLC, USA and UNI-CHEM, respectively. Distilled water and ethanol were the solvents used to dissolve PVA and curcumin, respectively. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Aldrich, USA for the use in the evaluation of antioxidant activity.

3.2 Microorganisms for in vitro studies

Pure bacterial cultures of *Escherchia coli (E.coli)* and *Pseudomonas aeruginosa (P. aeruginosa)* and *multiple resistant staphylococcus Aureus (MRSA)* were gathered from Mycovirus Research Lab, National University of Sciences and Technology (NUST), Islamabad for the investigation of anti-microbial activity. The cultures were preserved at a temperature of 4°C.

3.3 Solution preparation of PVA and curcumin

5% and 0.5% aqueous solutions of molecular weight 72,000 Da and 100,000 Da polyvinyl alcohols (PVA) were prepared respectively with the help of a magnetic stirrer at 80°C (for 72,000 Da) and 200°C (for 100,000 Da). The mixture was stirred until a homogenous solution was obtained. 0.65 %, 1.09% and 2.19% w/w curcumin concentrations were stirred in ethanol at 80°C to obtain a homogenous mixture. PVA low molecular weight and high molecular weights and curcumin were mixed together at room temperature in a ratio of 5:3:1. Three various solutions were prepared having the same ratio of both the PVA while concentration of curcumin was changed only.

3.4 Optimization and Fabrication of Curcumin based PVA electrospun mats

Electrospinning process was used for the fabrication of curcumin loaded PVA mats. All the prepared solutions were optimized to achieve a smooth and an error free fibrous mat. Composition with 0.65% w/w curcumin was optimized at a voltage of 26 kV at the syringe, 67 mm tip-to-collector distance and 1ml/h flow rate. The rest two compositions were optimized at the same distance as that of the first one with a voltage of 21 kV. The collector was miniaturized according to the needs of the research. The fabricated mats were preserved in vacuum to keep them from moisture and temperature.

3.5 In vitro Drug release and Degradation studies of curcumin based PVA mats

In order to investigate drug release and degradation of the drug loaded polymeric films, total immersion method was used with phosphate buffer solution (PBS) as the release medium. Curcumin release profile of curcumin loaded PVA nanofibers was assessed through Ultra violet - Visible spectroscopy. A portion of curcumin loaded PVA nanofibrous mat of measurable size (1 cm^2) was placed in 3mL of phosphate buffer solution (pH= 7.4) at 37∘C. On the first day, for first 6h, the buffer solution was removed after every 15-minute intervals and replaced with 3ml of fresh PBS. The drug release was monitored on daily basis for a week. It was then observed on alternate days until 4 weeks. Curcumin showed maximum absorbance at a wavelength of 426 nm which was recorded using UV-Vis spectrophotometer (Systronics2202). The release tests were carried out in triplicate and average values were calculated (Suwantong, 2007; Dhurai, B., et al., 2013; Sun, 2013; Ghafoor et. al, 2016).

The degradation rate was measured by weighing the sample at regular time intervals and the data was recorded. Samples were weighed in wet state before and after the addition of buffer solution using a weighing balance and the weights were subtracted and recorded (Ghafoor, B., Ali, M. N., 2016). The degradation tests were carried out in the sets of three and a mean value was calculated and graph was plotted.

3.6 Antioxidant activity testing of curcumin based PVA mats

The antioxidant activity of curcumin loaded PVA nanofibers was assessed with 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. 0.1 mM ethanolic solution was prepared (4.4 mg in 100 ml ethanol). Each electrospun mat of size 1cm^2 was dissolved in an ethanolic solution of DPPH for

30 minutes in an incubator at 37°C. The absorbance was observed at a wavelength of 517 nm after 10, 20, 30 and 40 minutes of incubation. Antioxidant activity of curcumin and PVA only was investigated separately as well. DPPH in ethanol was used taken as positive control. Ethanol was used as blank. Ascorbic acid was used as a standard. Decrease in absorbance was calculated. The results of triplicate tests were averaged.

Following formula was used to calculate the radical scavenging activity of DPPH.

DPPH scavenging effect (% inhibition) = $\{(A^0 - A^1)/A^0)*100\}$ (Shen Q, et al., 2010; Blois, M.S, 1958; Xie, J., & Schaich, K. M., 2014).

Where, A^1 represents the absorbance of the reference and the extract sample and A^0 shows the absorbance of the control reaction.

3.7 Antimicrobial testing of Curcumin based PVA mats

In order to investigate the antimicrobial activity, disc diffusion method was used. 28 g of Nutrient agar (pH 7.4), microgen, Central Drug House (P) Ltd, India, was prepared in 1000 mL of distilled water. Nutrient agar was poured into Petri plates post autoclaving. Inoculation was performed on nutrient agar with bacterial inoculums (0.1 mL) from the preculture of bacterial strains. $1cm^2$ sized electrospun mats of all compositions were tested. *Escherchia coli, multi resistant staphylococcus aureus* and *pseudomonas aeurogenosa* were the test bacterial strains. All the plates were placed in incubation for 24 h at 37 °C. Inhibition zones were measured in millimeter. Three tests of each composition were performed and their means were calculated.

3.8 Characterization of Curcumin based PVA mats

3.8.1 Morphological analysis: Scanning electron microscopy (SEM)

For the analysis of SEM was conducted. VEGA3 TESCAN Analytical scanning electron microscope (JEOL, Tokyo, Japan) was used for the assessment of the surface morphology of the curcumin/PVA mats at an activation voltage of 5 kV.

3.8.2 Fourier Transform Infrared (FTIR) Analysis

Fourier transformed infrared (FTIR) spectroscopy was conducted using a Perkin Elmer spectrophotometer of spectrum 100 FTIR for the investigation of the chemical and physical interaction between curcumin and PVA. It was carried out at 256 scans with a resolution of $8cm^{-1}$. FTIR was performed for 1 final composition only.

3.8.3 X-ray Diffraction (XRD)

For the investigation the degree of crystallinity and amorphous domain in the fibrous mats, x-ray diffraction was performed. X-ray diffraction patterns were obtained over the 2ɵ range of 10-40°.

4. Results and Discussion

4.1 Degradation and drug releases

Evaluation of degradation profile of curcumin based PVA mats was done by weighing the weight loss of the samples at preset time intervals **(Figure 1**). Increase in weight was observed, 0.089 grams from 0.002 grams, in the first 30 minutes due to absorption of the phosphate buffer solution which was followed by gradual weight loss. On day 5, an increase in weight was observed again, it reached to 0.05 grams but it was lesser than the weight in the first 30 minutes and again it was followed by slow weight loss until day 30. PVA appears to have swelling properties, owing to which it absorbed the buffer solution that resulted in weight increment and then it started losing mass since it became solvated (Kenawy, El-Refaie, Abdel-Hay, 2007; Sun, X. Z., et al, 2013). The gradual weight loss occurred due to the increase in drug content as compared to the polymer (Jannesari et al., 2011). Since, a combination of two various polymers was used, so the first 30 minutes show the absorption of water by PVA low molecular weight and the second weight increment shows absorption by high molecular weight PVA. The profile was evaluated for four weeks. Readings were in triplicates and their means were recorded.

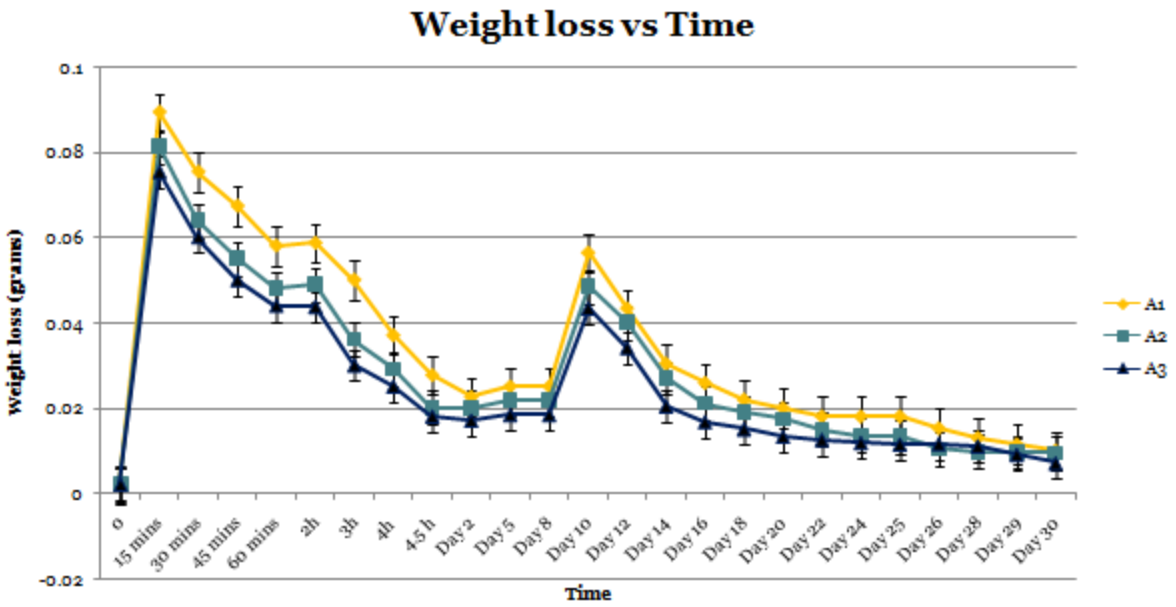

Figure 1: Weight loss of curcumin/PVA mats with 0.65% (A1), 1.09% (A2) and 2.19% (A3) w/w curcumin concentration.

PVA complexes usually present with a sustained drug release an initial burst release of the drug. Higher the drug content, greater will be the burst release. The similar mechanism occurred in the present study. An initial burst release was observed in all the three types of mats having curcumin concentrations 0.65%, 1.09% and 2.19%. With the absorption of the buffer solution, the aggregates of curcumin present on the surface of the mat were detached and hence released. The entrapped curcumin in the PVA nanofibers was released in correlation with the polymeric degradation. The first 30 minutes represented the drug release by low molecular weight PVA with the mechanism of diffusion, hence the initial burst release while the degradation of the polymer was also involved in the slow drug release period until day 5. In accordance with the results of degradation studies, the polymer again absorbed phosphate buffer solution owing to the presence of PVA of higher molecular weight. A second burst release was observed due to the curcumin aggregates left on the surface of the polymeric films which was again followed by a sustained release until day 30 (Figure 2). Mats with higher concentration of drug showed minimized drug release in the sustained release period. The trend of the drug release was found

to be similar for all drug concentrations (Sun, X. Z., Williams, G. R., Hou, X. X., & Zhu, L. M., 2013).

The results of x-ray diffraction reflected the presence of crystalline curcumin in the fibers. Higher drug concentration will have higher amount of crystalline curcumin present in the fibers. Curcumin is found to be hydrophobic and has a poor solubility in the buffer solution, so post diffusion drug release and higher lattice energies were required to release the drug, hence the sustained drug release (Fallah et al, 2016; Sun et al., 2013). The addition of high molecular weight PVA also added to a delayed degradation time.

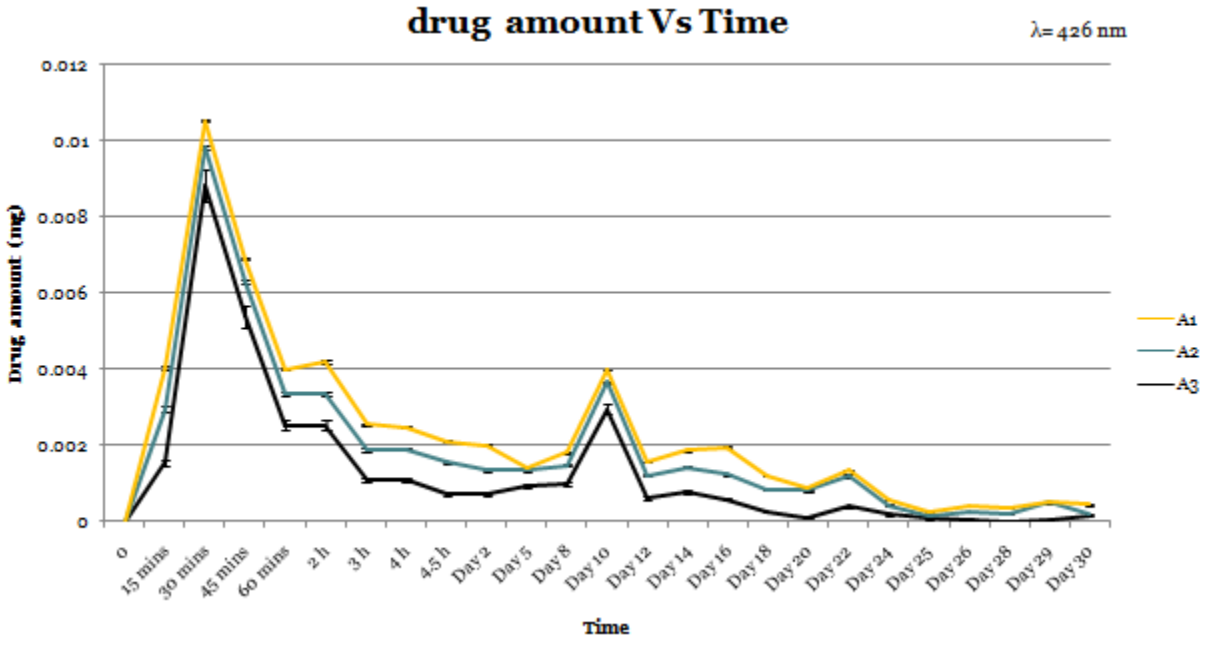

Figure 2: Drug release of curcumin/PVA mats with 0.65% (A1), 1.09% (A2) and 2.19% (A3) w/w curcumin concentration.

4.2 Antimicrobial activity of Curcumin based PVA mats

For all the compositions, curcumin showed maximum activity for *multiple resistant staphylococcus aureus (MRSA),* moderate for *Escherchia coli (E.coli)* and least activity for *Pseudomonas Aeruginosa (P. Aeruginosa).* The means of inhibition zones of *MRSA, E.coli* and *P. Aeruginosa* for 0.65 % w/w, 1.09% w/w and 2.19% w/w curcumin concentrations are represented in **figure 4** (Saeed, S. M., Mirzadeh, H., Zandi, M., & Barzin, J., 2017).

Figure 3: Pictorial demonstration of antimicrobial activity of curcumin/PVA mats with various curcumin concentrations against MRSA.

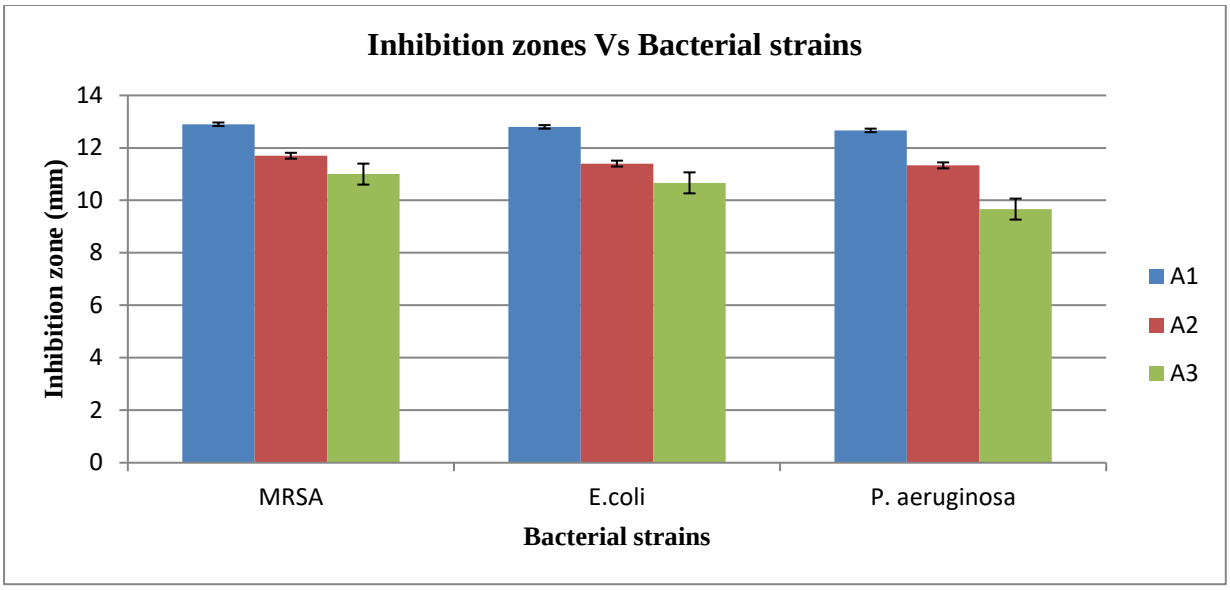

Figure 4: Antimicrobial activity of curcumin/PVA mats with curcumin concentration as 0.65% (A1) w/w%, 1.09% (A2) w/w% and 2.19% (A3) w/w%.

4.3 Antioxidant activity

1, 1-diphenyl hydrazine (DPPH) is a purple colored free radical having maximum absorption at a wavelength of 517 nm. After reaction with the free radical scavenger, DPPH is reduced to DPPH-H (hydrazine) while its color is changed to yellow from purple. Decrease in absorbance was calculated in %Antioxidant activity (%AA) by the following formula.

DPPH scavenging effect (% inhibition) = $\{(A^0 - A^1)/A^0)*100\}$

The %AA of the curcumin loaded PVA nanofibers was evaluated using DPPH assay. Absorbance was observed at certain time intervals at 517 nm wavelength.

PVA was used as negative control. Curcumin only and DPPH (in ethanol) were used as positive controls. Decrease in absorbance showed increase in %AA. % Antioxidant activity was increased with increasing time intervals. The maximum activity was shown by the composition with 2.19% w/w of the drug, 0.65% showed the least activity and 1.09% w/w showed moderate activity **(figure 5, figure 6, and figure 7)**. However, the activity of curcumin appeared to be decreased after binding with PVA as the same concentrations of curcumin showed higher antioxidant activity in comparison with the curcumin loaded fibrous mats **(figure 5, figure 6, and figure 7)**. Absorbance for positive control (DPPH only) was taken as 1 at 517nm.

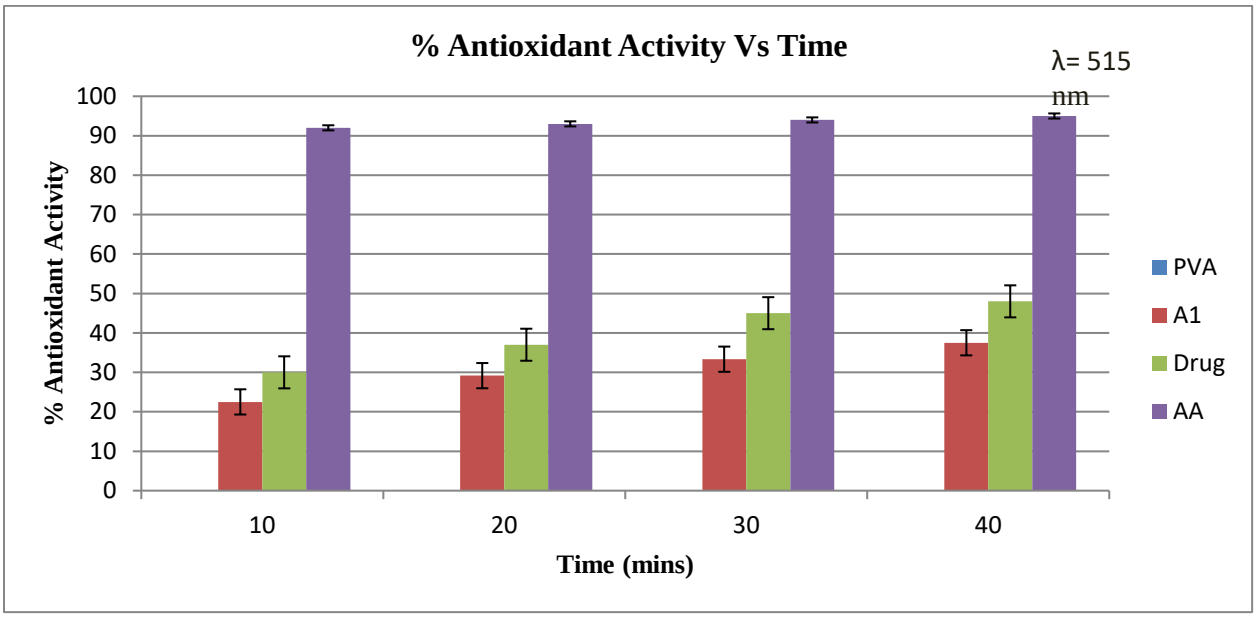

Figure 5: % Antioxidant activity of curcumin/PVA mats with 0.65% w/w (A1) curcumin concentration. AA represents ascorbic acid and drug shows the antioxidant activity of curcumin alone.

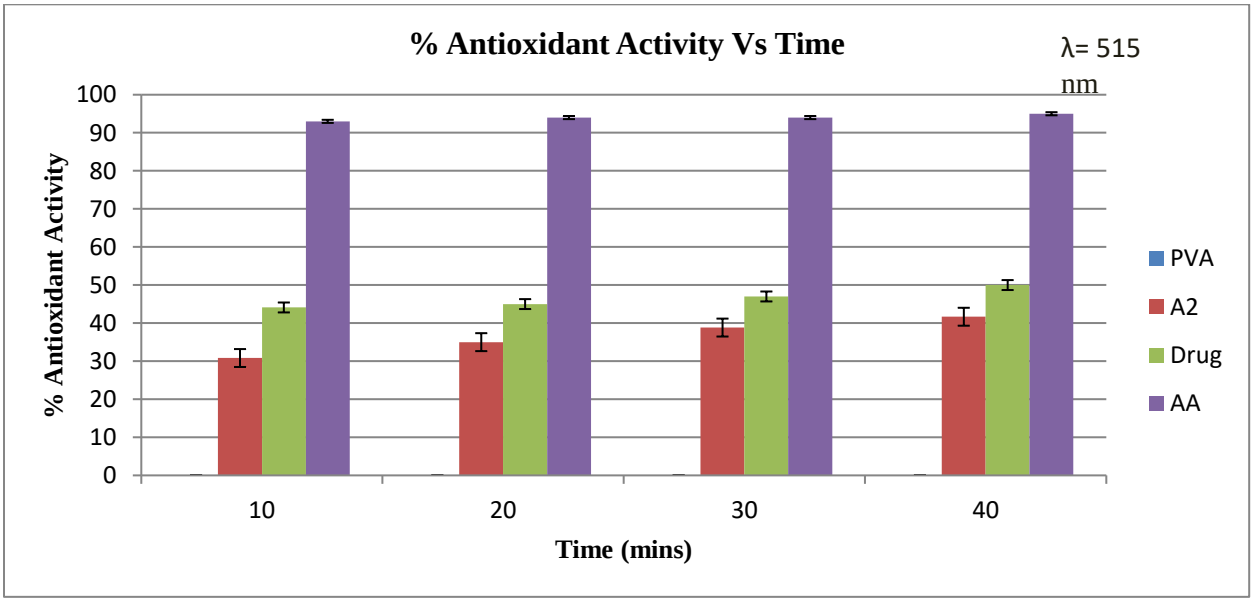

Figure 6: % Antioxidant activity of curcumin/PVA mats with 1.09% (A2) w/w curcumin. Drug shows the antioxidant activity of curcumin alone and AA represents ascorbic acid and.

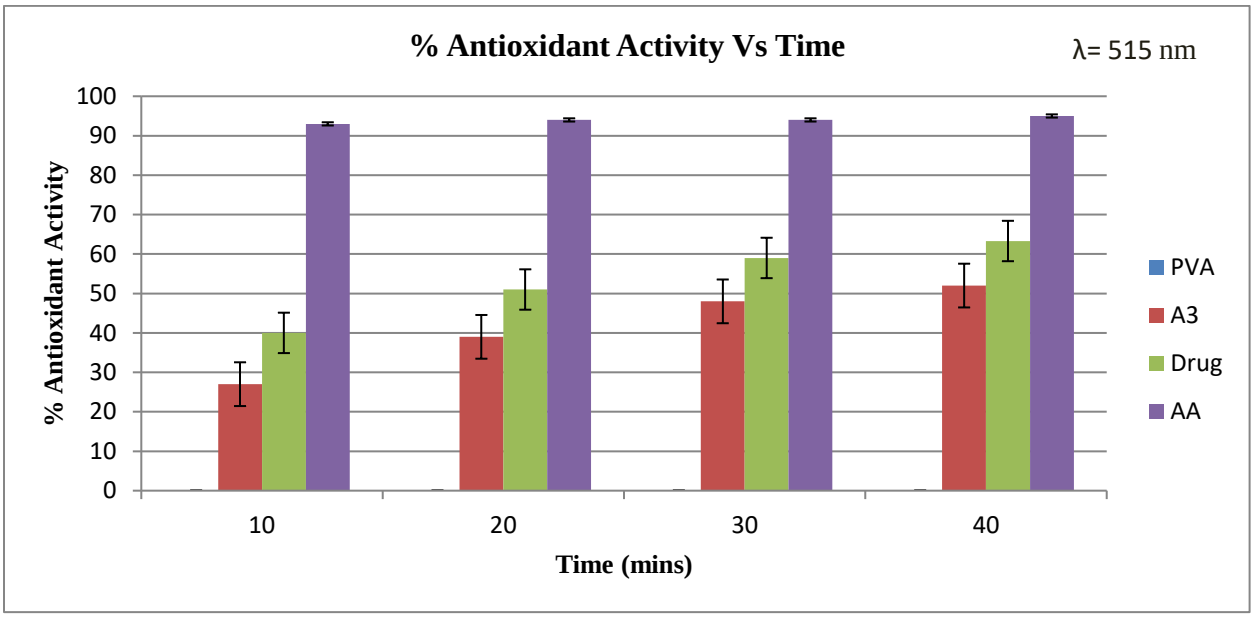

Figure 7: % Antioxidant activity of curcumin/PVA mats with 2.19% (A3) w/w curcumin content. AA represents ascorbic acid and drug shows the antioxidant activity of curcumin alone.

4.4 Fourier transformed infrared (FTIR) spectroscopy:

For PVA, OH stretching and hydrogen bonded OH stretching was observed between 3000-3650 cm-1and 3200-3570 cm^{-1}, respectively. Carbonyl stretching, C-H stretching was observed at 1734-1735 cm^{-1} and 2850-3000 cm^{-1}(CH2/CH stretching was at 2941/2912 cm^{-1}). Near 1100 cm^{-1} terminal vinyl groups were observed. Characteristic curcumin peaks were observed at 1143-1152 cm^{-1}, 1265-1279 cm^{-1}, 3085-3552 cm^{-1} and 1512-1588 cm^{-1} for C-O-C stretching, C-O stretching, phenolic OH stretching and benzene ring C=C stretching ,respectively (Kim and coworkers, 2008; Sun, X. Z, 2013; Sasipriya, K., 2013; Ravikumar, R., Ganesh, M., 2017). The peaks were approximately similar to the peaks reviewed in literature of PVA/ curcumin nanofibers. A peak at 3316 cm-1 was observed which shows hydrogen bonded OH stretching. C-H Alkyl stretching is observed at 2915 cm-1 and carbonyl stretching was observed at 1733 cm-1. Wavelengths 1246 and 1090 cm-1 showed carbonyl and C-O-C stretching. A shift from 1279 cm-1 and 1152 cm-1 was observed in C-O and C-O-C stretching **(figure 8)**.

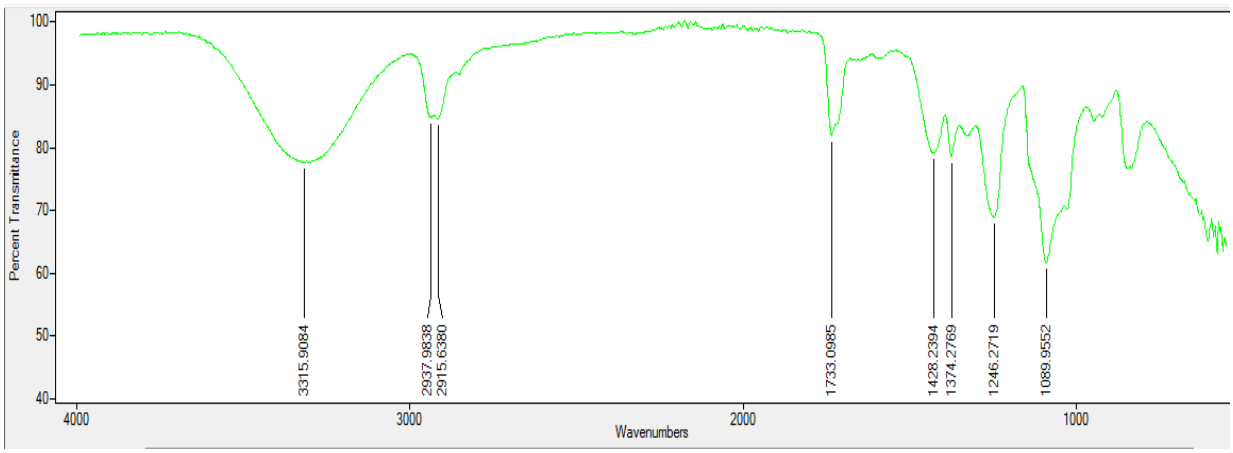

Figure 8: FTIR of curcumin/PVA mats of 0.65% w/w curcumin.

4.5 Morphological analysis: Scanning electron microscopy (SEM)

Surface morphology of the 0.65% w/w curcumin/PVA mat was analyzed **(figure 9)**. The SEM images appeared to have thin and round and thick and flat fibers. Flattened and thick fibers were observed due to slow evaporation rate of the solvent and reformation of inter and intra chain interactions among PVA which are para-crystalline and amorphous domains which break almost completely at higher temperatures but reform upon cooling when the solvent is evaporating or when the jet is accelerated from the Taylor cone (Koski, A., Yim, K., 2004). Also, flattening of the fibers with increased diameter was observed with high polymer concentration and high molecular weight which increases the viscosity of the solution and decreases the evaporation rate of the solvent. Bead formation indicates presence of high curcumin content in the films (Sun, X. Z., Williams, G. R, 2013).

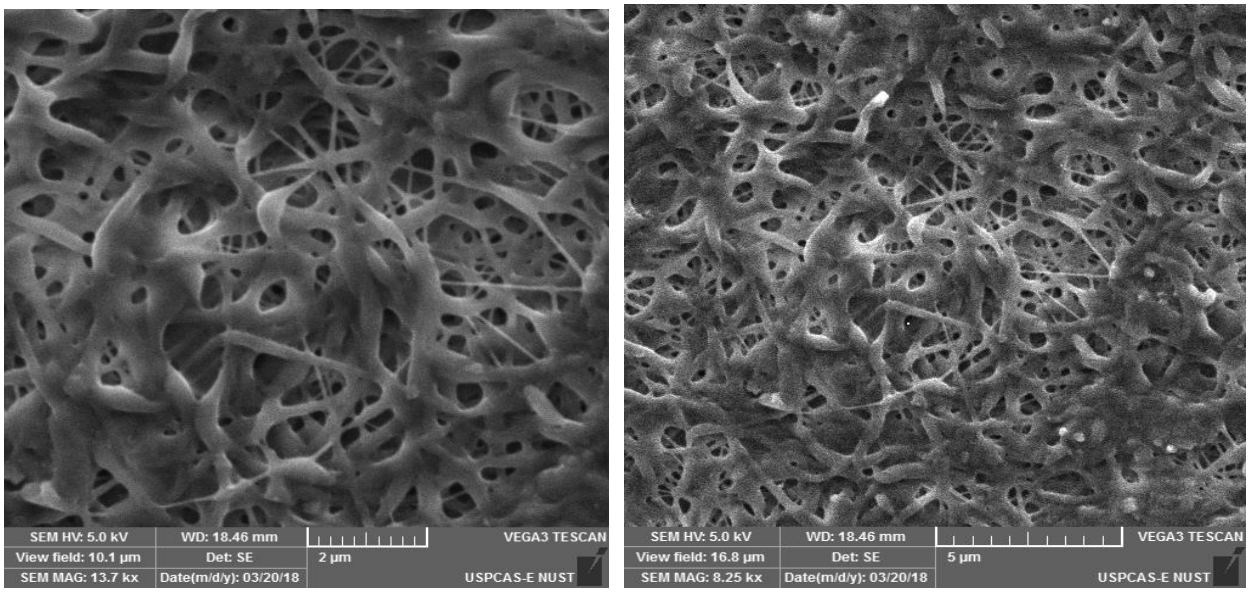

Figure 9: Scanning electron micrograph of curcumin/PVA mats of 0.65% w/w curcumin.

4.6 X-ray diffraction of Curcumin based PVA mats

XRD patterns were obtained over the range of 2ɵ 10°-40°. A broad characteristic peak of PVA was observed between 17° and 25° due to its semi-crystalline nature. Diffraction pattern exhibited two sharp peaks at 11.41° and 23.17° which showed the presence of crystalline curcumin in nanofibers **(Figure 10)**. Small diffraction peaks attributed to the presence of raw curcumin (Chen, J., Dai, W. T., 2013; Sun et al., 2013).

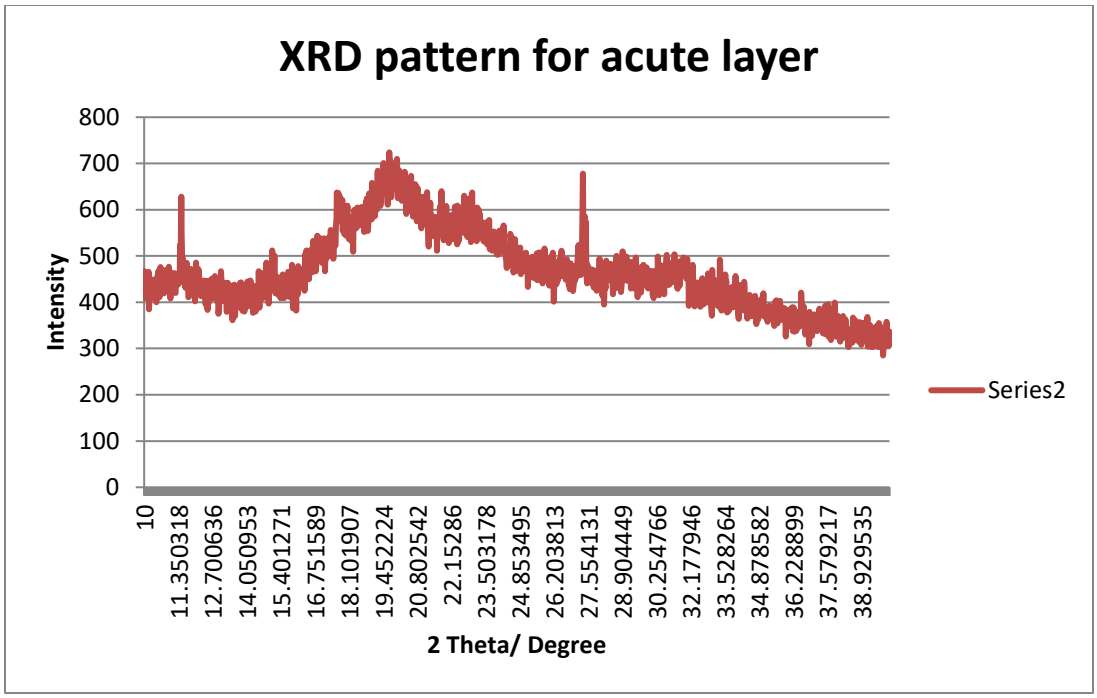

Figure 10: X-ray diffraction pattern of curcumin/PVA mats of 0.65% w/w curcumin.

Curcumin based polymeric e-spun mats are flexible and can be used in endovascular environment for the treatment of post-stent complications i.e. delayed endothelialization and late in-stent restenosis. The curcumin release in the first week was crucial for the treatment of acute events that occur after stent deployment which are acute inflammation and acute thrombosis (Iakovou, 2005). Antioxidant property of curcumin is a strong quality for the regulation of endothelial functions (Motterlini, 2000). And gradual release of the drug allows the films to be potentially effective for late events post-stent implantation i.e. late in-stent restenosis.

5. Conclusion

Curcumin loaded PVA nanofibers were fabricated using electrospinning process. Curcumin was used due to its strong antioxidant and antimicrobial properties. PVA/curcumin complex was used in order to attain a biodegradable and biocompatible stent graft which should be capable of treating the problems of delayed endothelialization and late in-stent restenosis. The compositions were tested for antioxidant and antimicrobial activities. *E. coli* and *P. aeruginosa* and *MRSA* were the bacterial strains used. 0.65% curcumin loaded PVA composition showed the best results in relation to its degradability, drug release and antioxidant activity. These nanofibrous mats have a potential use in the treatment of cardiovascular diseases, like coronary artery disease.

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Design and development of drug eluting stent grafts for the treatment of coronary diseases

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