DEVELOPMENT OF POLY VINYL ALCOHOL-CELLULOSE COMPOSITES FOR WOUND DRESSING APPLICATIONS

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CERTIFICATE

This is to certify that the thesis entitled **"Development of Poly Vinyl Alcohol-Cellulose Composites for Wound Dressing Applications"** is a bonafide work done by ARTATRANA TANDI (111BM0013) which has been submitted for partial fulfillment of the requirements for the degree of Bachelor of Technology (B.Tech) in Biomedical Engineering at National institute of Technology, Rourkela. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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ABSTRACT

Healing of wounds is a complex physiological process which involves four stages: Homeostasis, Inflammation, Proliferation and Remodeling. Wound dressings are often used to promote healing of wounds and prevent further infections at the wound site. An ideal wound dressing materials should be able to absorb wound fluids and exudates, provide a moist environment at the wound site and it must be non-toxic and biocompatible. In this study, PVA-Cellulose hydrogel composites incorporated with two chemical drugs (Streptomycin and Ciprofloxacin) and two natural drugs (turmeric and tridax extract) were developed for wound dressing applications. The structure, morphology and composition of the samples were characterized using scanning electron microscopy (SEM), X-ray diffraction study (XRD) and Fourier transform infrared spectroscopy (FTIR). And the wound healing characteristics of these samples were analyzed through swelling studies, water vapor transmission rate analysis, gel fraction studies and antibacterial studies. Furthermore the mechanical properties of the samples were also analyzed to determine the strength of the composites. As per the SEM results the surfaces of the samples were found to be uneven which validates good adherence property. The XRD results indicated semi-crystalline nature and the FTIR spectrum confirmed the presence of drugs in the samples. Due to the incorporation of drugs the swelling rate of the composites were prolonged and their mechanical properties were enhanced. The results of gel fraction studies and water vapor transmission rate analysis indicated PCtri and PCC can be used for moderate exudating wounds while PCS and PCtur can be used for low exudating wounds. The antibacterial behavior of PCC and PCS was maximum and the samples with natural drugs also showed a comparative antibacterial activity. According to the results of the above studies, the samples were found to satisfy several properties of an ideal wound dressing material.

Keywords: Wound healing, exudates, gel fraction, composites

CHAPTER 1: INTRODUCTION

"Wound" refers to a specific type of injury in which the dermis of the skin lose its structural integrity resulting in cut or break in continuity of the skin tissue. A wound is also characterized by contusion caused by trauma, and involves damage to blood vessels, capillaries and venules. Wound healing or repair is a physiological response which involves tissue reconstitution by which the body tries to replenish the lost cells or damaged cells of a wound. Wound healing primarily involves four stages as shown in the following flow chart.

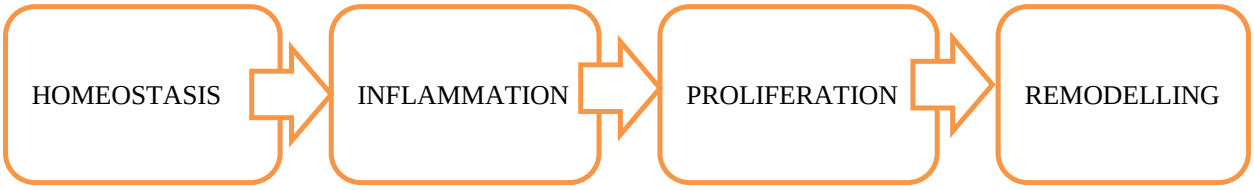

Figure 1 – Schematic diagram of wound healing process

Homeostasis is the first stage of wound healing which involves adherence of platelets to the site of injury which along with the clotting cascade proteins like thrombin and fibrin forms stable clots through a mesh of cross-linked proteins, thus sealing the damaged blood vessels. This essentially stops the active bleeding. Subsequently, after homeostasis, inflammation of wound healing takes place which is causes swelling of the wound with a reddish appearance along with pain, inflammation and sensation of warm in the area where wound has occurred. The reddish appearance and warm sensation is a result of dilation of vessels present in the wound area. In order to prevent secondary infections at the wound site a mesh of fibrin and platelet is formed. This mesh acts as a barrier and within this mesh the exudates from the wounds are accumulated resulting in the swelling of the wound. Removal of tissue debris and destruction of foreign organisms is done by a large number of cells present in the wound exudate which primarily includes granulocytes, monocytes and macrophages. Pain and inflammation in the wound site is caused by the release of kinins which causes the blood vessels to dilate. Due to inflammation the blood vessels become leaky which leads to the release of plasma and PMN's (polymorphonucleocytes) into the tissues surrounding the wound. These released PMN's or neutrophils provide the first line of defense against infection by causing phagocytosis of

microorganisms [1]. Proliferative stage involves a series of processes which includes angiogenesis in which new blood vessels are formed from vascular endothelial cells, tissue formation, epithelialization in which epithelial and fibroblast cells migrate to the wound area, wound contraction and collagen deposition. Migration of epithelial cells from the wound margins leads to the formation of a thick epidermal cap following which cell proliferation occurs. During cell proliferation, the strength of the skin tissue is enhanced due to the formation of granulation tissue along with formation of collagen and growth of lymphatic vessels and capillaries. In the remodeling phase of wound healing the strength of the tissue increases and formation of scar occurs [1]. Thus, wound healing happens to be a complex and dynamic process and if the normal pathway is not followed then the wound becomes chronic.

Wound management is one of the important areas of health care which focuses upon efficient and rapid healing of wounds. Wound dressings play a crucial role in the management and promotion of wound healing. Wound dressings are adjunct or pads which are applied to the wound to facilitate optimal healing and prevent secondary infections. An ideal wound dressing material should have following properties [2-4].

- It should be able to maintain a moist surrounding around the wound site
- Provide protection to the wound from secondary infections
- It should be able to absorb fluids and exudates released from the wounds
- It should be able to reduce necrosis of the wound surface
- Should be able to prevent dryness of the wound site
- Should be able to stimulate the growth rate of the tissue.
- It should be elastic, non-toxic, biocompatible and biodegradable

Wound dressings can be classified into various types like gauze and cotton dressings hydrocolloid dressings, alginate dressings, hydrofiber dressings, foam dressings, antimicrobial dressings and hydrogel dressings. The conventional gauze and cotton dressings are associated with several disadvantages like inability to prevent microbial infections, poor absorption of wound exudates, causing skin irritation, poor oxygen permeability to wound site, causing pain, trauma and bleeding on replacement, creating a dry environment at the wound site. Therefore, gauze dressings are no longer used for wound dressing applications. Hydrocolloid dressings are used as a primary dressing material for moderate or low exudative wounds since it consists of

carboxy metylcellulose, gelatin and pectin which form gels when brought under contact with wound exudates. Hydrocolloid dressings are found to be promoting epithelializing and granulating stages wound healing. Alginate dressings derived from seaweeds, formed into pad or ropes/ribbons to be used as a primary dressing material. Moreover alginate dressings can be used in bleeding wounds since they are haemostatic due to the presence of calcium ions. Hydrofiber dressings are made up of textile fibers with sodium alginate. It has been found that these dressings possess properties of both alginate and hydrocolloid dressings and when exposed to wound fluid they form gel. However hydrofiber dressings have certain drawbacks which include aggressive adhesion and cohesive gelling [5]. The foam dressings are highly absorbent in nature consisting of polyurethane foam and are used in cases of high exudative wounds. Hydrogel dressings are found to be very effective in maintaining a moist environment at the wound site and their non-adherent nature enables easy replacement without causing any pain or trauma.

1.1 Hydrogels

Hydrogel constitutes water soluble polymers which form a three dimensional network conferring to its high water retaining capacity and porous structure. Hydrogels can therefore be defined as networks of cross linked polymers which can retain a large amount of water and attain equilibrium. Various physical and chemical linkages within the components are responsible for the formation of network like structure of hydrogels. The presence of crystalline regions and physical interactions such as hydrophobic forces and electrostatic forces leads to the formation of physical linkages whereas the chemical linkages are characterized by formation of covalent bonds. Many polymers of hydrophilic nature can form hydrogels. Some of the common polymers are poly ethylene glycol (PEG), poly vinyl alcohol (PVA) and polyacrylic acid. Three principal methods are used for the synthesis of hydrogels and they are physical crosslinking, chemical crosslinking and radiation based methods. In case of physical cross linking method freeze and thaw cycles are used which gives a crystalline product whereas in chemical crosslinking a large number of crosslinking agents are used for network formation. Some of the common crosslinking agents include acetaldehyde, glutraldehyde and formaldehyde. In radiation based crosslinking method the polymer network formation occurs due to creation of free radicals when the polymer is exposed to high energy radiations. The three dimensional structural arrangement of hydrogel is due to formation of cross-linked networks within the constituent polymer. The extent of

crosslinking within the hydrogel influences its physical and mechanical properties such as shear modulus, swelling ratio and diffusion coefficient of the embedded molecules [6-8].

Hydrogels are found to meet various characteristic requirements of a wound dressing material such as absorption of wound fluids and exudates, immediate control of pain, good permeability to oxygen, provides control of drug dosage, easier to replace and act as a barrier to bacteria and other microorganisms [4]. Hydrogel show good permeability. But there are a few drawbacks associated with these hydrogel systems. These hydrogels possess weak or poor tear strength and show limited resistance to mechanical deformation [9]. In order to overcome these limitations hydrogels are usually used as composites which gives sufficient mechanical strength without causing any significant change in its properties. Thus, suggesting that these hydrogel composites can be used as potential wound dressing materials.

1.2 Hydrogel composites with Polymeric matrix

Hydrogel composites consist of two components: the polymer matrix and the reinforcing material. The resultant composite materials formed by the addition of reinforcing fillers to the polymer show better mechanical strength. Moreover the composites are found to be chemically resistant and thermally stable. Various types of reinforcing materials are used such as nanoclays, carbon nano tubes, carbon nanofibers, nanoaluminum oxide, nanosilica etc. In this study poly vinyl alcohol is used as the polymer matrix and cellulose is added to it as a reinforcing material to synthesize the drug loaded hydrogel composites.

1.3 Poly Vinyl alcohol (PVA)

Poly vinyl alcohol (PVA) is a synthetic polymer formed by free radical polymerization of vinyl acetate. PVA is water soluble, biodegradable, biocompatible, and non-toxic and is highly flexible by nature. These features of PVA make it suitable for wound dressing applications. PVA is also used for biomedical applications as contact lenses, artificial meniscus, medical sutures, and drug delivery devices. Moreover poly vinyl alcohol is used in food, adhesive, resin and cosmetics industrial sectors [10-11]. Poly vinyl alcohol hydrogels can be synthesized by physical crosslinking, chemical crosslinking and radiation based methods. Physical method is usually

preferred over chemical method since during application; no residual toxicity is produced through physical method whereas it is prominent in the chemical method. In case of physical crosslinking the PVA hydrogels are formed through repeated freeze and thaw cycles PVA solution. In the freeze cycle, formation of ice in the solution occurs which is followed by polymeric network formation and crystallization which continues until a complete crystallite formation takes place. In the process of thawing, melting of the formed ice leads to the formation of pores which ultimately results in hydrogel formation [12]. It has been found that the composites synthesized by physical method have better swelling behavior and elasticity than the composites formed by chemical methods [13]. However their wound dressing applications is often limited due to their poor mechanical properties. In order to raise sufficient mechanical strength various reinforcing fillers are used. In this study cellulose is used as the reinforcing material.

1.4 Cellulose

Cellulose is a polysaccharide made up of linear chain of glucose molecules held together by 1, 4 β-glucosidic linkages. Cellulose is a structural component of the plant cell wall and can also be found as an important constituent in natural fibers such as cotton and linen. Due to its purity and uniformity in its structure cellulose is used in manufacturing audio membranes, electronic paper and medical materials [14]. Cellulose membrane is found to be highly nano-porous by nature which enables transfer of drugs into the wound site and simultaneously it also serves as an effective barrier against external infections[14]. The unique nanostructure of the microbial cellulose membrane gives good mechanical strength and remarkable physical characteristics.

1.5 Streptomycin

Streptomycin is a bactericidal antibiotic which is derived from *Streptomyces griseus.* It acts against mycobacteria and belongs to the class of aminoglycoside drugs. It has a broad spectrum of activity. Streptomycin is used for treating diseases like infective endocarditis, plague, tuberculosis etc. Streptomycin acts as a protein synthesis inhibitor. It causes death of microbes due to misreading of codons as it binds to 16S rRNA of bacterial ribosome. Streptomycin is

used to for sterilization of wound present on the surfaces prior to skin grafting. Thus streptomycin is a good antibacterial drug to cure wounds by preventing further infections.

1.6 Ciprofloxacin

Ciprofloxacin is broad spectrum antibiotic of the fluoroquinolone class. It is freely soluble in water and has a short elimination half-life of about four h along with a narrow absorption window. Ciprofloxacin is approved for use in treatment of joint infections, infections of urinary tract and infections of lower tract of respiratory system. Ciprofloxacin belongs to the class of fluoroquinolones drugs and have broad spectrum of activity. It is available in both oral and intravenous forms and show excellent tissue penetration. Ciprofloxacin is widely preferred drug for healing wounds due to its low inhibitory concentration for bacteria which cause wounds and also it has low frequency of spontaneous resistance.

1.7 *Tridax procumbens*

Tridax procumbens is commonly known as tridax daisy. It has antiviral and antioxidant property and is widely known for its anti-inflammatory and wound healing activities. The juice obtained from the leaves of tridax is used to arrest bleeding from bruises and cuts. This juice retards scar formation and granulation [15]. However it accelerates two phases of wound healing namely epithelialization and collagenisation. Aerial parts of tridax also contain a new flavonoid procumbenetin which is known to be used in traditional medications for its antifungal, anticoagulant and wound healing activity [15-16].

1.8 Turmeric

Curcuma longa which is commonly known as turmeric have several therapeutic properties such as antibacterial and anti-inflammatory activities [17]. Rhizomes are the active part of turmeric which contains curcumin and turmeric oil. It has been found that curcumin is responsible for the anti-inflammatory and analgesic activity of turmeric [18]. And the volatile turmeric oil isolated from *curcuma longa* also poses antibacterial and anti-inflammatory properties. Studies have shown that turmeric contains several proteins, fats and vitamins which play a crucial role in

tissue regeneration and wound healing [19]. The presence of several proteins and vitamin A along with the anti-inflammatory feature in turmeric mimic the fibroblastic activity of wound healing leading to the early synthesis of collagen fibers [20]. Recent wounds and bruises are often treated by applying the juice of fresh rhizome of turmeric.

CHAPTER 2 LITERATURE REVIEW

Wound dressings play a crucial role in the management and promotion of wound healing. They should be attributed with ideal characteristics such as thermal insulation, absorption and retention of wound fluids and exudates, provide protection from further infections, prevent dehydration of wound, allow exchange of gases and on removal it should not cause any pain or trauma [21]. Studies have shown that hydrogel composites are credited to satisfy desirable wound dressing characteristics. The hydrogel are able to control pain, are easily replaceable and their transparency allows continuous monitoring of healing process. Moreover control of drug dosage can be achieved by using hydrogel composites as dressing materials [4]. However the use of hydrogel is often limited due to the fact that they have poor tear strength and can be easily deformed [9]. In order to overcome these limitations hydrogels are usually used as composites which gives sufficient mechanical strength without causing any significant change in its properties. And thus hydrogel composites can be potential wound dressing materials. Water held within the polymer network of hydrogel cause a plasticizing effect which is responsible for its poor tear strength and easily deformable nature. Hydrogels have domination of their structural properties and permeability due to their structural feature.

Renewable and desirable properties of synthetic polymer, PVA has led to its wide use in pharmacy and medicine for delivery of drugs, wound dressings and healing of wounds. Due to good water-soluble, biodegradable, non-carcinogenic and biocompatible characteristics of PVA it has been used to blend with natural and synthetic polymers to enhance wound dressing applications. It has been found that the physiochemical and mechanical properties of blended polymer is enhanced on blending with PVA [22].

Cellulose can hold a large amount of water because of the large surface area which arise s from its unique nano-morphology. Studies have shown that cellulose is highly conformable and display great elasticity. Cellulose shows remarkable performance as wound healing system due to the fact that the size of microbial cellulose fibril is too small. Moreover cellulose allows transfer of drugs or antibiotics into the wound site due to their highly nano-porous nature and at the same time they act as potential barriers against external infections [14].

In an approach combined therapy with drugs of different pharmacological activities has been used for optimal healing of wounds. Studies have shown that in streptomycin and diclofenac loaded film dressings, streptomycin can cause prevention as well as treatment of wound infections with minimal inflammatory response due presence of diclofenac [23]. In a report it has been found that when the concentration of streptomycin is around 200 mg/cc, the tissues which are wounded heal smoothly without any interference and no further damage of tissues occur. In the absence of any slough at the wound site, streptomycin is found to get rid of persistent gram negative bacilli infections [24]. It has also been found that the activity of streptomycin against young and actively growing cultures is more than that to older cultures. This property of streptomycin can avoid secondary infections at the wound site.

Wound healing is significantly modified with the presence of ciprofloxacin drug. A review has shown that topical application of ciprofloxacin prevented spreading of *P. aeruginosa*. Ciprofloxacin is known for its low minimal inhibitory concentrations against wound causing gram negative and gram positive bacteria. It has also been found that there is low spontaneous resistance of bacteria against ciprofloxacin.

Properties of turmeric have widely been investigated since it is a common traditional Indian medicine. Studies have led to several inherent properties of turmeric such as antimicrobial activity, pharmacological and chemotherapeutic activities which are significant for wound healing. Reduction in post-surgical inflammation is one of the best-explored features of turmeric. Studies have shown that turmeric reduces formation of blood clumps for the aversion of atherosclerosis. Turmeric has also been used topically on the skin surface to reduce irritation associated with inflammation and allergies.

Tridax is a known medicinal herb and is commonly used for treatment of skin diseases. It has been found that tridax can be used for several therapeutic applications for its antiviral, antioxidant, antibiotic and wound healing activities. The juice formulation of tridax is found to be very effective in arresting bleeding from bruises and cuts. Moreover it has been validated that tridax juice retards the formation of scars and granulations [15]. However it accelerates two phases of wound healing namely epithelialization and collagenisation. A flavonoid compound is

found in the aerial parts of tridax which show antifungal, anticoagulant and wound healing properties[15-16].

The present research work aims to develop Poly vinyl alcohol-Cellulose composites incorporated with two chemical drugs (Streptomycin and Ciprofloxacin) and two natural drugs (turmeric and tridax extract) for wound dressing applications. The advantage of incorporating drugs into the poly vinyl alcohol-cellulose composites is to target two different phases of wound healing: prevent bacterial infection and reduce inflammation, to achieve rapid wound healing. The structure, morphology and composition of the samples were characterized using scanning electron microscopy (SEM), X-ray diffraction study (XRD) and Fourier transform infrared spectroscopy (FTIR). And the wound healing characteristics of these samples were analyzed through swelling studies, water vapor transmission rate analysis, gel fraction studies and antibacterial studies. Furthermore mechanical properties of the samples were also analyzed to determine the strength of the composites.

CHAPTER 3 MATERIALS AND METHODS

3.1 Materials

Polyvinyl alcohol (PVA) and cellulose were purchased from Himedia. The chemical drugs, Streptomycin and Ciprofloxacin were purchased from SRL, India. Wild *Tridax procumbens* was obtained from the locality. The commercially available turmeric powder was procured and used in this study.

3.2 Methods

3.2.1 Preparation of PVA-cellulose composite by cyclic freeze thawing method

The PVA-cellulose composite was prepared by physical crosslinking method involving repeated freeze and thaw cycles. Accordingly 5g of PVA (10% w/v) was dissolved in 50ml of distilled water with continuous stirring at 90°C. After 2h of stirring 2.5g (5% w/v) cellulose was added. The prepared solution was poured into a glass mold and freezed at -20°C for 22h. The sample was then thawed at room temperature (37°C) for 2h. This process of freezing and thawing was repeated for three cycles to get the composite sample.

3.2.2 Preparation of drug loaded PVA-cellulose composite by cyclic freeze thawing method

Drug loaded PVA-cellulose composites were prepared by cyclic freeze-thaw method. Following similar procedure as explained earlier PVA-Cellulose solutions were prepared by dissolving 15g PVA (10% w/v) and 7.5g cellulose (5% w/v) in 150ml of distilled water for each sample. The obtained PVA-Cellulose solution is poured into three different beakers with 50ml solution in each beaker. Then, 0.05g (0.1% w/v) of Streptomycin, ciprofloxacin and turmeric were mixed with the PVA-Cellulose solution kept in each beaker separately and all the solutions were vortexed for 1h. The prepared solutions were poured into three glass molds separately and freezed at -20°C for 22h followed by thawing at room temperature for 2h. This process of freeze-thaw is repeated for three cycles, leading to the formation of the drug loaded composite samples.

3.2.3 Preparation of herbal extracts

Tridax procumbens leaves were collected from the herbs. The leaves were collected from the plants. In order to remove dust particles and residual moisture the leaves were thoroughly washed with distilled water and dried in sun. From this 20g leaves were powdered by lyophilisation using liquid nitrogen. The leaves were freezed in order to make coarse powder. Then 3.5g of coarse powder was taken for the extraction process. The extraction process was carried out using soxhlet apparatus for 15 cycles. Ethanol was used as solvent for the extraction process.

3.2.4 Preparation of PVA-cellulose Tridax composite by cyclic freeze thawing method

Cyclic freezing-thawing method was used for the preparation of PVA-cellulose tridax composite. Similar procedure was followed to prepare 45ml PVA-cellulose solution by dissolving 5g PVA (10% w/v) and 2.5g (5% w/v) cellulose in distilled water. Then, 5ml of tridax extract solution was prepared by mixing 0.05g of Tridax extract in distilled water. The obtained solution is then added to the PVA-cellulose solution and vortexed for 1h. Then the prepared solution was poured into a glass mold. Then this mold containing the sample was freezed at -20°C for 22h and followed by its thawing at 37°C for 2h. This process of freeze and thaw was carried out for three cycles to obtain the composite.

Table 1: Sample composition and their coding

SAMPLE COMPOSITION	SAMPLE CODE
PVA+5% Cellulose	PC
PVA+5% Cellulose+0.1%bStreptomycin	PCS
PVA+5% Cellulose+0.1%Ciprofloxacin	PCC
PVA+5% Cellulose+0.1%Tridax	PCtri
PVA+5% Cellulose+0.1%Turmeric	PCtur

3.2.5 Scanning electron microscopy (SEM)

At first, the PVA-Cellulose composite samples were vacuum dried in a desiccator to avoid shrinkage. Followed by vacuum drying, platinum coating is done in order to avoid charging of the samples. To study the surface morphology the dehydrated samples were then observed under SEM (Nova NANOSEM 450).

3.2.6 X-ray diffraction studies

Rigaku Ultima IV diffractometer was used to carry out the X–ray diffraction study of the composite samples. PVA-Cellulose composite samples with chemical and natural drugs were scanned from 15 to 60^{o} at a scanning speed of 5^{o}/min.

3.2.7 Fourier transform infrared spectroscopy (FTIR)

Perkin Elmer IR spectrophotometer was used to determine the FTIR spectra of composite samples. The FTIR measurements were recorded between the range of 400 cm^{-1} to 4000 cm^{-1}.

3.2.8 Swelling Studies

In order to carry out swelling studies the samples were cut into small pieces and then weighed to record their initial weights (W_i). In this study phosphate buffer saline (PBS) is used as the swelling medium. To analyze the swelling behavior of the samples were kept in PBS solution at 37^{o}C. To record the final weights of the samples (W_f) after swelling the samples were taken out from the PBS solution at regular intervals of time. Proper care was taken to ensure that no PBS was present during weighing. The process was continued until the final weights of the samples attain saturation. The following formula was used to calculate the swelling percentage:

$$Swelling\ percentage = \left[\frac{(W_f - W_i)}{W_i}\right] \times 100\ \%$$

3.2.9 Water vapour transmission rate (WVTR)

Cylindrical plastic bottles were taken and 15ml of water was added to it (Figure 2). The samples were cut in a circular shape with a diameter of 3 mm which is greater than the diameter of the bottle so that the sample can fully cover the mouth of the plastic bottle. The cut sample is used as a cap on the mouth of the bottle. A suitable adhesive agent was used to seal the samples. Then the initial weight of the setup is taken (W_i) and after that the setup is kept in an incubator at 37^{o}C for 24 h. After 24h the final weight of the setup is recorded (W_f). The following formula was used to calculate WVTR [4]:

$$WVTR = 10^6 \times \left[\frac{(W_f - W_i)}{24 \times A}\right] \; g/m^2 \; / \; h$$

where A is surface area of circular sample used.

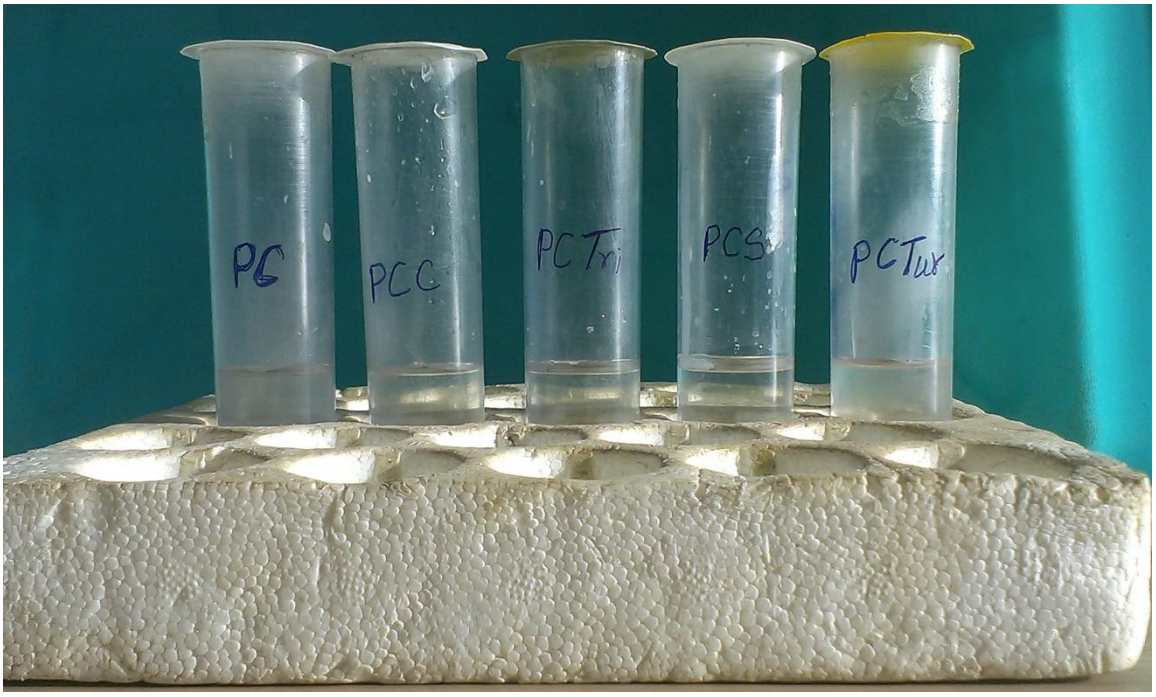

Figure 2 - Experimental setup for WVTR

3.2.10 Gel fraction studies

Preweighing of the samples was done and the samples were then dried under vacuum until no change in its mass is observed. To rinse away unreacted species nearly identical weight of another slice of the same sample was immersed in PBS solution. Subsequently the immersed

samples were removed from PBS solution and dried at room temperature until constant weight of the dried mass is attained. The gel fraction is calculated by using the following formula [4, 25].

$$G(\%) = \frac{W_i}{W_d} * 100$$

Where w_i is the dried insoluble weight and w_d is the normal dried weight of the samples.

3.2.11 Mechanical testing

Tensile strength measurement of the developed composites was carried out on a universal testing machine (ElectroPuls E1000, Instron, UK) as per ASTM D3039. A load cell of 250 N was used and a strain rate of 2 mm/min applied and measurements were taken. Testing was carried out in triplicate.

3.2.12 Antibacterial study

Nutrient agar medium were prepared on petri plates. The bacterial culture was spread using spread plate method and the petri plates were then inoculated with 100 µl overnight cultures. In the study 1 ml volume tips were used to punch the petri plates with wells. PVA-Cellulose samples with chemical and natural drugs were cut in circular shapes with a diameter of 1 cm and the petri plates were incubated at 37^0C. Bacterial strains *S.aureus* (gram positive) *and E.coli* (gram negative) were used to study the antibacterial activity of the samples.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Scanning electron microscopy

The surface morphology of the drug incorporated PVA-Cellulose samples were studied by scanning electron microscopy. The SEM micrographs of the samples are shown in Figure 3.

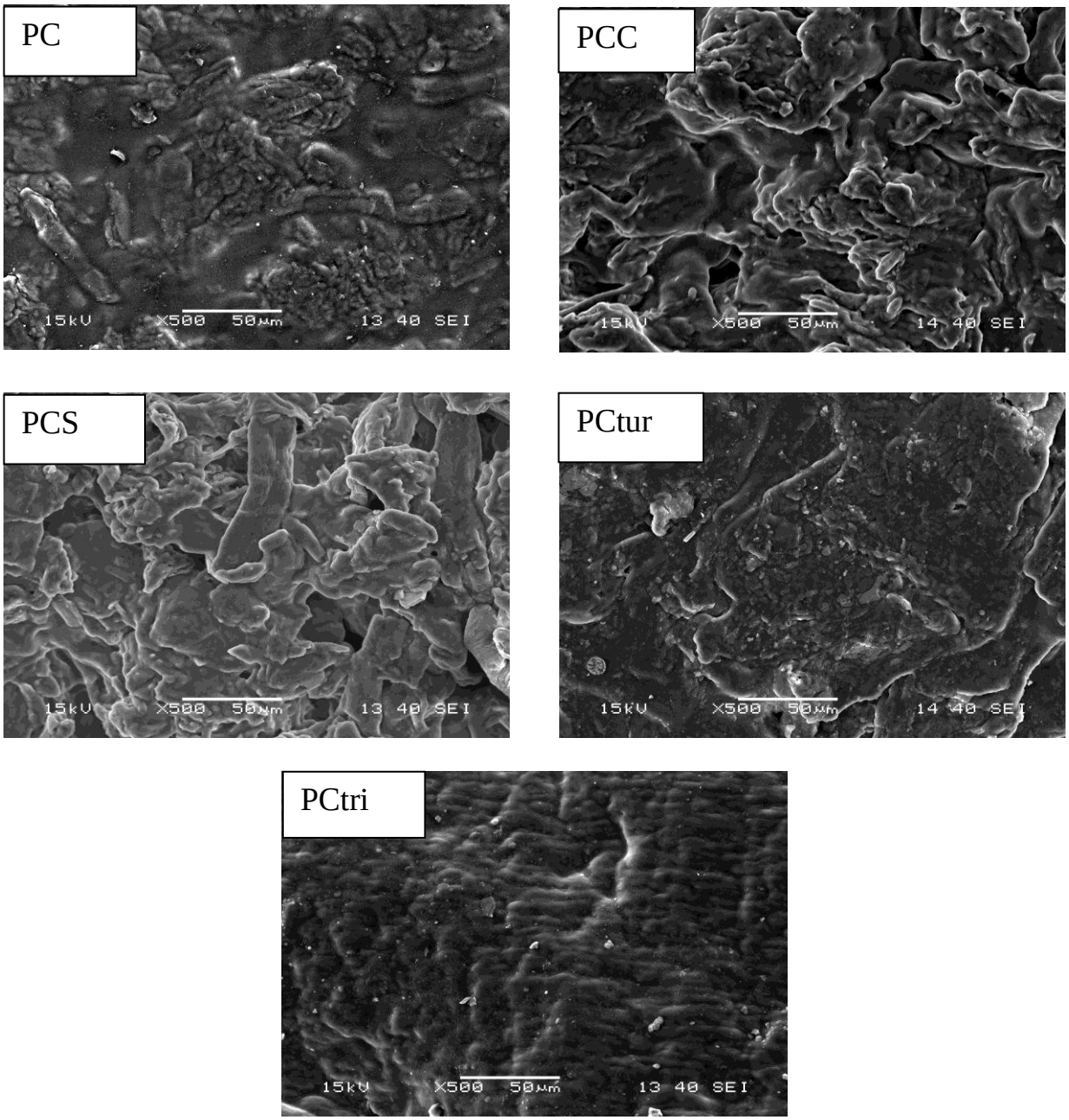

Figure 3 - Scanning electron micrographs of PVA-Cellulose composites

The concentrations of polymer matrix and the reinforcement used along with their interaction with each other define the surface morphology of the composites. The reinforcing material should be properly dispersed in the polymer matrix as it determines the physical, chemical and mechanical properties of the composite. The SEM micrographs of all the composite samples showed uneven surfaces. Comparing all the samples, PCtri have showed the highest amount of surface smoothness which may be due to proper mixing of tridax extract with the PVA-Cellulose polymer matrix. The surfaces of PCC and PCS incorporated with chemical drugs showed an uneven drug distribution while the surfaces of PCtur and PCtri incorporated with natural drugs were smoother. This can be attributed due to the reason that the chemical drugs, streptomycin and ciprofloxacin were crystalline in nature whereas in case of natural drugs, turmeric was amorphous in nature and tridax was used in the form of extract.

4.2 X-ray diffraction studies

The XRD patterns of the samples were recorded to detect formation of any phase and to investigate if any contamination has occurred during synthesis. The XRD patterns of drug incorporated PVA-Cellulose composites were obtained (Figure 4). PVA showed its characteristic diffraction peak at $2\theta = 19.5^{o}$ and 40.06^{o}. The XRD pattern of pure PVA shows reflections and diffuse scattering which is the characteristic of amorphous and crystalline phases of semi-crystalline polymers[26]. The overall structure of the samples could be semi-crystalline as per the XRD patterns. The peaks obtained at $2\theta=23.1^{o}$ and $2\theta=34.9^{o}$ corresponds to cellulose. The peaks for the drugs which are incorporated in the samples are not found due to their incorporation less concentration (0.1%).

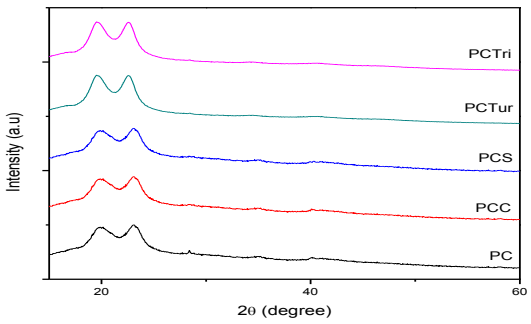

Figure 4 - XRD pattern of PVA-Cellulose composites

4.3 Fourier transform infrared spectroscopy (FTIR)

FTIR is a technique used for the identification of unknown chemical compounds and functional groups present in a material. The FTIR spectrum of the PVA-Cellulose composite samples is shown in Figure 5. Several characteristic bands were obtained from the FTIR spectrum of the samples corresponding to various functional groups and chemical bonds. The band around 3500 cm^{-1} corresponds to O-H stretching vibrations of PVA. C-H stretching of alkyl groups from PVA resulted in bands at 1010 cm^{-1} and 1392 cm^{-1}. Further, the band around 1540 cm^{-1} corresponds to C=C stretching found in turmeric. Due to the incorporation of the drug, there is a band at 1680 cm^{-1} corresponds to C=O stretching vibration found in ciprofloxacin. Also, the addition of streptomycin to PVA-Cellulose composite resulted in a band at 1650 cm^{-1} which is owing to the deformation vibration of –NH groups of streptomycin. In case of PCtri samples, there was a band at 1470 cm^{-1} which corresponds to C-H band found in tridax. The results of FTIR suggest the presence of the drug incorporation in the composite matrices.

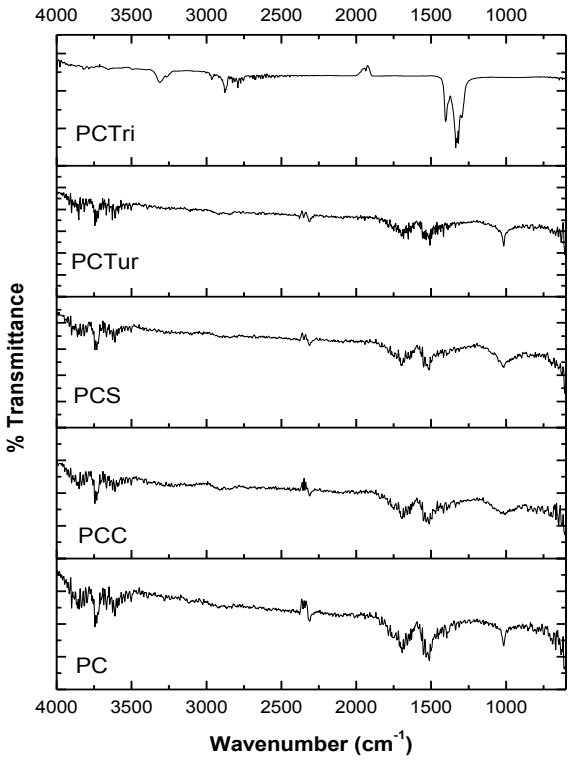

Figure 5 – FTIR spectrum of PVA-Cellulose composites

4.4 Swelling studies

Wound dressings should have a capacity to absorb wound fluids and exudates. It becomes necessary to characterize the swelling behavior of composites in order to decide upon its wound dressing application. Depending upon the swelling behavior composite hydrogels are used on a range of wounds from highly exudating wounds to dry wounds. Swelling studies also determine the period of replacement of the wound dressing. Considering the importance of swelling behavior of hydrogel composites swelling percentages of different composite samples have been calculated. PBS was used as swelling medium. Sample weights were observed at intervals of 2 h for 10 h at 37^0C. The equilibrium state of swelling was achieved within 10 h. Swelling percentage curves of the PVA-Cellulose composites is shown in Figure 6.

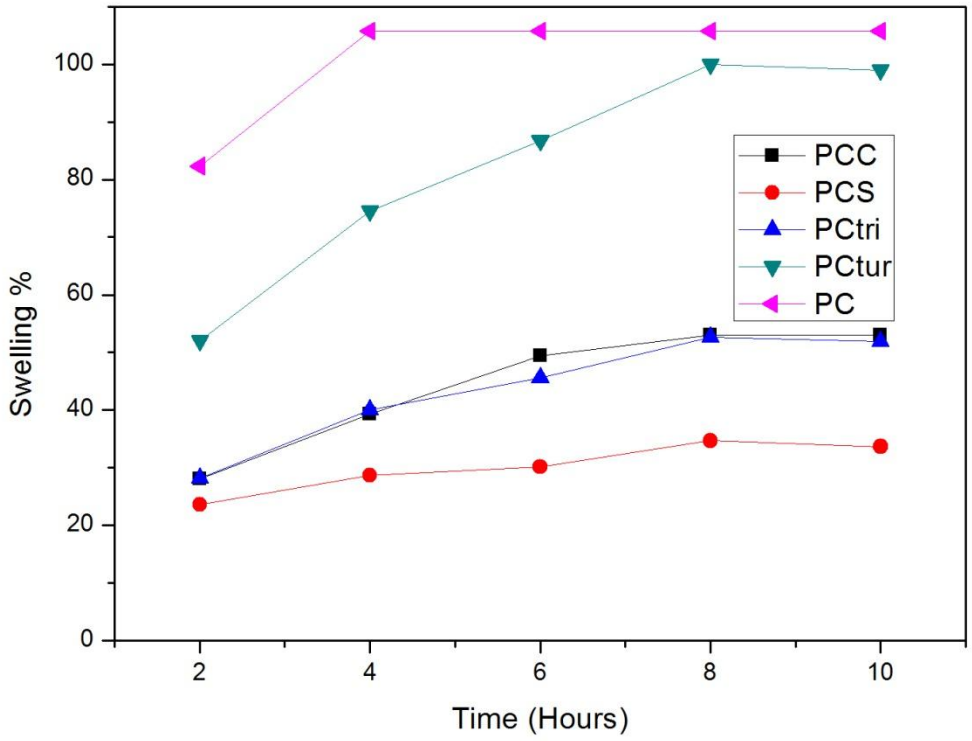

Figure 6 - Swelling percentage curves of PVA-Cellulose composites

The swelling profiles of the drug loaded samples clearly indicate a linear increase in the swelling percentages. It has been found that the control sample PC attains equilibrium swelling percentage within two hours. However in case of drug loaded samples the time for attaining equilibrium swelling percentage is prolonged. This indicates gradual release of drugs into the swelling medium. In case of PCtur, it can be seen that there is a steep increase in swelling percentage in the first two hours of the study. Subsequently, after few hours (4-8h), a linear increase in swelling % was observed. This swelling behavior later achieved constancy as the composite matrix completely swelled indicating its complete saturation. The sample PCS has shown least amount of swelling which can be attributed as surface pores being blocked by streptomycin. In the first two hours, the swelling behavior of PCtri and PCC are almost same. With the passing of time (after 4 to 6 h), the swelling percentage of PCC dominates over PCtri. However, no significant difference in swelling behavior is seen in case of PCC and PCtri (in the interval of 8 to 10 h). Therefore, this indicates that both the samples have a similar swelling behaviour.

4.5 Water Vapor Transmission Rate

Water vapor transmission rate (WVTR) characteristics of a wound dressing determine the ability of the material to absorb and transmit the wound exudates and fluids to the external environment. Ideally a wound dressing must have WVTR values which neither allows for lowering of wound moisture nor allows for excessive water retention. A low WVTR wound dressing allows for greater amount wound fluids to be in the wound environment which increases the chances of secondary infections in the wound area and retard the wound healing process. On the other hand, a high WVTR wound dressing allows faster drying of the wound [4]. The process of transport of the exudates determines the amount of moisture provided by the dressing to the wound. Maintenance of a moist environment is one of the ideal wound dressing characteristics to promote optimal healing and prevent further infections.

Table 2 Water vapor transmission values obtained for various PVA-Cellulose composites

SL NO.	SAMPLE CODES	WVTR VALUES IN $g/m^2/h$
1	PC	6.539
2	PCC	8.747
3	PCS	7.728
4	PCtur	6.539
5	PCtri	8.303

The water vapor transmission rate (WVTR) values for normal skin and diseased skin have been reported to be 8.5 $g/m^2/h$ to 11.6 $g/m^2/h$ [27]. The WVTR values obtained for different PVA-Cellulose composites incorporated with different chemical and natural drugs are shown in Table 2. The WVTR values of PCC and PCtri are in the range of WVTR values followed by PCS. The water vapor transmission rate of PC and PCtur are found to be in the lower range of WVTR values. PCC, PCS and PCtri hydrogel composites could be used in moderate exudate wounds. PC and PCtur hydrogel composites could be used in low exudate and fluid releasing dry wounds. From the results, it can be concluded that PCtri and PCC may be used in wound dressing application since their WVTR values lies in the range of normal skin and diseased skin.

4.6 Gel fraction studies

PVA hydrogels formed by repeated freeze thaw cycles may not have entire cross-linking between the molecules. So besides gel, certain portion of PVA macromolecules remains in the uncross-linked in the network [25]. The amount of gel formed depends on the type of polymer and the number of freeze-thaw cycles. The results of the gel fraction study (Table 3) indicate PC with highest gel fraction and PCS with lowest gel fraction among the samples. Thus, PC shows better cross-linking within the polymer matrix. The gel fraction of PCtri is found to be around 78% which is an ideal gel fraction value for wound dressing applications. The gel fraction data of PCtri and PCS do not differ significantly which may be due to effective cross-linking within the polymer matrix. It has been found that the gel fraction data of all the samples are in

accordance of their swelling behavior. From the results, it can be concluded that the sample PCtri, PCtur and PCC may be used in wound dressing applications.

Table 3 Gel fraction results of PVA-Cellulose composites

SL NO.	SAMPLE CODES	GEL FRACTION (%)
1	PC	91.66
2	PCC	77.14
3	PCS	56
4	PCtur	85
5	PCtri	78.12

4.7 Mechanical testing

Tensile strength measurement characterizes the mechanical properties of the material. The results obtained from mechanical testing are shown in Table 4. And Figure 7 shows a typical stress strain curve of PVA-Cellulose composite. It has been found that there is an increase in the tensile strength of the samples due to the incorporation of drugs which indicate effective crosslinking within the samples. According to the results, the tensile strength of PCtri is found to be maximum. The high tensile strength of PCtri could be due to the homogenous dispersion of tridax and cellulose in the PVA matrix, which helps the effective stress transfer in the composite leading to high tensile strength. The tensile strength of PCC is found to be closer to that of PCtri but with a higher young's modulus. PCS sample have shown least amount of extension at break (strain) and energy at break (toughness), thus it can be easily broken without spending much energy. The extension at break (strain) is found to be maximum in PCtri sample which make it more brittle with more strength as compared to other samples. Moreover, it was also found that the sample PCtri has high energy at break (toughness) which is due to the homogenous dispersion of tridax and cellulose in the PVA matrix. This results in large interfacial area and hence increases the amount of energy required to break the sample. From Table 4, it can be

concluded that the sample PCtri shows good strength and toughness when compared to other samples. Thus, it may be used for wound dressing applications.

Table 4 Mechanical testing results of PVA-Cellulose composites

Samples	Tensile strength(MPa)	Young's modulus (MPa)	Extension at break (mm)	Energy at break (J)
PC	19.8	278.64	27.52	2.35
PCC	25.5	1142	3.88	0.44
PCS	22.78	867.76	1.76	0.15
PCtur	23.63	436.97	14.89	1.44
PCtri	27.40	252.54	44.18	5.4

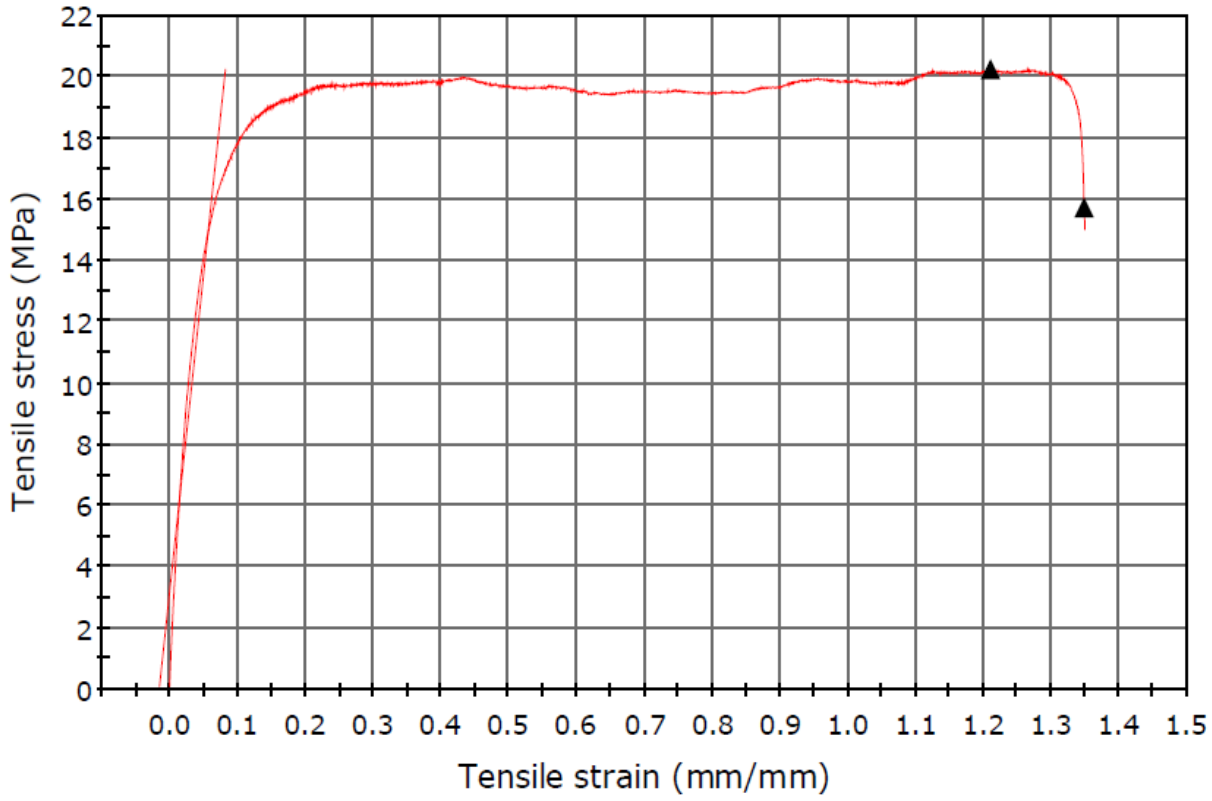

Figure 7 – Typical stress-strain curve of PVA-Cellulose composite

4.8 Antibacterial study

A large number of microorganisms reside in our body and their role is to help in normal functioning and maintenance. On the occurrence of a disease these microorganisms take advantage of the situation to cause infection and are called as opportunistic pathogens. These microbes can cause secondary infections in case of chronic wounds or in the condition where skin has been damaged and wounds prevail. If the wounds are not protected from secondary infections then the time for wound healing increases. So, antibacterial study is important to validate the property of wound dressings to prevent wound infections and promote faster healing. In this study nutrient agar diffusion assay was used to evaluate the property of PVA-Cellulose composites incorporated with different natural and chemical drugs against gram negative *E. coli* and gram positive *S. aureus.* The zone of inhibition assay on solid media is used to determine the antibacterial effects of the samples. The results are shown in Table 5 and figure 8 shows the antibacterial behavior of PCtri, PCC and PCS against gram negative bacteria *E.coli*. On testing the samples against *S. aureus it* was found the PC has shown no zone of inhibition and PCC has shown maximum zone of inhibition. It is due to the fact that PC does not have any antibacterial component whereas PCC contains ciprofloxacin which is a broad spectrum antibiotic drug. The sample PCS have also shown a significant zone of inhibition against *S. aureus* due to the presence of streptomycin which is a bactericidal antibiotic with broad spectrum activity. The sample PCtri showed a significant zone of inhibition and PCtur showed a small zone of inhibition against *S. aureus.* Similar trend was observed when the samples were tested against *E. coli* with PCC, PCS and PCtri showing maximum zone of inhibition. PCtri have shown significant zone of inhibition against both gram positive *S. aureus* and gram negative *E. coli* bacteria. Both the strains showed high sensitivity towards PCtri and the sensitivity was comparable to PCC and PCS. There was no significant difference in zone sizes produced by PCC, PCS and PCtri against both *E. coli* and *S. aureus.* Both the strains were resistant to PCtur as evident by small zone of inhibition. So it can be concluded that PCC, PCS and PCtri show good antimicrobial activity and thus they can be used for wound dressing application.

Table 5 Antibacterial activity of PVA-Cellulose composites towards *S. aureus and E. coli*

SAMPLE CODES	DIAMETER OF ZONE OF INHIBITION (cm)	
	S. aureus	***E. coli***
PC	0	0
PCC	3.5	3.4
PCS	2.5	2
PCtur	1.5	1.4
PCtri	2.1	1.8

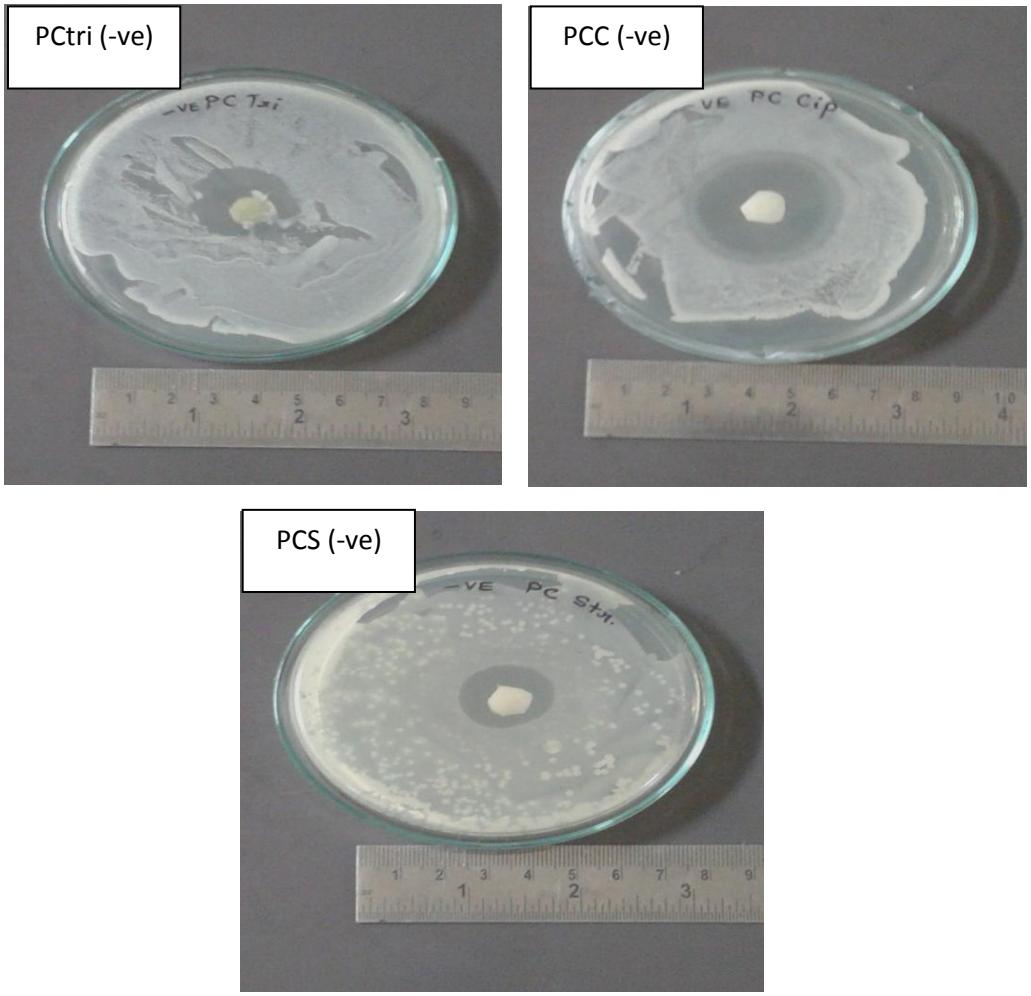

Figure 8 – Antibacterial behavior of PVA-Cellulose composites

SUMMARY AND CONCLUSION

PVA-Cellulose composites incorporated with chemical and natural drugs were prepared by cyclic freeze thaw method. The synthesized PVA-Cellulose composites were characterized using scanning electron microscopy, X-ray powder diffraction and Fourier transform infrared spectroscopy. The wound dressing characteristics were also evaluated by performing swelling studies, Water vapor transmission rate studies, antimicrobial studies and gel fraction studies. The mechanical properties of the samples were analyzed through tensile strength measurement on a universal testing machine. Micrographs of all the samples obtained from scanning electron microscopy have shown ample roughness which will help in good adherence of the samples to the wound and the samples with natural drugs showed a smoother surface than that of the samples with chemical drugs. The X-ray diffraction studies of the PVA-cellulose composites showed characteristic diffraction peak for PVA and cellulose. However no peaks for drugs are obtained since they were used in a very scanty amount (0.1%) and XRD cannot identify if the concentration is less than 2%. FTIR studies of the sample revealed the presence of several characteristic bands corresponding to various functional groups and chemical bonds present in the samples. The water vapor transmission rate studies indicated that PCtri and PCC may be used in wound dressing application since their WVTR values lies in the range of normal skin and diseased skin. Swelling and gel fraction studies further confirmed that the samples entitled PCtri, PCtur and PCC could be used as wound dressing materials. The tensile strength measurement showed the sample PCtri has good strength and toughness when compared to other samples. Thus it can be used for wound dressing applications. The antibacterial study also indicated that PCC, PCS and PCtri exhibited good antimicrobial activity and thus they can be used for wound dressing applications. According to the results obtained from the characterization techniques the samples were found to satisfy wound dressing properties.

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