SYNTHESIZATION AND CHARACTERIZATION OF NATURAL BIOCOMPATIBLE COMPOSITE HAVING SUSTAINED DRUG RELEASE MECHANISM FOR TOPICAL AND SUBCUTANEOUS APPLICATIONS

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Synthetization and Characterization of Natural Biocompatible Composite Having Sustained Drug Release Mechanism for Topical and Subcutaneous Applications

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MS Biomedical Sciences and Engineering

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“Dedicated to my exceptional parents and adored siblings whose unconditional support, cooperation and motivation led me to this wonderful accomplishment.”

Abstract

The study focuses on the development of a novel natural biocompatible composite with an ability to release drug at a constant rate. Topical drug delivery being used now a days has yet to achieve its potential in place of oral drug delivery and injections. Basil seed gum having absorption property has been used for many pharmaceutical applications. CMC and PVA being biocompatible, has been used for wound dressing for a decade. In this project, the composites in the ratios PVA/Gum 1:3, CMC/Gum 3:1, CMC/PVA/Gum 3:1:3 were synthesized. Swelling analysis of these composites was done along with characterization through FTIR, XRD, SEM and tensile testing. Hermal seed extract was incorporated in the composites because of its antimicrobial and anti-inflammatory property. Also, the *in vitro* testing was done to evaluate the drug release profile and anti-bacterial activity of the composite. The CG31-H composite showed uniform uptake of PECF solution and sustained release of hermal extract while in PG13-H and CPG313-H composites the swelling and release of drug started decreasing. The mechanical testing revealed that the composite having PVA have good tensile strength. Therefore, these composites can be used for wound dressing as it contains natural anti-bacterial agent to counter the antibiotic resistant bacteria.

Key Words: PVA, drug release profile, *in vitro* studies, swelling studies, antibacterial, biocompatible

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List of Abbreviations

PVA	Poly (vinyl alcohol)
CMC	Carboxymethyl cellulose
BSG	Basil Seed Gum
DMF	Dimethyl formamide
PECF	Pseudo-extracellular fluid
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning electron microscopy
FTIR	Fourier transform infrared
XRD	X- Ray Diffraction
CG31	Composite of CMC and Basil seed gum in the ratio 3:1
PG13	Composite of PVA and Basil seed gum in the ratio 1:3
CPG313	Composite of CMC, PVA and Basil Seed Gum in the ratio 3:1:3

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1. INTRODUCTION

For many years, people have placed substances on the skin for therapeutic effects and, in the modern era, a variety of topical formulations have been developed to treat local indications (Prausnitz and Langer 2008).

Topical drug delivery has made an important contribution to medical practice, but has yet to fully achieve its potential as an alternative to oral delivery and hypodermic injections (Prausnitz and Langer 2008).

Newer occlusive dressings speed up re-epithelialization, stimulate collagen synthesis, create a hypoxic environment at the wound bed to promote angiogenesis, decrease pH at wound surface and create an environment inhospitable to bacterial growth, which decreases the rate of wound infection (Paudel, Milewski et al. 2010). They have an edge over gauze dressings in terms of patient comfort, convenience and compliance as well as better cosmetic results because of reduced scarring.

In the past, traditional drug formulations (ointments, solutions, suppositories, emulsions) were used for treatment of various diseases. Newer drug design with essential characteristics like precise amount of drug release at a specific rate, or delivery of drug at pharmacological action site at a rate per the biological process are developed in last few years. When drugs are combined with macromolecular compounds the release rate of drugs in the body is modified and may result in prolonged action. Polymer-drug composites have been prepared in the past years which has attracted a lot of attention as they have many advantages over free drugs i.e. low drug consumption, prolonged action and sustained release of drug into the body(Buhus, Popa et al. 2007).

Natural polymers like PVA, CMC, gelatin, chitosan, etc. are widely used in pharmaceutical industry as adhesives, adjuvant and emulsifying agents (Akbari, Ghoreishi et al. 2015). These natural polymers possess water-binding capacity, gelation property, low cost and non-irritating as they are biocompatible in nature. They can be used as lining for artificial hearts and artificial skin, for preparation of contact lenses and biosensor membranes and drug delivery media (Gomes, Azevedo et al. 2012, Sim, Figueiras et al. 2012). Moreover, they also have functional groups that can be modified chemically thus provide a wide variety of products with adjustable chemistries and properties (Gomes, Azevedo et al. 2012, Akbari, Ghoreishi et al. 2015).

The biological active components can be immobilized on substrates with support materials like water soluble polymers, hydrogels that possess swelling properties, fibrous or woven membranes and porous structures(Buhus, Popa et al. 2007).

PVA (Polyvinyl alcohol) is a most extensively used polymer for many biomedical applications because of its suitable physical and chemical properties, biocompatibility, easy degradation and non-toxic nature. It has been used for wound dressings, catheters, contact lenses and coatings for sutures (Walker, Young et al. 2007, Ghafoor, Ali et al. 2016)

On the other hand, Na-CMC is an amylose (carbohydrate)which consists of many hydroxyl and carboxylic groups that intent to introduce absorption behavior in the polymer. The absorption of water and moisture results in many excellent properties like, high water content, good degradation and low cost (El Salmawi 2007).

Osmium basilicum or basil plant that is commonly found in Central and South America, Africa and Asia. For a long time, the seeds of this plant has been used for treatment of various diseases such as inflammation, dyspepsia, diarrhea and colic ulcer and other diseases (Hosseini-Parvar, Matia-Merino et al. 2010). The polysaccharide layer in their outer pericarp of basil seed swells when they are soaked in water. Mucilage, extracted from these seeds are concentrated and dried and can be used further for various applications that include as a powerful candidate for various pharmaceutical formulations (Malviya, Srivastava et al. 2011). Prajapati et al. (Prajapati, Jani et al. 2013) studied the used of mucilage and gums for the making drug delivery systems (Akbari, Ghoreishi et al. 2015).

Hermal seeds have been used as a popular healing agent by people in since very long. These possess many therapeutic effects and is pharmaceutically beneficial because of the presence of secondary metabolites such as alkaloids, flavonoids, phenols, tannins, minerals and volatile oils (Ahmad, Hussain et al. 2013). Sharaf et al. and Prashanth and John reported the presence of alkaloids, flavonoids and anthraquinones as a major constituent in hermal seed extract (Sharaf, El-Ansari et al. 1997, Prashanth and John 1999).

The aim of this study was the synthetization and characterization of natural composite films that possess natural antimicrobial agent and can release it in a sustained manner. In this study, CMC, PVA and Basil seed gum were used for the preparation of a nature composite and hermal seed

extract as an anti-microbial agent. Swelling tests were done through gravimetric method and anti-bacterial activity of the composite was also checked. The structural analysis of the composite was also assessed through FTIR, XRD and SEM.

2. LITERATURE REVIEW

The use of natural polymers in many biomedical applications is an emerging field of current research because of their biocompatible nature. During the last twenty years, biodegradable polymers are used for many applications. The degradation process is carried out by microorganisms or the by chemical decomposition by the body fluids. In the degradation process the complex organic molecules are converted into simpler ones (Chandra 1982, Nair and Laurencin 2007).

Degradable polymers are being used for drug delivery in various areas of research especially through skin which is very challenging. Advanced technologies have resulted in several drugs being administered subcutaneously including hydrophilic drugs, hydrophobic drugs having small molecules and macromolecules. This has advantage over tradition dosage routes i.e. convenient delivery of drug, pain free self-administration. It also eliminates the disadvantage of multiple dosage regimes, regular application of drug and maintaining a constant drug concentration in plasma in case of oral dosing or injections (Paudel, Milewski et al. 2010).

In the past, traditional drug formulations (ointments, solutions, suppositories, emulsions) were used for treatment of various diseases. Newer drug design with essential characteristics like precise amount of drug release at a specific rate, or delivery of drug at pharmacological action site at a rate per the biological process are developed in last few years. When drugs are combined with macromolecular compounds the release rate of drugs in the body is modified and may result in prolonged action. Polymer-drug composites have been prepared in the past years which has attracted a lot of attention as they have many advantages over free drugs i.e. low drug consumption, prolonged action and sustained release of drug into the body (Buhus, Popa et al. 2007).

2.1 Properties of Poly (vinyl alcohol) (PVA)

PVA is a water soluble and biocompatible polymer (Chiellini, Corti et al. 2003) which forms stable hydrogels and elastic gels through repeated freezing and thawing method or crosslinking chemically or physically (Nuttelman, Mortisen et al. 2001). For the use of PVA in pharmaceutical and in the areas of medicine, it must be crosslinked through chemical or physical means. Crosslinking can also be done by irradiation (Peppas and Merrill 1977, Shaheen and Yamaura 2002). The PVA hydrogels can be cross linked by difunctionally

crosslinking agents such as formaldehyde, glutaraldehyde, acetaldehyde and other monoaldehydes. In the presence of sulfuric acid, methanol these cross-linking agent forms acetal bridges between the hydroxyl group of PVA chains. However, the residual amounts of crosslinking agents are present in the PVA hydrogel. Removal of these residue material is very time-consuming process. However, when these residues are no removed PVA cannot be further used for biomedical or pharmaceutical applications. The crosslinking agents contain toxic chemicals that are released when they are in contact with the body. The crosslinking methods can be replaced with electron beam or gamma irradiations (DİLAVER 2011).

PVA (Polyvinyl alcohol) is a most extensively used polymer for many biomedical applications because of its suitable physical and chemical properties, biocompatibility, easy degradation and non-toxic nature. It has been used for wound dressings, catheters, contact lenses and coatings for sutures (Walker, Young et al. 2007, Ghafoor, Ali et al. 2016).

2.2 Properties of Carboxymethyl Cellulose (CMC)

Cellulose is expected to become the main chemical resource of the future considering it is the most widely available renewable resource on earth. (Schurz 1999, Eichhorn, Young et al. 2005). In addition to this there is an increasing demand for sustainable and environmentally friendly products and cellulose is a great precursor for the development of new functional materials for a broad range of applications (Klemm, Heublein et al. 2005). There is immense potential to prepare hydrogels using cellulose because of the abundant hydroxyl groups present in cellulose. There are many favorable properties associated with hydrogels such as biodegradability, hydrophilicity, biocompatibility, non-toxcicity, low cost and transparency. Due to their attractive structures these hydrogels have wide applications such as in tissue engineering (Vinatier, Gauthier et al. 2009), controllable delivery system (Chang, Duan et al. 2010), blood purification (Ye, Watanabe et al. 2003), sensor (Sannino, Pappada et al. 2007), and chromatographic supports (Xiong, Zhang et al. 2005). Figure 1 shows prospects for the various applications of cellulose based hydrogels. Cellulose based products are expected to replace a lot of petroleum based products in the future (DİLAVER 2011).

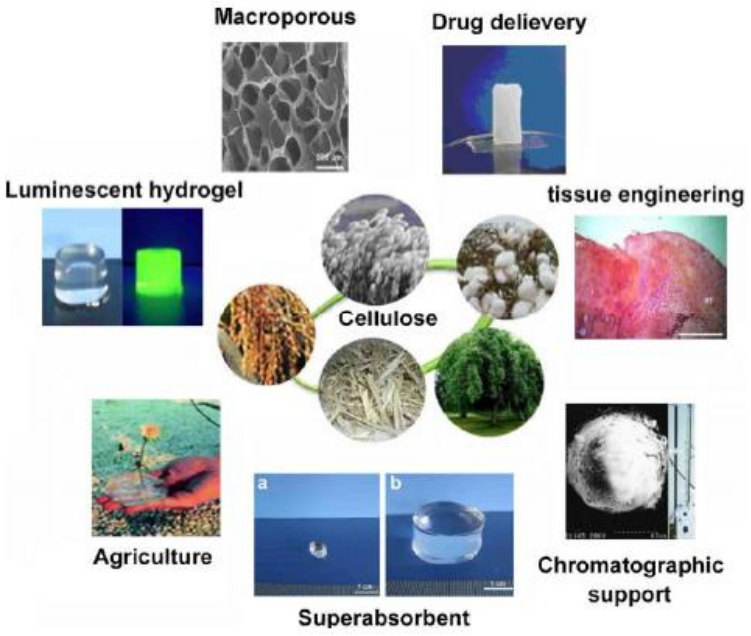

Figure 1 Application of Cellulose based Polymers

As mentioned earlier the numerous hydroxyl groups in cellulose make hydrogen bonding easy, therefore cellulose hydrogels can be easily prepared via physical cross-linking using cellulose solutions. However, this same phenomenon of extended hydrogen bonding makes dissolution of cellulose very difficult in common solvents (Edgar, Buchanan et al. 2001). To overcome this issue water soluble cellulose derivatives can be used which have the added advantage of being biocompatible. These derivatives can be used as thickeners, emulsifiers, binding agents, suspension aids, film formers, surfactants, stabilizers and lubricants. They can also be used as additives in pharmaceutical, food and cosmetic industries (Weng, Zhang et al. 2004).

One prominent example of such a derivative is Carboxymethyl cellulose (CMC). CMC is a non-toxic, highly biocompatible and biodegradable derivative. It is a water soluble, low cost ionic polysaccharide that contains abundant carboxyl and hydroxyl groups. Another added feature is its ability to exhibit Ph sensitivity (Charpentier, Mocanu et al. 1997, Mitsumata, Suemitsu et al. 2003).

One of the most widely studied conversion methods is carboxymethylation. Carboxymethylation is simple and leads to a variety of products with promising features. The general procedure involves the activation of the polysaccharide with aqueous alkali

hydroxide usually sodium hydroxide. Monochloroacetic acid or its sodium salt is then used for further conversion to yield carboxymethyl polysaccharide derivative the process which is generally known as the Williamson ether synthesis (Heinze 2005). CMC also behaves as a typical polyelectrolyte and is therefore also categorized as a cellulose ether.

2.3 Properties of Basil Seed Gum

A novel hydrocolloid is extracted from seeds of basil herb and is known as Basil seed gum (BSG) or alternatively as Ocimum bascillum. It is commonly used as a thickening and gelling agent in the food industry (Hosseini-Parvar, Matia-Merino et al. 2010). BSG contains xylan, glucan and glucomannan and is therefore referred to as hetero-polysaccharide(Rafe and Razavi 2013). Chemically BSG is comprised of two major fractions i.e. an acid stable core and a linked xylan with acidic side chains. The acid stable core glucomannan (43%) has a glucose/manose ratio of 10:2. The acidic side chains make up the acid soluble portion. There is also a minor glucan fragment that comprises 2.31% of the total structure(Mirhosseini and Amid 2012).

Basil (O. basilicum L) is an herb that is found abundantly in central and south America, Asia and Africa. Basil seeds have been used traditionally to treat several diseases such as diarrhea, dyspepsia, colic ulcer, inflammation etc.(Hosseini-Parvar, Matia-Merino et al. 2010). The seeds are black, oval and covered in a polysaccharide layer. This outer layer turns into mucilage when the seeds are soaked in water. This mucilage can then be extracted , dried or concentrated to be used in further applications(Akbari, Ghoreishi et al. 2015).

Various studies have focused on the use of mucilages for the development of drug delivery systems. Prajapati et al. (Prajapati, Jani et al. 2013) reviewed how mucilages, natural gums and their altered forms can be used in the pharmaceutical industry to develop drug delivery systems. mucilage polysaccharide from waste of *Abelmoschus esculentus* for biomedical applications has been characterized by Archana et al. (Archana, Sabina et al. 2013). Characterization and in vitro drug release studies of *Terminalia catappa* gum were carried out by Meka et al. (Meka, Nali et al. 2012). Srinivas et al. (Srinivas, Prakash et al. 2003) study focused on O. basilicum as disintegrates in the formulation of dispersible tablets.

2.4 Properties of Hermal Seed

Pegnum hermala or Hermal Seeds are widely distributed in most parts of the world including the middle east, Pakistan and India. Moreover, they have now been introduced in America and Australia too (Asghari and Lockwood 2002, Yousefi, Ghaffarifar et al. 2009). By burning the seeds of *P. harmala* it can be used as a disinfectant and antiseptic and such is the practice in Iran (Fathiazad, Azarmi et al. 2006, Arshad, Zitterl-Eglseer et al. 2008).

Hermal seeds have the potential to be used in the treatment of a variety of diseases such as asthma, jaundice, colic and lumbago. It is also used a s a stimulant emmenagogue (Bukhari, Choi et al. 2008).

The seeds and the roots are most beneficial because they contain the active compounds which have been characterized as alkaloids (Mirzaie, Nosratabadi et al. 2007). Anti-tumor activity of P.harmala has also been identified (Goel, Singh et al. 2009). In addition to showing anti-tumor activity P.harmala also has anti-histaminic (Asghari and Lockwood 2002), vasorelaxant effect (Asghari and Lockwood 2002), wound healing (Derakhshanfar, Oloumi et al. 2010), anti-oxidant activity (Astulla, Zaima et al. 2008), leukemic healing (Zaker, Oody et al. 2007), immuno- modulator properties (Astulla, Zaima et al. 2008) and anti-inflammatory properties (Muhi-eldeen, Al-Shamma et al. 2008). In addition to the above-mentioned properties this plant has exhibited antifungals and antibacterial properties too. (Shonouda, Osman et al. 2008).

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

Hermal and Basil seeds were purchased from local market (Islamabad, Pakistan) and suitable seeds were collected and cleaned from sand and dust. Polyvinyl alcohol (PVA) (Mol. Wt. 72000 g/mol) was obtained from AppliChem Panreac (Darmstadt, Germant). Carboxymethyl cellulose sodium salt was purchased from Daejung Chemical Co. (Siheung, Korea). Ethanol (Mol. Wt. 46.07g/mol) used for extaction was obtained from Merck KGaA (Darmstadt, Germany). Sodium Chloride (NaCl) (Mol. Wt. 58344 g/mol), Sodium Hydrogen carbonate ($NaHCO_3$) (Mol. Wt. 84.01g/mol) and Sodium Phosphate monobasic dehydrate (NaH_2PO_4) (Mol. Wt. 156.01 g/mol) purchased from Sigma-Aldrich (USA) whereas Potassium Chloride (KCl) (Mol. Wt. 74.55g/mol) purchase from Haque Chemicals (Pakistan) were used for the preparation of Pseudo-extracellular Fluid (PECF). For anti-bacterial and anti-fungal Testing Tryptone (BioWorld, USA), yeast extract (MERCK, Germany), nutrient agar (MERCK, Germany), were used. Doubly distilled water was used for preparation of solutions.

3.2 Extraction from Plant material

3.2.1 Basil Seed Gum Extraction:

Basil seeds were soaked and swelled in Distilled water in a ratio 1:50 at room temperature for 2-3 hours. The seeds were blended until the mucilage is smooth and squeezed out through double folded muslin cloth. The extracted gum was further used for preparation of composite films (Razavi, Mortazavi et al. 2009).

3.2.2 Hermal Seed Extraction:

10g dry seeds were ground and soaked in 100 ml 96 % ethanol. Extraction was done in water bath at 30^{o} C for 4-5 days with continuous stirring at 150 rpm (Arshad, Zitterl-Eglseer et al. 2008). The extract was filtered and dried at 40^{o}C. The dried extract was then dissolved in DMF and used in composite films.

3.3 Solution Preparation

Carboxymethyl Cellulose (CMC) 1% solution was prepared by dissolving 1g CMC in 100ml distilled water at 70^{o}C. CMC was added slowly with continuous stirring for even distribution and avoiding formation of clumps.

Polyvinyl Alcohol (PVA) 5% solution was prepared by adding 5g of PVA in 100ml distilled water at 120^{o}C and stirred for 15-20 min until clear solution was obtained.

Hermal Extract Solution was prepared by dissolving 10g extracted powder in 50ml Dimethyl Formamide (DMF) purchased from TEDIA Company Inc, USA.

3.4 Synthesis of Composite Films

Different composites were synthesized by mixing 1% CMC, 5% PVA and Basil Seed Gum (BSG) in varying ratios. PVA/BSG and CMC/BSG composites were prepared in 3 different ratios i.e. 1/1, 3/1 and 1/3 whereas CMC/PVA/BSG composite was prepared in ratios 3:1:1, 3:1:2 and 3:1:3. The ratios were mixed such that final volume remains 40ml in all composites. The mixture was then poured in petri-plates and air dried for 2 days. The harvested films were than used for further testing.

3.5 Synthesis of Compsite Films with Hermal Extract

16ml of hermal extract solution was added slowly, in intervals, to the composites of (a) PVA/BSG, ratio 1:3 (PG13) (b) CMC/BSG, ratio 3:1 (CG31) and (c) CMC/PVA/BSG, ratio 3:1:3 (CPG313) and mixed manually with a glass stirrer to avoid clump formation. The mixture was casted in petri-plates and air dried for 2-3 days. The resultant films (PG13-H, CG31-H, CPG313-H) were used for further testing.

3.6 Swelling Analysis of Composite Films

Swelling analysis of the films was done by Gravimetric method i.e. by immersing a small piece (1cm x 1cm) of the film in pseudo-extracellular fluid (PECF pH 7.4) at 37^{o}C and measuring the weight at intervals. The dry weight of pieces was measured initially. The weight of the film was measured after an interval of 10 min for 80 mins. PECF solution was

prepared by dissolving 0.68g NaCl, 0.22g KCl, 2.5g $NaHCO_3$, 0.35g NaH_2PO_4 and keeping the pH to 7.4.

3.7 *In-vitro* Analysis of the Composite Films

3.7.1 Antimicrobial testing of Composite films:

Disc Diffusion method was used to evaluate the anti-microbial activity of the composite films. To investigate the anti-bacterial activity of the films sterilized nutrient broth agar was used as a growth medium for bacterial strains of *Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis).* Film discs of 6mm diameter were placed on the inoculated plates and incubated at 37^{o}C and results were recorded after 24 hours of incubation (Monte, Abreu et al. 2014).

During anti-microbial testing whatman filter paper no. 1(6mm diameter) was used as a negative control and commercial tetracycline (30ug) disc were used as positive controls for anti-bacterial testing. The bacterial culture strains were provided by Mycovirus Lab, NUST, Islamabad.

3.7.2 Drug Release Study:

The release of hermal extract incorporated in the films as an antimicrobial (2) and anti-inflammatory agent was assessed by UV-Vis spectrophotometry (Systronics 2202). A small piece of composite films weighing 0.03g were cut and placed in 15ml of PECF solution (pH 7.4) at 37°C (Lin, Chen et al. 2001). At an interval of 20 min, an aliquot of solution was withdrawn from PECF solution. After each sampling, the withdrawn solution was replaced with the same amount of fresh PECF Solution. The drug release profile of the collected samples was checked at λ max= 417 nm (Rajendra K. Patel 2015).

3.8 Material Characterization

3.8.1 Fourier Transform Infrared Spectroscopy (FTIR):

FTIR was performed to identify the chemical linkage between the functional groups of the components of the composite. FTIR spectroscopy (Perkin Elmer, spectrum 100 FTIR spectrophotometer) of the composite films was carried out in KBr mode, in wavenumber range of 4000 – 450 cm^{-s}, with 256 scans and at 8 cm^{-1} resolution.

3.8.2 X-Ray Diffraction Analysis (XRD):

The crystallinity of the composite films was assessed using STOE X-ray Diffractometer, with monochromatic Cu-K α radiation source (λ= 0.15418). The data was collected at 2θ between 5-60 degrees.

3.8.3 Scanning Electron Microscopy (SEM):

The surface morphology of the composite film was analysed using JSM-6490A Analytical scanning electron microscope (JEOL, Tokyo, Japan). The activation voltage under which the SEM images were collected was 20kV.

3.9 Mechanical Testing

TRAPEZIUM-X Universal Testing machine (AG- 20KNXD Plus) manufactured by Shimadzu Corporation was used to determine the mechanical strength of the composite films. Sample films were cut into films of 1cm width with gauge length 2 cm during testing. The samples were cut carefully to avoid notches and cracks. The tests were performed in triplicates and average value was obtained.

3.10 Statistical analysis

All tests were performed in triplicates and averages with standard deviation were obtained. The Statistical analyses of the results were done by using unpaired t-test in statistica software. The values that were $P < 0.05$ were considered statistically significant value.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Swelling Results of the Composite films:

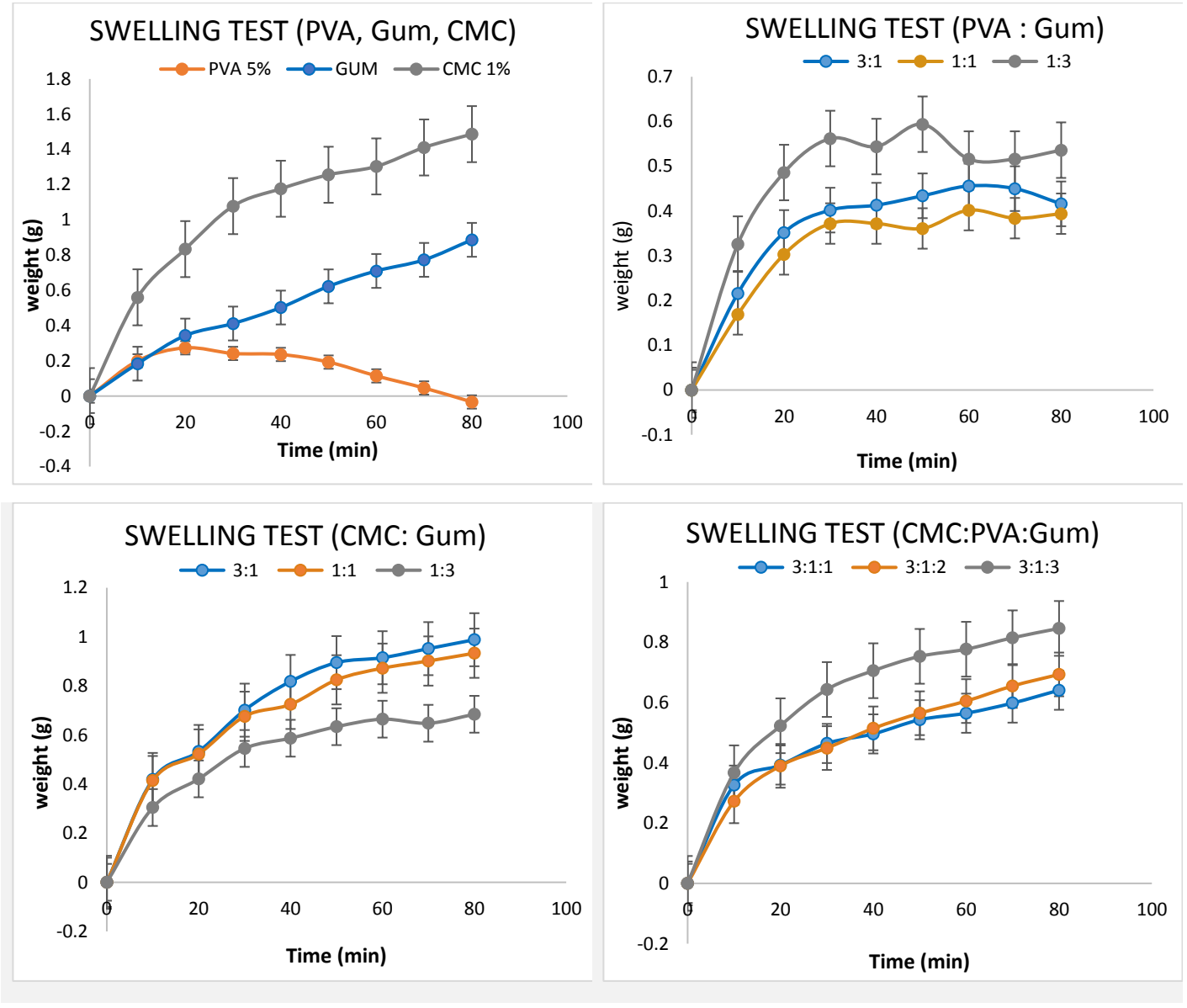

Figure 2 : Graphical Representation of increase in weight of the films when submerged in PECF Solution

Swelling analysis of individual components (CMC, PVA, Basil seed Gum) and the 3 compositions was done through Gravimetric method (figure 2). The swelling tests confirm the hydrophilic nature of the composite material. It can be observed however, that the ratio of gum in the composites affects the swelling profile. When swelling of the components was

done, CMC possess higher degree of swelling as compared to gum which possess a complex polysaccharide structure, while PVA was degraded during the same course of time.

In PVA/Gum swelling profile, it can be observed that when the ratio of gum was increased (PG13) the swelling of the film was also increased. It may be attributed to the amine groups present in gum which are present in more amount in case of higher gum ratio (Akbari, Ghoreishi et al. 2015). While when the amount PVA was increased (PG31), the downward curve shows the rapid degradative nature of the film. The structural integrity of the film was compromised which is indicated by the presence of a hump in the curve. PVA is degrading rapidly leaving behind gum which doesn't retain film forming property.

The swelling curve of the CMC/Gum composition shows that increasing CMC ratio increase the PECF uptake of the composition. The ratio 3:1 (CG31) hold more fluid because of free carboxyl and hydroxyl groups in the composite contrary to gum (Charpentier, Mocanu et al. 1997, Mitsumata, Suemitsu et al. 2003). The CG31 curve also shows stable increase in weight during latter half of the time.

All the three components under study were mixed together in different ratios as shown in bottom right graph. Here the ratio 3:1:3 (CPG313) hold more PECF uptake ability than rest of the ratios. This shows increase in weight at constant speed which will help in sustained release of drug in later section. The carboxyl and hydroxyl groups; present in abundance in CMC and gum; are responsible for this property.

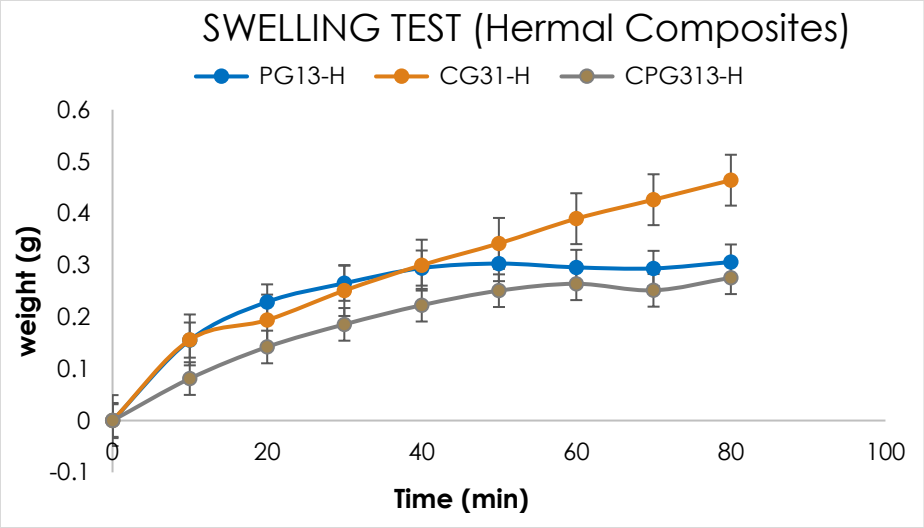

Figure 3 Graphical Representation of increase in weight of the films with Hermal Seed Extract when submerged in PECF Solution

Hermal extract was added in the composite ratios (CG31, PG13, CPG313) showing greatest swelling results as mention above. As in can be seen in figure 2, CG31-H illustrates maximum increase in weight in 80 minutes and PG13-H has reached to its equilibrium state but the swelling curve of CPG313-H shows lesser uptake of PECF solution when compared with the other two. This may be because the oligosaccharide layer of basil seed gum binds strongly with polymers with carboxyl and hydroxyl groups by forming H- bonds (Archana, Sabina et al. 2013).

4.2 Material Characterization Results:

4.2.1 FTIR

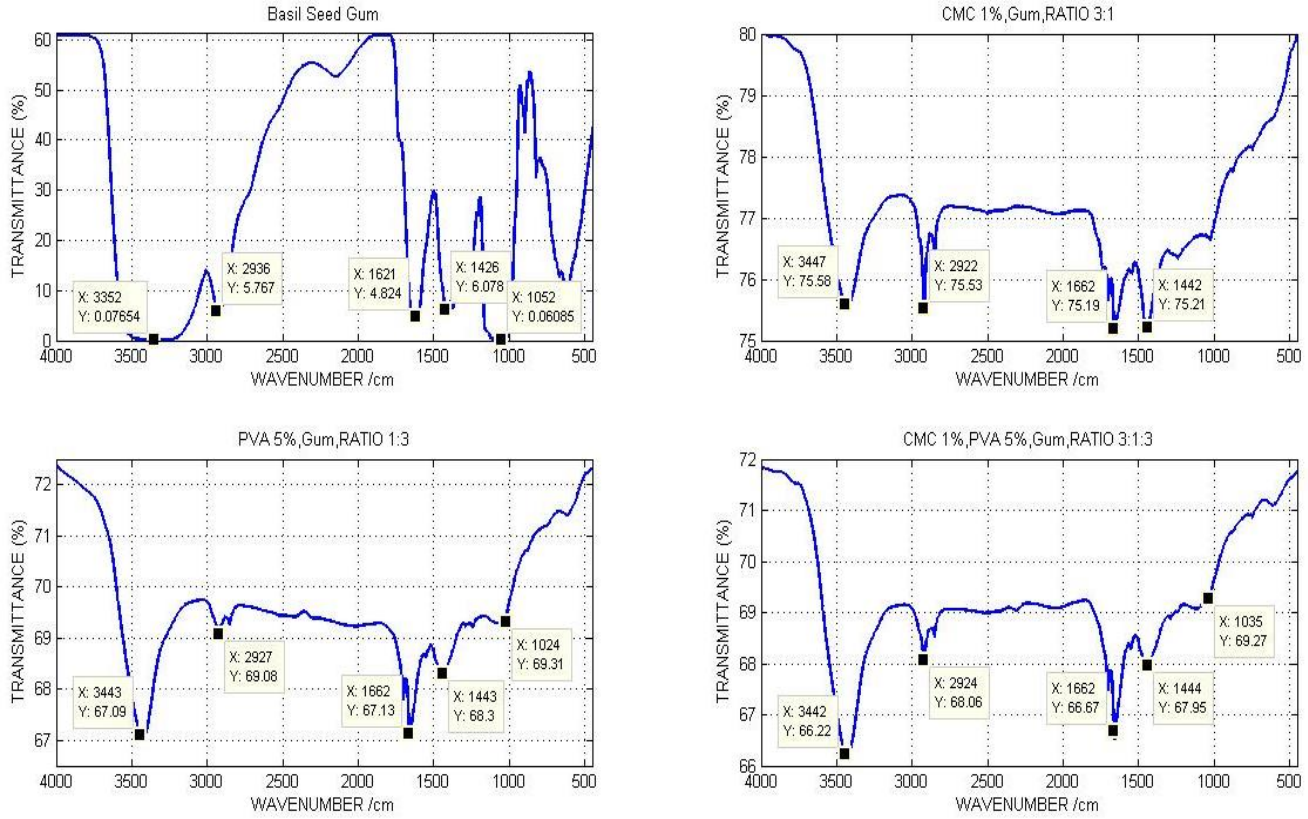

Figure 4 FTIR Spectra of Basil seed gum Composites with CMC and PVA

FTIR of the composite films was done for the functional group analysis of the composite film. The FTIR graph of pure basil seed gum shows the occurrence of peak at the 2936 cm−1 signifies C-H asymmetric stretching. The observed characteristic peaks at 3352 cm−1 owing to OH stretching of alcohol (or water absorbent) and N-H bond, at 1052 cm−1 owing to CO stretching of alcohol and at 2936cm−1 due to C-H stretching of alkyl group. The peaks at 1621 and 1426 cm−1 was observed in the present study for N-H primary amide and at 1052 cm−1 showed C-O-C stretching indicating the presence of aliphatic amines, was also detected (Gomes, Azevedo et al. 2012, Akbari, Ghoreishi et al. 2015). It can be infereed from these results that basil seed mucilage possess peptide cross-links and some amino sugars (Mishra, Clark et al. 2008).

The IR spectra of CMC/PVA composite films show characterstic absorption at 3400 cm-1 which shows the presence of OH stretching and H-bonding in the film. At 2918cm-1 and 2815cm-1 shows the presence of C-H stretching and presence of aromatic rings can be seen at 1630cm-1 and 1440cm-1. The peak at 1317cm-1 indicates C-O stretching due to presence of alcohols and carboxylic acid groups in the film (DİLAVER 2011).

When Basil seed gum was incorporated in the CMC/PVA and CMC and PVA IR peak obtained showed similar peaks as shown in figure 1. Here peaks in IR graphs of CMC:Gum (3:1), PVA:Gum (1:3) and CMC: PVA: Gum (3:1:3) can be seen at 3400-3447cm-1 which shows OH stretching and existence of H-bonds in the composite. The alkyl group presence in the composite film is shown by the C-H stretching; peaks in the ranges 2922-2927cm-1 and 2837-2855cm-1. The IR band at 1662cm-1 demonstrates the presence of C-C aromatic rings as in PVA and CMC and gum. Peak at 1052-1024cm-1 show the presence of CH-O-CH_2 stretching (Qiu & Yu, 2007; Ma, Xu, Fan & Liang, 2007).

After the incorporation of basil seed gum into CMC, PVA and CMC/PVA the peaks in the IR spectrum have become sharp as compared to the peaks of CMC and PVA (DİLAVER 2011). The peak at 2922cm-1 and 1442cm-1 in the CMC/Gum composite is deeper as compared to the peak in PVA/Gum and CMC/PVA/Gum composite. This may be because CMC is present in larger ratio thus indicating more amount of C-H stretching groups.

The peaks at 3352, 3447 3443 and 3442 cm-1 in all four graphs can be attributed to the hydrophilic nature of the composite. Thus, playing a key role in the swelling of the composite films. Moreover, literature suggests that the hydrophilic groups (carboxylic and hydroxyl) of polymers can strongly bind with oligisaccaride chains of the mucus layer of basil seed gum through hydrogen bonding (Archana, Sabina et al. 2013). Studies have suggested that basil seed mucilage when combined with polysaccarrides (like aliginate sulfate) gives good bioadhesive properties for biofilm formation and drug delivery systems (Akbari, Ghoreishi et al. 2015).

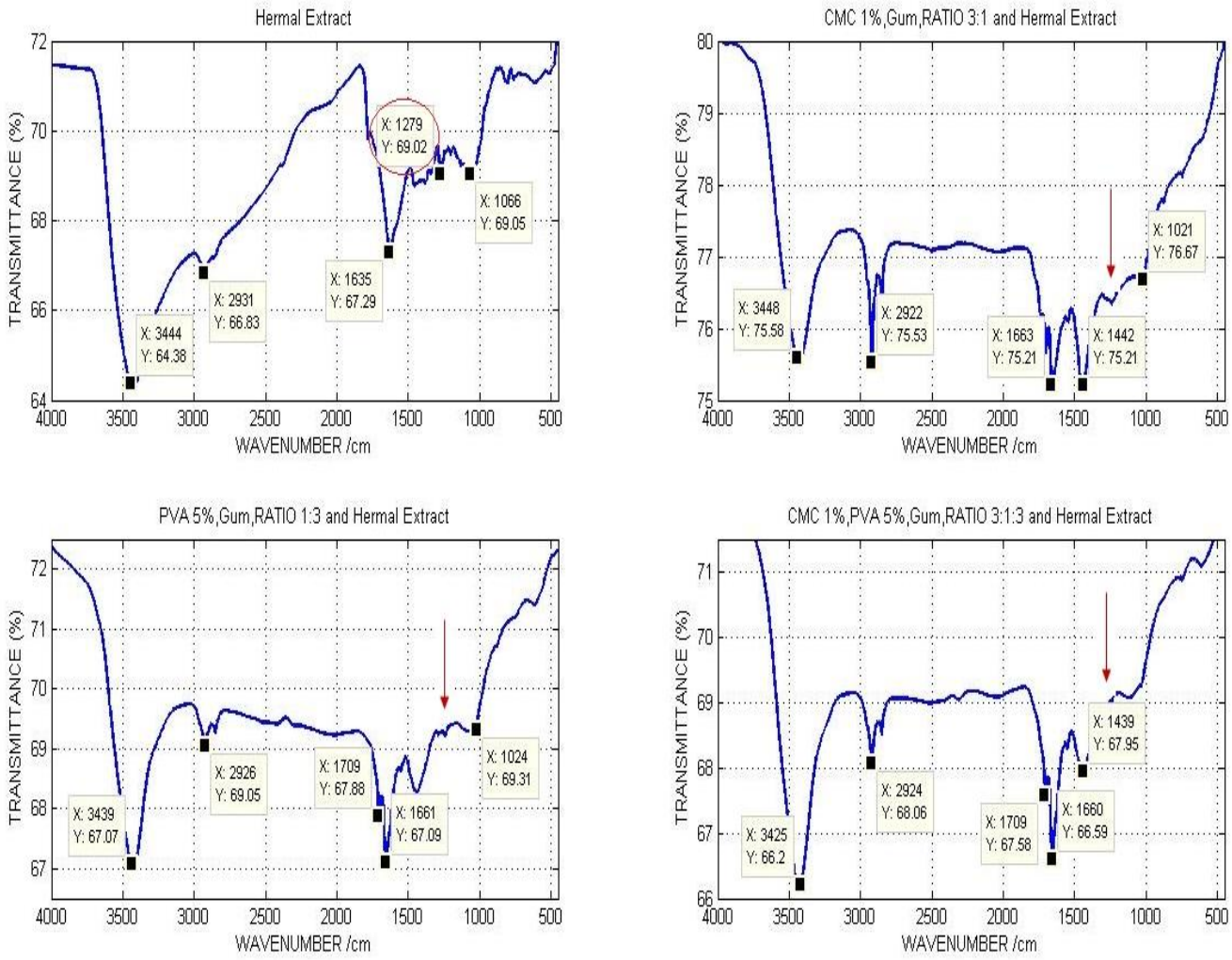

Figure 5 FTIR Spectra of the composites with Hermal Seed Extract

When we compare the IR graphs of the composite with (figure 4) and without hermal extract, we see no prominent change in the intensity or shifting of the peaks. Here we can say the hermal seed extract has been physically incorporated in the composite. Alkaloids; an anti-oxidant, anti-inflammatory and anti-microbial agent (Darabpour, Motamedi et al. 2011); consists of basic nitrogen atoms. The peak at 1279cm-1 in all graphs indicated the presence of aromatic amines because of C-N stretching (aromatic amines) (Ghafoor, Ali et al. 2016).

4.2.2 XRD:

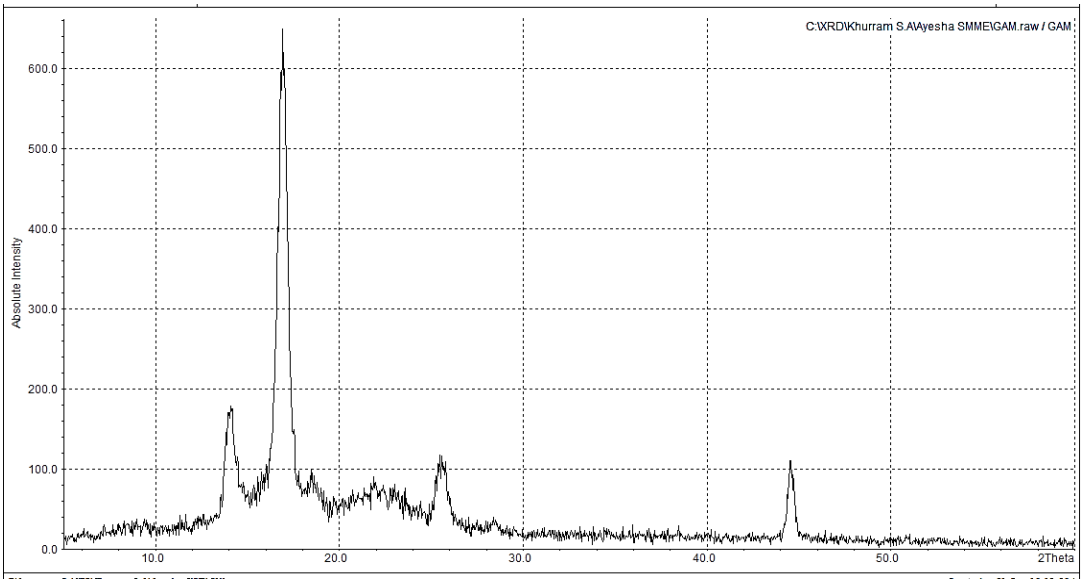

Figure 6 XRD pattern of Basil Seed Gum

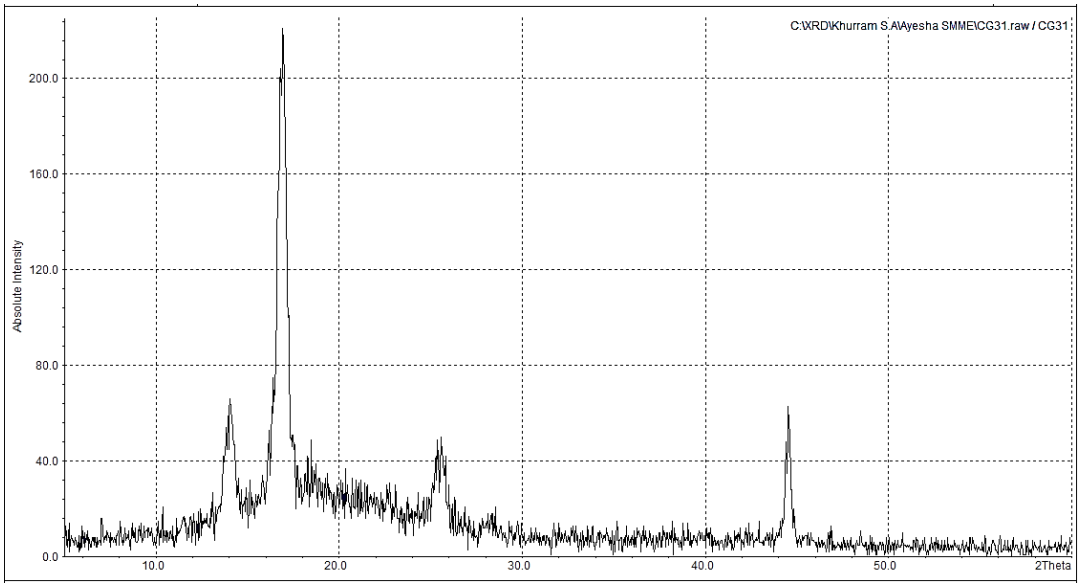

Figure 7 XRD pattern of CMC/Gum Composite (CG31)

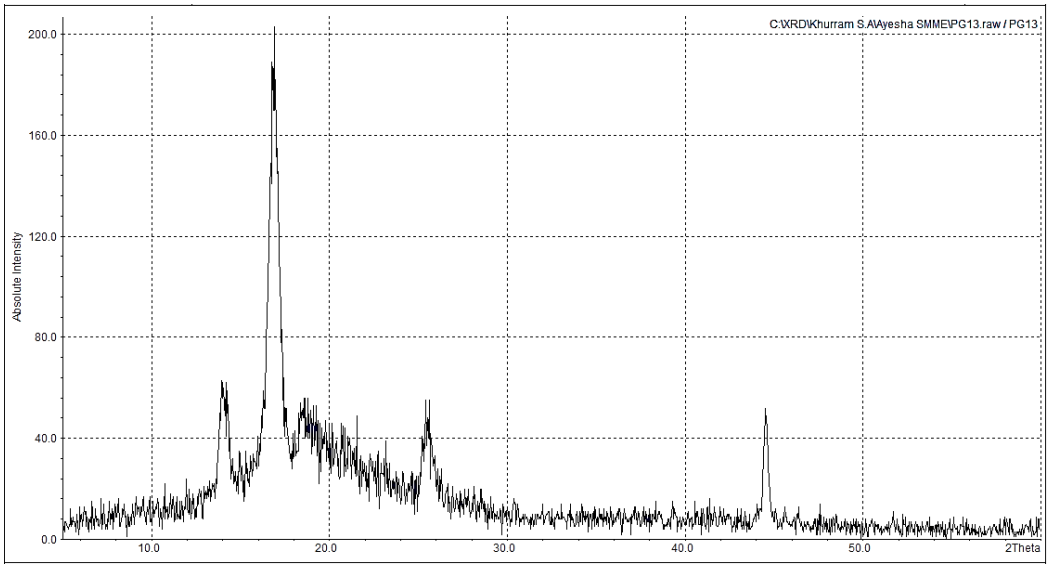

Figure 8 XRD pattern of PVA/Gum Composite (PG13)

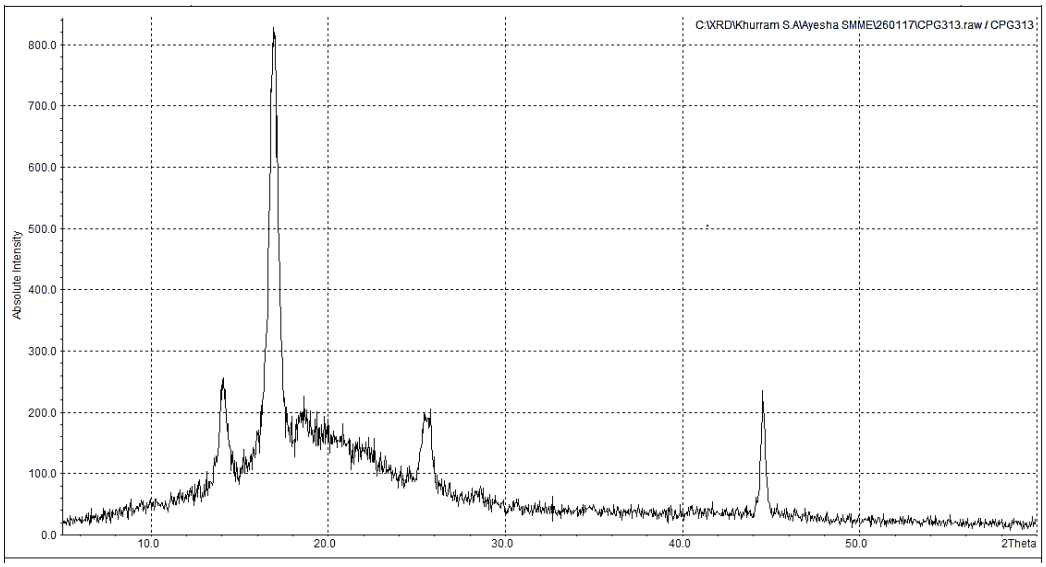

Figure 9 XRD pattern of CMC/PVA/Gum Composite (CPG313)

X-ray diffraction (XRD) pattern of basil seed gum shows a sharp peak at 20º associated with the high crystallinity. The diffractogram of NaCMC, PVA and NaCMC/PVA blend shows the characteristic peaks at 20.5º, 20º and 20º respectively (DİLAVER 2011). NaCMC is not completely amorphous in nature nor does basil seed gum, which is more crystalline in nature than CMC.

It can be observed in the XRD pattern of the composites that when basil seed gum was added in CMC and PVA, a sharp peak at absolute intensity of 17º was formed. It can be noticed that the peak intensity in CMC/Gum composite is a little more than

PVA/Gum composite and highest in CMC/PVA/Gum composite. CMC and PVA contains hydrophilic groups, such as carboxyl and hydroxyl groups, that bind strongly with the oligosaccharide chains of the mucous layer of basil seed gum (Akbari, Ghoreishi et al. 2015).

The sharp peaks of FTIR also contribute to the point of creation of hydrogen bonding resulting in more attraction and indicating toward the increase in crystallinity of the films when gum was added.

4.2.3 SEM Analysis:

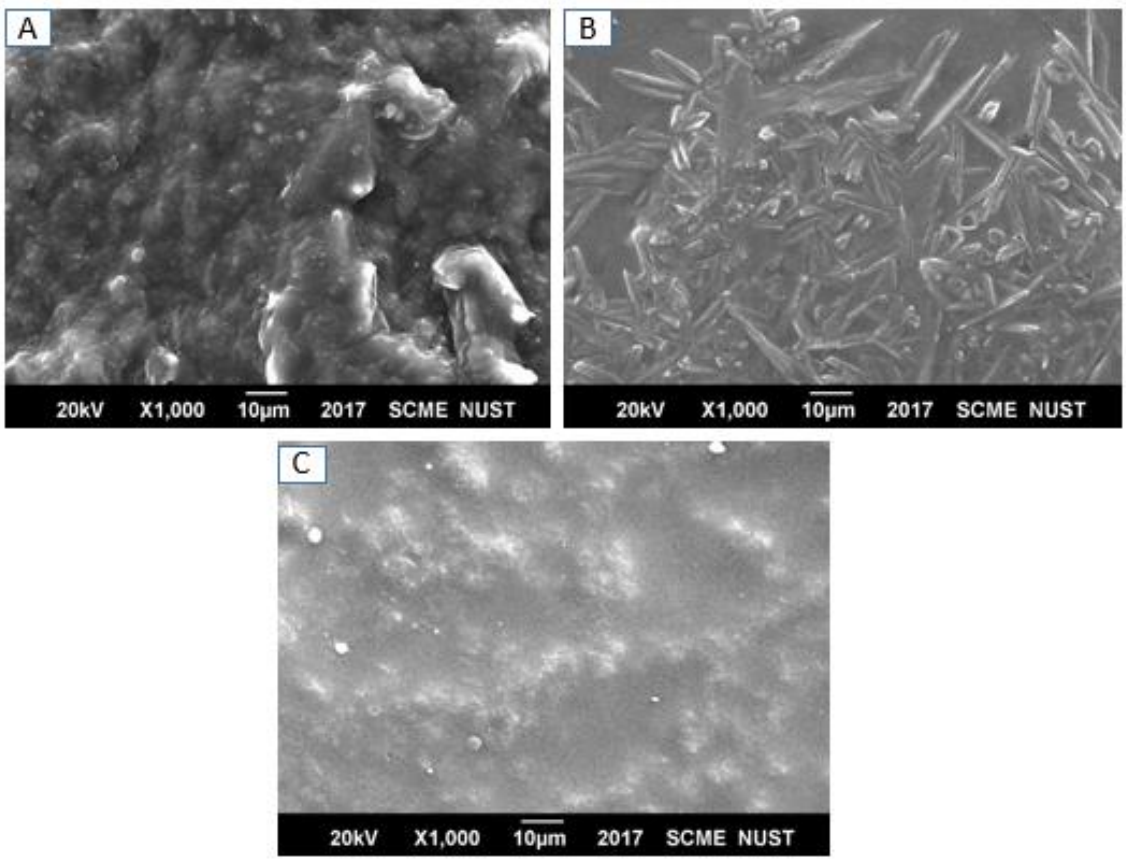

Figure 10 Surface Morphology of the Composite films with Hermal Seed Extract (A) CPG313-H (B) CG31-H (C) PG13-H at 20kV and 1000X magnification

The surface morphology of the composite films was assessed by Scanning Electron Microscopy as shown in figure 10. These images show a non-homogenity and roughness in the films. In figure 5 (A) semi crystalline nature of the film can be observed. The ridges and grooves on the surface contribute to the water uptake by the films. In the SEM image of CG31-H (figure 10B) needle like structures can be seen

which occur due to the occurrence of H-bonds (Archana, Sabina et al. 2013) and absence of PVA in this composite whereas, the SEM image of CPG313-H (figure 10C) shows similar groves and ridges as in PG13-H.

4.3 *In vitro* Results of the Composite Films

4.3.1 Antimicrobial Testing:

The composite films, placed on bacterial inoculated plates, gave zone of inhibitions that were recorded after 24 hours. Hermal extract released from the surface of the films contributed to the formation of zone of inhibitions. The maximum antibacterial activity was observed by PVA/Gum composite (PG13-H), as PVA is degrades quickly releasing hermal extract. Also, the structure of the films does not remain integrated and gum starts dispersing after the degradation of PVA during swelling of the films. While CG31-H has second largest zone of inhibitions with good structural integrity. CPG313-H formed smallest zones as compared to the other two because of the formation of hydrogen bonding between the gum and the carboxyl and hydroxyl groups of the polymers as well as of hermal extract (as discussed in swelling and FTIR analysis).

Arshad et al. demonstrated the Anti-bacterial activity of ethanolic extract of hermal seed and the zone of inhibition of *E. coli* 11–21 mm, *Staphylococci* sp. 14–18 mm was recorded. The solvent used for extraction do not have any anti-bacterial activity.(Arshad, Zitterl-Eglseer et al. 2008). In the current research the mean diameter of the zone of inhibition of the composite films is shown in the table 1.

Table 1 In-vitro Results of the Composite films with Hermal Extract

Zone of Inhibition (mm)				
	PG13-H	**CG31-H**	**CPG313-H**	**Tetracycline**
Bascillus	20.6	19.375	16.28	25.12333333
S. aureus	19.626	20.26	17.97	20.13666667
P. aeruginosa	19.77	16.68	15.49	21.20666667
E. coli	22.54	22.24	18.205	24.12

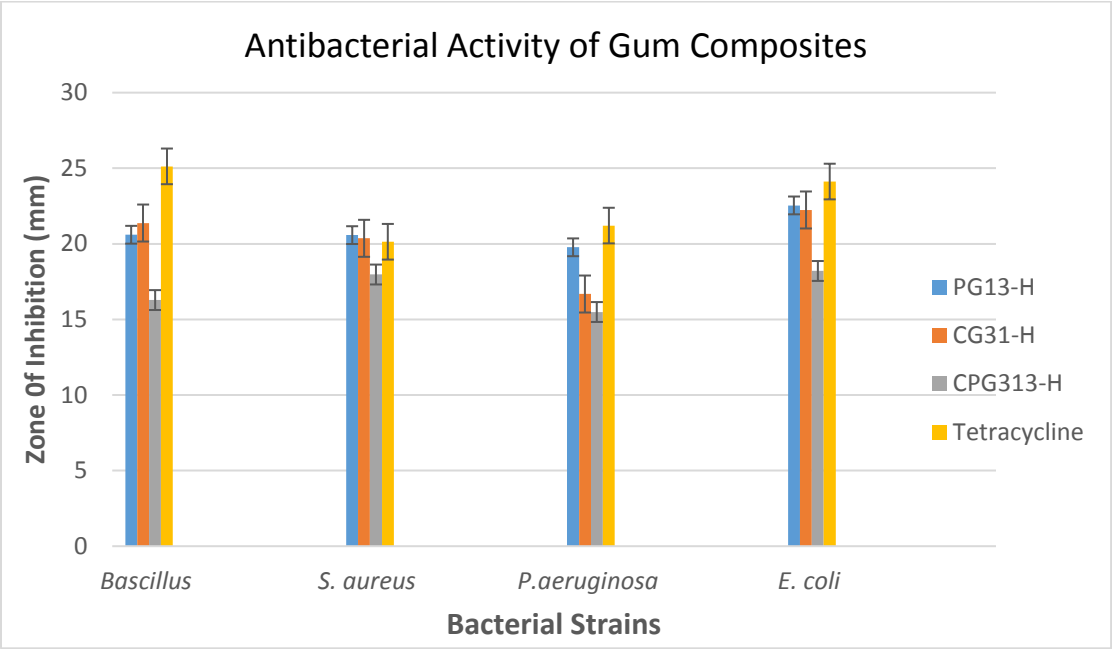

Figure 11 Graphical representation of antibacterial activity of different concentrations of composite films. Y axis shows zones in mm while x axis shows different bacterial strains used

4.3.2 Drug Release Study:

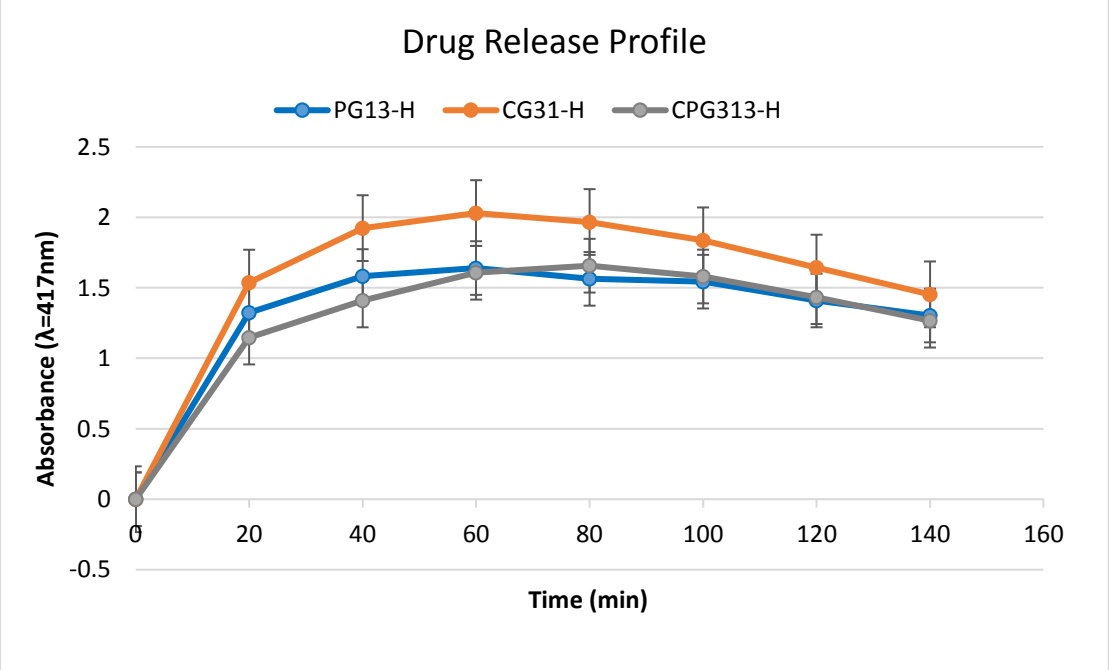

Figure 12 Drug release profile of Composite films with hermal extract. Y-axis represent absorbance at wavelength of 417nm and x-axis represent Time (min).

Alkaloids, one of the major constituents of hermal extract, play a key role in the antimicrobial activity (Schmeller and Wink 1998) which is known to show maximum absorbance at 417nm (Rajendra K. Patel 2015). Initially, burst release of hermal extract was observed till 20 min in all the composites followed by slow release (figure12). This may be because of the physical presence of hermal extract in the composite films (shown by SEM images and FTIR of the films). It can be seen from the drug release profile that CG31-H shows sustained release of hermal extract while drug release from PG13-H and CPG313-H starts decreasing earlier. The drug release process starts with diffusion process. The hydrogen bonding the exist between the carboxyl and hydroxyl group of CMC and mucilage of the gum (discussed in FTIR analysis) keep the hermal extract trapped that is released in controlled manner when these bonds undergo hydrolysis. These results also correspond to the swelling behavior of the composite films with hermal (figure 3) extract where increase in weight of CG31-H was consistent. The controlled release of hermal extract would be helpful for wound healing purposes by protecting against secondary infections.

4.4 Tensile Testing:

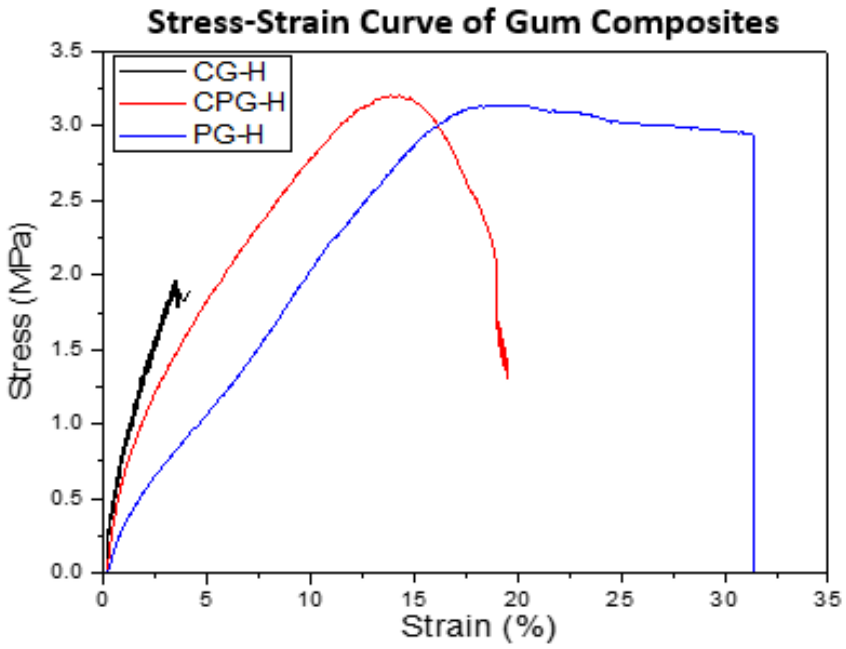

Figure 13 Stress-Strain curve of the Composite films

The tensile testing of Basil seed gum composites was done at room temperature. Stress-Strain curve of the composites is shown in the figure 13. As it can be seen in the graph that with the addition of PVA the mechanical properties of the composite were enhanced. S-S curve shows brittle to tough nature of the composite, as the stiffness and elongation at break were increased that gave it strong and tough property. The improvement in mechanical properties depends on the homogeneity/dispersion of the composite. Figure 13 shows that increasing the PVA content increases the tensile strength. It can be seen in the graph that PG-H shows more plastic region before breaking than CPG-H which contain less PVA content than PG-H. This shows the tough nature of PG-H. While CG-H shows very less or no plastic region and breaks abruptly thus showing its brittle nature. Xiao *et al.* studied the blends of CMC with polysaccharides and suggested the formation of hydrogen bonds which contribute to mechanical stability and can be controlled by blending ratio (Xiao, Lu et al. 2001).

5. CONCLUSION

This study focused on the application of the composite for topical and subcutaneous applications where accumulation of exudate causes maceration and enhances bacterial growth. Hermal extract can be very suitable to counter this problem. A detailed analysis on swelling and drug release property of Basil seed gum composites with PVA, CMC and hermal extract was done. The effect of functional groups, crystallinity, mechanical properties and antibacterial effect of the composite were also assessed in detail. According to antibacterial, drug release and swelling tests results, CMC/Gum 3:1 with hermal extract showed sustained Drug Release. These composites can be further studied as coating material and wound healing applications.

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