Original Article

Preparation and Characterization of Wound Healing Composites of Chitosan, Aloe Vera and *Calendula officinalis* – A Comparative Study

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ABSTRACT

Several natural polymers have application in terms of therapeutic treatment of ailments. Chitosan, Aloe Vera and *Calendula officinalis* have proven wound healing properties individually. It was theorized that a combination of the biopolymer and the plant extract will show an improvement in wound healing properties. Thus, composite films of Aloe Vera extract in Chitosan and *C. officinalis* petal extract in Chitosan were prepared and evaluated to ascertain the applicability for wound healing. The chemical composition and mechanical, morphological, and surface characteristics including the antibacterial activity were also evaluated. The optimum conditions for obtaining a maximum percentage of composition were standardized. Their mechanical properties, water absorption characteristics and compatibility were analyzed by tensile tester, FTIR and SEM analyses. The composite films containing aloe vera had higher water absorption capacity and antimicrobial activity as compared to those containing *C. officinalis* petal extract. The films containing *C. officinalis* petal extract showed higher tensile strength as compared to those containing Aloe Vera. With the above results, it was concluded that a composite of 25% Aloe Vera in Chitosan can be used as a wound dressing amongst all the combinations.

Keywords: Chitosan, Aloe vera, *Calendula officinalis* , composite films, wound healing, FTIR, SEM.

INTRODUCTION

Wound healing is a dynamic process of regenerating dermal and epidermal tissue and the performance requires a dressing that can change as the wound progresses towards healing. Wound healing is the body's natural process whereby the body restores the

injured part to as near its normal condition as possible. Though wound healing takes place naturally on its own, some of complications like sepsis, disruption of tissue and skin layer, extension of infection to adjacent and interior organs occur in major cases. To prevent extensive loss and damage to the tissue, skin grafting and biological dressings were developed. The ability of the skin to repair itself after a minor wound is remarkable, but when the damage is severe or occurs in large amounts of skin area, proper and immediate coverage of wound surface with an adequate dressing is needed to protect the wound and accelerate wound healing. Ultimately the immediate wound coverage, temporary or permanent, is one of the principal goals of wound management. For this films made with biomaterials are becoming popular due to many advantages[1]. Some dressings simply adsorb exudate or wound fluid and therefore are suitable for application to a variety of different wound types whereas, other function have limited range of indications. The major advantages of natural polymers are good cyto compatibility[2]. It is also biodegradable in nature and do not require any surgery for removal of polymers[3].

Many sophisticated dressing are available and are made of materials like polyurethane, salts of alginic acid and other gelable polysaccharides namely starch and carboxymethyl cellulose. They may be used alone or in combination to form products as diverse as films, fibrous products, beads, hydrogels or adhesive gel forming wafers more commonly called hydrocolloid dressings[4].

Biomaterials are natural polymers which are biodegradable. These are used in regenerative medicine, implantable materials, controlled release carriers or scaffolds for tissue engineering. Cellulose, chitin, chitosan, collagen and gelatin are widely used natural polymers. Natural polymers when used as drug delivery carriers, they are degraded into biologically accepted compounds, often through the process of hydrolysis, which leave the incorporated medications behind. The major advantages of natural polymers are good cytocompatibility, biodegradable and do not require any surgery for removal of polymers[30].

Biological dressings like fibrin glue, gelatin sheets, chitosan films and collagen are popular for acceleration of wound healing. These polymers when used in combination such as fibrin-gelatin, fibrin-chitosan, show better results, than when used alone[1].

The main objective of this work was to prepare a composite containing both wound healing materials and water-absorbing materials. In this composite, chitosan and marigold act as wound healing materials and the aloe vera moieties act as hydrogels. However, these biocomposites have been proposed to be used as temporary biological dressings on open wound surfaces and have to be changed periodically.

Chitosan

Chitosan is obtained from the deacetylation of, chitin a naturally occurring and abundantly available (in marine crustaceans) biocompatible polysaccharide. However, applications of chitin are limited compared to chitosan because chitin is structurally similar to cellulose but chemically inert. Acetamide group of chitin can be converted into amino group to give chitosan, which is carried out by treating chitin with concentrated alkali solution. Chitosan is a linear copolymer of d-glucosamine and N-acetyl d-glucosamine in a β-(1-4) linkage, in which glucosamine is the predominant repeating unit. Chitin and chitosan represent long chain polymers having molecular mass up to several million Daltons. It is easily processed into powders,

paste, films, 3D-scaffolds, nanofibers, and nanoparticles[5].

Chitosan is nontoxic, biocompatible and biodegradable. It is used in drug delivery, cell delivery systems, and orthopedics, wound healing, ophthalmology, pharmaceuticals and bone healing[46]. It enhances the function of polymorphonuclear cells, macrophages and fibroblastic proliferation of migration. It exhibits antimicrobial activity against bacteria, fungi, and yeast. It is hypoallergenic, has rapid blood clotting property, haemostatic and acts as fat attractor by binding to dietary lipids[1]. Chitosan is known for its haemostatic properties, also possesses other biological activities and effects macro phage function that helps in faster wound healing. The biological properties include bacteriostatic and fungistatic properties which are useful for open wound treatment. Chitosan which contains wound dressings has been found to generate optimal healing environment for the wound underneath[33].

Chitosan have been found to possess beneficial biological properties including hemostasis[6], antimicrobial activity stimulation of healing[7], tissue-engineering scaffolds[48], cell culture[8] and drug delivery[9].

Chitosan is a novel polymer which has a wide application in drug delivery systems, hydrogels and wound dressing. It is mostly obtained from chitin, the polymer second most abundant in nature next to cellulose. It stimulates cell proliferation and histoarchitectural tissue organization. Chitosan helps in wound healing mainly due to its biocompatibility, biodegradability, antimicrobial nature and also its bacteriostatic and fungistatic properties. The polycationic nature of chitosan and interaction between the positively charged chitosan and the negatively charged cell wall of the microorganism leads to the leakage of intracellular components and thus the death of the organism. Chitosan known for its biodegradability as it can be metabolized by certain human enzymes like lysozyme. Various biomedical applications like implantations, injections, or tropical ocular applications is due to its biocompatibility[10,34,35]. It was revealed that for chitosan film was employed as wound dressing, as it should be durable, stress resistant, flexible, pliable, and elastic, also easy to apply and remove without incurring any trauma during dressing changes[31]. The mechanical properties of the films are critical and should possess reasonable tensile properties, which could bear the stresses exerted by different parts of the body having varying contours. Moreover, the dressing must be rapidly and uniformly adherent and conform to wound bed topography and contour to prevent air or fluid pocket formation. Good adherence reduces pain, facilitates decontamination, prevents peripheral channeling into the wound by bacteria, and promotes bonding to tissues[32]. The dressing must be an absolute barrier to bacterial ingress and could prevent egress of wound organisms to the surface of dressing. Furthermore, the dressing is preferably permeable to water vapor to the extent that a moist exudate under the dressing is maintained without pooling, but excess fluid absorption and evaporation leading to desiccation of the wound bed is prevented. In addition, the dressing must be compatible with body tissues, be nontoxic, non-antigenic and non-allergenic[11]. Chitosan (3% w/v) in acetic acid solutions was prepared by stirring overnight using magnetic stirrer. The resulting solution was filtered through a sintered glass filter to remove the extraneous matter, followed by casting on glass plate and dried in an oven at 60°C for 24 hours. After drying, the transparent film was carefully peeled off from the glass plate. The films were stored in an air tight glass container maintained at room temperature of 25±1°C and relative

humidity of 60-65% until further investigations. The tensile testing provides an indication of the strength and elasticity of the film, which can be reflected by tensile strength and elongation at break. It is suggested that films suitable for wound dressing should preferably strong but flexible[12]. Chitosan based hydrogels exhibit good biocompatibility, low degradation and processing ease. The ability of these hydrogels to swell and dehydrate depend on composition and environment which has been exploited to facilitate a range of applications such as drug release, its biodegradability and ability to form hydrogels[12.36,37]. It was reported that powdered chitin, chitosan or whole crab shells were not effective in any of the tests, but solution of chitosan in acetic acid inhibited the bacterial strains and the fungus[13,38,39].

Aloe Vera

Aloe Vera is a plant whose extracts have been used in herbal medicine and in alternative medicine for their rejuvenating, soothing and healing properties[41]. The aloe plant is the source of two herbal preparations: aloe gel and aloe latex. Aloe gel is often called Aloe Vera and refers to the clear gel or mucilaginous substance produced by parenchymal cells located in the central region of the leaf. Diluted aloe gel is commonly referred to as Aloe Vera extract. The gel is composed mainly of water (99%) and mono- and polysaccharides (25% of the dry weight of the gel)[10]. The properties of aloe vera in accelerating wound healing in burn victims have been studied extensively[43]. Aloe Vera promoted complete healing of burn wounds. Several studies and clinical trial have assessed the effectiveness of aloe vera in the treatment of skin burns[4]. It was found that aloe vera was found to exhibit anti-inflammatory effects, and the histological examination showed early epithelialization and also showed complete wound healing and left a protective layer on skin after drying and providing some protection to the wound[29]. In a study, with good replication, of healing after a precise skin hole punch demonstrated the anti inflammatory properties of the aloe gel as a dressing material, which lead to rapid healing. In a detailed study with aloe vera as a wound dressing along with polyethylene gel showed to accelerate wound healing and re-epithelialization, following full-face dermal abrasions[10].

Both topical and oral aloe vera gel has been shown to significantly stimulate collagen synthesis in dermal wounds. Aloe Vera contains 75 essential nutrients necessary for wound healing. It was revealed that it possess the moisturizing properties and enhances clot formation and useful in maintaining the wound temperature and increasing blood flow to the skin. It also promoted oxygenation, proved anti-inflammatory properties as well as immune-modulating and antimicrobial activity. It has also been shown to increase cell proliferation[14]. The preparation of aloe vera gel is very easy, the leaves were rinsed thoroughly in distilled water. The mucilaginous leaf gel was scraped out from the leaf and the gel was thoroughly homogenized and then filtered[15]. *In vitro* bacteriological examinations were carried out testing various percentages of aloe vera solutions against tissue cultures of four common pathogens – *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa* and *Eschericha coli*. It was found that all these microorganisms were killed within twenty four hours of exposure to high levels of aloe vera (85%)[16]. The mupirocin loaded polymeric composite containing aloe vera, showed more than 98% of reduction in wound area after 12th day. It was studied that mupirocin incorporated chitosan films

with aloe vera may be excellent new dressing for wound occlusion/tissue repairing and could be used for effective treatment of all types of wound[17,40]. The evidence suggests that the primary sites of action for aloe vera are epithelial tissues and their action on surfaces and membranes, rather than solid organs, may account for some of the healing properties[18-20]. In the case of the immune system, aloe vera exerts an effect on the cytokine system, resulting in immune modulation[21-23]. It was revealed that the mannose-6-phosphate, a major constituent of aloe vera gel, either directly or indirectly stimulates fibroblast activation, an important factor in the wound healing process or plays a significant role in the biological activity of aloe vera[18]. It was revealed that a triple antibiotic ointment (containing polymixin B sulfate, bacitracin and neomycin sulfate) and an aloe vera extract gel were evaluated for their effects on open wound healing of wounds created under anesthesia in 15 Beagle dogs. It was found that the primary difference between the two medications was noticed at 7 days when the aloe-treated wounds had a smaller unhealed area than did untreated control wounds and wounds treated with antibiotics[24].

Calendula officinalis

Calendula officinalis, or pot marigold, is a common garden plant belonging to the Composite family. Native to Southern Europe, *Calendula* grows up to 60 cm in height and produces large yellow or orange flowers. The flowers are the part of the herb used medicinally, either in the form of infusions, tinctures, liquid extracts, creams or ointments, or in one of a number of skin and hair products available over-the-counter across the globe. Calendula has been considered beneficial in reducing inflammation and promoting wound healing. It has been used to treat a variety of skin diseases and has been seen effective in treatment of skin ulcerations, eczema, juvenile acne and dry phthiriasis[25]. For centuries, *Calendula* flowers have been used to treat a number of clinical conditions, specifically, the treatment of dermatological disorders. Whilst the many chemical constituents within *Calendula* and the numerous actions of the plant suggest that *Calendula* may be effective in treating a myriad of complaints, there is currently insufficient clinical evidence to support the use of pot marigold in conditions other than cutaneous lesions. Calendula is one of several herbs used traditionally to treat conjunctivitis and other eye inflammations as it helps to reduce the swelling and redness of eye infections. It was also believed that calendula may have some anti-spasmodic action, and as such it has been used to relieve menstrual cramps[26]. In a study, the effects of oral and topical application of *Calendula officinalis* flower extract on excision wounds made in rats were checked. The parameters assessed were the days needed for re-epithelization and percentage of wound closure. The hydroxyproline and hexosamine content in the granuloma tissue of the wound was also measured and the data indicated to have potent wound healing activity of *C. officinalis* extract[27]. The results of an antimicrobial studies of the plant extracts of *C. officinalis* showed that it possesses antimicrobial activity against commonly encountered microorganisms in humans. It was also shown that the action is non toxic[27]. The experimental study revealed that *C. officinalis* presented anti-inflammatory and antibacterial activities as well as angiogenic and fibroplastic properties which acted in a positive way on the inflammatory and proliferative phases of the healing process (Paulo N. M. *et al*, 2012). *C. officinalis* gel can effect collagen deposition and wound healing, but not all of its concentrations because it has both a dual

and opposite effect in different concentrations and the low concentrations have no effects and high concentrations have cytotoxic effects[28].

MATERIALS AND METHODS

Powdered Chitosan of low molecular weight (~150 kDa) with a degree of deacetylation above 85% and PEO of high molecular weight (~ 900 kDa) were purchased from Aldrich Chemical Co. India. Aloe Vera plant and *C. officinalis* were purchased from a green house. All other reagents used were of analytical grade.

Preparation of 3% Chitosan solution

A 3% (w/v) Chitosan solution was prepared by dispersing 1.5g of powdered chitosan in 50ml of 0.3N acetic acid solution. It was stirred using magnetic stirrer for 1 hour and then it was sonicated for 10 minutes in the Sonicator.

Preparation of Aloe vera extract

An aloe vera leaf was obtained and surface sterilized with ethanol. The outer, green dermis of the leaf was peeled off using a sterile blade. The inner flesh (gel) was cut into small pieces, weighed and 25g of this was taken. 50ml of distilled water was added to it. This mixture was then boiled for 10 minutes. After cooling to room temperature, it was filtered using Whatmann Filter Paper No. 1 and immediately used.

Preparation of Marigold petal extract

Marigold flowers were obtained and washed under tap water. They were then rinsed with distilled water and dried in a hot air oven for 1 hour to remove the moisture. One liter of deionized water was boiled and 15g of dried flower petals were added and then it was left to stand for 15 minutes. The extract was sterilized by filtering through Whatman Filter Paper No. 1, and at the same time insoluble materials were removed.

Microorganisms

For the evaluation of antimicrobial activity, the gram positive bacteria *Bacillus subtilis* was obtained from SRM University, Chennai. It was maintained in nutrient agar media.

Preparation of films

Solvent casting technique

3 ml of the 3% chitosan solution was taken in 6 Nunc Cell Petri dishes (~3cm diameter) and the aloe vera extract was incorporated to a percentage of 5%, 10%, 15%, 20%, 25% and 30% (v/v) respectively. The petri dishes were kept in a 37°C sterile incubator for 48 hours for drying and film formation. 3 ml of the chitosan solution was taken in 6 Nunc Cell Petri dishes (~3cm diameter) and the marigold petal extract was incorporated to a percentage of 5%, 10%, 15%, 20%, 25% and 30% (v/v) respectively. The Petri dishes were kept in a 37°C sterile incubator for 48 hours for drying and film formation. 3 ml of the 3% chitosan solution was taken in a Nunc Cell Petri dish and was kept in a 37°C sterile incubator for 48 hours for drying and film formation. 3 ml each of the aloe vera extract and the marigold petal extract were separately taken in a Nunc Cell Petri dish and were kept in a 37°C sterile incubator for 48 hours for drying and film formation. The films were then carefully removed with a blade and placed in sterile, labelled bags and stored in a deep freezer for future use.

Characterizations of films

Water absorption capacity

It is of utmost importance, if they are used for biological applications and wound healing. It is used to measure the capacity of the films to absorb wound exudates. The water absorption capacity of the Chitosan-Aloe vera films and the Chitosan-Marigold

films was measured by swelling the films in distilled water at room temperature. The dimensions of the films were taken as 1cm x 1cm to maintain equal surface area. They were pre-weighed and then immersed in 15ml. distilled water. The films were withdrawn from the water every hour upto 6 hours. The wet weight of the films were determined after first blotting with a filter paper followed by blowing with a stream of air to remove surface water and immediately weighing the films. Every time after noting the weight, the film was placed in fresh water.

- The swelling ratio was calculated using the equation:

$$(\%)\ E_{sr} = ((W_s - W_d) / W_d) \times 100$$

Where, E_{sr} is the water absorption (%wt) of the films,

W_d and W_s are the weights of the samples in the dry and swollen states respectively.

- The equilibrium water content (EWC) was calculated from the following equation:

$$\%EWC = ((W_e - W_d) / W_e) \times 100$$

Where, W_e represents the weights of the swollen states at equilibrium

Tensile Strength

Tensile strength measures the ability of the films to withstand rupture, mechanical pressures or the force required to break the film. Tensile strength of the films was determined by using the Instron tensile testing machine at SDDC section in CLRI Chennai, India. It was expressed in MPa units.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy was used to identify the chemical structure of the composite films and the possible interactions between their components. To carry out the FTIR analysis, the films were sent to the Sophisticated Analytical Instrumentation Facility (SAIF), Indian Institute of Technology - Madras (IIT-Madras), Chennai.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy is carried out to obtain the microstructure of the composite films at the nano scale level. The surface morphology of the composite films can be viewed using SEM. Based on the results of above characteristics; some of the films were selected among the prepared composite films and sent for SEM analysis to Anna University, Guindy, Chennai.

Evaluation of antimicrobial activity

The measurement of the antimicrobial activity of individual chitosan films, the two plant extracts and the selected films was done by disc diffusion method using the gram positive bacterium *B. subtilis*. The zone of inhibition was determined by placing a definite size of the film into discs made in inoculated solidified nutrient agar medium in a petriplate. Petriplates were incubated for 24 hours at 37°C. This was done in triplicate with each film and an average diameter of zone of inhibition was noted.

Statistical Analysis.

All measurements were done in triplicate with individually prepared films as the replicated experimental units. Significant differences between two groups were determined using least significant difference (LSD) test in the SAS program (SAS, 2000).

RESULTS AND DISCUSSION

Film formation

It was revealed that the aloe vera plant extract and the marigold petal extract do not form films. All the composites that involved chitosan were able to form film. It was proved that chitosan had film forming characteristics.

The water absorption capacity of chitosan-aloe vera films and chitosan-*calendula* films decreased with increasing concentration of the plant extract. The overall water absorption capacity of the aloe vera containing films was better than that of the *C. officinalis* containing films. This may be due to the gelatinous nature of the polysaccharides in aloe vera. The EWC (Equilibrium Water Content) of the films containing aloe vera extract in chitosan reduced gradually as the proportion of aloe vera extract in the films increased. A similar result was observed for the films containing *C. officinalis* petal extract. This may be due to the negligible film-forming capability of the plant extracts and the fact that they were water extracts, which may have influenced the porous structure of the chitosan film. All the films reached their respective maximum water absorption within the second hour of introduction into the aqueous environment.

Mechanical properties

The tensile strength of the pure chitosan film was much higher than that of the composite films. This may be due to the influence of the aqueous plant extract on the cross-linking and mechanical integrity of the chitosan structure. For the Aloe Vera containing films, the tensile strength increased gradually upto a maximum tensile strength of 21.41 MPa, which was exhibited by the film containing 25% of Aloe Vera extract in Chitosan and then dropped again. A similar trend was seen for the films containing *C. officinalis* petal extract. The maximum tensile strength (28.05 MPa) was displayed by the film containing 15% *C. officinalis* extract in Chitosan. On the whole, *C. officinalis* extract containing films displayed improved mechanical properties over the films containing Aloe Vera extract.

Fourier Transform Infrared analysis

The infrared spectrum of pure Chitosan as shown in Figure 4 was in the wavelength range 622.03 cm^{-1} to 3917.02 cm^{-1}. There are four main functional groups that are characteristic to Chitosan, as shown in Table 2.

The presence of broad bands at 3249 cm^{-1} is attributed to intramolecular hydrogen bonding. The presence of strong to medium intensity band was also observed at 1638.02 cm^{-1} which confirms the presence of an amino group.

When this spectrum is compared to that of the film containing 25% of Aloe Vera in Chitosan (Figure 5), it is observed that the peaks corresponding to amino group and hydroxyl group have become broader and deeper. This indicates a possibility that the extract has interacted with the free amino and the hydroxyl group of Chitosan. Also, a peak characteristic to Aloe Vera was observed at 1538.88 cm^{-1}, which indicates the presence of aromatic nitro compounds.

When the spectrum of pure Chitosan is compared with that of 15% *C. officinalis* in Chitosan, as in Figure 6 a similar change is observed. The peaks corresponding to the amino group and hydroxyl group have become deeper and broader and a peak shift is observed. The amino peak shifts from 1638 cm^{-1} to 1640 cm^{-1}. Also, the hydroxyl peak shifts from 3249 cm^{-1} to 3209 cm^{-1}.

The surface morphology of pure Chitosan (Figure 7) was studied using SEM. The surface structure of pure chitosan is smooth and uneven. From the micrograph under magnification, the surface of chitosan films shows randomly distributed microstructures. This structure suggested that chitosan film particles might have failed to crystallize. (D. Sruthi *et al.*). The surface morphology of chitosan – aloe vera films drew two observations. One was the randomly distributed microstructures, and another was a certain kind of nanotubes

(Figure 8). The surface morphology of Chitosan – *C. officinalis* showed randomly distributed microstructures as shown in the Figure 9.

CONCLUSION

Based on tensile strength, the film containing 15% *C. officinalis* petal extract in Chitosan has the most promising mechanical property. The film containing 25% aloe vera extract in Chitosan also has a high tensile strength, but it is not as high. The EWC of the 15% *C. officinalis* film is higher than that of the 25% aloe vera film. However, both show good swelling characteristics by reaching their maximum water absorption capacity within the second hour of introduction into the aqueous environment. Based on the FTIR analysis, it was revealed that the aloe vera extract interacts with the amino group and the hydroxyl group of Chitosan. Similarly, the Marigold petal extract also interacts with the amino group and the hydroxyl group of Chitosan. Chitosan-Aloe Vera films show a slight advantage over Chitosan-Marigold films in terms of bacteriostatic activity. However, this might be due to the low activity of Chitosan-Marigold against *B. subtilis* (gram positive) specifically. In conclusion, a Chitosan film containing 15% of *C. officinalis* extract and a similar film containing 25% of aloe vera extract have extremely good properties for use as a wound healing composite. The composite films are given to the Veterinary College for testing on the wounds of dogs. Further studies may be done into creating a composite containing the aforementioned percentages of both extracts and analyzing its efficacy as a wound dressing for diabetic foot sores.

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Table 1. Tensile strength parameters of prepared films

Sample ID	COMPOSITION	Maximum Load (N)	Tensile Strength (MPa)	Elongation at Break(%)
C1	1% chitosan	30.48	33.87	39.83
CA 5	1%Chitosan +5% Aloevera	11.89	16.98	14.61
CA 10	1%Chitosan +10% Aloevera	10.49	13.11	5.83
CA 15	1%Chitosan +15% Aloevera	8.57	12.25	11.61
CA 20	1%Chitosan +20% Aloevera	10.81	18.01	14.17
CA 25	1%Chitosan +25% Aloevera	12.85	21.41	25.56
CA 30	1%Chitosan +30% Aloevera	8.81	17.62	11.22
CC 5	1%Chitosan +5% *calendula*	14.81	18.55	9.72
CC 10	1%Chitosan +10% *calendula*	19.46	20.27	18.56
CC 15	1%Chitosan +15% *calendula*	22.44	28.05	45.39
CC 20	1%Chitosan +20% *calendula*	15.41	22.01	23.78
CC 25	1%Chitosan +25% *calendula*	12.6	18	16.72
CC 30	1%Chitosan +30% *calendula*	9.05	15.09	16.67

Table 2. Functional compounds of chitosan analyzed using FTIR

S. No.	Wavenumber (cm^{-1})	Functional compound
1.	1019.90	Cyclohexanes (C-C)
2.	1638.02	Amines (NH_2)
3.	2920.5	Alkyl (CH_2)
4.	3249.52	OH Intramolecular Hydrogen Bonding

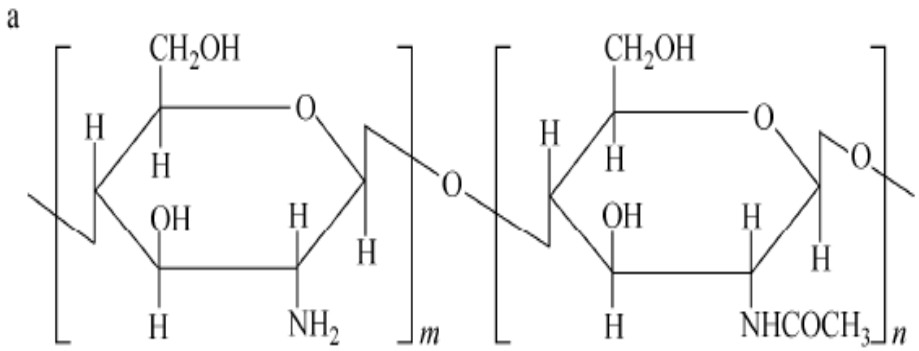

Figure 1. Chemical Structure of Chitosan

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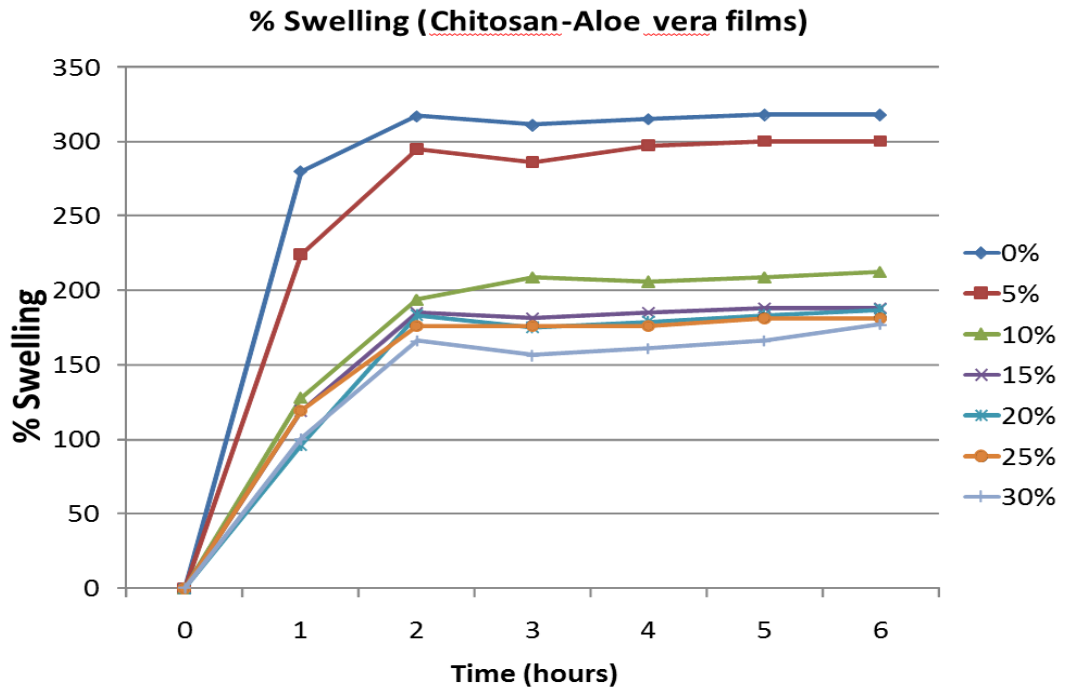

Figure 2. Water absorption capacity of chitosan-aloe vera flims

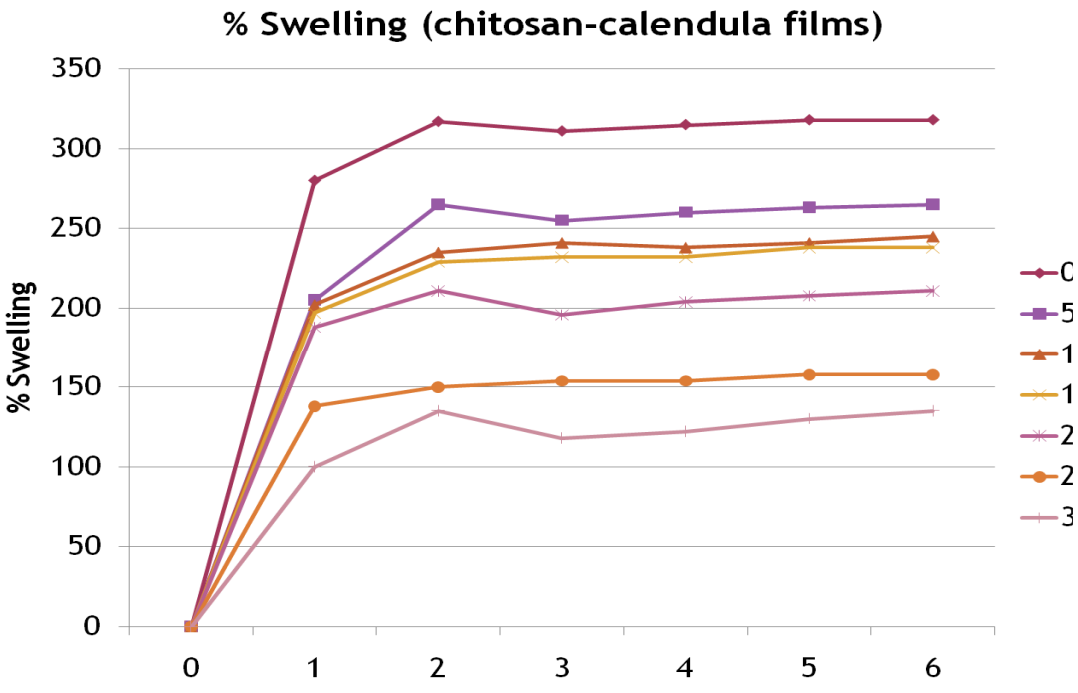

Figure 3. Water absorption capacity of chitosan *calendula* films

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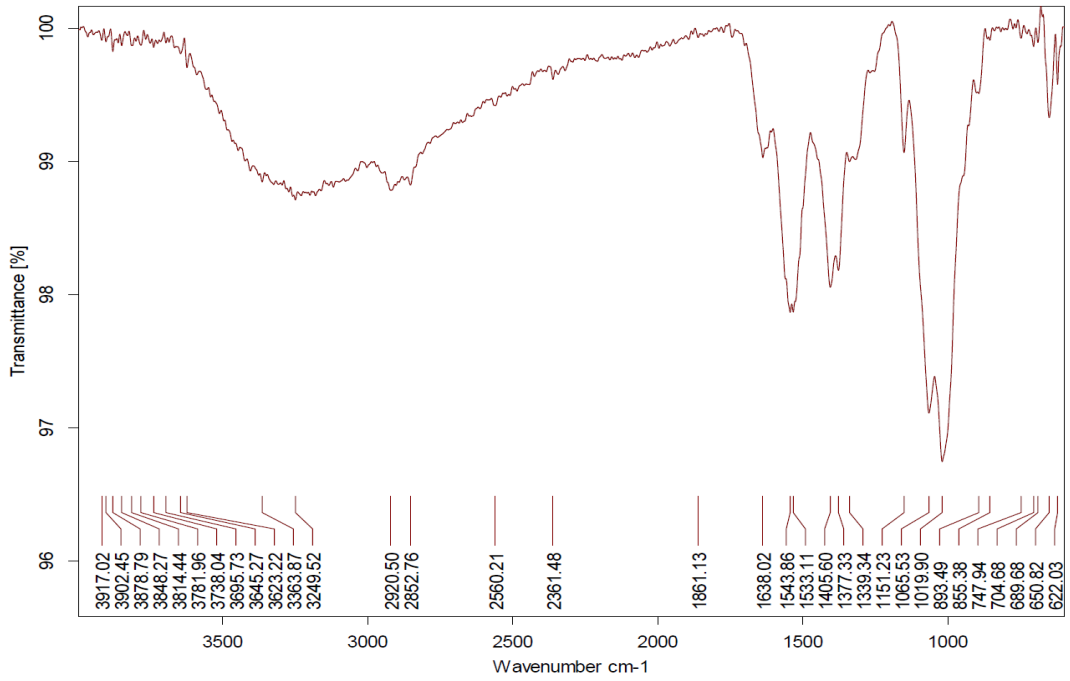

Figure 4. Fourier Transform Infrared spectrum of Chitosan film

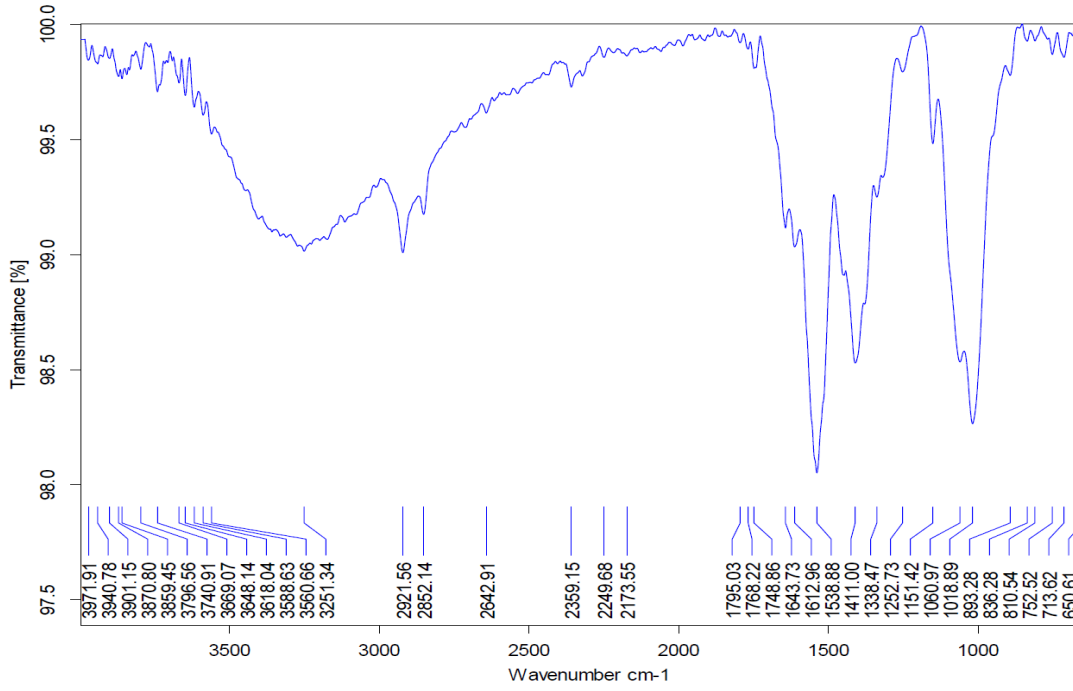

Figure 5. Fourier Transform Infrared spectrum of Aloe Vera in Chitosan film

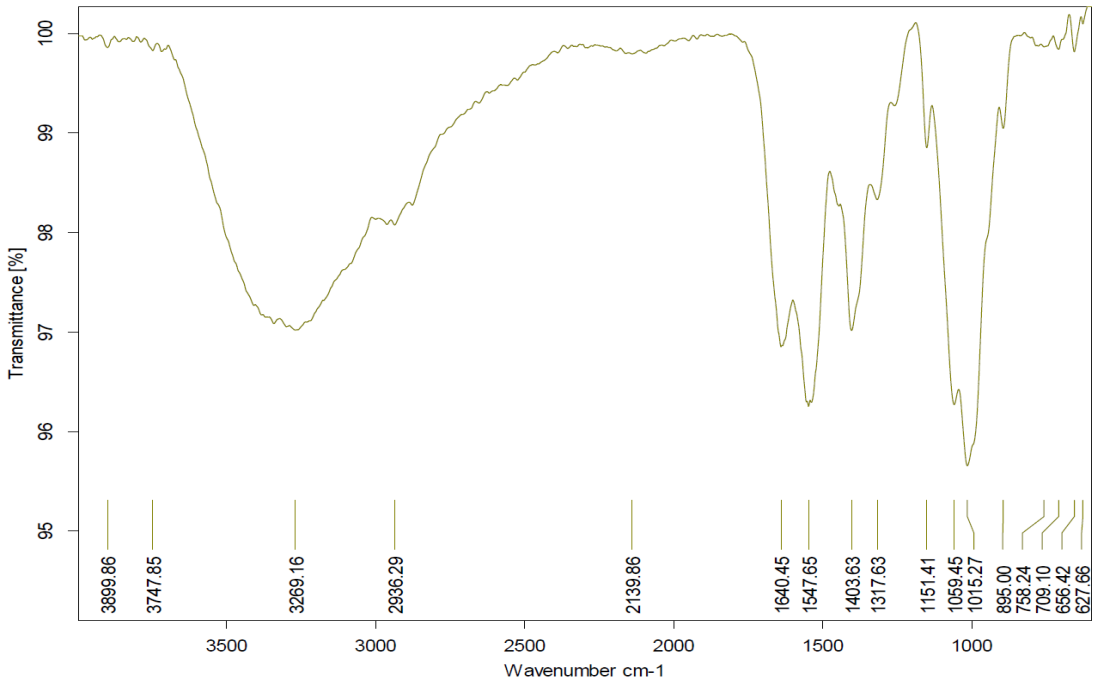

Figure 6. Fourier Transform Infrared spectrum of *C. officinalis* in Chitosan film

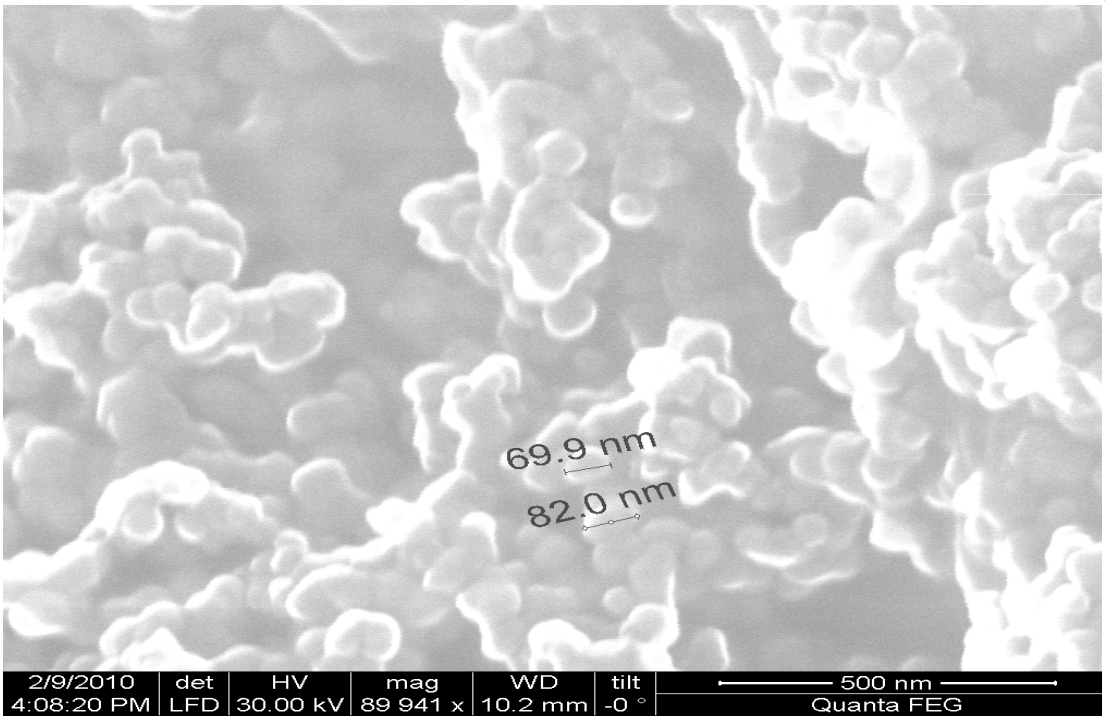

Figure 7. SEM image of chitosan

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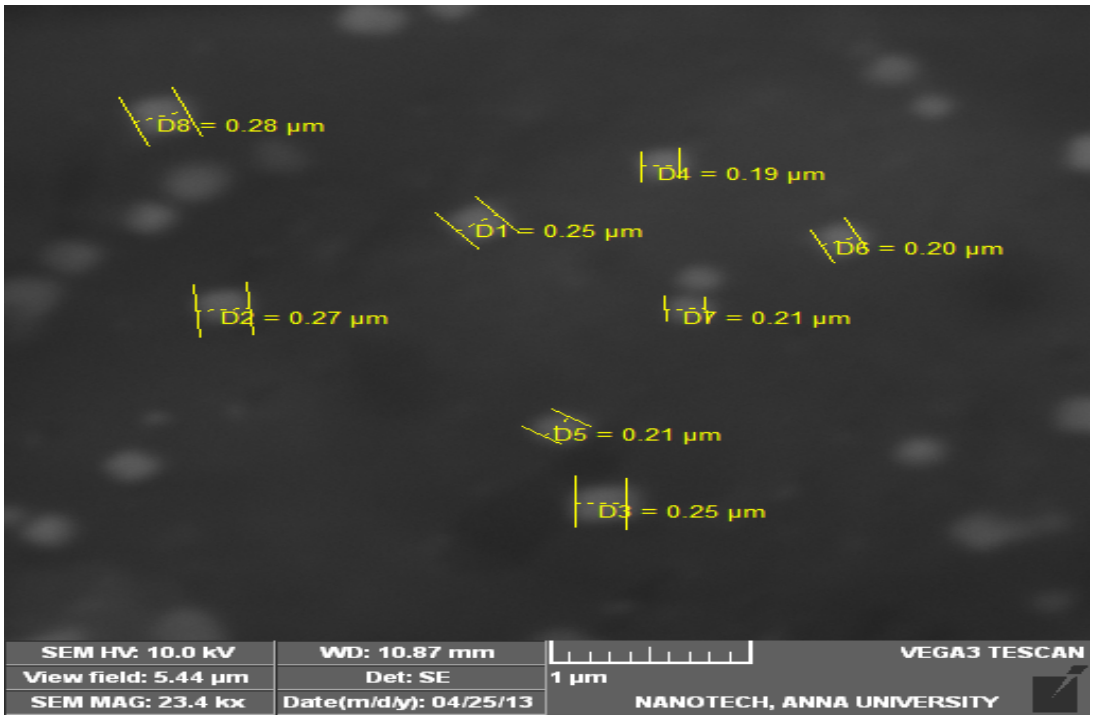

Figure 8. SEM results of Aloe Vera - Chitosan film

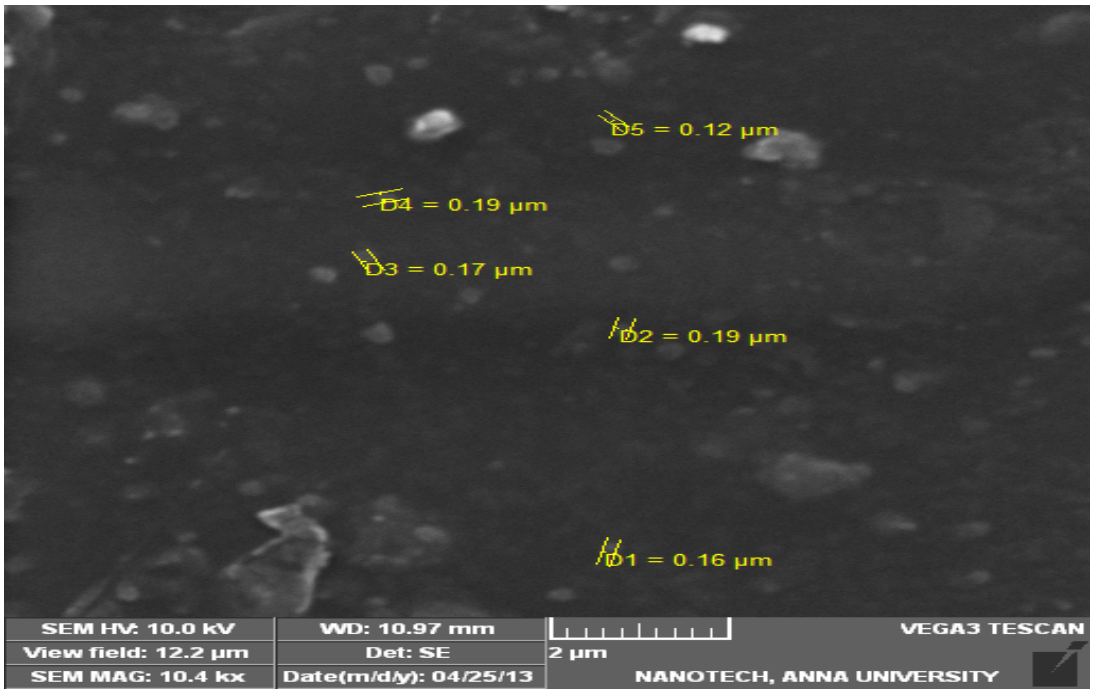

Figure 9. SEM results of *C. officinalis* - Chitosan film