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WOUND DRESSING PRODUCTION WITH ESSENTIAL OILS AND INVESTIGATION OF ANTIMICROBIAL PROPERTIES

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LOCATION ANALYSIS OF THE EMERGENCY SERVICE CENTERS OF A CASE COMPANY

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LIST OF SYMBOLS

C	Celsius
cm	Centimeter
g	Gram
h	Hour
ha	Hectare
kg	Kilogram
l	Liter
min	Minute
ml	Milliliter
mm	Millimeter
nm	Nanometer
v/v	Volume per volume
w/w	Weight per weight
λ	Wavelength
°	Grad
µl	Microliter
Å	Angstrom
rpm	Revolution per minute

LIST OF ABBREVIATIONS

App	Appendix
CFU	Colony Forming Unit
DSC	Differential Scanning Calorimetry
DTA-TG	Differential Thermal Analysis and Thermogravimetric Analysis
DTG	Derivative Thermogravimetry
E. Coli	Escherichia Coli
EDX	Energy Dispersive X-Ray
EO	Essential Oil
FT-IR	Fourier Transform Infrared Spectroscopy
FWHM	Full Width Half Maximum
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer
ISSC-MAP	International Standard of the Sustainable Nature Collection of Medicinal and Aromatic Plants
PBS	Phosphate Buffer Solution
PVA	Poly Vinyl Alcohol
PVP	Poly Vinyl Pyrrolidone
S. Aureus	Staphylococcus Aureus
SEM	Scanning Electron Microscopy
SR	Swelling Ratio
TEM	Transmission Electron Microscopy
TSI	Turkey Statistical Institute
UV-Vis	UV-Visible Spectrometer
W_i	Initial Weight
W_f	Final Weight
XRD	X-Ray Diffraction

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ABSTRACT

WOUND DRESSING PRODUCTION WITH ESSENTIAL OILS AND INVESTIGATION OF ANTIMICROBIAL PROPERTIES

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MSc. Thesis

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The medicinal wound dresses are more practical in our modern world instead of old fashion medicinal wound dresses such as synthetic or natural bandages or cotton gauges. Today's medical researches increases rapidly, as a result of that medicinal wound dresses are usual textiles or products from the other chemical materials and biomaterials such as sponges, films, gels, hydrocolloids, and pastes. Medicinal wound dressing is an important production of biomedical materials; because the ideal medicinal wound dressing should excess exudates, prevent infection, provide moist environment, excess exudates, allow water vapor and oxygen exchange, comfortable, be thermally insulating, removable without causing trauma, be non-allergenic and non-toxic and be cost effective.

The sick-abed or diabetic people have hard healed wounds and the wound healing is complex process and it contains the interactions between cells, growth factors and extracellular matrix components. The inflammation is delaying cause in the healing process, for that reason, researches of antibacterial medicinal wound dresses takes important place in medical developments.

Antimicrobial activities of the essential oils have been known from Avicenna. Essential oils are aromatic, hydrophobic, volatile liquids obtained from plant seeds, fruits, flowers, leaves, and roots by expression, distillation, or solvent extraction. Essential oils show antifungal, antibacterial, antioxidant, insecticidal and antiviral properties depend on their biological active compounds. Antimicrobial activities of medicinal wound dresses can be increased by using essential oils as an assistant production component of wound dresses. Gentian species are known in medicinal world. Three types of gentian plants such as Bluebottle, Common Centaury and St John's Wort is used in the experiments.

Chitosan is found in the form of gels, composites, films, nanofibers, scaffolds and non-wovens. Chitosan has been used as a helper in regeneration of wounded tissues due to their significant physicochemical and biological properties such as biocompatibility, biodegradability, antibacterial, non-toxicity and hydrating specialties. For that reasons; chitosan is chosen as a raw material in this study. Gram negative bacteria Escherichia coli (E. Coli) and Gram positive bacteria Staphylococcus aureus (S. Aureus) is used in antibacterial tests.

According to this study, production of chitosan based wound dresses by essential oils and their antimicrobial activities, water swelling tests, thicknesses and thermal analysis with Differential Thermal Analysis and Thermogravimetric Analysis (DTA-TG) were examined. Also analysis of X-Ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) were examined on the films. The essential oils which are obtained from gentian species were examined with Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and UV-Visible Spectrometer (UV-Vis).

As a result of this study, Blubottle essential oil addition with (0.5% w/w) gave the best antibacterial effect for both E. Coli and S. Aureus, the increasing ratio of Common Centaury essential oil in chitosan films increased the antibacterial effect too.St John's Wort essential oil addition results closely to Bluebottle. Swelling ratio decreases with essential oil addition. Other characterizations found suitable with literature.

Key words: Wound dressing, antibacterial, chitosan, essential oils, St John's Wort, Bluebottle, Common Centaury

ÖZET

ESANSİYEL YAĞLAR İLE YARA ÖRTÜSÜ ÜRETİMİ VE ANTİ BAKTERİYEL ÖZELLİKLERİNİN İNCELENMESİ

Gizem BALKAN

Kimya Mühendisliği Anabilim Dalı

Yüksek Lisans Tezi

Tez Danışmanı: Doç. Dr. Emek MÖRÖYDOR DERUN

Günümüzde kullanımı yaygın olan modern yara örtüleri; eski tarz yara bantları, gazlı bezler ve sentetik bandajlar ya da pamuklu örtülere göre daha verimlidirler. Yara örtüleri üzerine yapılan yeni araştırmalarda yara örtüleri formları genellikle köpükler, filmler ve hidrokolloid jellerdir. Yara örtüleri biyomedikal filmlerin arasında büyük önem taşıyan bir üründür çünkü bu filmler yarayı enfeksiyondan korumalı, kanın dışarı sızmasını önlemeli, ortamın nemini korumalı, gaz geçişine izin vermeli, ciltten kolay ve yara oluşumuna izin vermeden çıkarılabilmeli, toksik ve alerjik olmamalı ve ucuz olmalıdır..

Yatalak hastalar veya diyabetik insanların, zor iyileşen yaralarının iyileşme süreçleri karmaşıktır. Yaranın iyileşme süreci, hücre büyümesine, hücre dışı matriks bileşenlerine ve bunlar arasındaki etkileşimlere bağlıdır. Açık yaraların enfeksiyon kapması yara iyileşme sürecini olumsuz etkileyeceği için anti bakteriyel örtüler tıbbi gelişmelerde araştırılan en önemli çalışmalardandır.

İbn-i Sina'dan günümüze kadar bitkilerden elde edilen esansiyel yağların yara iyileşme sürecini hızlandırmak için kullanıldığı bilinmektedir. Esansiyel yağlar distilasyon ya da soxhlet ekstraksiyonu ile bitkilerin çiçeklerinden, tohumlarından, yapraklarından, meyvelerinden ve köklerinden elde edilen hidrofobik aromatik uçucu sıvılardır. Uçucu bitkisel yağlar sahip oldukları biyolojik aktif bileşenler nedeniyle, antibakteriyel, anti-mantar, anti-viral, antioksidan ve böcek öldürücü özelliklere sahip olduğu bilinmektedir. Yara örtülerinin anti bakteriyel özellikleri, üretilecek olan filmlerin içine eklenecek olan bitkisel esansiyel yağlar ile arttırabilir. Bu yüzden yapılacak olan araştırmada farklı türlerdeki kantaron yağı, yara örtüsüne eklenecek olan bitkisel yağlar olarak seçilmiştir.Bu türler, Sarı kantaron, Kırmızı kantaron ve Mavi kantaron'dur.

Kitosan kompozitler genellikle, jeller, nanofiberler, filmler ve doku iskelesi şeklinde kullanılırlar çünkü doğada kolaylıkla bulunurlar, kolay çözülürler, biyouyumludurlar, toksik değildirler, antimikrobiyal ve nemlendirici özellikler gösterirler, bu nedenlerle mükemmel biyolojik ve fiziko-kimyasal özelliklere sahiptirler. Bu yüzden kitosan yaralı dokunun iyileşme sürecinde kullanılabilecek bir malzemedir. Bu yüzden kitosan bu çalışmada hammadde olarak seçilmiştir. Gram negatif bakteri Escherichia coli (E. Coli) ve Gram pozitif bakteri Staphylococcus aureus (S. Aureus) bu çalışma sırasında antibakteriyellik testlerini yapmak için kullanılmıştır.

Bu yüksek lisans projesinde, kitosan bazlı kantaron esansiyel yağları içeren biyofilmlerin üretimi sağlanacaktır. Üretilen filmlere antimikrobiyal aktivite testi, şişme testi ve termal analiz karakterizasyon testleri Termogravimetrik yöntemle (DTA-TG) ile yapılmıştır. Ayrıca üretilen filmlerin, X-Işını kırınımı (XRD), Fourier Kızılötesi Spektroskopisi (FTIR) analizleri filmlere yapılmıştır. Bitkilere İndüktif eşleşmiş plazma optik emisyon spektroskopisi (ICP-OES) ile ve üretilen yağlara da Ultraviyole ve görünür ışık (UV-Vis) absorpsiyon spektroskopi karakterizasyon testleri yapılmıştır.

Sonuç olarak yapılan bu çalışmada Mavi kantaronun kütlece %0.5 yağ oranı ile hazırlanan filmlerin E. Coli ve S. Aureus bakterilerinin ikisine de en iyi antibakteriyelliği gösterdiği bulunmuştur. Ayrıca Kırmızı kantaronun filmlerdeki artan yağ içeriği ile antibakteriyelliğini arttırdığı gözlemlenmiştir. Sarı kantaron antibakteriyel özelliğini Mavi kantaronunkine benzer olarak göstermiştir. Kitosan filmlere yağ eklenmesi filmlerin şişme yüzdelerini artan yağ oranı ile azaltmıştır. Yapılan diğer karakterizasyon analizlerinin ise yapılan literatür çalışmalarıyla uyumlu olduğu gözlemlenmiştir.

Anahtar Kelimeler: Yara örtüsü, antibakteriyel, kitosan, esansiyel yağlar, sarı kantaron, kırmızı kantaron, mavi kantaron

CHAPTER 1

INTRODUCTION

1.1 Literature Review

The damage of normal skin tissue function and structure is called as wound [1]. The healing process of wound is a very important and complex, it contains three main phases, which are sequential, overlapping and distinct such as proliferation, tissue regeneration and inflammation [2]. Every day and year, myriads of people suffered from different types of epidermal damage such as burn, ulcer or other traumatic injuries, which company with considerable amount of cost on treatment [3]. Wound dressing is really important protector and healing material in treatments. The cost of treatments is decreased with taking precautions such as using wound dressing. Sometimes wound dressing would be more expensive but when it is considered in a long time period, it becomes more expensive than treatment procedure.

Wound dressing is external protector which is used for protection from external environment. The environment must sustain moist environment around the tissue damage (wound). It assists the wound healing and shorten the regeneration period [2].

The environment contains bacteria, fungi and viruses, wherefore open wounds are sensible from that inflammatory creatures. Occasionally, the conservation of wound is substantial. Essential oils have been known since Avicenna with their antimicrobial activities.

Essential oils are the volatile oil products of plants and have a significant odor. More or less three thousand essential oils are known in the world, and around 300 of which are commercially substantial [4].

Chitosan is one of the biomaterials which is used in wound dressing applications. It has special characteristics. For example, biodegradable, non-toxic, hemostatic and

biocompatible. Also it is widespreadlly used in drug delivery systems, and tissue engineering scaffolds [1].

1.2 Objective of the Thesis

Wound dressings is a textile cover in medical and surgical practice such as the wounds on the skin and skin trauma. Today, the production of wound dressings performed by many companies; It is used to keep clean the wound during the healing process. Wound dressing selection has considerable importance according to the infection status of the patient and the wound type. In general, cotton gauze is used as a wound covering since it creates good absorbance properties and soft nature. However, the cotton gauze cannot maintenance of moist wound environment that accelerates wound healing and cannot provide the necessarily antibacterial area. Modern wound dressing should be body compatible, antibacterial, provide moisture and gas passage, cheap, antitoxic, not show allergic reactions. This kind of wound dressing should be synthetic, semisynthetic or non-synthetic.

In this study, the desired wound dressing was produced chitosan-based with different gentian plants. Chitosan is known as a biopolymer material, and gentian plants are known with their antibacterial properties. In the researches of gentian plants, they are known as antidepressant, antipyretic, therapeutic stomach wounds, partial relieving skin discoloration of skin, acting antivirals and they are used as wound healing assistant with their anti-bacterial properties. Chitosan was manufactured from chitin. Chitin is found in shells of crustaceans which are many in oceans and it is a biopolymer which is found more than cellulose in the world. Chitosan is nontoxic material which is used in food industry because of its antibacterial properties and eatable film structure.

As a raw material, chitosan and gentian plant's essential oils were used in this study. For that reason, the produced wound dressings would bring a new option to the wound dressing applications and market because of their features which are listed.

Chitosan and gentian plants should prevent an antibacterial area where the wound keeps away the bacteria with creating a barrier which will accelerate the healing process. Also chitosan is a biopolymer, as a result of this it cannot damage the wound and it is nontoxic for human body.

The wound dressing which is produced by studies of this project, shows an antibacterial property by itself and it would create a barrier to bacteria growth to accelerate the time to treat the wound of the body without requiring the use of any drug. Therefore, antibacterial wound dressing manufacture, it is particularly important.

In this thesis, different ratios of essential oils were added to chitosan-based films and the films were produced by ultrasonic bath. The oil content of films was studied as an antibacterial property. For to find optimum wound dressing films, different times of reaction, reactions under different temperatures with different ratios of essential oils were compared. After that the characterization of films were analyzed.

1.3 Hypothesis

Wound dressing materials have been used for many years. Before common Era years, the wounds were wrapped with leaves and plant essences, these are the most basic steps of wound dressing which is actually today's emerging generation. Wound dressing should be biocompatible, shouldn't be mutagenic, toxic, allergic and carcinogenic.

Many materials may come to mind when wound dressing called, firstly cotton gauze or plaster. However, non-sticking a plaster on the wound and leave the wound as an open to air should not provide the wound to heal faster, the healing of wound according to the shape and condition of it, but the aeration of the wound is important. The patients who's in hospitals are open to inflammatory bacteria, wound infection it is a very common condition and is a factor that affects the healing of the wound to keep bacteria away from the wound. In short, wound dressing must speed up the healing of skin damages in our body and closing the top of the wound to protect it from external factors and should have an antibacterial system.

The raw material chitosan is dissolved in acidic medium, under 6.5 pH, it cannot be dissolved in water, it is biomaterial and nontoxic material for human body. Dissolution of chitosan decreases by increasing pH. According to this study different pH values of acidic acid solution were tested as solvent of chitosan and the best solution was choosen after few studies.

This study is aimed to increase the antibacterial property of producing chitosan biopolymer films with addition of gentian plant's essential oils. Under this project, the soxhlet extraction method is chosen to obtain essential oil from gentian plants. Pretreated

gentian plants (granulated) taken to the extractor and enough solvent (ethanol) was added to get essential oil from the plant. As a result of this process, essential oil with ethanol in mixture form was obtained. The addition of essential oils to the chitosan-acetic acid mixture needs ethanol as a solvent, so after soxhlet extraction method there is no need an addition of ethanol to the essential oils because it is taken from the extractor as a mixture of it and directly used in the experiments.

After the transaction is obtained to chitosan- acetic acid and ethanol-essential oil mixtures were mixed under some ratios and they left to react at specific temperatures and time for the reaction to occur in an ultrasonic bath.

After the obtained results of the tests to be conducted, reaction temperature and time were chosen as optimum and other reactions were undergone some standards. And after reaction the mixture of films was taken to petri dishes for drying.

After drying the characterization tests were applied to chitosan-essential oil based films. These are UV-Visible Spectrometer (UV-Vis), Differential Thermal Analysis and Thermogravimetric Analysis (DTA-TG), Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES), Fourier transform infrared spectroscopy (FT-IR) and X-ray Diffraction (XRD) devices. Also antibacterial tests were done with Staphylococcus aureus (S. Aureus) and Escherichia coli (E. Coli) bacteria because these are gram positive and gram negative respectively, and swelling test should be taken in consideration.

After at all, results of all these analyses were evaluated and antibacterial optimum product should be chosen as the best product.

CHAPTER 2

GENERAL INFORMATION

2.1 The Skin and Wound

The skin, the largest single organ in the body. The skin covers the entire body, protecting the deep tissues from being injured, drying out, or being invaded by bacteria, fungi, viruses and other foreign bodies. These organisms are everywhere and are routinely found lying on the skin surface and deep in its grooves and glands; they never, however, penetrate through the skin. Germs cannot pass through the skin unless it is broken by injury; thus, the skin provides a constant protection against outside invaders. So, it protects the body in environment. The skin helps to regulate body temperature by aiding in the elimination of water and various salts. The skin senses heat, cold, touch, pressure, and transmits that information to the brain and spinal cord [5], [6].

The protective functions of the skin are numerous. Over 70 percent of the body is composed of water. The water contains a delicate balance of chemical substances in solution. The skin is watertight and serves to keep this balanced internal solution intact [6].

The energy of the body is derived from metabolism (chemical reactions) that must take place within a very narrow temperature range. If the body temperature is too low, these reactions cannot proceed, metabolism ceases, and the body dies. If the temperature becomes too high, the rate of metabolism increases. Dangerously high temperatures producing too high a metabolic rate can result in permanent tissue damage and death [6].

The major organ for regulation of the body temperature is the skin. Blood vessels in the skin constrict when the body is in a cold environment. In a cold environment, constriction of the blood vessels shunts the blood away from the skin to decrease the amount of heat radiated from the body surface. When the outside environment is hot, the vessels in the

skin dilate, the skin becomes flushed or red, and heat radiates from the body surface. Also, in the hot environment, sweat is secreted to skin surface from the sweat glands. Evaporation of the sweat requires energy. This energy, as body heat, is taken from the body during the evaporation process, which causes the body temperature to fall. Sweating alone will not reduce body temperature; evaporation of the sweat must also occur [6].

The skin (Figure 2.1) consists of two layers: the outer layer (superficial epidermis) and the inner layer (dermis). The epidermis is composed of several layers off cell varies in the thickness in different parts of the body (the palms and the soles of the feet are thickest), and its dead cells are constantly worn off. At the base of epidermis is the germinal layer, which continuously produces new cells that gradually rise to the surface. On the way to the surface these cells die and form the watertight covering. The epidermal cells are held together securely by an oily substance called sebum, which is secreted by sebaceous glands of the dermis. The outermost cells of the epidermis are constantly rubbed away and then replaced by new cells produced by the germinal layer. The deeper cells in the germinal layer also contain pigment granules that (along with the blood vessels lying in the dermis) produce skin color. The epidermis varies in thickness in the different areas of the body. On the soles of feet, the back, and the scalp it is quite thick, but in some areas of the body the epidermis only 2 or 3 cell layers in thickness. The watertight seal provided by the epidermis prevents the invasion of bacteria and other organisms. The deeper part of the skin is the dermis which contains the specialized skin structures and it is separated from the epidermis by the layer of germinal cells. The dermis has a rich supply of blood vessels and nerve endings. Hair grows from the dermis through opening called hair follicles. Sweat glands and oil glands in the dermis empty onto the surface of the epidermis through pores in the skin. Beneath the dermis is the subcutaneous layer (under the skin), which is well supplied with fat cells and blood vessels [5], [6].

Sweat glands (perspiration) occur nearly all parts of the skin. It produces sweat for cooling the body. The sweat discharged onto surface of the skin through small pores, or ducts, that pass through the epidermis onto the skin surface. Sweat contains essentially the same minerals as blood plasma and urine, but it is more dilute. Normally, only traces of the waste products excreted in urine are in sweat. But when sweating is profuse or when the kidneys are diseased, the amounts of such wastes excreted in the sweat could be considerable. Several mineral salts are removed from the body in sweat. Chief among those in quantity is sodium chloride (the same mineral as common table salt) [5], [6].

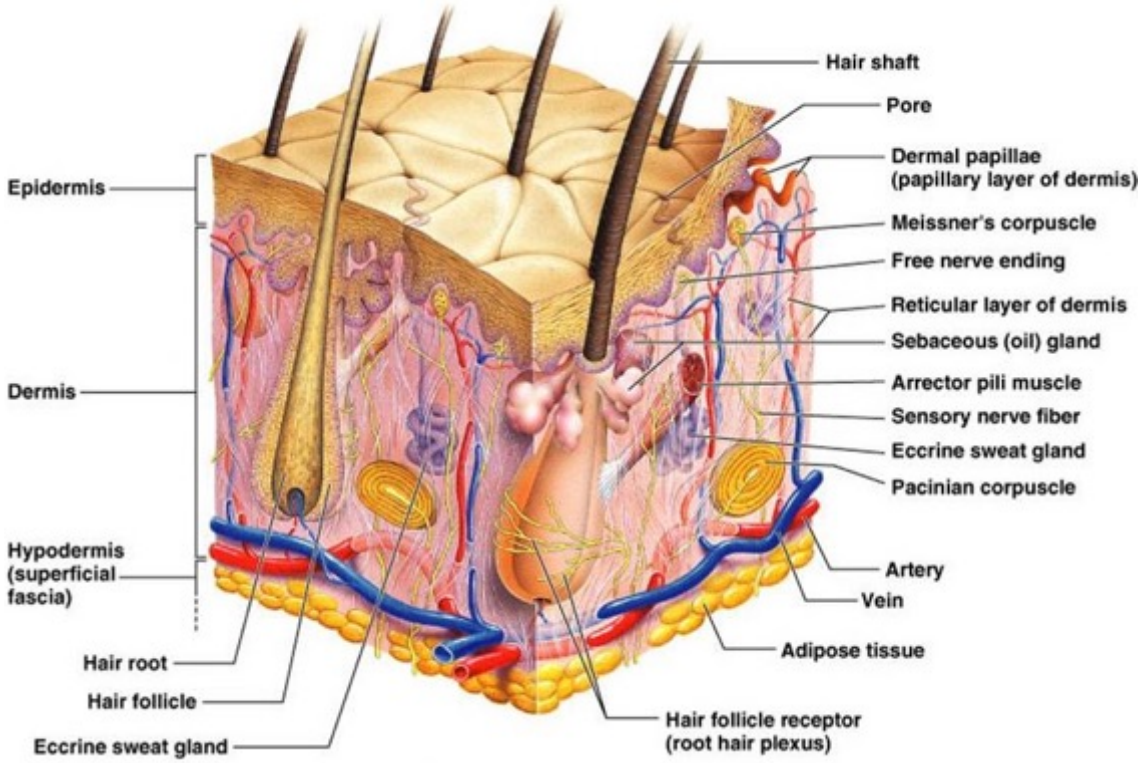


Figure 2.1 The skin layers [7]

The sebaceous glands produce sebum, the oily material that seals the surface epidermal cells. The sebaceous glands lie next to the hair follicle to the skin surface. In addition to providing waterproofing for the skin, sebum keeps the skin supple so that it does not crack. Hair follicles are the small organs that produce hair. There is no follicle for each hair connected with sebaceous gland and also with tiny muscle. The muscle serves to pull the hair into an erect position when the individual is cold or frightened. All hair grows continuously and is either cut off or worn away clothing [6].

Blood vessels provide nutrients and oxygen to the skin. The blood vessels lie in the dermis. Small branches extend up to the germinal layer. There are no blood vessels in the epidermis. A complex array of nerve endings also lies in the dermis. The specialized nerve endings are sensitive to environmental stimuli: they respond to these stimuli and send impulses along the nerves to the brain [6].

Beneath the skin, immediately under the dermis and attached to it, lies subcutaneous tissue. The subcutaneous tissue is largely composed of fat. The fat serves as an insulator for the body and as a reservoir to store energy. The amount of subcutaneous tissue varies greatly from individual to individual. Beneath the subcutaneous tissue lie the muscles and the skeleton. The skin covers all of the external surface of the body. The various orifices

(openings to the body) including the mouth, nose, anus, and vagina are not covered by skin. Orifices are lined with mucous membranes. Mucous membranes (Figure 2.2) are quite similar to skin in that they provide a protective barrier against bacterial invasion. Mucous membranes differ from skin in that they secrete mucus, a watery substance that lubricates the openings. Thus, mucous membranes are moist whereas the skin is dry. A mucous membrane lines the entire gastrointestinal tract from the mouth to the anus [6].



Figure 2.2 The mucous membrane of the mouth [8]

2.2 Wound

The soft tissues are the first line of defense against most injuries, they are often damaged. Soft tissue injuries or wounds are divided into two types: open and closed [6].

2.2.1 Wound Species

An Open Wound is one in which there is a break in the surface of the skin resulting in external bleeding or the mucous membrane. It may allow bacteria to enter the body, causing an infection. However, once the protective skin layer has been violated, the wound becomes contaminated and may become infected. These two problems (bleeding and contamination) must be addressed in the treatment of open soft tissue wounds. There are several types of open wounds. With an abrasion, the top layer of skin is removed, with little or no blood loss. In these kind of injury cause the loss of a portion of the epidermis and the part of the dermis as a result of the skin being rubbed or scraped across a rough or hard surface. Blood may ooze from the injured capillary blood vessels in the dermis, but the abrasion usually does not penetrate completely through the dermis (Figure 2.3). Abrasions tend to be painful because the nerve endings often are abraded along with the skin. Ground-in debris may be present. This type of wound can be serious if it covers a

large area or becomes embedded with foreign matter. Other names for an abrasion are scrape, road rash and rug burn [5], [6].



Figure 2.3 Abrasion [9]

A laceration is cut skin with jagged, irregular edges. The cutting object may leave a smooth or jagged wound through the skin and may penetrate into subcutaneous tissue This type of wound is usually caused by forceful tearing away of skin tissue (Figure 2.4) [5], [6]. Incisions tend to have smooth edges and resemble a surgical or paper cut. The amount of bleeding depends on the depth, the location, and the size of wound (Figure 2.5) [5].



Figure 2.4 Laceration [10]

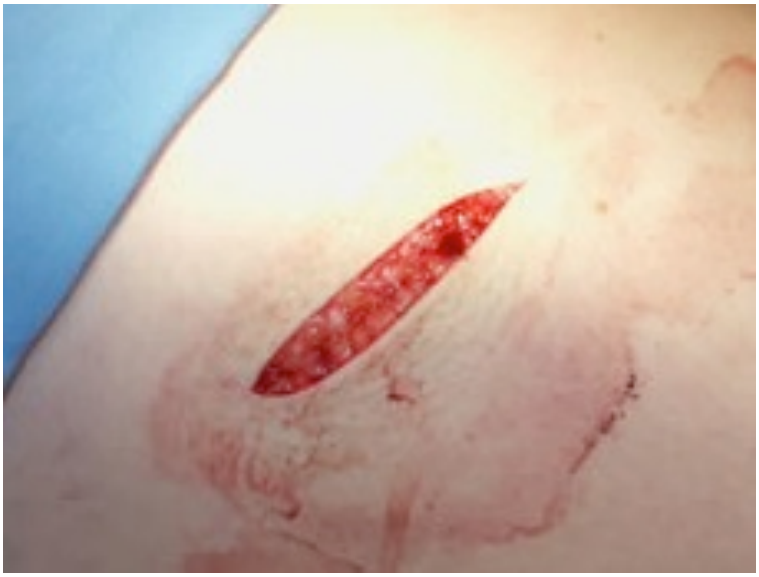


Figure 2.5 Incision [10]

Punctures are usually deep, narrow wounds in the skin and underlying organs such as a stab wound from a nail or a knife (Figure 2.6). The object causing the injury may remain impaled in the wound. The entrance is usually small, and the risk of infection is high. External bleeding from a puncture wound is usually not severe because the entrance is the wound is relatively small. However, the wounding object may injure structures deep within the body and cause rapid, fatal bleeding if the wound occurs in the chest or abdomen [5], [6].



Figure 2.6 Puncture wound [11]

With an avulsion, a piece of skin is torn loose and is hanging from the body or completely removed. Avulsed tissues ordinarily separate at normal anatomical planes, usually between the subcutaneous tissue and the muscle fascia. If the avulsed part remains

attached only by a small pedicle of skin, the circulation to the flap may be jeopardy. This type of wound can be bleed heavily and it comes from the bed of the wound (Figure 2.7).

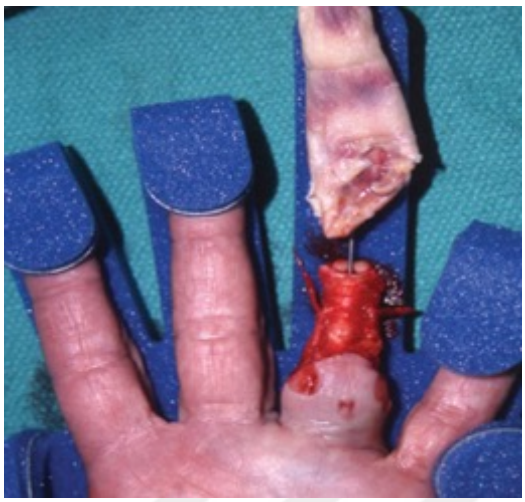


Figure 2.7 Amputation of finger [12]

Avulsions most often involve ears, fingers, and hands (Figure 2.8). An amputation involves the cutting or tearing off of a body part, such as finger, toe, hand, foot, arm, or leg [5], [6].



Figure 2.8 Avulsion of ear [13]

A blister is a collection of fluid in a “bubble” under the outer layer of skin. Repeating rubbing of a small area of the skin will produce a blister. Blisters are so common that many people assume they are a fact of life. However, blisters are avoidable, and life for many people could be more comfortable if they knew to treat and prevent blisters (Figure 2.9). Rubbing – as between a sock and a foot – causes stress on the skin’s surface because underlying supporting tissue remains stationary. The stress separates the skin into two layers, and the resulting space fills with the fluid. The fluid may collect under or within

the skin's outer layer, the epidermis. Because of differences in skin, blister formation varies considerably from person to person [5].



Figure 2.9 Blister on the finger [14]

A closed wound is one in which soft tissue damage occurs beneath the skin or mucous membrane surface, but the surface remains intact. It happens when a blunt object strikes the body. In other words, the skin is not broken, but tissue and blood vessels beneath the skin's surface are crushed, causing bleeding within a confined area. There are three types of closed wounds:

- Bruises and contusions occur when blood collects under the skin in the injured area. The victim will experience pain and swelling (immediately or within 24 to 48 hours). As blood accumulates, a black and blue mark may appear.
- A hematoma is a clot of blood under the skin. There may be a lump of bluish discoloration.
- Crush injuries are caused by extreme forces, which can injure vital organs and bones without breaking open the skin. Crush injuries may indicate an underlying problem such as fracture. Signs and symptoms include discoloration, swelling, pain, and loss of use [5], [6].

A blunt object that strikes the body will crush the tissue beneath the skin. This injury is called a contusion (a bruise). The epidermis remains intact. Damage beneath the epidermis will extend to varying depths, depending on the force of injury. In the dermis, cells are damaged and small blood vessels usually torn. Varying amounts of edema fluid and blood leak into the damaged area. This leakage produces swelling and pain. As blood

accumulates in the damaged area, a characteristic discoloration occurs. Usually, the discoloration is black or blue and is called an ecchymosis (Figure 2.10) [6].



Figure 2.10 A closed tissue injury, a contusion of leg [15]

When large amounts of tissue are damaged beneath the outer layer of the skin, large blood vessels may tear and cause rapid bleeding. A pool of blood called a hematoma will collect within the damaged tissue. A hematoma (Figure 2.11) occurs whenever a large blood vessel is damaged. The formation of hematoma is not limited to soft tissue injuries; it can also occur following a fracture or when blood vessels to any organ in the body are damaged. When a large bone such as the femur or pelvis fractures, the hematoma that forms may contain more than a liter of blood [6].



Figure 2.11 The hematoma on the woman's breast [16]

Closed tissue injuries are characterized by a history of injury, pain at the site of injury, swelling beneath the skin, and ecchymosis. They may be mild or quite extensive [6].

Burns occur in every age group, across all socioeconomic levels, at home and in workplace, and in urban, suburban, and rural settings. It has been estimated that about 70% of all burn injuries occur in the home, with house fires responsible for the majority of fire deaths. Most burn victims are injured as a result of their own actions [5], [6].

The highest-risk age groups for burn injuries are children younger than 5 years and adults older than 55 years. Both groups may have limited ability to recognize and escape from a fire or burn incident. In addition, their relativity thinner skin predisposes them to more serious injuries. Death and complications increase dramatically for burn victims older than 55 years owing to the likelihood of preexisting health problems and their immune system's decreased ability to fight infection [5], [6].

Skin death and injury occur as the applied heat exceeds the body's ability to disperse the heat; that point starts about 113°F. The amount and depth of skin damage depend on the heat's intensity, the duration of contact, and the skin's thickness [5], [6].

Types of Burns;

Burn injuries can be classified as thermal (heat), chemical, or electrical.

- Not all thermal (heat) burns are caused by flames. Contact with hot objects, flammable vapor that ignites and causes a flash or an explosion, and steam and hot liquid are other common causes of burns. Just 3 seconds of exposure to water at 140°F can cause a full-thickness (third degree) burn in an adult. At 156°F, the same burn occurs in 1 second.
- Chemical burns, a wide range of chemical agents can cause tissue damage and death on contact with the skin. As with thermal burns, the amount of tissue damage depends on the duration of contact, the skin thickness in the area of exposure, and the strength of the chemical agent. Chemicals will continue to cause tissue destruction until the chemical agent removed. Three types of chemicals; acids, alkalis, and organic compounds are responsible for most chemical burns. Alkalis produce deeper, more extensive burns than acids.
- Electrical burns, the injury severity from contact with electric current depends on the type of current depends on the type of current (direct or alternating), the voltage, the area of the body exposed, and the duration of contact. Electricity can induce ventricular fibrillation (a type of cardiac arrest), cause respiratory arrest,

or “freeze” the victim to the electrical contact point with powerful muscle spasms that increase the length of exposure. Victims of low-voltage electrical injuries may no skin burns at all but might still have cardiac or respiratory arrest.

Burns have been described as first degree, second degree, and third degree injuries. The terms superficial, partial thickness, and full thickness are often used by burn-care professionals because they are more descriptive of the tissue damage.

- First degree (superficial) burns, affect the skin’s outer layer (epidermis). Characteristics include redness, mild swelling, tenderness and pain. Healing occurs without scarring, usually within a week. The outer edges of deeper burns often are first degree burns (Figure 2.12).



Figure 2.12 First degree burn [17]

- Second degree (partial thickness) burns, extend through the entire outer layer and into the inner skin layer. Blisters, swelling, weeping of fluids, and severe pain characterize these burns, which occur because the capillary blood vessels in the dermis are damaged and give up fluid into surrounding tissues. Intact blisters provide a sterile, waterproof covering. Once a blister breaks, a weeping wound results, and the risk of infection increases (Figure 2.13).

- Third degree (full thickness) burns are severe burns that penetrate all the skin layers into the underlying fat and muscle. The skin looks leathery, waxy, or pearly gray and the sometimes charred. It has a dry appearance because capillary blood vessels have been destroyed and no more fluid is brought to the area. The skin does not blanch after being pressed because the area is dead. The victim feels no pain from a third-degree burn because the nerve endings have been damaged or

destroyed. Any pain felt is from surrounding burns of lesser degrees. A third degree burn requires medical care and the removal of dead tissue and often a skin graft to heal properly [5], [6]. From the Figure 2.14, differences of burning can be shown.



Figure 2.13 Second degree burn [18]



Figure 2.14 Comparison of the different degrees of burns [19]

2.3 General Pathology

The normal cell is limited to a fairly narrow range of structure and function. These structure and function hinged on its genetic programs of metabolism, specialization and differentiation is restricted by neighboring cells. Also the metabolic substrates availabilities are restricted too. Nonetheless, maintaining a steady state called homeostasis and it is able to managed normal physiologic demands. Some pathologic stimuli and severest physiologic stresses may bring series of morphologic and physiologic cellular adaptations. During the adaptations, the alerted homeostasis is achieved, protected the viability and altering its functions to responds of stimuli (Figure 2.15) [20].

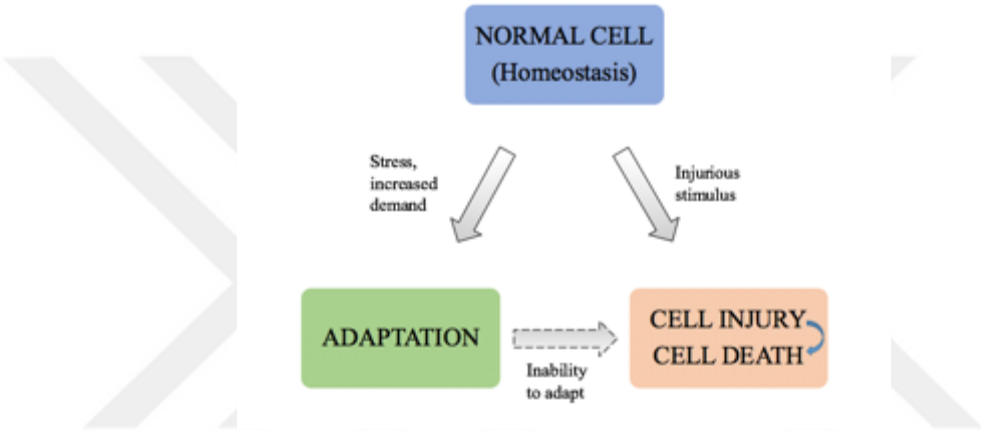


Figure 2.15 The cellular response stages to injurious stimuli and stress [20]

The hyperplasia is the adaptive response of increasing number of cells, or an increasing of individual cell sizes called as hypertrophy. On the contrary, atrophy is a decrease in the cell sizes and functions depends on the adaptive response. In certain circumstances, when the cell of the skin is exposed to stress or an injurious agent and if the adaptive response limits of stimulus are exceeded, a sequence of incidents follows that is termed cell injury. Up to a certain point, cell injury would be reversible but if the stimulus continues or from the beginning is severe enough, the cell come up to a "point of no return". At this point, the cell suffers irreversible cell injury. Cell death would be ultimately occurring. From the beginning of normal cell, adaption, cell injury and cell death can be considered as stages of normal functions of cell structure. The ultimate result of cell injury is cell death. It is most crucial incident in the progress of disease of any organ or tissue. The cell death results from diverse causes, involves lack of blood flow, toxins, infection and immune reactions [20].

When the cell is stressed severely that it is no longer able to adapt or the cell is exposed to naturally damaging agents, as a result of these the cell injury occurs. Injury can be resulted as a cell death or it is progressed through a reversible stage. The general evaluation of morphological changes in injury of cell shown in Figure 2.16 [20].

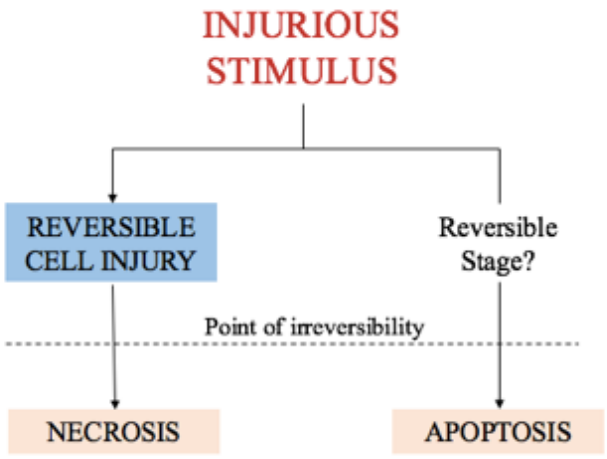


Figure 2.16 Stages of cell injury and death [20]

The biochemical alterations might be divided into 2 stages; these are reversible cell injury and irreversible cell injury. When the destructive stimulus is removed, injury is revealed as morphologic and functional changes which are reversible. The characteristics of reversible injury are cellular swelling, adenosine triphosphate (ATP) depletion and reduced oxidative phosphorylation caused by changes in water influx and ion concentrations. Irreversible injury ends with cell death. When the damaging continues, the injury becomes irreversible and it cannot be recover again. The cells which are irreversibly injured, continually undergo morphological changes, and they are recognized as cell death. There are two types of cell death which are necrosis and apoptosis. Their difference is in their mechanism, roles in disease, morphology and physiology (Figure 2.17 and Table 2.1) [20].

When damage is to acute on the membrane, the cell digests with entrance of lysosomal enzymes to cytoplasm. As a result of necrosis, cellular contents leak out and the cell death occurs. Especially, DNA damaged noxious stimuli caused another type of death, which is named as apoptosis, it is characterized by nuclear dissolution without entirety loss of membrane completeness. However, necrosis is pathologic process as always, but apoptosis serves lots of normal functions and it is not about only the cell injury. Although apoptosis and necrosis have common mechanism and overlaps between these two ways.

Therewith, at least some kinds of stimuli may cause either necrosis or apoptosis, depending on the duration and intensity of the stimulus, the rapidity of the cell death process, and the biochemical disorderedness induced in the damaged cell [20].

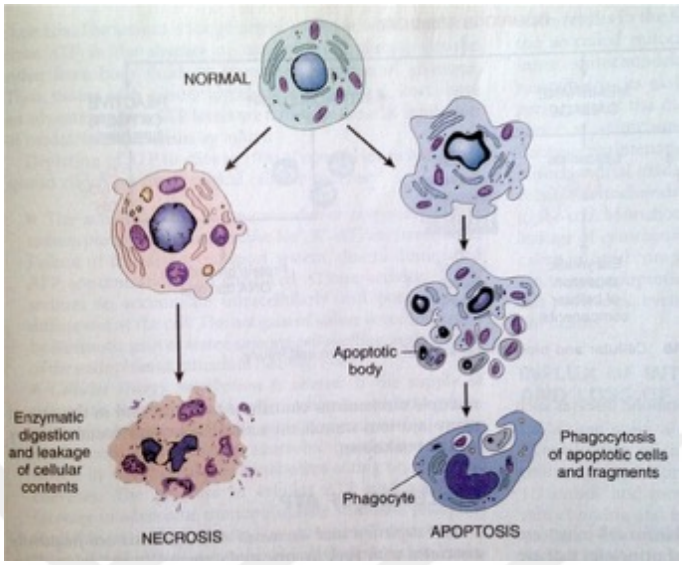


Figure 2.17 Mechanism of necrosis and apoptosis [20]

Table 2.1 Features of necrosis and apoptosis [20]

Feature	**Necrosis**	**Apoptosis**
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis→karyorrhexis →karyolysis	Fragmentation into nucleosome size fragments
Plasma membrane	Disrupted	Intact; altered structure, especially orientation of lipids
Cellular contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent inflammation	Frequent	No
Physiologic or pathologic role	Invariably pathologic (culmination of irreversible cell injury)	Often physiologic, means of eliminating unwanted cells; may be pathologic after some forms of cell injury, especially DNA damage

2.3.1 Tissue Renewal and Repair

The body's sufficiency to repair tissues and replace dead or injured cells after inflammation is serious to survival. If some injurious agents damage the normal tissues and cells, the host cells respond by series of events which are setting in motion and they serve to eliminate injurious agents, involves the damage and get ready the surviving cells for replication. The tissue damage's repairs caused by wounds, surgical resection, and various types of chronic injuries separated into two processes such as healing and regeneration (Figure 2.18) [20].

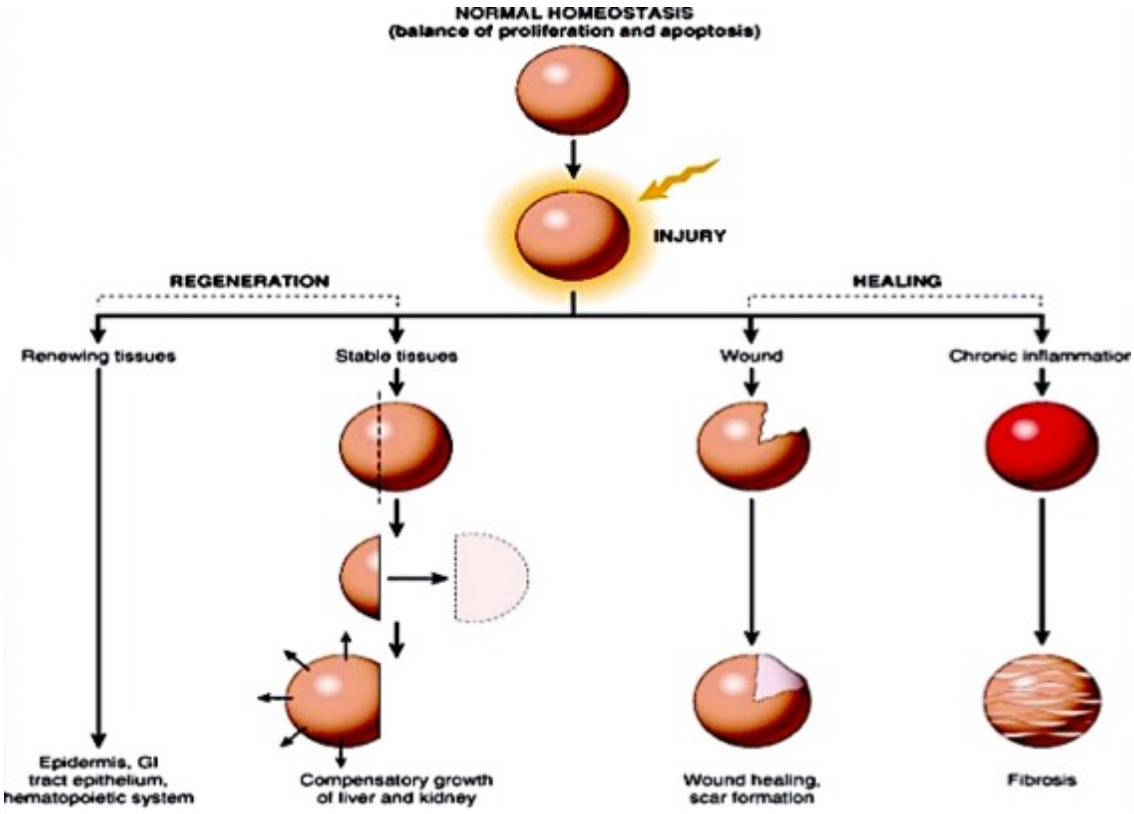


Figure 2.18 Tissue response to injury [20]

Regeneration indicates to growth of tissues and cells to switch lost structures such as the progress of an amputated part in amphibians. In mammals, whole complex tissues and organs occasional regenerate after healing. The period is generally applied to operations such as kidney and liver after, respectively unelated nephrectomy and hepatectomy. This operation relies of compensatory growth than regeneration. The epithelia of the skin, hematopoietic system and gastrointestinal tract that tissues with high proliferative capacity, renew their selves perpetual and can regenerate after injuries, as can as the cells of tissues aren't destroyed [20].

Healing is a tissue reply to a wound (usually in the skin), to cell necrosis in organs or to inflammatory period in internal organs incapable of regeneration. In this wide definition, it also includes circumstances such as atherosclerosis, noted to be an interference to heal injuries of the arterial wall. Healing includes of variable quantity of two significant processes such as the identification of fibrous tissue and scar formation or regeneration. Superficial wounds like cutaneous wound which damages only the epithelium can be healed by epithelial regeneration. Excisional and incisional wounds which are damaged the dermis, heal via the generation of a collagen scar. Also scarring happens in the myocardium after infarction. In this process, original tissue is not reproduced and is replaced by collagen. Inflamed tissue conditions of the peritoneum, pericardium and pleura frequently heal via scar formation. So it forms adhesions between parietal layers and the visceral of these tissue. The progress of a dense, fibrosis in the pericardium may cause an important condition which called constrictive pericarditis. Chronic injuries, inflammation and persistent may cause scarring in internal organs. Example, they result as stomach ulcers, and it is caused by chronic infection Helicobacter pylori [20].

In adult tissues, the standards of cell populations are confirmed by the rates of cell differentiation, proliferations and cell death by apoptosis. Figure 2.19 demonstrate these processes and depicts that remain cell numbers might result from either decreased cell death or increased proliferation. Cell proliferation might be stimulated by pathological and physiologic conditions [20].

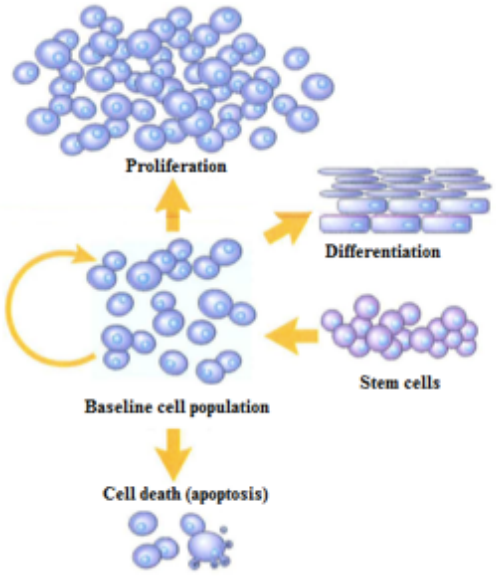


Figure 2.19 The mechanism of regulating cell populations [20]

Not all injuries give result in persistent damage, some of them cleared up with nearly perfect return of normal function and structure. Superficial skin damages are healing with entire restitution of normal structures. In some types of inflammatory terms, restitution can be occurring, as an example, lobar pneumonia, which is abundant inflammatory exudate is reabsorb. Mostly, the inflammatory and injury response might become organized with generation of granulation scarring and tissue. Even though, it is not perfect, scarring ensures a constant patch that lets the residual parenchyma to approximatively functioning. Occasionally, however, when the scar is so situated or too large, it might inspire permanent dysfunction, as with healed myocardial infarct. In this circumstances, the fibrous tissue not just result with a loss of preexisting contractile muscle. Also composes a persistent burden to the overworked permanent muscle. Scarring and tissue destruction is commonly result of chronic inflammatory and persistent injury. The processes of repair and inflammation underscore the significant capacity of the body. It restores itself. The repair responses after inflammation and injury is shown in Figure 2.20 [20].

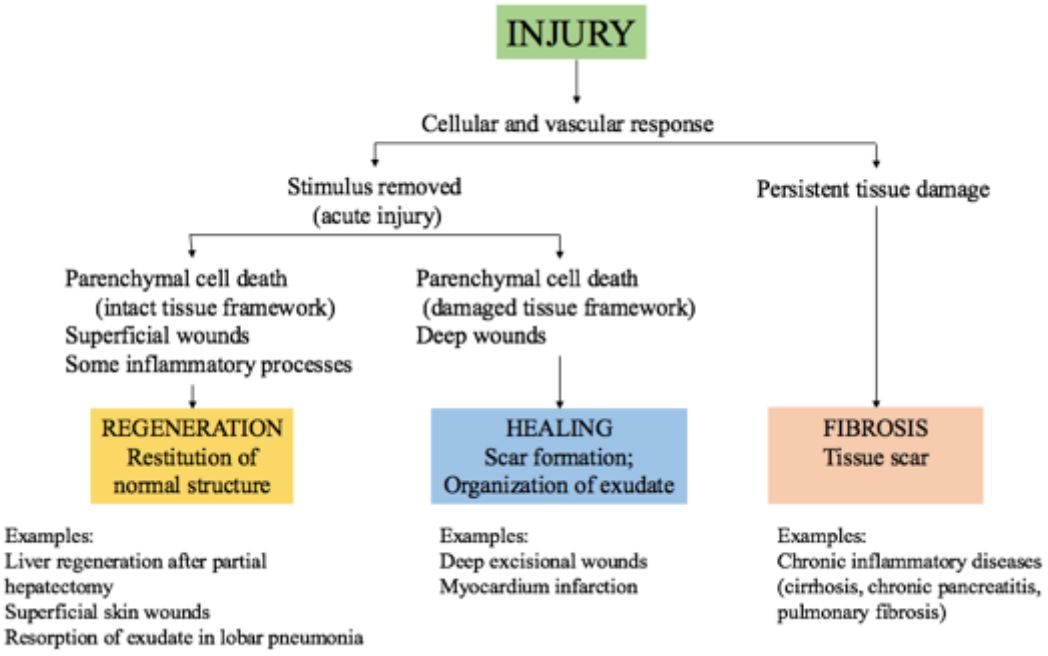


Figure 2.20 Repair responses [20]

2.4 Wound Dressing

For long years, wounds were covered with cotton and gauze bandages instead of wound care products. In 1960's researches on human and animals showed that, the wound

healing was 3 times faster in humid environment than dry environment. After that researches, the wound care products were developed faster, also in last quarter of a century the wound care products had more multifariousness [21].

However, gauze bandages still more common to use than modern products. Gauze bandages, does not protect the heat on the wound surface. The heat decrease on the wound surface causes the local vasoconstriction, hypoxia, decreasing in leucocyte mobility and phagocytic activity. Also gauze bandages couldn't prevent the passing microorganism's to wounds. According to, the researchers showed that microorganisms can pass though 64 layer of gauze bandages. The passing of microorganism can be easier when gauze bandages get wetted [21].

The wounds which were covered with gauze bandages causes more infection on the wound than covered with transparent film or hydrocolloid films. The researchers showed that the changing of dress a wound with gauze bandages spread more bacteria to air medium. Wet-dry gauze bandages can be used for removing the dead cells, but these operation causes the pain and damages the healthy cells too (Figure 2.21). It is known that many advanced wound care products more expensive than gauze bandages and cotton but when all costs are taken into account gauze dressing for use in wound healing can be considered more expensive [21].

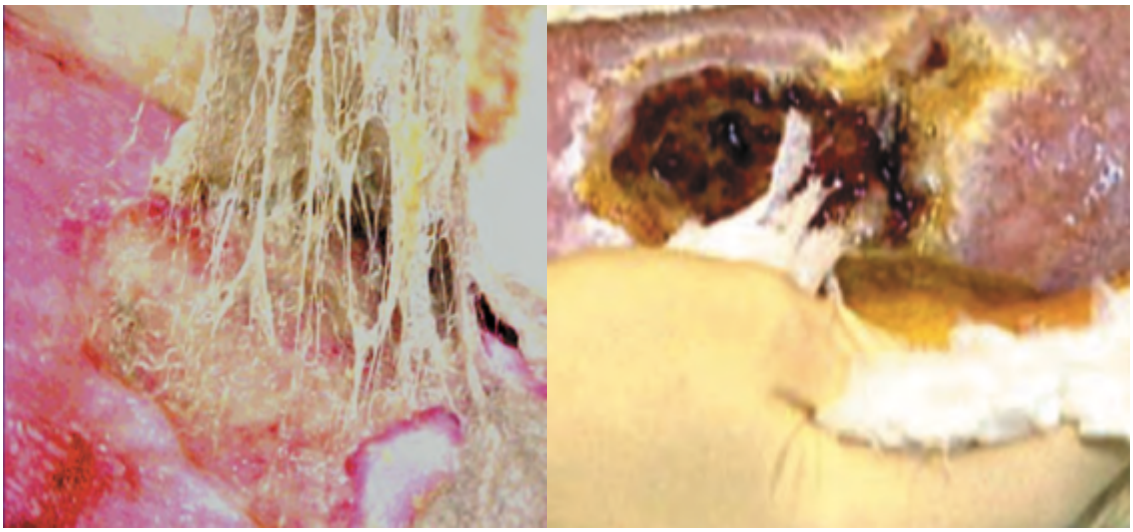


Figure 2.21 The wound which is dressed by gauze bandages and cotton [21]

2.4.1 Wound Dressing Species and Specialties

For all types of wounds need different type of wound dressing. For that reason, recognizing the wound has important role for choosing the wound dresses. The right

chosen of wound dressing will cure the wound in the best way and rapidly. In these days, there is various wound care products have been using in medical applications. The wound dresses which are using prevalently are shown in below:

- Dry Gauze Bandage: The usage areas of dry gauze bandages are surgical wounds and the few bleeding small wounds after 24 hours of surgical incision. It absorbs the exudate and drained. It protects the area dry gangrene which cannot be debrided. Also it used as a second dressing product. It cannot be used in the wounds which needs humidity area, is painfully and bleeding wounds.
- Wet-Dry Gauze Bandage: It is using for absorbs the exudate, drainage and fill the dead tissue. Also it uses for debride wet necrotic wounds. It is not useful for painfully and bleeding living wounds. Also it cannot be used for local thickness wounds and the wounds which needs humidity environment.
- Transparent Cohesive Films: It provides humidity environment to wounds and it is antibacterial, the bacteria cannot pass through the films to wounds. It can be used for superficial wounds, the wounds which has small exudates and protecting of the injured skin. It cannot be used in the high exudate wounds, the sensitive skin's which are damaged during the changing of dressing, the wounds which has sinuses and complete thickness wounds (Figure 2.22) [21].

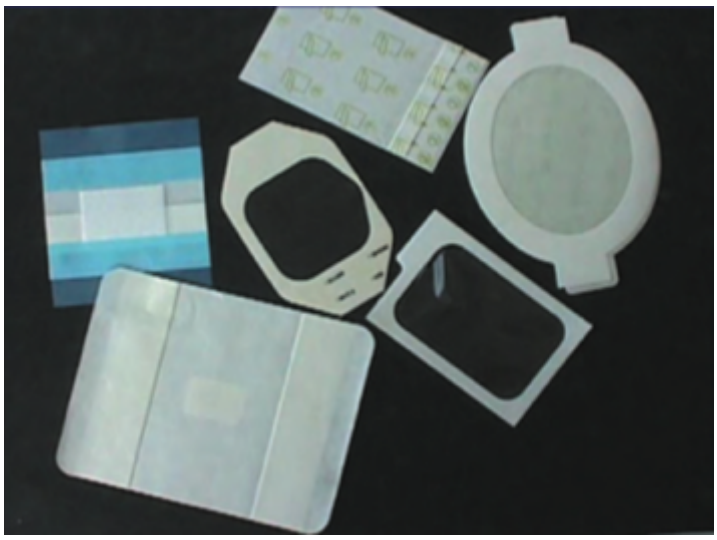


Figure 2.22 Transparent cohesive films [21]

- Hydrogel Dressings: The gel dressings have a cooling property. They can decrease the pain and inflammation. They cannot stick to skin because of that they need to have second dressing material. It can be used in the wounds which have small

exudates, irritations, small burnings, local thickness wounds, radiation burnings, the wounds which needs humidity environment, donor areas, first and second type of burnings, dead tissues. These kind of wound dresses cannot be used in the exudative wounds, third degree burnings, infected wounds, fungi infected wounds, medium type of burnings, cavity type wounds (Figure 2.23) [21].

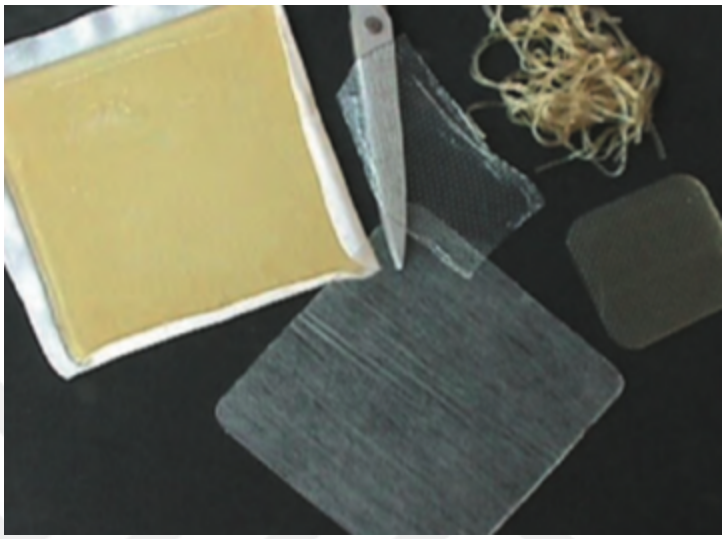


Figure 2.23 Hydrogel dressings [21]

- Alginate Dressings: These dressings cannot stick on the skin because of that they used for infected wounds and the wounds which has lot of exudate. Also they can use in the cavity wounds. They can be used for fill the dead tissues in the exudate accumulation and to help for otolith debridement. It is not useful for third degree burning wounds, heavily bleeding wounds and dried wounds. Also if the skin has sensibility for alginate and collagens, they cannot be used (Figure 2.24) [21].



Figure 2.24 Alginate dressings [21]

- Odor Absorptive Dressings: These kind of dressings have a thin active carbon layer to absorb the bad odor of the wounds. They are used with the other dressing types. The use of the odor absorptive dressings is the absorption of odor in the necrotic wounds. Also they can be used in the palliative care of terminal period patient's running wounds as a relaxing and comfortable material. They can be used in the medium degree drainage infected and not infected wounds but not in the dry and superficial wounds [21].
- Collagen Dressings: They increase the collagen store in the incipient wound bed. These kind of dressings should be in the form of pad, gel or particular sized. They are used for fill the dead tissue gap, absorption of exudates and provides humidity environment. They are useful in the drainages such as fistula or cavity wounds, partial layer wounds and second degree burnings. They are not useful if the skin has sensible for the material and if the wound is dried (Figure 2.25) [21].

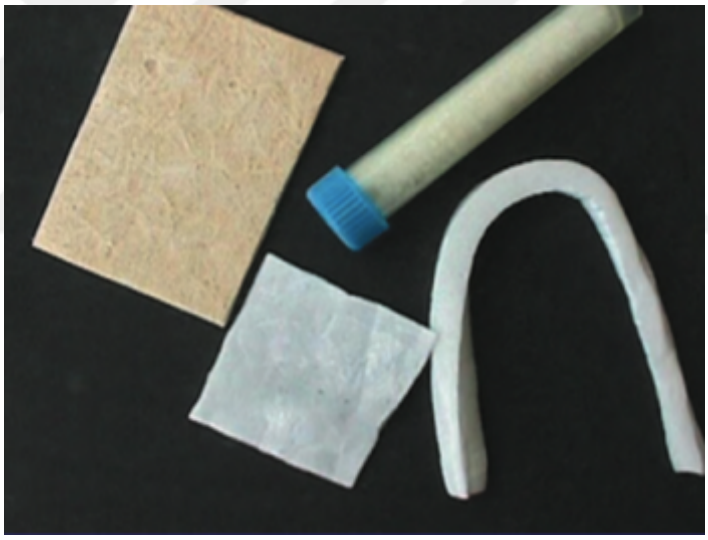


Figure 2.25 The collagen wound dressings [21]

- Foam Dressings: They are made from polyurethane foams and most of them are antibacterial. They provide thermal insulation and moisture. Some foams are not tacky and require a second dressing. They are useful with medium degree and large amount of exudate wounds. Also these kind of dressing can be used as secondary dressing in the deep wounds to ensure more absorption of exudate and drainage. The dressing is used under the drainage wound's care to ensure impression of compression dress. Dry wounds, exudates with minimal partial thickness wounds, arterial ischemic necrosis are not suitable for foam dressings. (Figure 2.26) [21].

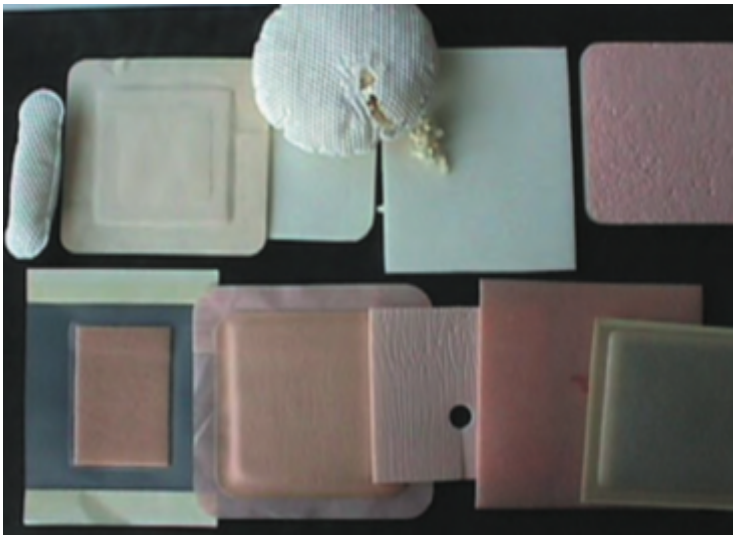


Figure 2.26 Foam dresses [21]

- Hydrocolloid Dressings: These kind of cohesive dressings don't allow to passing of oxygen neither water. It composes hypoxic wound bed for otolithic debridement and ensures fibroblast proliferation. It can be used with the wounds which have slightly-medium exudate, granulation wounds, and partial thickness wounds. Also they are used in cases where the skin is protected from irritation. They should not be used in the wounds which have infected sinuses, deep cavity, and large amount of exudate. Also they cannot be used with 3rd degree burns, when the skin around the wound is damaged (Figure 2.27) [21].



Figure 2.27 Hydrocolloid dresses [21]

- Noncohesive Dressings: Without applying in the wound bed, it can provide a superficial area on the wound. They are used as a primary wound dressing but they need secondary wound dresses on the application. They are used to reduce bacterial proliferation in the superficial wounds and also they are used with skin

grafts, donor sites, abrasions and lacerations. The noncohesive dressings' usage are not available in the individuals who has antibacterial sensitivity and the wounds which have excess quantity of exudate [21].

- Complex (Combined) Dressings: These are the complex products which are containing two or three products and they designed to provide many features with a single cover, also most of them have cohesive parts. The usage of complex dresses are not as much as primary and secondary dresses. It is used for the partial and full thickness wounds and the wounds which have medium sized and surplus drainage. They are not being used in the wounds that exposed tendons, muscle and bone, the wounds with severe drainage, untreated infected wounds, the third degree burns and also the patients who has sensitivity to the ingredients of the dresses such as alginate and polyurethane [21].

2.5 Wound Dressing Selections

The choose of wound dressing is according to patient's wound type, wound specialties, the stage wound healing and what is needed at this stage and all these characteristics should take into account when the wound care products are chosen. The ideal wound dressing should have these specialties which are given below:

- Increases the wound healing
- Provide humid environment for wound
- Without the permission of the wound let dry and more exudate and toxic substances should move away from media
- Avoid the smell
- Protect the heat of the wound surface
- It should not allow the passage of microorganisms of the air to the wound surface
- Allow the gas and oxygen passage to the wound to help the cell migration and the cell division
- Protect from the hematoma and hypertrophic scar formation
- It should not harm the wound during dressing changes

- It should not be causing trauma and tissue damage in the skin which is near the wound
- The wound should prevent the appearance from the outside
- Pain reduction
- It should be easily found, implemented and inexpensive product [21].

2.6 Modern Wound Dressing Applications

Zhao, et al. (2014), produced chitosan/sericin based composite nanofibers by electrospinning. The nanofibers were characterized with SEM, FT-IR, XRD and TGA. Also, the composite nanofibers showed good antibacterial activity against both of Gram-negative and Gram-positive bacteria. The chosen Gram- positive bacterium Bacillus subtilis and Gram-negative bacterium is Escherichia coli [1].

Archana, et al. (2014), was prepared wound dressing material from poly vinyl pyrrolidone (PVP), chitosan and silver oxide nanoparticles. They characterized films with their mechanical properties, thermal behavior, antibacterial properties and morphological properties and wound healing properties. Both chitosan and silver oxide reveals antibacterial activity. The chosen bacterium for Gram negative is E. Coli and for Gram positive is S. Aureus. The swelling tests was performed with phosphate buffer solution (PBS) with 7.4 pH. The films which contains PVP, chitosan and silver oxide nanoparticles showed good results for wound healing applications and higher swelling capacitated films shows good antibacterial activity this is an important factor too [2].

Zhang, et al. (2015), studied wound dressing from carboxyl-modified poly(vinyl alcohol) crosslinked chitosan hydrogel. Their hydrogel films are produced with different concentrations of PVA-COOH and chitosan. They were crosslinked via formation of amide linkages. In swollen and dry state of crosslinked hydrogen films of mechanical properties were increased with high swelling ratio. PBS is used in swelling tests and square shaped 2cm x 2 cm of films immersed in solution. The device characterizations analyzed with FT-IR and XRD. Oxygen permeability and water vapor consideration of films sustain a moist surrounding over wound bed [3].

Liakos, et al. (2013), studied natural polymeric composite films which are made with essential oils of cinnamon, eucalyptus, lavender, peppermint, globulus juvenile, tea tree, lemongrass and lemon. Essential oils embed in sodium alginate matrix, as a result of this,

the films have significant antifungal and antimicrobial properties. The bacterium is studied in this study, E.Coli and as a fungi Candida albicans [4].

Kavoosi, et al. (2014), produced poly (vinyl alcohol) (PVA) films with addition of Zataria multiflora essential oil and studied antibacterial and antioxidant potential. Films moisture absorption, water vapor permeability, water solubility and water swelling potentials are searched. Films presented good antifungal and antioxidant properties. The films were singly tested against four fungi; Aspergillus niger, Paecilomyces variotii, Aspergillus oryzae and Trichoderma harizanum [22].

Yari, et al. (2013), works on polyurethane membranes with addition of castor oil for wound dressing application. The characterizations analyzed with FT-IR, dynamic mechanical analysis (DMA) and energy dispersive X-Ray (EDX). Antibacterial tests of produced membranes was studied against both S. Aureus and E.Coli [23].

Mehdizadeh, et al. (2012), studied starch-chitosan composite with addition of Thymus kotschyanus essential oil with different concentrations from 0 to 2%. Their antioxidant and antimicrobial properties substantially increased with essential oil addition. Antibacterial activities are tested for Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus and Escherichia coli cultures [24].

Gu, et al. (2013), produced chitosan/silk fibroin mix membranes crosslinked with alginate di-aldehyde. They studied the biocompatibility and physical properties of the wound dressing membranes. These were water vapor permeability, water absorption rate, stability in saline solution and scanning electron microscopy (SEM) images. Therefore, the addition of alginate di-aldehyde can can improve the stability of membranes and promote cell proliferation [25].

Öztürk, et al. (2005), work with alginate/chitosan sponges that includes model antibiotic such as ciprofloxacin which is used in wound and/or burn treatments. Gelation of sodium alginate and then lyophilization was made sponges. After that sponges crosslinked with calcium chloride and at last coating with chitosan. Sponges were characterized as in vitro drug release behavior, morphology, antimicrobial activity and water uptake. Only the E. Coli bacterium is used in antibacterial activity tests. The death rate of bacterium is increased by increasing the concentration of ciprofloxacin in sponges. These kind of wound dressing usage in burn and wound dressing application is an important alternative [26].

Guan, et al. (2007), studied with a new inorganic antimicrobial particle which is added into collagen-chitosan mixture to, minimize wound infection that caused by bacteria, and improved antibacterial properties of wound dressing. Mechanical properties, swelling properties, antimicrobial properties, cytotoxicity and release rate of this new material was examined. The bacterium is chosen as E. Coli only. As a result of this study, antimicrobial property increased with addition of new particle [27].

López-Mata, et al. (2015), produced chitosan films with addition of cinnamon bark oil with different ratios such as 0%, 0.25% and 0.5% by an emulsion method. All films were characterized by physical properties such as water vapor permeability, solubility, microstructure and optical property and its antioxidant properties. Microstructure was analyzed with scanning electron microscopy (SEM). Cinnamon oil addition decreased the solubility of new produced films but it reduced the water vapor permeability. Also cinnamon oil gives a yellow color to films and it improve the transparency of new produced films. Also it acts as a well blocker of ultraviolet radiation [28].

Altiok, et al. (2010), studied chitosan films with thyme oil addition by solvent casting method. The films characterized by oxygen transmission, water vapor permeability, and mechanical properties. Also morphologies of films were analyzed with SEM, FT-IR, TGA and differential scanning calorimetry (DSC). In addition, antioxidant and antimicrobial activities of the films were examined too. The bacteria are chosen as gram positive Staphylococcus aureus and gram negative Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa. The antimicrobial tests undergo afar diffusion method and different ratios of thyme oil were tested with these bacteria. The antimicrobial tests showed that even minimum thyme oil concentration in films has antibacterial effect [29].

Wang, et al. (2012), composed hydrogel sheets by chitosan, gelatin and honey. The sheets were produced as a burn wound dressing. Hydrogel sheets had significant antibacterial efficiency to E. Coli and S. Aureus, also chitosan and honey have good efficiency separately. Hydrogel sheets are not irritant to body and skin, also, nontoxic. And they studied healing process on animal such as rabbit. It remarkably promoted burns healing [30].

Mi, et al. (2001), produced a wound dressing from asymmetric chitosan membranes with immersion precipitation phase inversion method. Macro porous sponge like sublayer supports skin surface of top layer of the skin. Using of pervaporation method with phase

separation process' modification was controlled the porosity of sponge like sublayer's porosity and thickness of the intensive skin surface. The membrane showed excellent oxygen permeability, controlled evaporative water loss and incited fluid drainage but it could prevent external microorganisms. The bacteria for antimicrobial test is chosen as Staphylococcus aureus and Pseudomonas aeruginosa. The membrane helped healing process and the skin healed quickly [31].

Archana, et al. (2013), produced nano dressings with titanium oxide, chitosan and pectin. The antimicrobial, biocompatible of dressings and in vivo healing properties were determined. The characterizations of nano dressing were made by TGA, FT-IR, DSC, TEM and SEM. The wound healing properties determined on male albino rats. Other properties such as antibacterial abilities, swelling ratios, water vapor transmission rate and hydrophilic nature, wound closure rate tests were done for wound healing applications. Antibacterial activity of inhibition effect of chitosan–titanium dioxide–pectin dressing material studied with E. Coli, S. Aereus, P. Aeruginosa, B. Subtilis. A. Niger. The addition of titanium oxide increased inhibition zones and healing efficiency [32].

Xu, et al. (2014), studied with tannic acid/chitosan/pullulan composite nanofibers. The antibacterial activity test made on gram negative bacterium E. Coli. The nanofibers were developed with help of force spinning method and fibers were crosslinked. The water absorption capability, structure, morphology, water solubility, and thermal properties of the fibers were characterized. The fibers provided good water absorption ability. And produced nano fibers had good potential for intricate and deep wound healing [33].

Rubilar, et al. (2012), studied with grape seed extract, carvacrol and chitosan in different ratios. The films were prepared by solvent casting method. Structural, mechanical, barrier and color properties of films are interpreted. The carbon dioxide and water vapor permeability, tensile strength, oxygen permeability, crystallinity properties were determined. As a result of this, with addition of functional compounds this films were alternatives to synthetic materials [34].

Moradi, et al. (2012), antioxidant chitosan films were produced with combination of Zataria multiflora Boiss essential oil and grape seed extract. The properties of swelling, color, antioxidant, wettability and physico mechanical were investigated. The addition of essential oil and seed extract increased water vapor transmission rate. Only grape seed

extract added films had high swelling ratio. Essential oil gives yellowish and seed extract gives grey color to films [35].

2.7 Bacteria's for Antibacterial Tests

Bacteria's cannot be seen by naked eye, they are seedless and chlorophyll free with 1-6-micron structure species. They increase their number by dividing, there is harmful and beneficial bacteria's in the world. Bacteria's are different from the animals or plants; they have an important role in living creature because of their biochemical effects. Generally, bacteria's divided by some categories such as pathogenic bacteria, yeast bacteria, digester bacteria, nitrogenous bacteria. They can be live under high pressures and temperatures. It refutes changing the structure of dead organisms and are used as a support for other organisms to become organic matter. These kind of bacteria are beneficial one's [36], [37].

Bacteria have a cell membrane but they have lack membrane-enclosed organelles and membrane-bound nuclei, they are prokaryotes. Bacteria are connected by a cell wall generally inhere in peptidoglycan, a polymer of complex amino acids and sugars. There are two types of bacteria which have different forms of cell wall such as a thick wall enclosing the cell membrane which keeps crystal-violet stain that are called as Gram positive bacteria, or a thin cell wall compress between two phospholipid bilayer membranes that are called as Gram negative bacteria. Bacteria can be classified by shape, gram stain (gram positive or gram negative) and form of respiration. Most bacteria have long helical filaments and whip, these filaments stretching out from the cell surface, that is enable bacteria to transfer their environment. Also some other bacteria have pili, this is another type of surface projection, that can attach bacteria to host cells. Depends on the host for suitable growth conditions, many bacteria can synthesize their own RNA, DNA and proteins.

Healthy people may be colonized by as much as 10^{10} bacteria in the mouth, 10^{12} on the skin, and 10^{14} bacteria in the gastrointestinal tract [20].

Bacteria:

- It is prokaryotes.
- It has the enzyme system, and a unique metabolism.
- The organelles of the Nucleus and membrane systems are not available.

- Leader molecule is naked DNA and it found in the nuclear field.
- A cell wall containing fat and carbohydrates.
- Some of them has capsule forms.
- In cytoplasm, DNA, RNA, ribosomes, polyribosomes, glycogen and fat drops are located.
- Some of them formed in the cell membrane folds and has a bearing thylakoids and mesosome. Mesosome types can carry oxygen in respiratory enzymes. Thylakoids can carry chlorophyll.
- Flagellum (whip) which is actively moving.
- They live single or in colonies.

Classification:

According to types;

- Round (Coccus)
- Rod (Bacillus)
- Comma (Vibrio)
- Spiral (Spirillum)

According to colony formation;

- Monococcus: Single cells
- Diplococcus: Binary cells
- Streptococcus: As chain cells
- Staphylococcus: Grape bunch shaped

According to the gram staining;

- Gram positive: paintable
- Gram negative: non-paintable

According to respiration format;

- Anaerobic bacteria: oxygen free respiration

- Aerobic bacteria: oxygen respiration [36], [37].

Gram negative bacteria don't absorb crystal violet dye during the gram staining. Alcohol washed gram positive bacteria has blue gray color after washing but in gram negative bacteria has lost blue gray color. During the gram stain, crystal violet color adding, after that against stain added and as a result of this, gram negative bacteria are painted red pink (Figure 2.28) [38].



Figure 2.28 Gram negative bacteria [39]

Most of the gram negative bacteria is pathogen, and it will be highly capable of doing disease.

- It has thinner peptidoglycan in cell walls than gram positive bacteria.
- There is lipopolysaccharide membrane outside the peptidoglycan layer.
- Outer membrane cells have pores for specific molecules.
- It has periplasm between peptidoglycan layer and outer membrane.
- Teichoic acid or lipoteichoic acid is not present in gram negative bacteria.
- It has cytoplasmic membrane.
- S layer is attached to outer membrane.
- Flannel is 4 ringed.

Gram negative bacteria cell wall is thin, only about 10% of the cell wall consists of peptidoglycan material. Lipopolysaccharide found on the outer surface is quite dense. In this group of bacteria, cell surface is covered with a capsule. This is due to the structure

of lipopolysaccharide toxic substances, more children are involved in these bacteria into the bloodstream and cause sepsis faster discomfort [38].

Gram positive bacteria, has purple blue or black color under microscope after gram stain process (Figure 2.29). These bacteria colored with gram stain because of this they called gram positive. The reason of color is due to hold the crystal violet iodine mixture in cell walls. These bacteria do not have outer membrane out of the cell wall. When the cell wall is thick, it has high power to keep the stain. There is a S layer membrane in Gram positive bacteria such as gram negative bacteria. The S layer will be attached to the peptidoglycan layer in gram positive bacteria. The distinctive feature of these cells is teichoic acid in the cell wall. The lipid portion of this acid promotes combination of the peptidoglycan layer of the cell.

- It has polysaccharide capsule.
- It has cytoplasmic membrane.
- It has very thick cell membrane.
- If it has flagellum, there is 2 ring for protection, in gram negative bacteria there is 4 ring. The reason for this is that the membrane layer in bacteria [40].

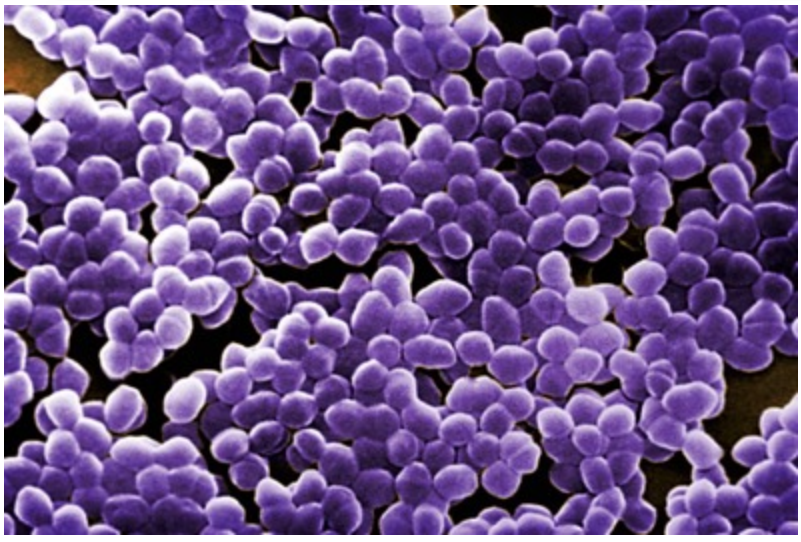


Figure 2.29 Gram positive bacteria [41]

Figure 2.30 shows molecules on surface of Gram positive and Gram negative bacteria contains in pathogenesis [20].

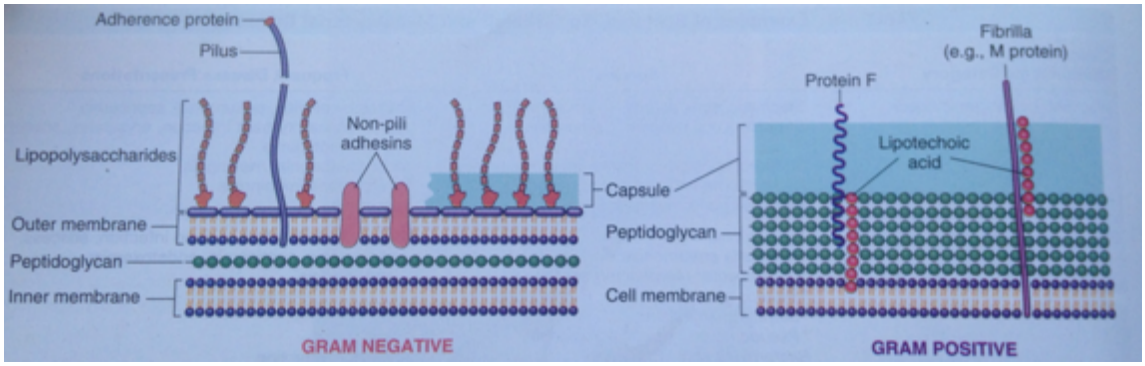


Figure 2.30 Gram negative and Gram positive bacteria [20]

Staphylococcus Aureus (S. Aureus) is one of gram positive bacteria that infection by pyogenic cocci, which cause Abscess, pneumonia, cellulitis, and septicemia. Escherichia Coli (E. Coli) is one of gram negative bacteria that common inflammation causes urinary tract infection, abscess, septicemia, wound infection, endocarditis and endotoxemia. S. Aureus organisms are nonmotile, pyogenic, gram positive cocci that grapelike clusters. This S. Aureus bacteria cause a myriad of skin lesions such as carbuncles, boils, scaled skin and impetigo, also cause pneumonia, osteomyelitis, food poisoning, toxic shock syndrome and endocarditis (Figure 2.31) [20].

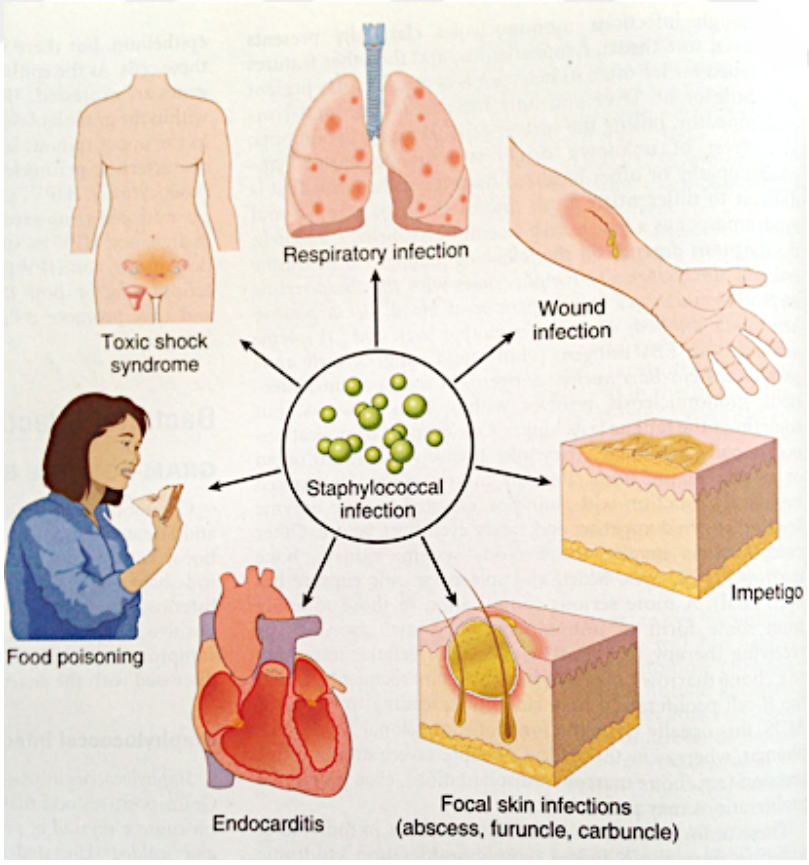


Figure 2.31 Staphylococcal infections [20]

2.8 Medicinal and Aromatic Plants

Medicinal and aromatic plants are used in traditional and modern medicine such as prevention of diseases and as drugs for the treat of maintaining health and disease. At the same time, the plants are utilized as a condiment in nutritional supplements, herbal teas, and taste products. The plants find wide use in various branches of industry such as perfumery and cosmetics also as insecticides, body care products, and brighteners. Some parts of medicinal and aromatic plants are dried and measured under some standards to use with beneficial advantages. These parts of plants are root, root stems, tubers, stems or woody structure, bark, leaves, flowers, fruit, seeds and herb [42].

Medicinal and aromatic plants find widely usage area in many fields, the plants are biological, cultural and industrial resources. Demand for these resources have increased significantly in recent years and it continues to increase. The value of these kind or aromatic and medicinal plants are increasing for reasons such as their versatile effects and lack of side effects in the medical and health field because the active substance obtained synthetically has side effects and they are not natural [42].

In the World 50000 to 70000 kinds of plants are known to use in traditional and modern medicine. Also the plants species used in botanical and cosmetics industry are not included in this number. In 2007 the "International Standard of the Sustainable Nature Collection of Medicinal and Aromatic Plants (ISSC -MAP) "created [42].

The plants which are collected from nature does not have same standardization, because of that all collected plants have different qualities and the active agents has different rates. For that reason, this kind of plants have to need cultivation. Table 2.2 is shown the number of plant species, medicinal plant species number and rates in some countries and the world [43].

At the beginning of 20^{th} century, the innovations of technological developments, social and political changes have led to a sharp reduction in the use of medicinal plants. At the same time the mass production with synthetic drugs in the pharmaceutical industry has been quite effective in reduce of medicinal plants [44].

But nowadays, developed countries in particular are already turning to herbal treatment resource. A significant portion of the drugs used in treatment is a natural sourced drugs. The natural sourced drugs usage rate is 60% in developed countries and 4% in developing

countries. Approximately 80% of the world population uses traditional medicine and medicinal plants to return to health [45].

Today, the number of medicinal and aromatic plants used in the world is around 20,000 according to the World Health Organization. Still, 4000 of these drugs are widely used and already 2000 of medicinal plants merchandise in the world and 500 from Western Europe. In recent years, the world's herbal drugs annual trade volume is around 18-20 billion dollars and it is estimated to increase further in the coming years. In the world, essential oil export value in 2008 is amounted to 2.5 billion dollars [42].

Table 2.2 The number of plant species, medicinal plant species number and rates [43]

Countries	**The number of plant species**	**The number of medicinal plant species**	**%**
China	26092	4941	18.9
India	15000	3000	20.0
Indonesia	22500	1000	4.4
Malaysia	15500	1200	7.7
Nepal	6973	700	10.0
Pakistan	4950	300	6.1
Philippines	8931	850	9.5
Sri Lanka	3314	550	16.6
Thailand	11625	1800	15.5
USA	21641	2564	11.8
Vietnam	10500	1800	17.1
Turkey	9222	500	5.4
Average	13366	1700	12.5
World	422000	52885	

2.9 Medicinal and Aromatic Plants in Turkey

As a natural structure, three old continents (Europe, Africa and Asia) qualifications merged and Turkey's ecological and biological diversity of nature leaves behind other places in 40° north longitude. As an indication the wide range of climate and topography of Turkey, there have three plants region such as the Mediterranean, the Euro-Siberian and Iran-Turan. Each of these regions has its own endemic species and natural ecosystems [46].

Turkey's flora has the response of the many herbal products of developed countries such as herbal medicine, food additives, cosmetics and perfume industry constitutes. The existing 9222 plant species in species diversity in terms of natural herbal products, 2991 plant species are endemic species [44], [46].

Turkey, which hosts to 75% of plant species in Europe, about one to third of which are endemic [47]. Endemic plants are only one of their specific geographic regions of our country up to 2282 and found that only show the spread in that area. The demand for medicinal and aromatic plants in the world market is increasing every day a little more. Medicinal and aromatic plants are collected from the Aegean, Marmara, Mediterranean, Eastern Black Sea, Southeastern Anatolia region [44]. Table 2.3 is shows distribution of Turkey endemic plants by region.

Table 2.3 Distribution region of Turkey endemic plants [47]

Region	**The number of endemic plants**
Mediterranean	862
Eastern Anatolia	471
Central Anatolia	335
Black Sea	277
Aegean	171
Marmara	102
Southeastern Anatolia	102
Total	2282

In Turkey, domestic and foreign trade in medicinal and aromatic plants have 347 types of including species and subspecies but only 139 species of them are export [48]. Table 2.4 is shows the Turkey's export figures of medicinal and aromatic plants.

Table 2.4 Turkey's export figures of medicinal and aromatic plants [42]

Plant Name	**2007**		**2008**		**2009**	
	Amount (ton)	**Value (1000$)**	**Amount (ton)**	**Value (1000$)**	**Amount (ton)**	**Value (1000$)**
Cilantro	41	51	19	71	31	76
Cumin	4209	9228	2367	6832	5822	12146
Fennel	1057	2125	1945	3793	1217	2541
Fenugreek	94	156	51	74	54	79
Mahaleb	109	908	102	897	159	1305
Licorice	249	266	67	125	311	471
Carob	3623	5264	518	838	3130	3091
Linden	80	1116	27	313	179	1428
Sumac	965	1176	1175	1771	1245	2143
Rosemary	432	1019	573	1587	620	1671
Black Sesame	45	163	38	139	32	109
Mint	153	505	125	326	309	1085
Bay Leaf	7519	20301	3934	20020	9079	24336
Thyme	11308	39493	9683	42878	11475	28662
Sage	1530	4479	373	1541	1545	6048
Aniseed	2003	4704	2658	9350	2053	8616
Total	33417	90954	26655	90555	37261	93807

Despite being one of most important countries in the world in terms of medicinal and aromatic plants, medicinal and aromatic plants export in Turkey is not yet at the desired level. According to 2009 data, about 94 million US dollars in export of medicinal and

aromatic plants was performed, but the level of imports was 9 $ million. Table 2.5 shows the Turkey's import figures of the medicinal and aromatic plants [42].

Table 2.5 Turkey's import figures of the medicinal and aromatic plants [42]

Plant Name	**2007**		**2008**		**2009**		**Import from important countries**
	Amount (ton)	**Value (1000$)**	**Amount (ton)**	**Value (1000$)**	**Amount (ton)**	**Value (1000$)**	
Bay Leaf	12	87	41	111	177	339	USA, Georgia, Poland, Syria
Thyme	2341	4456	851	2486	460	1198	Poland, Morocco, Albania, Chile
Sage	564	1018	1155	2928	547	1551	Albania, USA, Bosnia-Herzegovina
Aniseed	276	483	82	248	170	503	Syria, USA, Spain
Cilantro	304	138	202	183	149	80	Bulgaria, Ukraine, Afghanistan
Cumin	688	1270	507	903	259	582	Syria, USA, India
Fennel	111	116	266	386	185	210	Syria, Germany, Macedonia
Soapwort	550	212	484	201	293	150	Afghanistan
Ginseng root	3	37	3	51	1	18	China, USA, Germany
Licorice	3	121	5	88	51	104	Italy, Iran, Chine
Carob	648	605	673	1048	1255	784	Cyprus, Egypt

Table 2.5 Turkey's import figures of the medicinal and aromatic plants (cont'd)

Plant Name	2007		2008		2009		Import from important countries
	Amount (ton)	Value (1000$)	Amount (ton)	Value (1000$)	Amount (ton)	Value (1000$)	
Linden	113	523	137	1163	35	185	Chine, Germany, Bosnia-Herzegovina
Sumac	274	27	140	14	26	33	Afghanistan, Iran, Jordan
Turmeric	384	191	109	150	346	264	India, Iran, China
Rosemary	387	375	553	613	480	590	Morocco, Tunisia
Black Sesame	1167	1195	1505	1254	1058	831	Syria, India, Ethiopia, Iran
Cinnamon	829	500	670	413	898	581	Indonesia, Singapore, Vietnam
Clove	136	137	83	99	228	253	Singapore, Brazil, Mexico
Ginger	247	172	235	215	705	450	China, Thailand, India
Coconut	18	45	4	22	21	82	Indonesia, Singapore
Curry	39	35	102	81	148	98	India
Cardamom	2	7	11	45	-	-	Guatemala, Singapore, India
Total	9551	11750	7818	12702	7492	8886	-

Turkey has 18th rank out of 110 countries of the exporting medicinal plants. Turkey was ranked in the 5th in exports from East and South East European countries and was ranked 8th in imports. Turkey's 16 types of plants which are important in exports, their export value in 2001 was realized as 52$ million, then it was increased to 94 million dollars in 2009. As well as oil rose of in trade in medicinal and aromatic plant species as cumin, oregano, anise, fennel, coriander, mint and black sesame's culture is made and the production of these plants is very limited compared to other crops [48].

In general, medicinal and aromatic plants is being exported in the raw state, creating low added value. Cleaning, sorting, grading, packaging, wholesale and retail operations are also applied in a limited number of spices and herbal tea production. Some cultured medicinal and aromatic plant's production area and amount values in Turkey, has recorded by Turkey Statistical Institute (TSI). Medicinal and aromatic plants which grow in Turkey is shown in Table 2.6 [42].

In the world, Turkey is most important exporting country of qualified bay and Turkey is one of the few countries that export the bay leaf. Also Turkey meet the requirements of approximately 90% of the world at bay. Forest secondary production has important role in medicinal and aromatic plants because bay leaf is one of them and also gentian plant is another forest secondary plant. Turkey's forest secondary products amounts are shown in Table 2.7 [49].

Table 2.6 Medicinal and aromatic plants which grow in Turkey [42]

Plant Name	**Year**	**Cultivated Area (ha)**	**Production (ton)**	**Efficiency (kg/deca)**
Aniseed	2007	12291	8006	65.1
	2008	11880	8594	72.3
	2009	11918	9472	79
Cumin	2007	18327	9159	49.9
	2008	18351	8879	48.4
	2009	19011	14533	76
Thyme	2007	6075	5350	88.1
	2008	8413	10082	119.8

Table 2.6 Medicinal and aromatic plants which grow in Turkey (cont'd)

Plant Name	**Year**	**Cultivated Area (ha)**	**Production (ton)**	**Efficiency (kg/deca)**
Thyme	2009	8496	12329	145
Fenugreek	2007	55	36	66
	2008	188	195	104
Mint	2007	-	9376	-
	2008	-	9824	-
Dill	2007	-	2637	-
	2008	-	2677	-
Hops	2007	284	1433	502
	2008	284	1555	548
	2009	326	1650	506

Table 2.7 Turkey forest secondary products amount [49]

Plant Names	**Total Area (ha)**	**Total Amount (kg)**
Bay Leaf	130617	298948953
Rosemary	6641	5579250
Sage	18031	264558
Linden	210	51712
Fern	2176	266287
Calbee	18031	264558
Rockrose	93041	32564619
Daisy	255	7969
Upland meadow	4636	260885
Carob	19654	2742481
Lavender	5	6255

Table 2.7 Turkey forest secondary products amount (cont'd)

Plant Names	**Total Area (ha)**	**Total Amount (kg)**
French Lavender	668	1089250
Stringer	504	17873
Gentian	2303	296869

2.10 Gentian Species

In the world's mild areas have substantial gentian species, around 400 different species. Gentian species naturally spread in our country and 45 of them are endemic and belonging to Turkey. In Turkey most known gentian species is St John's Wort with yellowish blossoms and other species of gentian is Common Centaury with pinky red blossoms. These gentian plants can be annual or perennial herbaceous plant structure. Different types of gentian plants are used in public as medicinal plant for treatment of costive, worm in bowel and antispasmodic. Also it can be used as wound healer, burn healer and antiseptic. Dried plant flowers boiled with water and drink as herbal tea and also immersed in olive oil and extracted oil is used on wounds. According to researches gentian plants have efficient activated components such as phenolic components and various terpenoits, as chemical substances hyperforin and hypericin has important roles, because the antidepressant activity of gentian plant comes from these two chemical substances. Mineral materials are important for plants to survive. Main elements of gentian plant to survive are nitrogen (N), potassium (K), phosphorous (P) and the others iron (Fe), copper (Cu), manganese (Mn), boron (B), magnesium (Mg), cobalt (Co), calcium (Ca) and they have important roles in plants life [50].

2.10.1 St John's Wort (*Hypericum Perforatum*)

Gentian plant such as St John's Wort (Figure 2.32) is most known plant in America and Europe as medicinal plant which is used in depression treatments. St John's Wort is known as antidepressant, antimicrobial and antiviral activities [50].

From Siberia to Europe, from North Africa to our country, which has a wide distribution in Central Asia it is also known as common gentian plant. The plant can be found in Hakkari to the Aegean Sea and altitude up to 2500 m above sea level. The plant is 10-110

cm tall, perennial herbaceous plants which is blossoming in April to September. It prevails in swamp and watery areas [51].



Figure 2.32 St John's Wort [52]

2.10.2 Bluebottle (*Centaurea Cyanus*)

Bluebottle (Figure 2.33) plant is one of the gentian plant. It has more than 500 species in the World. In Turkey's flora 61.1% of bluebottle including in endemic area and the 192 taxon of bluebottle can be represented in West, Southwest, Central Anatolia naturally. Bluebottle known with the names such as Bachelor's buton, Cornflower and Knapweed. For years, it is used as chest tightness, fever reducer, diarrhea cutter, pain killer of stomach, menstruation retarder, appetizer. Some types of bluebottle can be used for hemorrhoids, malaria, wound healing and abscess healing. Different researches showed that, bluebottle plant has antibacterial, antioxidant, antiulcerogenic, anti-inflammatory, cytotoxic and antiprotozoal activities. Bluebottle plant has 15 cm to 60 cm tall with branched from the bottom, annual plant. Leaves sparsely hairy, hairless below the plant. The plants flower has blue color on the top [53].

The blossoming period is on April to June in spring to summer period. The plant can be found in pinewood area, stony slopes, garden fields and near the roads that it shows the distribution of sea level up to 1500 m [53].



Figure 2.33 Bluebottle [54]

2.10.3 Common Centaury (*Centaurium Erythraea*)

Common Centaury (Figure 2.34) is seen as herbaceous plant to grassy tree-form structure. The centaury plant has more than 400 species which includes annual and multiyear grower plant. The *Centaurium Erythraea* is one of the medical plant which is grown in Turkey especially in Southeast Anatolia (Kilis, Gaziantep, Adıyaman and Malatya). The blossoming period of Common Centaury plant is on April to June. The plant can be found in dried calcareous stony slopes and steppe areas which has altitude between 360 to 900 meters [50].



Figure 2.34 Common Centaury [55]

2.11 Chitosan

Reams of chitosan studies and its potential on different applications have been reported since 1859, when Rouget founds out the deacetylate forms of chitin as natural polymer [56]. Chitin is raw material of chitosan, chitin is a long chain polymer and it is found in many areas in natural world. Chitin is the main component of the exoskeletons of arthropods, shell of cancers, insects and other crustaceans. Also the cell walls of fungi, beaks of mammals, internal shells of octopuses, squids and cephalopods [57].

Chitosan polymers are semi-synthetically that have unique structures with forms of amino polysaccharides. The structure has unique multidimensional properties, myriad variety of applications in industrial and biomedical areas with its complicated functionalities [58], [59].

Chitosan is a linear β-(1-4) linked polymer composed of 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose residues (Figure 2.35). Chitosan is derived from chitin with a partial deacetylation, and they are biodegradable, biocompatible, nontoxic, hydrating agents and antimicrobial [60], [61], [62]. The deacetylation degree of Chitosan is exponible as a sign of the number of amino groups through the chains and it is estimating as the ratio of D-glucosamine to the sum of N-

acetyl D- glucosamine and D-glucosamine. To be a chitosan, deacetylated chitin might contain at least 60% of D-glucosamine residues [61], [62]. Chitin doesn't have an opportunity to find a place in industry such as chitosan because of poor solubility in organic solvents and aqueous solutions [63].

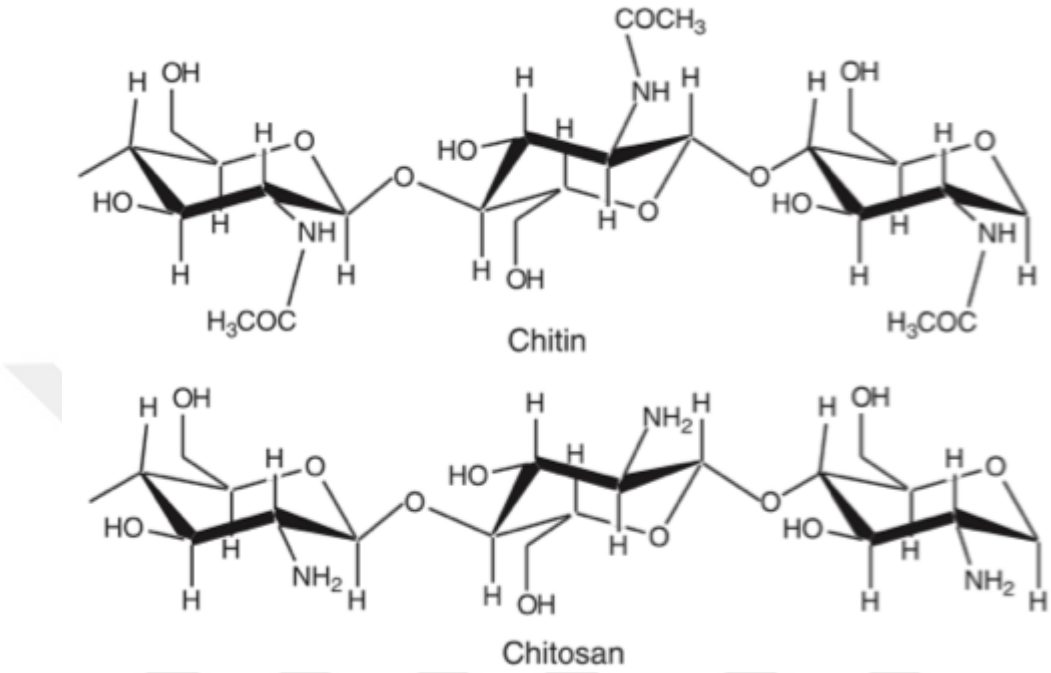


Figure 2.35 Structure of chitin and chitosan [60]

Chitosan will slowly be depolymerized to release N-acetyl-β-D-glucosamine. This commences fibroblast proliferation. It assists in ordered collagen deposition and also, stimulates enhanced level of hyaluronic acid synthesis as natural at the wound area. It prevents faster scar prevention and wound healing [60].

2.12 Extraction Method

Extraction is one of the decomposition method such as distillation. Extraction process can be divided into two groups such as traditional extraction method and new methods. Soxhlet extraction and maceration process is a traditional method with long time processes and large amounts of polluting solvents are used in these processes. Supercritical fluid extraction and microwave extraction are developed in recent years, they are efficient and modern methods or extraction. Temperature is an important factor for an effective extraction. Volatile and semi-volatile compounds ingenerate between the temperatures 40°C-60°C and 80°-100°C. Increase in temperature values cause the formation of artifacts [64].

Solvent extraction is traditional extraction method; in this process the plants can be immersed in the solvent in room temperature or immersed in soxhlet equipment and boiled in organic solvent. In industrial operations, ethanol and hexane used as the organic solvent, in analytical laboratory studies ether and pentan-dichloromethane is used as a solvent. At the end of extraction, the recovered organic solvent was removed from the medium by distillation. The remaining portion, are essentials and fatty compounds. The advantage of this method from the distillation, is the use of low temperatures during the extraction method. Low temperature ensures that the forms a natural content of obtained essential oil than steam distillation. There are two disadvantages of solvent extraction. First of them is, densification process after extraction method caused a loss of low volatile compounds, second is the solvent that recovered cause pollution. This problem is important both economic and environment pollution (toxic properties). Pure and qualified solvents are expensive and large amount of solvents used in method [64].

2.13 Ultrasonic Bath

Ultrasonic vibrations are high frequency sound waves from the audible sound frequencies. In other words, the human ear cannot hear these sounds. The ultrasonic transducer which converts electrical energy into sound energy device that produces sound waves and the energy range of waves are higher than hearing of the ear. If the liquid in contact with a fluid transducer will vibrate with the same frequency. Sound waves rippling constitute expansion-compression zones moves through the liquid. Tiny bubbles in the liquid during the current expansion and consists gaps. This is called as acoustic cavitation (Figure 2.36) [65].

Acoustic cavitation is depending on the acoustic parameters such as temperature, gas content, driving pressure, frequency, and the liquid properties. There are many studies about these parameters of cavitation. Holzfuss et al. [66] found out that usage of dual frequency excitation would increase the cavitation intensity. Chen et al. [67] examined three types of sound waves such as rectangular, triangular and sinusoidal. They found that, sound pressure amplitude with fixed sound, rectangular wave provides high level for single bubble with the intensity of cavitation activity [68].

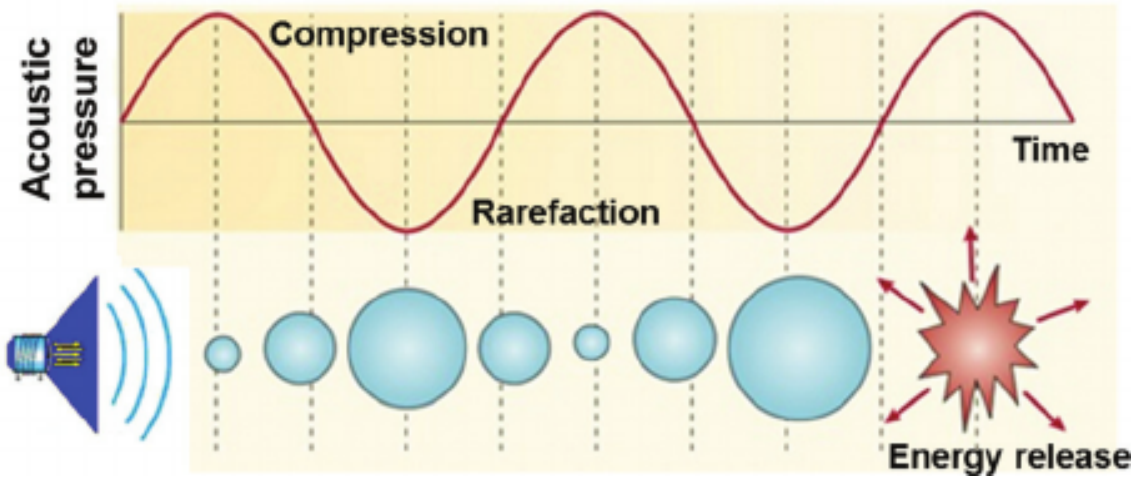


Figure 2.36 Acoustic cavitation [69]

CHAPTER 3

MATERIALS AND METHOD

3.1 Raw Materials

In these experiments, chitosan (low molecular weight) was used as a main material which was obtained from Sigma-Aldrich. The acidic solvent solution was made from acetic acid with distillated water at ratio of 3%. Acetic acid was obtained from Sigma Aldrich, ACS reagent with purity of ≥99.8%. Distillated water was produced with Gesellschaft für Labortechnik (GBL) mbH D-30938 Burgwedel Typ. 2004. The gentian plants were purchased from local markets in Turkey. In the extraction method ethanol was used as a solvent which was obtained from Rysseen Alcools S.A.S., France with purity of 96% pharma grade. The bacteria's Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) were obtained in the 15 ml of sterilized falcon tubes from Boğaziçi University, department of molecular biology and genetics. The nutrient broth was obtained from Merck Millipore with pH value:7.0 (8 g/l, H_2O, 37 °C after autoclaving) and solubility: 8 g/l under 20 °C. The nutrient broth agar petri dishes with 90 mm were obtained from Diatek, Hypet Media Nutrient Agar. In the swelling tests, Phosphate-buffered saline solution (PBS) with 7.2 pH was obtained from was obtained from ChemBio Laboratory Research in 500 ml bottle.

3.2 Preparation of Essential Oils

Essential oils were acquired with liquid-solid extraction method. The soluble components of solid materials are transferred to solvent solution. Soxhlet extraction is one of these liquid-solid extraction method which has flush reflux of solvent onto solid material. The dried gentian plants (Figure 3.1) were grinded and 3g of each plant was measured with analytical balance equipment (Ohaus Pioneer Analytical Balance) for each chemical

cellulose cartridge which was used in Soxhlet extraction equipment (Velp Scientifica Ser 148) (Figure 3.2).



Figure 3.1 The gentian species

Soxhlet extraction was carried out with ethanol extraction at 210°C with 3 steps. Soxhlet extraction has three fundamentals such as, immersion, washing and recover respectively. On the immersion step, dried plant specimens were heated up to 210°C with ethanol for one hour. Then, on the washing step, the ethanol was refluxed with flushing method for one hour again. At the end, last step was recover step, the ethanol was recovered to solvent chamber and stored for reuse.

The obtained essential oils were stored in amber glass bottles of 40 ml (Figure 3.3) in the fridge (Arçelik A++, +5°C ± 2°C) until is used for characterization analysis and production of wound dressing films.



Figure 3.2 Soxhlet extraction equipment



Figure 3.3 The bottled essential oils of gentian species

3.3 Preparation of Wound Dressing Films

The films are produced in the ultrasonic water bath (Figure 3.4- ISOLAB - 6 Liter). Ultrasonic bath; transmitting the ultrasonic wave through a liquid when the intensity of ultrasound wave is high enough, micron sized gas bubbles could be excited to vibrate, expand, and burst inward violently. This phenomenon is called as ‘‘acoustic cavitation”

[68], also this phenomenon ensures saving time in the experiments. Because of acoustic cavitation, molecules of chitosan and acetic acid was run into each other faster than magnetic saturation (Figure 3.5).



Figure 3.4 Ultrasonic bath



Figure 3.5 Dissolution of chitosan with acetic acidic in ultrasonic bath

Chitosan was dissolved under pH<6 [70] and according to these researches 10%, 5% and 1% (v/v) [70] of acetic acid solution would be the perfect solvent for best dissolution. In the experiments, 3%, %2 and %1 (v/v) of solutions were prepared and dissolution of chitosan was observed. All percentage of solutions were under pH-2 and all of these solutions dissolved chitosan but the 3% (v/v) was more acidic and dissolution was

observed in less time. For that reason, 3% (v/v) of acetic acid solution was chosen and used as a solvent solution of chitosan in the experiments.

About 0.5 g of chitosan powder were dissolved in 25 ml of acetic acid solution (3% v/v), according to Altiok 2010 [29]. Before starting to main experiments, temperature differences on dissolution were examined. The dissolution of chitosan was investigated at 80°C, 60°C, 40°C, 30°C and 20°C. Their dissolution parameters such as time (min), temperature (°C) and observation explications were shown in Table 3.1 In consequence of these pretests of dissolution, 20°C, 30°C and 40°C were chosen as acceptable temperatures. After that pretesting, the drying temperatures would be considering forward experiment parameters.

Table 3.1 Dissolution parameters of chitosan

Chitosan(0.5g) / **Temperature (°C)**	**Time**	**Observation**
80	120 min	The chitosan acetic acid solution stuck on the beaker's wall and it burned.
60	120 min	The chitosan acetic acid solution stuck on the beaker's wall and it burned.
40	150 min	The chitosan acetic acid solution stuck on the beaker's wall and it parched.
30	180 min	The few amount of chitosan acetic acid solution stuck on the beaker's wall and it just harden a bit.
20	270 min	The chitosan dissolved in acetic acid properly and it didn't stick on beaker's wall.

The essential oil ratios of gentian plant were chosen as 1%, 0.75%, 0.5% and 0.25% (w/w) of total weight, according to literature studies which was suggested for better antibacterial efficiency [4], [22], [29]. As it was mentioned before, all essential oils were obtained with soxhlet extraction method in laboratory conditions, also there was not any other chemical additions, just an ethanol which was used as a solvent in the experiments. Figure 3.6 shows the essential oils which are measured for 0.5% (w/w) addition to experiments.

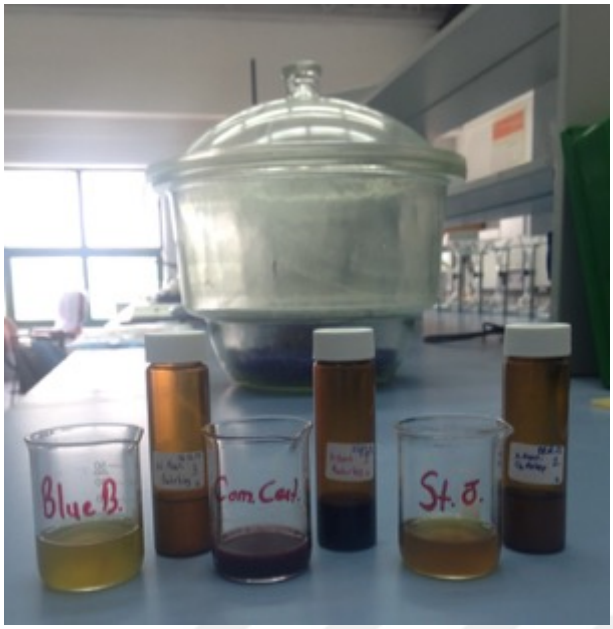


Figure 3.6 Bluebottle, Common Centaury, St. John's Wort, respectively

Besides that, blue bottle plant's essential oil (EO) was chosen as a pretesting material. Bluebottle plant's essential oil, which was the 0.5% (w/w) of total weight, was added in to chitosan acetic acid solution in ultrasonic bath. First experiments of chitosan-essential oil films were carried out under the five different temperature conditions which was shown on Table 3.2 and their codes are given in Table 3.3. The control group of chitosan films were carried out under the last three temperatures such as 40°C, 30°C and 20°C, because 80°C and 60°C temperatures were burned the films in the drying part in drying oven (Ecocell-55L).As a result of these observation, last three temperatures which were 40°C, 30°C and 20°C were chosen as pretesting temperatures for production of films. Drying conditions of chitosan-essential oil films were fixed to their reaction temperatures. The reaction time, temperature data's and drying condition observations of chitosan-essential oil films of pretesting experiments were shown in Table 3.2.

The obtained films were characterized and after pretesting experiments, the reaction under 20°C were chosen as an optimum reaction condition. Optimum condition had 4 hours (h) and 30 minutes (min) of dissolution time, 1 hour to 2 hour of reaction time in ultrasonic bath (Figure 3.7) and it ends with drying under room conditions for 5 days in fume cupboard. After that, dried films were stored in desiccators until it would use in characterization analyses and antibacterial testes. The experiment data set were shown in Table 3.4.

Table 3.2 Pretesting experiment parameters of chitosan-essential oil films

Temp. (°C)	**Chitosan (g)**	**Dissolution Time (min)**	**Reaction Time (min)**	**Drying Temp. (°C)**	**Drying Time (hour)**	**Observation**
80	0.5	120	60	80	24	Burned
60	0.5	120	60	60	24	Burned
40	0.5	150	60	40	36	Suitable
40	0.5	150	120	40	36	Suitable
30	0.5	180	60	30	60	Suitable
30	0.5	180	120	30	60	Suitable
20	0.5	270	60	20	120	Suitable
20	0.5	270	120	20	120	Suitable

Table 3.3 Data set of pretesting chitosan film and chitosan-essential oil film production

Temp. (°C)	**Chitosan (g)**	**% of EO**	**Dissolution Time (min)**	**Reaction Time (min)**	**Drying Temp. (°C)**	**Drying Time (hour)**	**Code**
80	0.5	0.5	120	60	80	24	B-80-60-0.5
60	0.5	0.5	120	60	60	24	B-60-60-0.5
40	0.5	0	150	90	40	36	R-40-90
40	0.5	0.5	150	60	40	36	B-40-60-0.5
40	0.5	0.5	150	120	40	36	B-40-120-0.5
30	0.5	0	180	90	30	60	R-30-90
30	0.5	0.5	180	60	30	60	B-30-60-0.5
30	0.5	0.5	180	120	30	60	B-30-120-0.5
20	0.5	0	270	90	20	120	R-20-90

Table 3.3 Data set of pretesting chitosan film and chitosan-essential oil film production (cont'd)

Temp. (°C)	Chitosan (g)	% of EO	Dissolution Time (min)	Reaction Time (min)	Drying Temp. (°C)	Drying Time (hour)	Code
20	0.5	0.5	270	60	20	120	B-20-60-0.5
20	0.5	0.5	270	120	20	120	B-20-120-0.5

Table 3.4 Data set of chitosan-essential oil film production

Chitosan (g)	Essential Oil	% of EO	Temperature (°C)	Reaction Time (min)	Code
0.5	Bluebottle	1%	20	60	B-20-60-1
0.5	Bluebottle	0.75%	20	60	B-20-60-0.75
0.5	Bluebottle	0.5%	20	60	B-20-60-0.5
0.5	Bluebottle	0.25%	20	60	B-20-60-0.25
0.5	St John's Wort	1%	20	60	S-20-60-1
0.5	St John's Wort	0.75%	20	60	S-20-60-0.75
0.5	St John's Wort	0.5%	20	60	S-20-60-0.5
0.5	St John's Wort	0.25%	20	60	S-20-60-0.25
0.5	Common Centaury	1%	20	60	C-20-60-1
0.5	Common Centaury	0.75%	20	60	C-20-60-0.75
0.5	Common Centaury	0.5%	20	60	C-20-60-0.5

Table 3.4 Data set of chitosan-essential oil film production (cont'd)

Chitosan (g)	**Essential Oil**	**% of EO**	**Temperature (°C)**	**Reaction Time (min)**	**Code**
0.5	Common Centaury	0.25%	20	60	C-20-60-0.25
0.5	Bluebottle	1%	20	120	B-20-120-1
0.5	Bluebottle	0.75%	20	120	B-20-120-0.75
0.5	Bluebottle	0.5%	20	120	B-20-120-0.5
0.5	Bluebottle	0.25%	20	120	B-20-120-0.25
0.5	St John's Wort	1%	20	120	S-20-120-1
0.5	St John's Wort	0.75%	20	120	S-20-120-0.75
0.5	St John's Wort	0.5%	20	120	S-20-120-0.5
0.5	St John's Wort	0.25%	20	120	S-20-120-0.25
0.5	Common Centaury	1%	20	120	C-20-120-1
0.5	Common Centaury	0.75%	20	120	C-20-120-0.75
0.5	Common Centaury	0.5%	20	120	C-20-120-0.5
0.5	Common Centaury	0.25%	20	120	C-20-120-0.25
0.5	-	%0	20	60	R-20-60
0.5	-	%0	20	120	R-20-120



Figure 3.7 Bluebottle-chitosan film, Common Centaury-chitosan film and St. John's Wort-chitosan film in ultrasonic bath, respectively

3.4 Swelling Tests

The film samples were cut into 1mm*1mm squared form and they were stored in desiccator until they used for swelling tests. Before testing specimens, their weight was measured and initial dry weight (W_i) was considered. Pre-weighted dry specimens of reference and essential oil based chitosan films were immersed in PBS solution at a given interval. PBS solution had 7.2 pH and 25 ml of solution was poured into 50 ml of falcon tubes for swelling tests. Specimen tubes were kept in room temperature for 9 hours at same day and after 1 hour, 3 hours, 5 hours, 7 hours and 9 hours the specimens were weighted respectively. The final weight (W_f) of specimens were measured after 3 days and it was kept in room temperature same as for 9 hours' test. All specimens were taken out of falcon tubes after the appointed hours such as after 1h, 3h, 5h, 7h and 9h. The swollen films weight was measured after removing overflow of PBS solution using a filter paper, the specimens were wiped between ordinary filter papers to remove surface PBS solution. Then the swollen film's weight was measured to determine final weights (W_f) after decided hours. The experiments were repeated for three times, then the average value was taken as swelling ratio (SR). Swelling ratio is percentage of weight gaining of each film. The mathematical equation for swelling ratio is given below;

$$SR = \frac{W_f - W_i}{W_i} \times 100 \quad (3.1)$$

In the above formula W_f and W_i are the weights of final and initial dressing films respectively and SR is the calculated swelling ratio of each specimen.

3.5 Antibacterial Tests

All bacteria were obtained from Department of Molecular Biology and Genetics of Boğaziçi University. The bacteria E. coli and S. aureus were obtained in 15 ml of falcon tubes with 6 ml of bacteria solution. The bacteria solutions were kept in fridge (Arçelik A++) at +4 °C ±1 °C until it would use for re-activing process. The reserved bacteria of E.coli and S. aureus stocks were re-activated in Nutrient Broth, 100µl of bacteria solution was infused to 15 ml of sterilized falcon tube, then it completed to 11 ml with Nutrient Broth solution, the prepared falcon tubes were incubated at 37°C for 24 hours by using shaker incubator at100 rpm (ZHWY-200B Incubator Shaker-Figure 3.8) After these incubation, from the bacteria suspensions, three serial, 1/100 dilutions were made with NaCl solution (8.5g /1Liter) and 100µl were plated on the Nutrient Agar petri dishes from the last dilution. After that the colonies on the agar plate were counted and original sample CFU/ml unit was calculated. The equation of CFU/ml measurement is given below:

$$\frac{CFU}{ml} = \frac{(number\ of\ colonies \times dilution\ factor)}{volume\ of\ culture\ plate} \tag{3.2}$$

The equation given above is calculation of the number of bacteria in 1ml (1000µl) of the original sample solution. According to control plates colony counts the original sample CFU/ml was found, then 100µl of bacteria solution was plated and spread on Nutrient Broth agar plate and reference and essential oil based film discs with 12 mm diameter were placed on the nutrient agar plates simultaneously. All planted plates were incubated (BLULAB 55 Liter Incubator- Figure 3.9) under 37°C for 24 h. The plates cultured with no addition of any films or antibiotics under the same condition were used as control. After the incubation of 24 hours the clear zones around the film discs were measured and wrote down as inhibition zone that indicate antibacterial property.



Figure 3.8 Incubator shaker

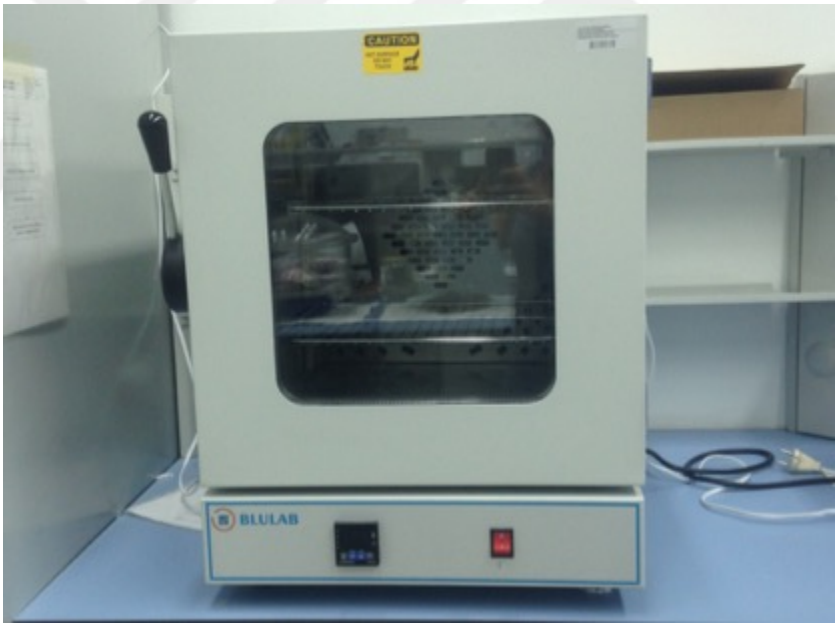


Figure 3.9 Incubator

3.6 Characterization of Experiments

In this study, Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES), UV-Visible Spectrometer (UV-Vis), Fourier transform infrared spectroscopy (FT-IR), Differential Thermal Analysis and Thermogravimetric Analysis (DTA-TG) and X-ray Diffraction (XRD) devices used for analyze raw materials and produced films of the study.

The analysis of ICP-OES, UV-Vis and FT-IR occurs through spectroscopy conditions under atomic level. The processes in spectroscopy depend on the interaction of the light, which named electromagnetic radiation, with a material and the pursuer energy transfer ends with three main processes which are absorption, fluorescence and emission.

The absorption process is transferred to the molecule or atom brings up them from the ground state to an excited state by the energy of the light, in the form of photons.

The fluorescence is absorbed energy in the system and weaken back to the ground state which is rapidly lost to the enclosing by clash. Occasionally the energy isn't missing in this way but is reemitted a few mile seconds later, which is named as fluorescence.

The emission, the material atoms or molecules are heated up to high temperatures within an electric discharge or a flame, the electrons are excited to higher energy levels. After that, with the emission of the radiation, they weaken to the ground state, the magnitude of which is more or less equivalent to absorbed energy. All these three processes become through spectroscopy.

Generally, the analytical techniques are based on the light interactions. The light interactions of substances at various wavelengths are used in different types of analysis and instruments. The entire light spectrum is shown in Figure 3.10, the light has magnetic and electrical components and it is known as "Electro-Magnetic Spectrum". Electro-Magnetic Spectrum is classification of electromagnetic waves of light according to their various frequencies and wavelengths. The figure xx shows all types of electromagnetic radiation of our universe [71], [72].

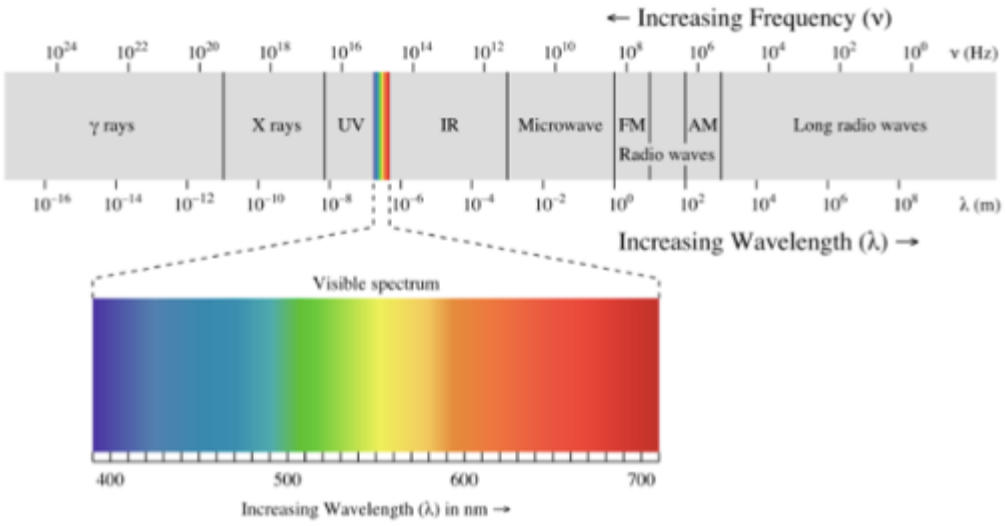


Figure 3.10 The electromagnetic spectrum [72]

The thermal analysis techniques virtually involve of a series of methods. The methods detect the changes in mechanical and physical properties of the given material by usafe of thermal energy of heat. The physical properties comprise temperature, mass, enthalpy, dynamic characteristics and dimension etc. According to these properties finding the integrity, thermal stability, crystallinity and purity of chemical materials can be evaluable. Occasionally, this technique can be used as determination of the composition of complex mixtures. In quality control in the process control of production field or material inspection this technique has been adopted as a standard requirement. It can be applied in lots of fields such as glass, metals, ceramics, polymer, semiconductors, foods, medicines and explosives [71].

3.6.1 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)

Atomic emission analysis by atomic emission spectroscopy creates a general method. The radiation is emitted by the atoms of measuring elements on the research and their atoms would be present in an excited state, generally following ionization. When a chemical element emits a characteristic radiation, it would be excited from the atomic state to appropriate conditions. These method is based on both qualitative and quantitative observations of general form of elemental analysis. This optical emission spectroscopy (OES) derived from sample is very complex because of that it leads to spectra with millions of spectral lines in beside the point by a continuum background. The ICP instruments are designed for these analysis includes several parts such as the device which is responsible for the carrying the sample in the form of excited or/and ionized atom based on gas plasmas, good quality optical bench which would be condition the analytical performances, a detector which includes a sensor and a computerized section, it is essential to control the all instrument. The parts of Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) instrument and the running principle is shown in Figure 3.11 [73].

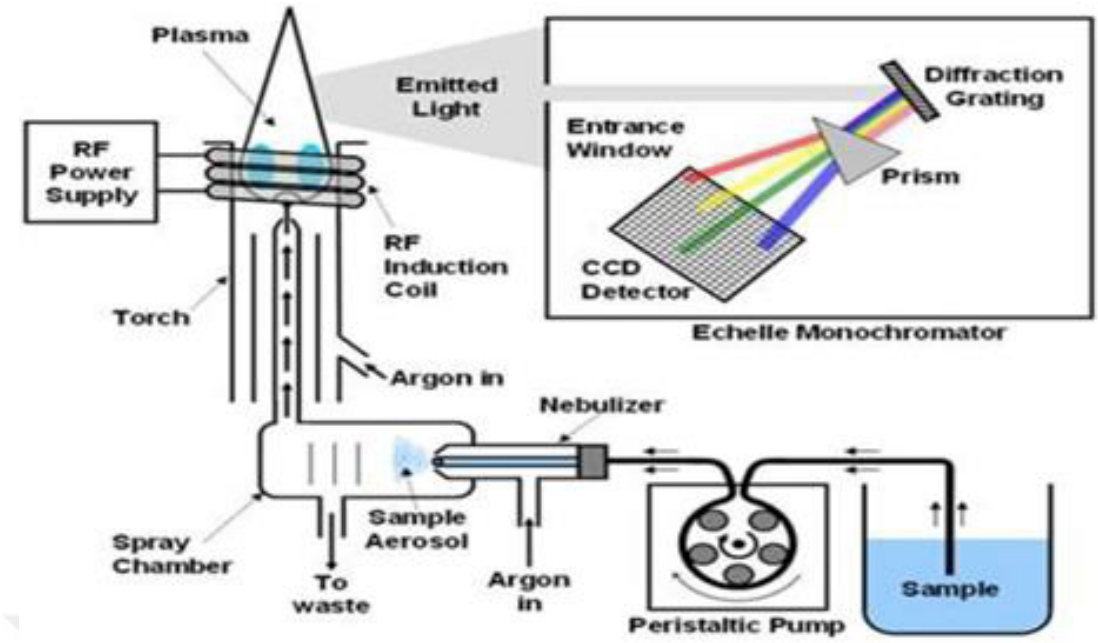


Figure 3.11 The ICP-OES Instrument parts and it's running principle [74]

Inductively Coupled Plasma (ICP) Optical Emission Spectrometer (OES) is one of the most required and significant analysis technique which is used for determining the trace elements in number of many sample types. It is an atomic emission technique, which is running in high temperature gas plasma and the atoms and ions present in the sample are excited. Thenceforth the plasma ensures very high temperature and thereby the energy, nearly all the atoms which is presented in the sample can be excited by the high temperature energy and this technique ending up with really high efficiency. The high temperature hotter source increases both excitation efficiency and atomization efficiency. Thus, even very small concentrations of metal ions and metals can be determined and accurately measured with this technique, for that reason the emission from the atoms are very intensive [71].

The ICP-OES analysis were done with Perkin-Elmer Optima 2100 DV model with an AS-93 auto sampler in the experiments (Figure 3.12). All samples and blank solutions were measured by ICP-OES twice.

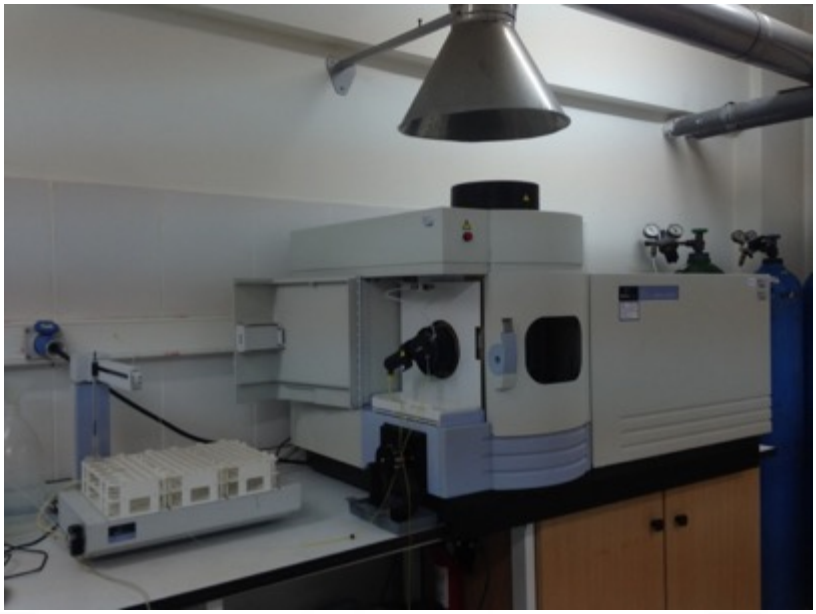


Figure 3.12 Inductively coupled plasma optical emission spectrometer (ICP-OES)

The grinded Gentian plants powder was prepared for ICP-OES using by microwave method. In this method, 0,1g of grinded Gentian plants were digested with 10ml of HNO_3 in microwave digestion system (Berghof MWS 3+) Figure 3.13, according to the digestion program presented in Table 3.5.

Table 3.5 Operating conditions for the microwave digestion system

Step	**Temperature (°C)**	**Ramp Time (min)**	**Hold Time (min)**
1	180	5	5
2	190	10	5
3	210	10	5
4	100	1	1



Figure 3.13 Microwave digestion system

3.6.2 UV-Visible Spectrometer (UV-Vis)

The absorption of electromagnetic radiation by matter has been studied comprehensively. The domain range was from the very near infrared to near ultraviolet (180 to 1100 nm). This type of the electromagnetic spectrum called as "UV/Visible" since it has recognizable radiation with human eye, generally proceeds few structural information but it is really useful for quantitative measurements. UV-Vis measurements are running on the Lambert-Beer Law. The representative diagram of UV-Vis instrument is shown in Figure 3.14. The measurements of absorbance level at some wavelengths determines the concentration of specimen solutions. This method is generally known as colorimetry. According to colorimetry, it applies not only to some absorbance wavelength spectrum of compounds possessing in that spectral region, also pursuer modification by some specific reagents which, leads to derivatives the warrant absorption measurement. The spectrum regions are conventionally divided into three parts such as near UV, visible and very near infrared and their wavelengths are between 185-400 nm, 400-700 nm and 700-1100 nm respectively. Usually commercial UV-Vis spectrophotometers contains the spectral range of 185 to 900 nm [73].

It includes the electromagnetic radiation of the absorption by the compound solutions in the visible and ultraviolet regions of the spectrum. According to electromagnetic radiation, the electronic structure of substances will change, the molecules and ions change through the excitations of non-bonded and bonded electrons [71].

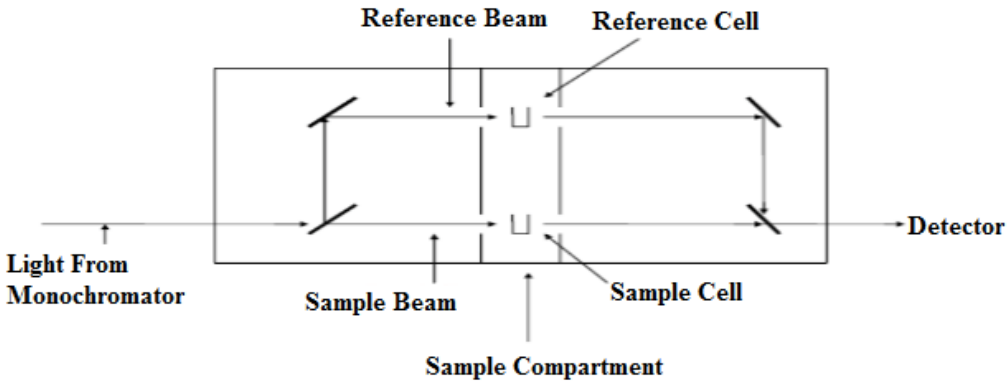


Figure 3.14 The representative diagram of UV-Vis [71]

The essential oils which were obtained from the Gentian plants was taken and diluted to 1/100 and 1/150 (Figure 3.15) before using in UV-Visible Spectrometer shown in Figure 3.16 (Perkin Elmer Lambda 35).

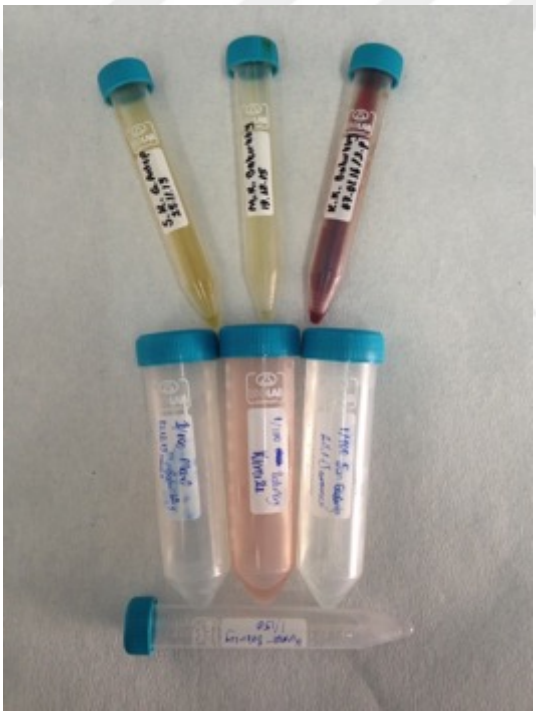


Figure 3.15 Diluted bottles of gentian plants



Figure 3.16 UV-Vis spectrometer

The spectrums of UV-Visible spectrometer were recorded from 235 to 610 nm. The glass and quartz cells can be used in the analysis if the measurement is made under visible region, for that reason quartz cell should be used in the measurements under ultraviolet region, because glass cell absorbs ultraviolet rays, according to these knowledge, quartz cell is used to determine spectrums of Gentian plants.

The UV-Vis Spectrometer has double-beam system, and it measures the transmittance of the specimen and reference solvent simultaneously. When one beam passes through the specimen, the other passes through the reference solution. Usually, spectrometers use mirrored rotating chopper wheel which has one or two mirrors to alternately direct light beam through the reference and sample cells. Also, it warrants the detector to compare two intensities transmitted by sample of reference solutions for the same absorbance wavelength. Double beam UV-Vis spectrometers permit differential measurements to be made between the analytical blank and sample [73].

3.6.3 Fourier transform infrared spectroscopy (FT-IR)

The Fourier transform infrared spectrometer (FT-IR) is an interferometric measurement which undertake a synchronical analysis of the all spectral region. In these type of instrument uses Michelson type interferometer of some kind of similar device, united to a specialized microprocessor for measuring and calculating the spectrum. This spectrometer has a single beam optical assembly with, as an essential component. It has

three parts such as moving mirror, a fixed mirror and the beam splitter [73]. But it was traditionally designed with double beam comprising IR source, grating monochromator, thermocouple detector, sodium chloride or potassium bromide cells and etc. but it is outdated because the light dispersed from the monochromator. Since, a newer technique which is known as FT-IR is in practice. As it mentioned before, it has single beam of undispersed light and the other parts of instrument is same as the traditional one [71].

It includes the electromagnetic radiation absorption with infrared region. The resulting spectrums is depending on the vibrational energy of molecules. In order that, generally all molecules have vibrations in the form of bending, stretching etc. The energy absorption evaluated in changing energy levels interrelating with them. It is a precious and formidable tool in identifying polar chemical bonds in the organic compounds such as OH, NH, CH and etc. with fine charge separation (strong dipoles) [71].

In FT-IR process, the light beam (undispersed) is passed through the sample. All wavelengths of the absorbance values are received with the detector contemporaneously. Fourier transform is known as a computerized mathematical manipulation of all these wavelengths. FT-IR instrumentation contains two mirrors, one moveable and one fixed with a beam splitter in between them to perform calculations of interference of light pattern. Before starting to scan the sample, a blank or a reference scanning is required. The simplified design of FT-IR instrument is shown in Figure 3.17.

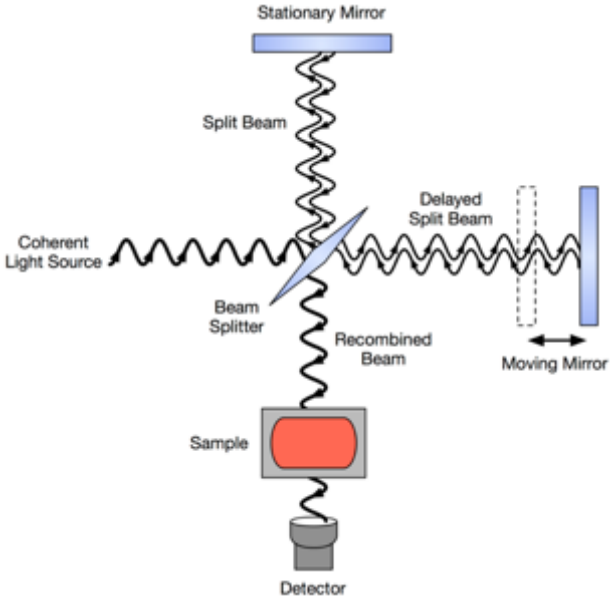


Figure 3.17 Schematic diagram of a Michelson interferometer, configured for FT-IR [75]

The films with 1%, 0.75%, 0.5%, 0.25% and 0% (w/w) essential oil contents and pure powdered chitosan were measured with FT-IR between 650 nm to 4000 nm. The FT-IR analysis were done with Perkin-Elmer Spectrum One FT-IR Spectrometer (Figure 3.18).



Figure 3.18 FT-IR instrument

3.6.4 X-ray Diffraction (XRD)

X-ray powder diffraction (XRD) is an instrumental technique that is usually used to study crystalline materials (Figure 3.19). The three-dimensional structure of non-amorphous materials is defined by regular, repeating planes of atoms that form a crystal lattice. When a focused X- ray beam interacts with these planes of atoms, part of the beam is transmitted, part is absorbed by the sample, part is refracted and scattered, and part is diffracted. When an X- ray beam hits a sample and is diffracted, we can measure the distances between the planes of the atoms that constitute the sample by applying Bragg's Law. Bragg's Law is $n\lambda=2d \sin\theta$, where the integer n is the order of the diffracted beam, λ is the wavelength of the incident X-ray beam, d is the distance between adjacent planes of atoms (the d-spacings), and θ is the angle of incidence of the X-ray beam. Since we know λ and we can measure θ we can calculate the d-spacings. The characteristic set of d-spacings and theirs intensity generated in a typical X-ray scan provides a unique "fingerprint" of the phases present in the sample. When properly interpreted, by comparison with standard reference patterns and measurements, this "fingerprint" allows for identification of the material [76].

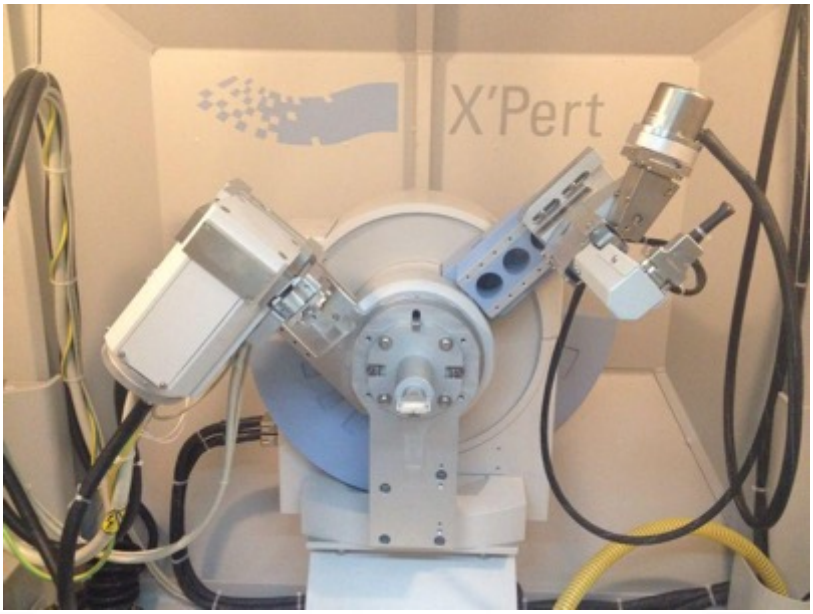


Figure 3.19 XRD Device

The characterizations of raw materials were determined with Philips PAN analytical X-Ray Diffraction (XRD) device under the 45kV and 40mA parameters with the X-Rays from the Cu-Kα tubes. The essential oil film and pure film samples were scanned from 5° to 60° , 2θ at a scanning rate of 0.06°min^{-1}. The film sample was shown in Figure 3.20.



Figure 3.20 The film in the XRD specimen vessel

3.6.5 Differential Thermal Analysis and Thermogravimetric Analysis (DTA-TG)

In this technique Differential Thermogravimetric (DTG), the sample is heated up to constant temperature or heated up at a constant rate under different atmospheres such as nitrogen (inert), air (oxidative). The change in sample weight is measured when the

heating occurs. This is an effective in quantitative analysis of thermal changes which is related to thermal reactions. Thermal reactions under the heat increases accompanied by mass difference, such as decomposition, desorption, gas absorption, evaporation and dehydration. In the heating process the temperature would be high as 1500°C inside the instrument furnace. The mass change of sample during the measurement is connected to microbalance, it has a significant role, because it affects the equilibrium of the balance. This imbalance is fed back to a force coil, which generates additional electromagnetic force to recover equilibrium. The amount of additional electromagnetic force is proportional to the mass change [71].

Differential Thermal Analysis (DTA) measures the temperature difference between a specimen and reference material. The materials are cooled or heated at constant temperature which is known as isothermal process, and the function of the temperature change is determined as DTA. In this technique the reference material and the sample are heated or cooled at the same time with constant rate. The difference of temperature change between the reference and sample are measured as a mathematical function. It gives information about the materials endothermic and exothermic behavior which studied under this technique. Figure 3.21 shows the instrument schema [71].

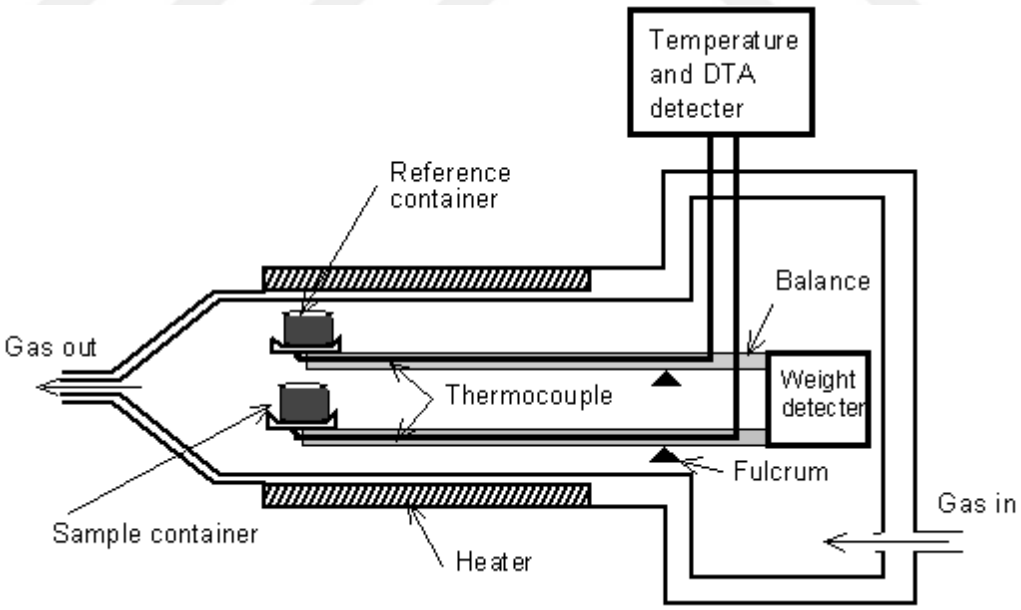


Figure 3.21 The DTA-TG instrument schema



Figure 3.22 TG-DTA instrument

In this study, Perkin-Elmer Diamond TG-DTA instrument is used in thermal analysis (Figure 3.22). Thermogravimetric (TG) measurement was performed under temperature range of 50–610°C with scan rate of 10°C/min under nitrogen atmosphere. Specimen weights are measured in the range of 3-4 mg in a platinum pan with adding of alumina.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 XRD Results of Chitosan Films

XRD results of chitosan films are separated by pretesting specimens and main specimens. All results showed that chitosan films have amorphous structure but some of them showed better crystallites.

Table 4.1 XRD results of chitosan films with different temperatures

R-40-90							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.6877	0.656	49.08	44	7.57176	38.97	39.49
2	25.8674	0.082	9.57	38	3.4444	60.76	61.58
3	**26.6241**	**0.0615**	**11.65**	**34.03**	**3.34819**	**98.68**	**100**
4	28.7525	0.123	9.91	24	3.10501	41.96	42.52
5	32.8148	0.123	5.59	15	2.72931	23.69	24.01
6	42.954	0.45	21.26	13	2.10391	24.61	24.94
R-30-90							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5419	0.2116	7.68	34	7.66704	54.4	82.83
2	**26.4902**	**0.2952**	**19.12**	**38.49**	**3.36482**	**65.67**	**100**

Table 4.1 XRD results of chitosan films with different temperatures (cont'd)

R-30-90							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
3	28.689	0.2362	6.75	29	3.11173	28.96	44.1
4	32.7112	0.3542	5.58	22	2.73772	15.96	24.31
5	42.7889	0.5904	17.22	14	2.11339	29.57	45.02
6	54.9024	0.864	9.6	7	1.67096	8.34	12.69
R-20-90							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.6129	0.9446	37.53	49	7.62033	40.27	43.4
2	24.8871	0.3542	8.9	42	3.57781	25.48	27.46
3	25.8854	0.1181	7.29	38	3.44204	62.59	67.45
4	**26.6288**	**0.1476**	**13.51**	**34**	**3.34762**	**92.79**	**100**
5	28.8192	0.1771	5.91	24	3.09797	33.82	36.45
6	32.8249	0.1771	4.88	16	2.7285	27.94	30.11
7	42.8186	0.72	22.8	10	2.11025	23.76	25.6

Chitosan film produced under 40°C has best peak height but that does not mean it has not amorphous structure, but for chitosan films 40°C comprise better crystallinity. From the Figure 4.1, the XRD patterns of chitosan films can be seen.

Pretesting of chitosan essential oil based films made with addition of 0.5% (w/w) bluebottle essential oil to chitosan-acetic acid solution, for to find best reaction temperature and time produced films' XRD analysis was determined. XRD results of reaction that undergoes 20°C, 30°C and 40°C with reaction times of 60 minutes and 120 minutes are shown in Table 4.2.

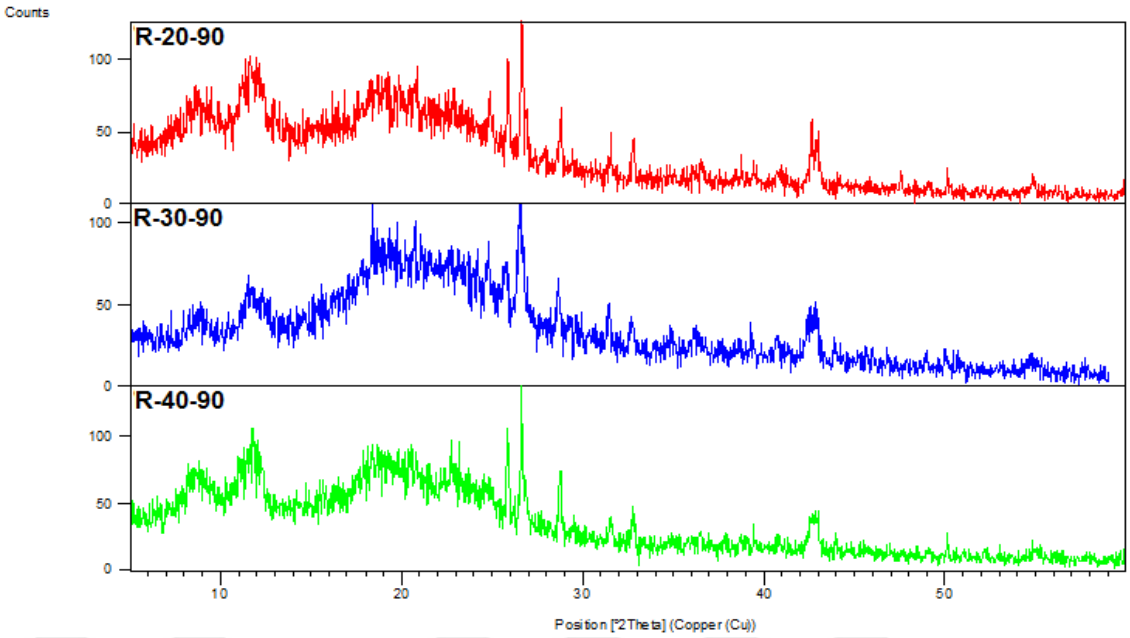


Figure 4.1 XRD patterns of chitosan films that are produced under different temperatures

Table 4.2 XRD results of pretesting chitosan films

B-40-60-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.0857	0.3001	8.42	45	7.98149	42.09	67.45
2	25.934	0.1771	7.29	39	3.4357	41.74	66.89
3	**28.7986**	**0.0866**	**3.6**	**26**	**3.10014**	**62.4**	**100**
4	42.9266	0.72	22.84	12	2.10519	23.8	38.13
B-30-60-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.3147	0.9446	21.88	41	7.82051	23.48	43.67
2	25.834	0.3227	11.49	45	3.44878	53.41	99.34
3	**26.572**	**0.1771**	**9.39**	**41.77**	**3.35464**	**53.76**	**100**
4	32.835	0.09	0.06	24	2.72768	1	1.86
5	42.6583	0.72	20.61	19	2.11781	21.47	39.94

Table 4.2 XRD results of pretesting chitosan films (cont'd)

B-20-60-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.6172	0.8266	26.29	47	7.61753	32.24	40.21
2	18.2274	0.9446	28.16	55	4.86722	30.22	37.68
3	25.8787	0.2362	9.79	39	3.44293	42.03	52.41
4	**26.5907**	**0.1771**	**14.01**	**36**	**3.35233**	**80.2**	**100**
5	28.7757	0.1771	4.97	27	3.10256	28.43	35.45
6	32.8085	0.3542	5.72	20.88	2.72982	16.37	20.41
7	42.8075	0.72	20.82	12	2.11077	21.69	27.04
B-40-120-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.4717	0.9446	28.76	46	7.71384	30.86	44.47
2	**26.6011**	**0.0886**	**6.06**	**38**	**3.35104**	**69.4**	**100**
3	28.7843	0.1771	6.3	26	3.10165	36.08	51.98
4	32.7888	0.3542	6.31	19.54	2.73142	18.04	26
5	42.8277	0.72	18.91	15	2.10982	19.69	28.38
B-30-120-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.6158	0.5904	23.97	44	7.61846	41.16	59.29
2	17.9533	0.9446	34.68	56.94	4.94088	37.21	53.61
3	25.8632	0.1771	8.2	42	3.44495	46.95	67.63
4	**26.6144**	**0.1181**	**8.09**	**38**	**3.3494**	**69.42**	**100**
5	28.7577	0.1771	6.09	28	3.10446	34.87	50.23

Table 4.2 XRD results of pretesting chitosan films (cont'd)

B-30-120-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
6	42.8508	0.72	18.2	15	2.10874	18.95	27.3
B-20-120-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5584	0.9446	28.56	41	7.65614	30.65	42.83
2	25.9422	0.1771	6.7	37	3.43463	38.34	53.58
3	**26.6805**	**0.1181**	**8.34**	**34**	**3.34125**	**71.56**	**100**
4	28.8615	0.1621	4.59	29	3.09353	42.5	59.39
5	42.8226	0.576	13.93	15	2.11006	18.13	25.34

At 20°C and reaction for 60 minutes gives highest peak height, also the reaction time 120 minute gives best peak with 20°C too. That means as a result of these pretesting period 20°C is chosen as optimum reaction temperature from XRD results. Also Figure 4.2 and Figure 4.3 shows XRD patterns of pretesting chitosan films with addition of 0.5% (w/w) bluebottle gentian plants essential oil.

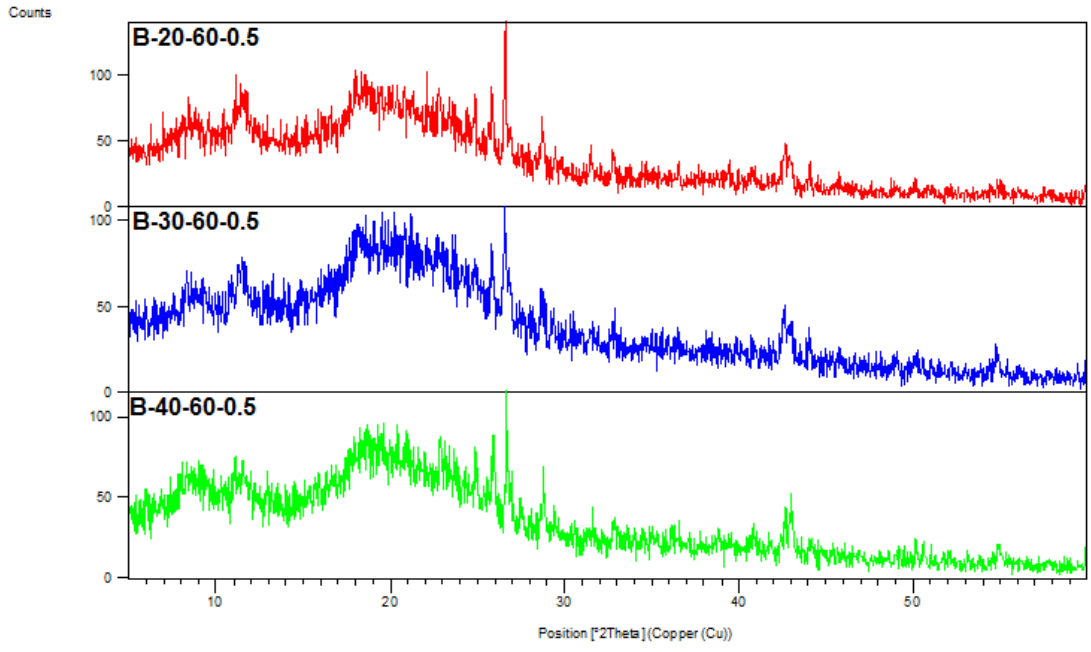


Figure 4.2 XRD patterns of 60 minutes' reactions

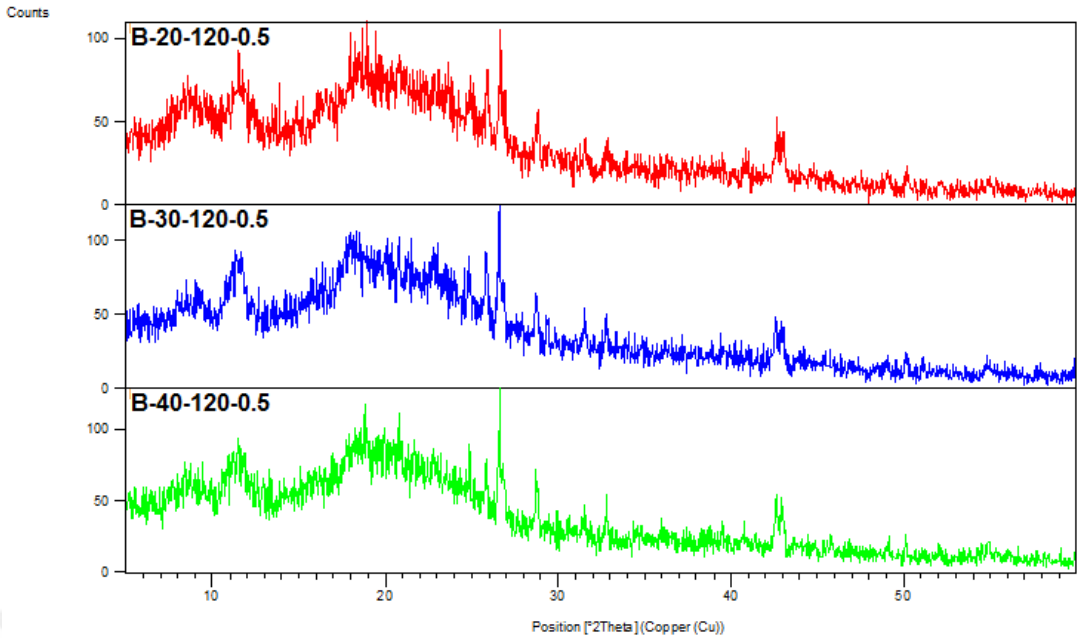


Figure 4.3 XRD patterns of 120 minutes' reactions

Main specimen of Bluebottle gentian plant's XRD results showed that best peak reached with addition of 0.5% Bluebottle essential oil, then 0.25%, 0.75% and 1% (w/w), respectively. Table 4.3 shows the XRD results of Bluebottle essential oil chitosan based films. Also Figure 4.4 shows the XRD patterns of same films.

Table 4.3 XRD results of chitosan films with addition of Bluebottle essential oil (60min)

B-20-60-0.25%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5742	0.7085	22.44	44	7.64575	32.11	35.53
2	18.5085	0.9446	28.15	47	4.79392	30.21	33.43
3	25.9309	0.1771	8.77	35	3.43611	50.17	55.51
4	**26.6456**	**0.1181**	**10.53**	**31.31**	**3.34554**	**90.38**	**100**
5	28.843	0.1771	4.4	22	3.09547	25.18	27.86
6	42.6247	0.864	34.28	10	2.1194	29.75	32.92

Table 4.3 XRD results of chitosan films with addition of Bluebottle essential oil (60min) (cont'd)

B-20-60-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.6172	0.8266	26.29	47	7.61753	32.24	40.21
2	18.2274	0.9446	28.16	55	4.86722	30.22	37.68
3	25.8787	0.2362	9.79	39	3.44293	42.03	52.41
4	**26.5907**	**0.1771**	**14.01**	**36**	**3.35233**	**80.2**	**100**
5	28.7757	0.1771	4.97	27	3.10256	28.43	35.45
6	32.8085	0.3542	5.72	20.88	2.72982	16.37	20.41
7	42.8075	0.72	20.82	12	2.11077	21.69	27.04
B-20-60-0.75%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5307	0.5904	14.91	41	7.67449	25.6	53.76
2	**26.6525**	**0.1771**	**8.32**	**38**	**3.3447**	**47.61**	**100**
3	28.8353	0.1771	4.05	29	3.09628	23.2	48.73
4	32.876	0.1771	3.31	20	2.72437	18.92	39.74
5	42.7664	0.7085	10.79	14	2.11445	15.45	32.44
6	55.0343	0.864	8.82	7	1.66726	7.65	16.07
B-20-60-1%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.2651	0.9446	20.26	48	7.85479	21.74	51,.08
2	**17.9759**	**0.9446**	**39.67**	**61**	**4.93472**	**42.57**	**100**
3	42.7939	0.864	19.6	19	2.11141	17.01	39.96

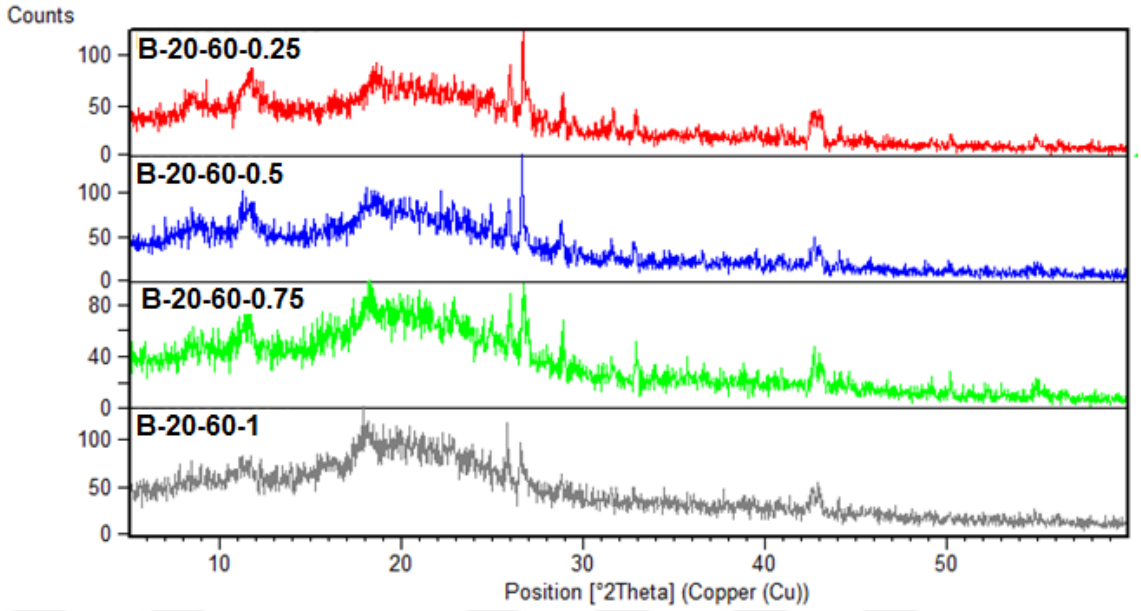


Figure 4.4 XRD patterns of chitosan films with Bluebottle essential oil (60 min)

Main experiments of Common Centaury gentian plants showed best crystallinity with addition of 0.5% of essential oil, then 0.25%, 1% and 0.75% (w/w) has highest peak heights, respectively. As are result of this, there is no relation crystallinity with ratios of essential oils of Common Centaury. Figure 4.5 shows the XRD patterns of chitosan films with addition of common centaury. The XRD results of common centaury based chitosan films are shown in Table 4.4.

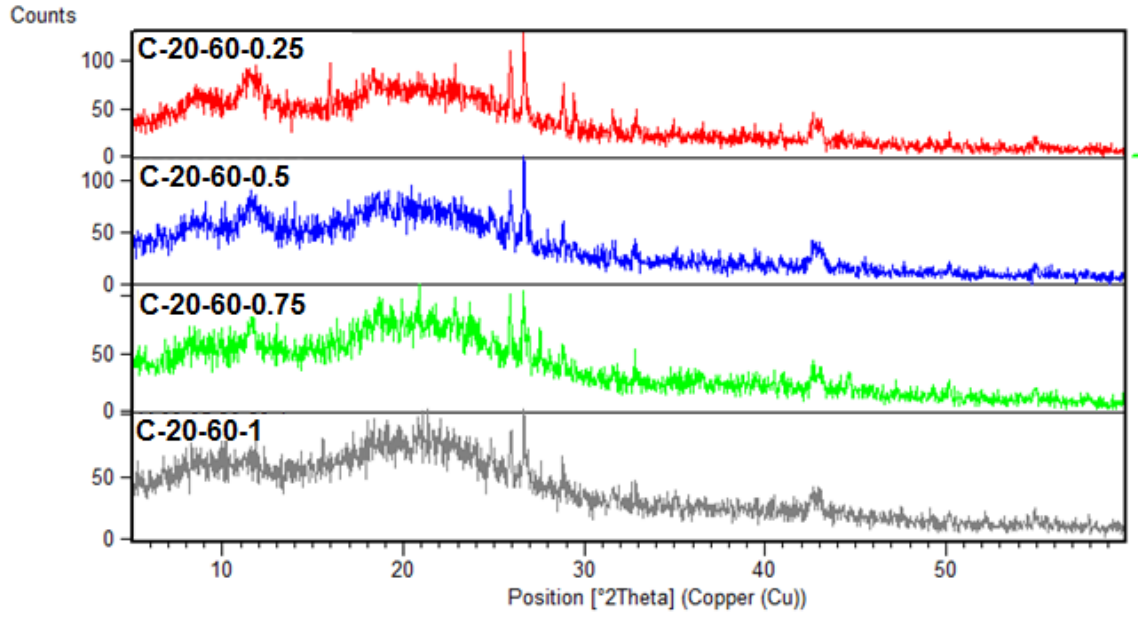


Figure 4.5 XRD patterns of Common Centaury based chitosan films (60 min)

Table 4.4 XRD results of Common Centaury based chitosan films (60 min)

C-20-60-0.25%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5444	0.9446	33.21	47	7.66538	35.64	52.1
2	15.9399	0.0886	3.27	46	5.56015	37.41	54.69
3	**25.9279**	**0.1181**	**7.97**	**40**	**3.4365**	**68.41**	**100**
4	26.6378	0.1181	7.54	37	3.3465	64.75	94.66
5	28.8182	0.1771	6.56	28	3.09808	37.53	54.86
6	32.8987	0.3542	5.62	19.88	2.72254	16.09	23.52
7	42.9713	0.864	23.1	14	2.1031	20.06	29.32
C-20-60-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5736	0.9446	24.24	49	7.64615	26.01	32.03
2	25.958	0.1771	6.6	41	3.43259	37.77	46.5
3	**26.6745**	**0.1181**	**9.46**	**37**	**3.34199**	**81.22**	**100**
4	42.811	1.008	29.08	12	2.1106	21.64	26.64
C-20-60-0.75%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5071	0.9446	22.73	48	7.69015	24.4	52.26
2	25.8991	0.1771	7.78	43	3.44025	44.54	95.42
3	**26.6193**	**0.1771**	**8.16**	**41**	**3.34879**	**46.68**	**100**
4	42.7781	0.864	17.51	16	2.11215	15.2	32.55

Table 4.4 XRD results of Common Centaury based chitosan films (60 min) (cont'd)

C-20-60-1%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	25.9105	0.1771	5.89	46	3.43876	33.69	65.65
2	**26.669**	**0.1771**	**8.97**	**43**	**3.34266**	**51.31**	**100**
3	42.9219	0.72	17.06	16	2.10541	17.77	34.63

St John's Wort has best crystallite peak with highest peak height at 0.25% (w/w) ratio of essential oil. After that 0.5% (w/w) essential oil has other high peak than 0.75% and 1% (w/w), respectively. Table 4.5 shows the results of XRD and Figure 4.6 shows XRD patterns of St John's Wort based chitosan films.

Table 4.5 XRD results of St John's Wort essential oil based chitosan films (60 min.)

S-20-60-0.25%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5693	0.9446	32.75	47	7.64896	35.15	37.87
2	24.9315	0.1771	6.95	46	3.57154	39.77	42.85
3	25.9113	0.1771	9.23	41	3.43866	52.81	56.9
4	**26.6541**	**0.1181**	**10.81**	**38**	**3.34449**	**92.82**	**100**
5	28.8188	0.1771	5.41	26,87	3.09802	30.95	33.34
6	31.5647	0.3542	5.96	19	2.8345	17.04	18.36
7	42.6385	0.864	29.82	12	2.11874	25.88	27.89
S-20-60-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.7132	0.9446	30.65	49	7.55529	32.89	46.37
2	**26.6552**	**0.1181**	**8.26**	**36.99**	**3.34436**	**70.93**	**100**
3	31.6256	0.3542	6.55	21	2.82918	18.76	26.45

Table 4.5 XRD results of St John's Wort essential oil based chitosan films (60 min.) (cont'd)

S-20-60-0.5%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
4	42.9072	0.72	22.51	13	2.1061	23.45	33.06
S-20-60-0.75%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.4857	0.9446	29.77	56	7.70444	31.95	45.31
2	**26.5714**	**0.1181**	**8.21**	**38**	**3.35472**	**70.51**	**100**
3	28.7257	0.2362	7.74	30	3.10784	33.2	47.09
4	42.8223	0.3542	8.03	14	2.11182	22.97	32.57
5	54.8703	0.864	9.83	7	1.67186	8.53	12.1
S-20-60-1%							
	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	Backgr. [cts]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	11.5435	0.7085	23.34	54	7.666	33.4	92.47
2	**18.5067**	**0.7085**	**25.24**	**58**	**4.79438**	**36.12**	**100**
3	26.8047	0.7085	18.93	39	3.32605	27.08	74.98
4	42.7508	0.72	24.31	15	2.11344	25.33	70.12

For reaction time of 120 minutes XRD patterns and XRD result tables are given with Appendix A. St John's Wort had highest peak result with 0.5% (w/w) addition of essential oil then, 0.25%, 1% and 0.75% (w/w), respectively. Bluebottle had highest peak result with 0.25% (w/w) addition of essential oil then, 1%, 0.75% and 0.5% (w/w), respectively. Common Centaury also had highest peak 0.25%, then 0.5%, 1% and 0.75% (w/w), respectively. All results showed that time of reaction and essential oil ratio does not have any relations between crystallinity.

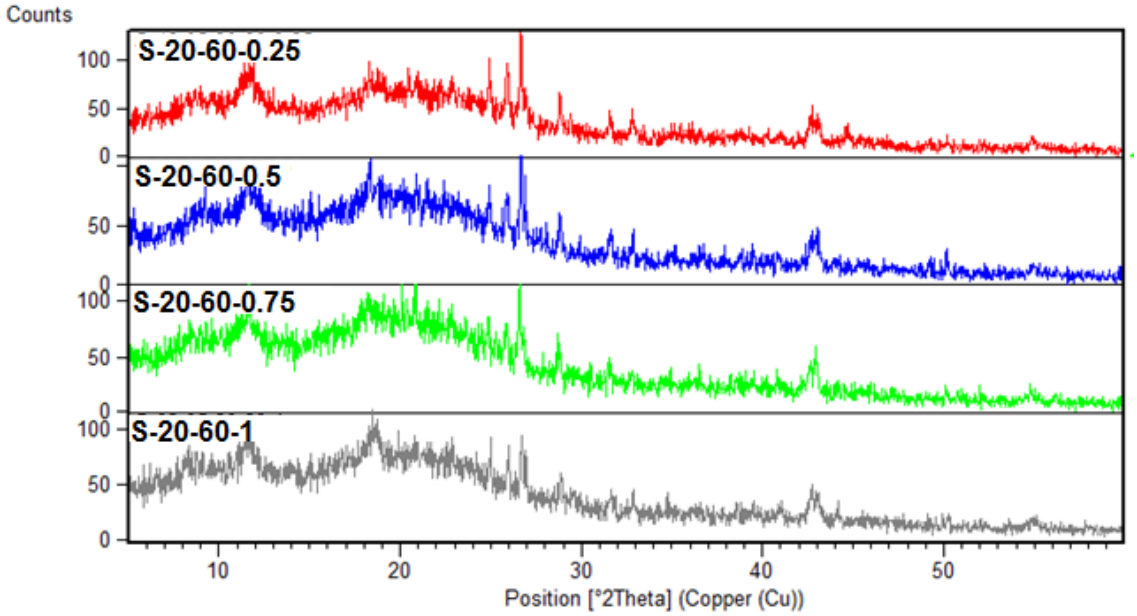


Figure 4.6 XRD patterns of St John's Wort essential oil based chitosan films (60 min)

4.2 FT-IR Results of Essential Oil Based Chitosan Films

Pure chitosan powder and chitosan films which are produced under different temperatures are characterized by FT-IR and their characteristic peaks such as polymerization peaks were determined. As a result of this peaks at 3354 and 1308 cm^{-1} reduced their volumes and new peaks occurs around 2290, 1540, 1400 and 1250 cm^{-1}, all these peaks occurs with every temperatures of reactions, so that is just a polymerization peak of chitosan. Figure 4.7 shows FT-IR peaks of pure chitosan and chitosan films.

Pretesting films of reaction time with 60 minutes and 120 minutes does not showed any different peaks from pure chitosan films that means there is no negative effect of essential oils to polymerization.

As a result of researches of literatures, the peak between 650 to 900 cm^{-1} due to OH or NH_2 bending of chitosan [2], the peak between 1000 to 1160 cm^{-1} is due to CO stretching [1], [3], the peak at 1239 cm^{-1} is belonged to δNH bending [1], the peak around 1409 to 1471 cm^{-1} is carboxylate stretching [77], [78], the peaks around 1300 $cm^{-1}$is belongs to CN bending [3], [78], the peaks between 1600 to 1700 cm^{-1} shows CO stretch of acetyl groups [2], [79], the peak at 1450 cm^{-1} belonged to in plane NH bending [1], and the peaks around 2921 and 2867 cm^{-1} due to CH stretch. All FT-IR analysis done with ATR, and peaks under 650 cm^{-1} are not involves the graphs.

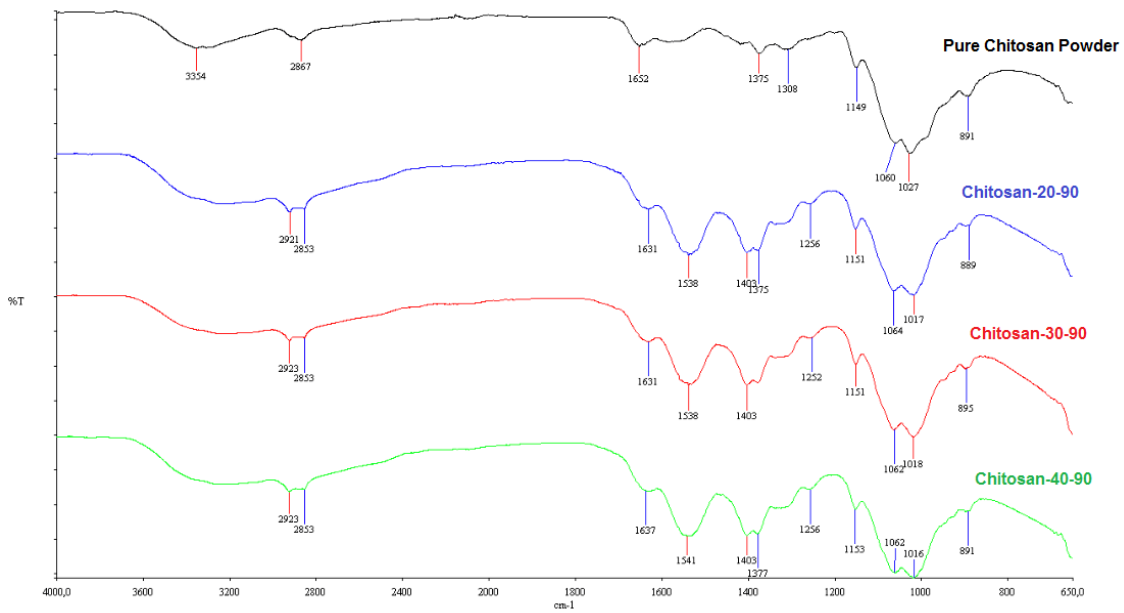


Figure 4.7 FT-IR result of pure chitosan and chitosan films

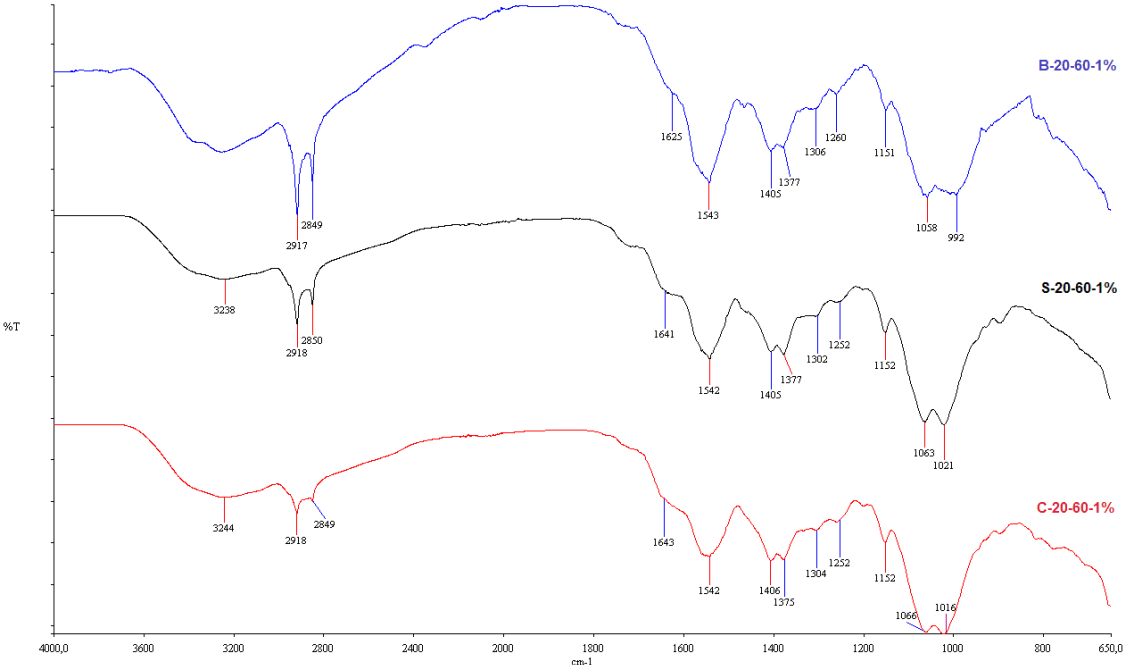


Figure 4.8 FT-IR results of three different gentian plant based chitosan films with ratio of 1% (w/w) with 60 minutes of reaction time

All essential oils from Bluebottle, Common Centaury and St John's Wort have similarly FT-IR peaks, there is no significant differences between oil ratios and oil types, neither with reaction times. For that reason, Figure 4.8 shows different types of essential oil based chitosan films with addition of 1% (w/w) essential oil with reaction time of 60 min and Figure 4.9 shows different types of films with different essential oils with addition of

0.25% (w/w) with reaction time of 120 minutes. Other FT-IR results are given with appendix B.

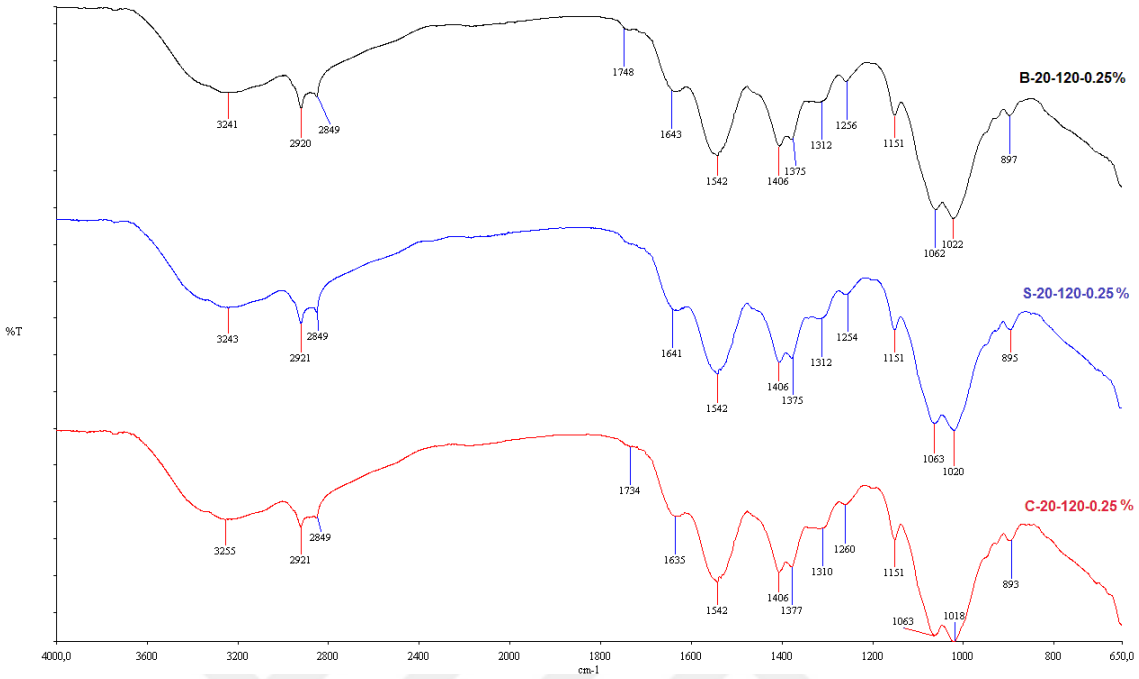


Figure 4.9 FT-IR results of three different gentian plant based chitosan films with ratio of 0.25% with 120 minutes of reaction time

4.3 Results of DTA-TG Analysis

The chitosan film's DTA-TG analysis showed 2 steps of degradation and total weight loss was 69.253%. At the first area of first step, the bound water left the structure of chitosan film. The weight loss of this step measured as 15.864% and DTG peak occurred at 49.50°C. At the first area of second step, saddle type reaction occurs and DTG peak at this reaction was at 182.17°C and weight loss measured 7.350%. Main reaction's DTG peak occurred at 281.19°C with 34.650% loss of weight. The saddle reaction which followed the main reaction occurred at 365.62°C and weight loss measured 12.261%. Figure 4.10 shows the DTA-TG analysis of chitosan film with reaction time of 60 minutes.

C-20-60-1 specimen's total weight loss is 63.283%. Thermal degradation determined with 3 steps, first step was 10% of weight loss and DTG peak occurred at 127.31°C. The weight loss at this step caused by bound water of chitosan films and bound ethanol. The weight loss at second step increased and the weight loss was 40.246% and DTG peak occurred at 266,68°C, this loss is caused by degradation chitosan polymer structure. The

last step's weight loss depends on the bounded oil structure of polymer films. The weight loss was 12,870% and DTG peak occurred at 439.10°C. Figure 4.11 shows DTA-TG analysis of C-20-60-1 specimen.

Also Table 4.6 shows DTG peak temperatures and weight loss measurements of B-20-60-1, S-20-60-1, C-20-60-1, B-20-120-1, S-20-120-1 and C-20-120-1.

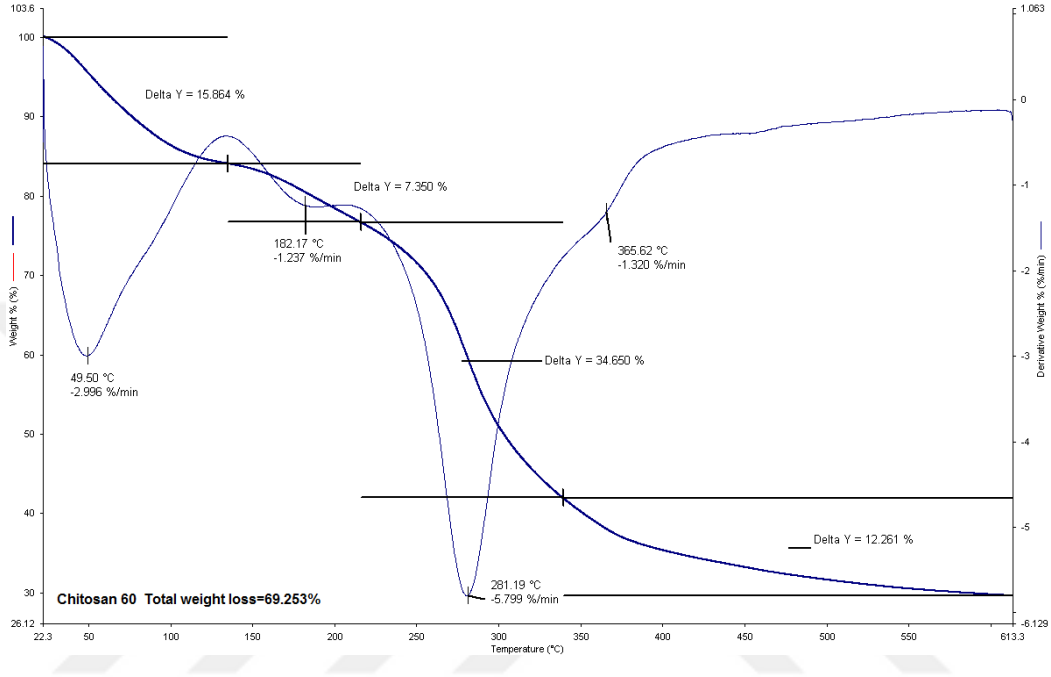


Figure 4.10 DTA-TG analysis of chitosan film (60 min)

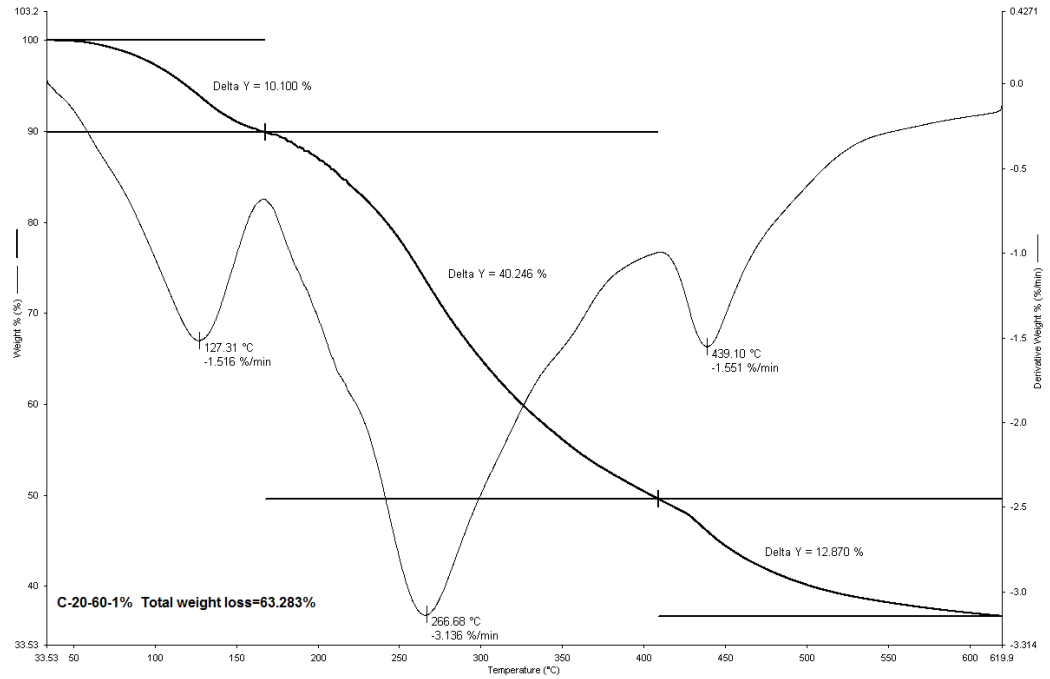


Figure 4.11 DTA-TG analysis of C-20-60-1

Table 4.6 DTA-TG results of 60 minutes' and 120 minutes' reactions with 1% addition of three type of gentian plant

Code	1st step		2nd step		3rd step	
	Weight loss (%)	DTG peak (°C)	Weight loss (%)	DTG peak (°C)	Weight loss (%)	DTG peak (°C)
B-20-60-1	13.668	130.87	39.658	267.69	13.280	454.87
S-20-60-1	8.087	101.88	40.472	280.92	18.165	403.50
C-20-60-1	10.100	127.31	40.246	266.68	12.870	439.10
B-20-120-1	14.325	131.89	36.662	270.74	15.112	436.05
S-20-120-1	9.318	112.56	37.470	273.80	18.079	420.28
C-20-120-1	11.615	126.29	39.772	265.66	16.332	441.64

When DTA-TG analysis compared with types of essential oils, Bluebottle, St John's Wort and Common Centaury has closely same weight loss ratios. Their DTG peak temperatures were matching with each other but there are some minor differences between all temperatures. After all, DTA-TG analysis, the degradation temperatures increased with essential oil types, St John's Wort, Common Centaury and Bluebottle, respectively. The degradation reactions which are caused by bound water of film and essential oil connection of films are shown slowly with St John's Wort essential oil addition. At this comparison, degradation of chitosan structure of films was compatible with each other for each essential oil. (Figure 4.12 and Figure 4.13)

From the Table 4.6 the temperatures and weight loss ratios of 1% essential oil added films can be seen.

For addition of 0.25% (w/w) essential oil to chitosan films, showed DTA-TG analysis result such as chitosan films. There is no 3rd step of reaction which is caused by bounding of essential oil to film structure. But Common Centaury has dense consistency, for that reason, 0.25% (w/w) essential oil added film of Common Centaury had a small DTG peak at 3rd step of degradation of films. (Figure 4.14)

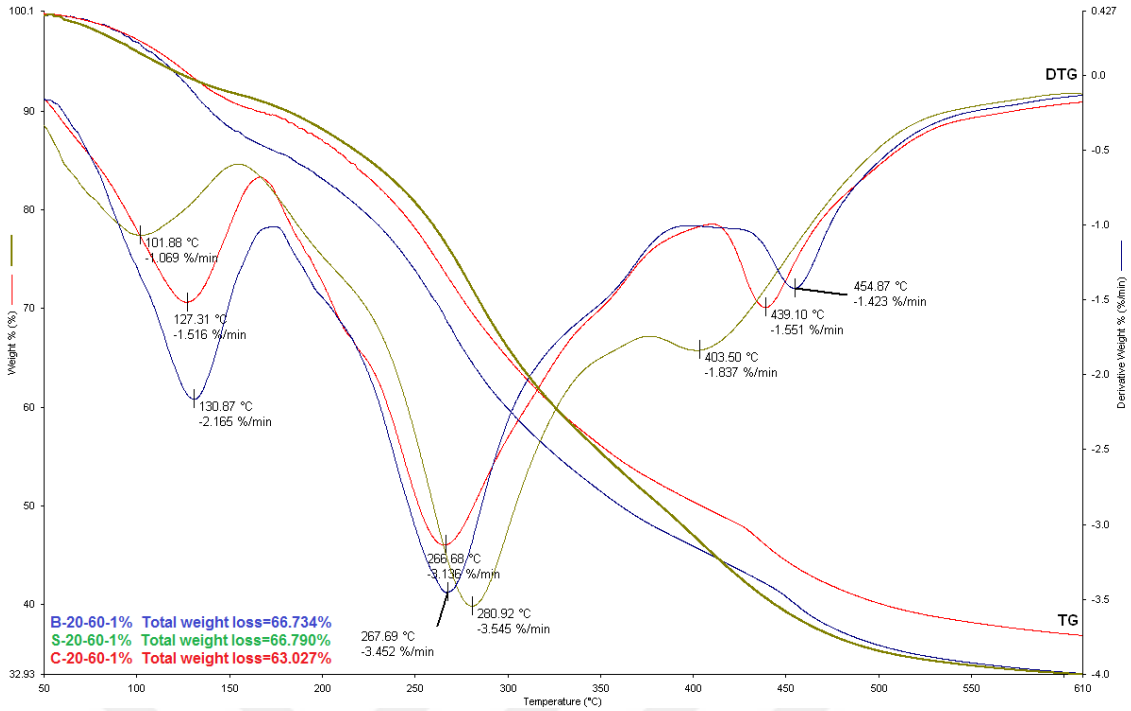


Figure 4.12 DTA-TG analysis of B-20-60-1, S-20-60-1 and C-20-60-1

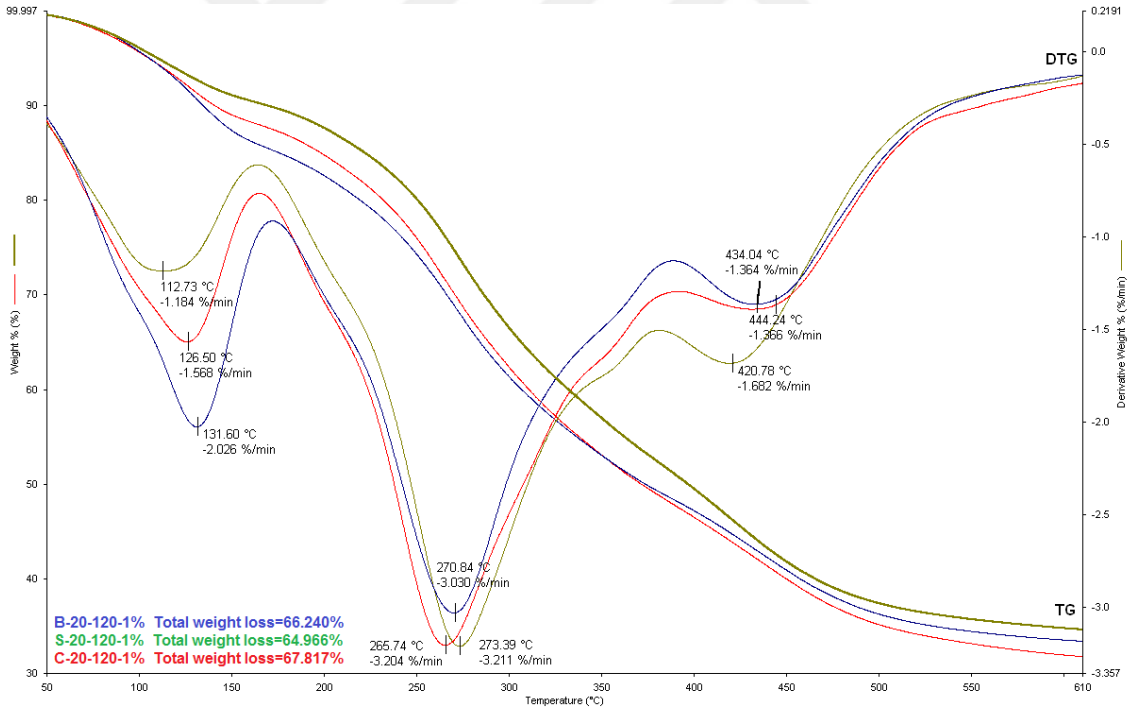


Figure 4.13 DTA-TG analysis of B-20-120-1, S-20-120-1 and C-20-120-1

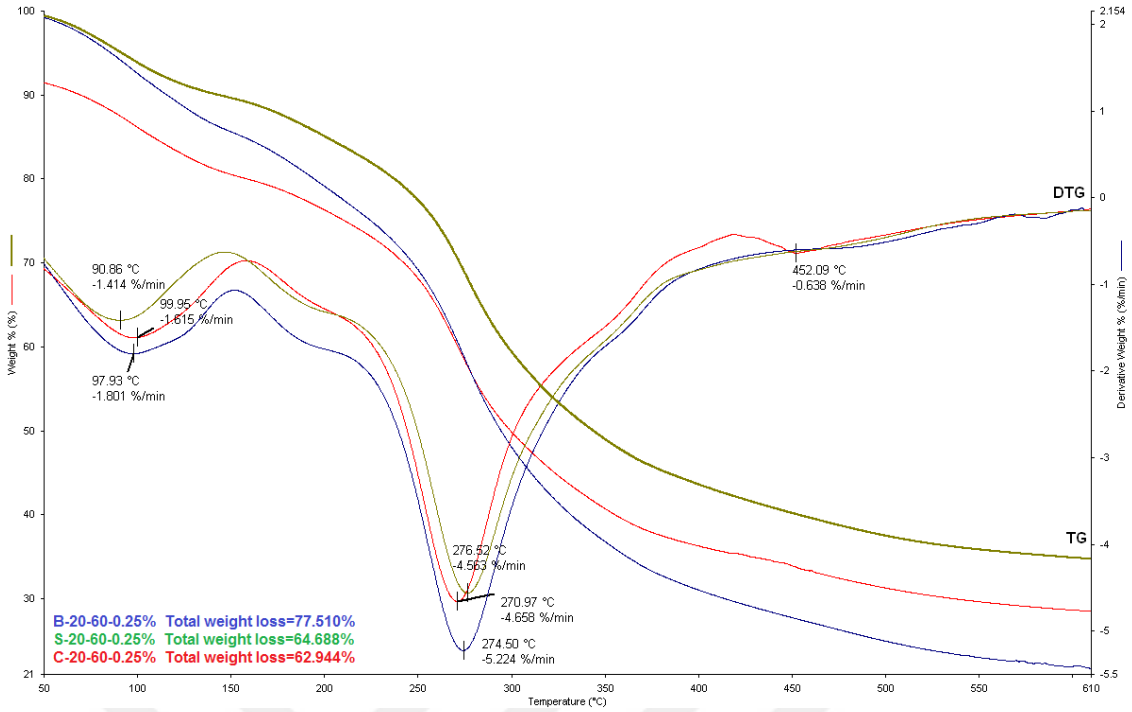


Figure 4.14 DTA-TG analysis of B-20-60-0.25, S-20-60-0.25 and C-20-60-0.25

Table 4.7 DTA-TG results of 60 minutes' and 120 minutes' reactions with 1% (w/w) addition of three type of gentian plant

Code	1st step		2nd step		3rd step	
	Weight loss (%)	DTG peak (°C)	Weight loss (%)	DTG peak (°C)	Weight loss (%)	DTG peak (°C)
B-20-60-0.25	13.715	97.93	63.749	274.50	-	-
S-20-60-0.25	9.758	90.86	54.903	276.52	-	-
C-20-60-0.25	11.432	99.95	44.070	270.97	7.377	452.09
B-20-120-0.25	11.757	66.45	54.408	277.64	-	-
S-20-120-0.25	9.241	94.90	59.974	278.04	-	-
C-20-120-0.25	10.640	84.08	53.359	278.88	-	-

Ratio scanning of each gentian essential oil in itself was determined by DTA-TG. In Ethanol amount was increased with addition of increased essential oil ratio, as a result of this, first step of degradation reaction was speeding and DTG peak temperatures were increasing. Second step of degradation which is caused by chitosan structure in polymer films were slow down because of increasing essential oil addition.

Table 4.8 Bluebottle essential oiled films with different ratios at 60 minutes' and 120 minutes' reaction time

Code	1st step (°C)	2nd step (°C)	3rd step (°C)
B-20-60-1	130.87	267.69	454.87
B-20-60-0.75	128.84	275.32	457.41
B-20-60-0.5	118.16	275.32	453.34
B-20-60-0.25	97.93	274.50	-
B-20-120-1	131.89	270.74	436.05
B-20-120-0.75	130.58	271.35	474.85
B-20-120-0.5	121.14	264.41	452.59
B-20-120-0.25	66.45	277.64	-

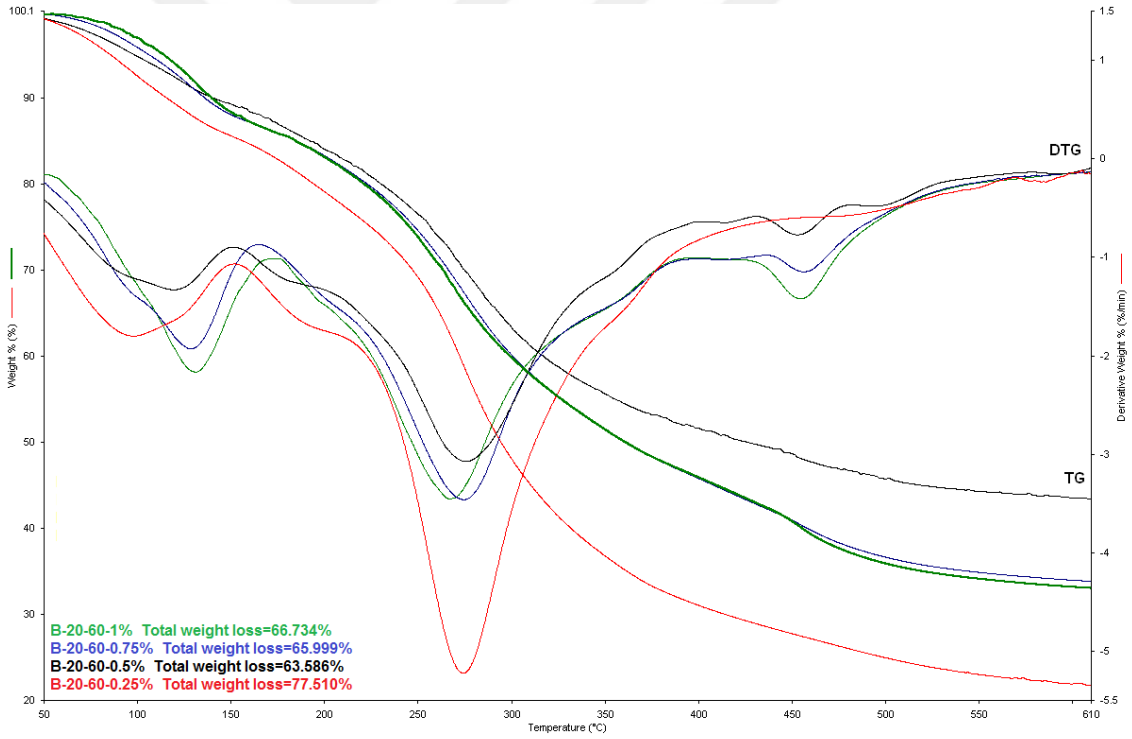


Figure 4.15 DTA-TG analysis of Bluebottle essential oiled films with different ratios at 60 minutes' reaction time

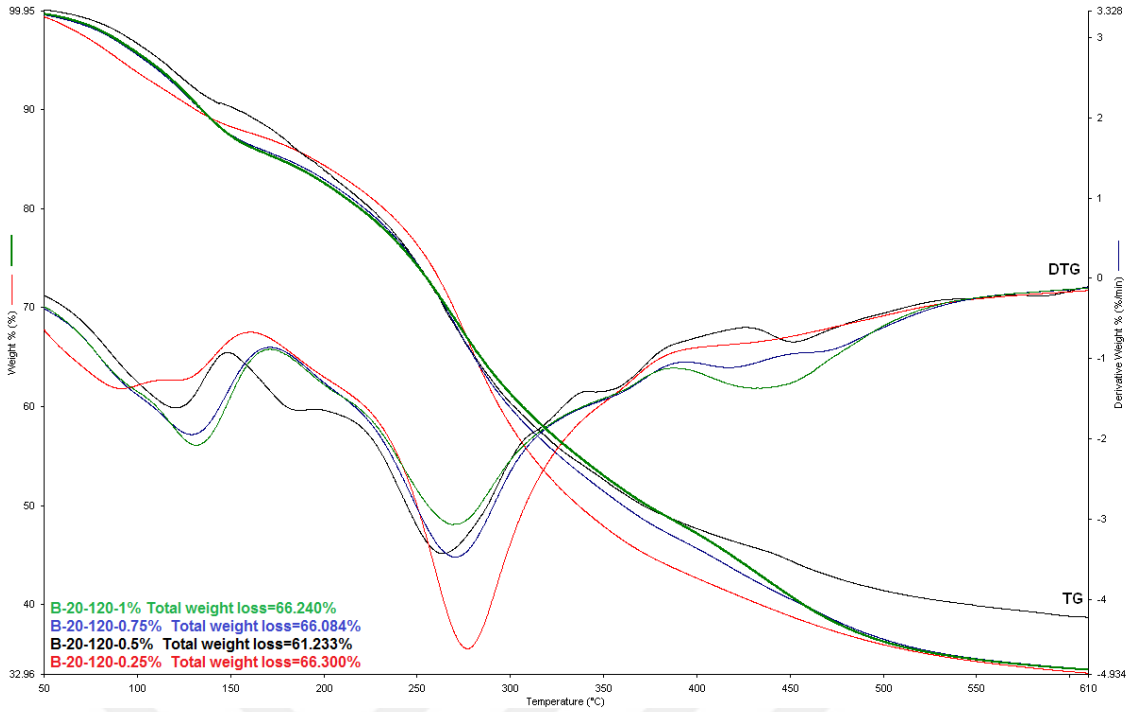


Figure 4.16 DTA-TG analysis of Bluebottle essential oiled films with different ratios at 120 minutes' reaction time

From the Table 4.8, Figure 4.15 and Figure 4.16, the characterization of Bluebottle essential oil based chitosan films are determined. The ratio of 0.25% (w/w) essential oil has similar DTG peaks with chitosan films. That means the 3rd step of degradation caused by essential oil which is connected with bounds to chitosan film structure. At 120 minutes of reaction time process showed slowly degradation reactions than 60 minutes of reaction time. These DTA-TG results were same with all types of essential oil additions.

Figure 4.17 and Figure 4.18 belongs to St John's Wort essential oil based chitosan films with different ratios and temperatures. The table 4.9 shows the temperatures of DTG peaks for each step of St John's Wort essential oil addition in chitosan films.

Figure 4.19 and Figure 4.20 shows Common Centaury essential oil based chitosan films with different ratios and temperatures. The table 4.10 shows the temperatures of DTG peaks for each step of degradation of films.

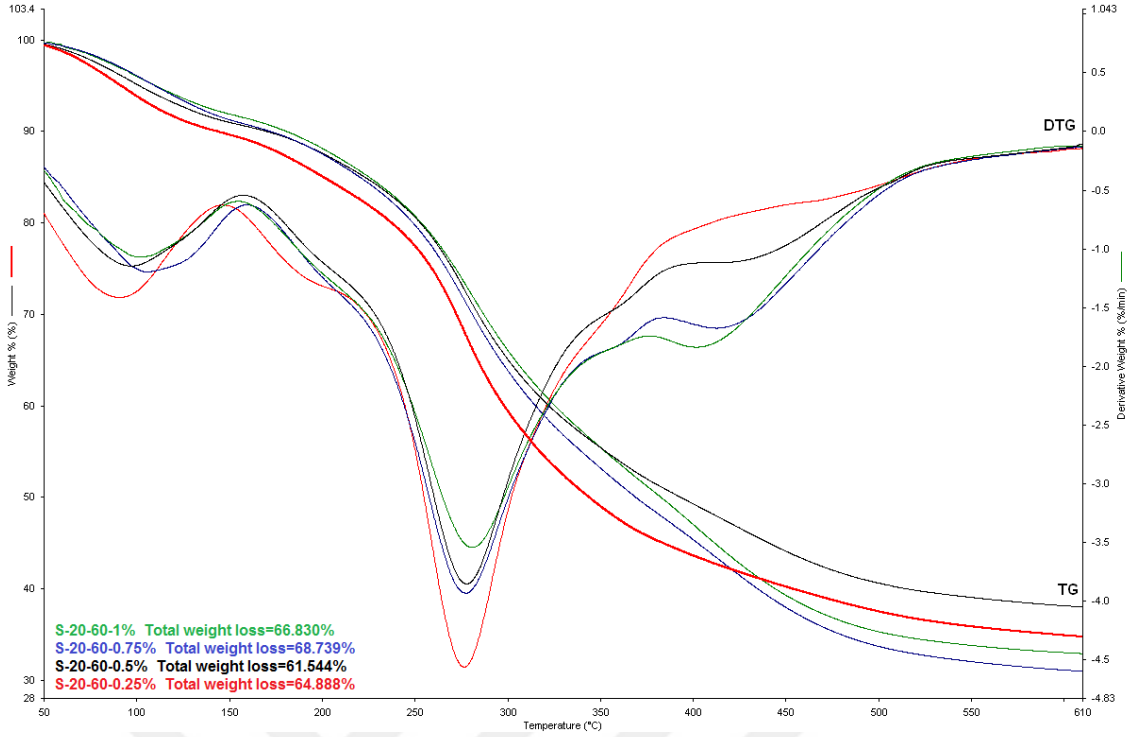


Figure 4.17 DTA-TG analysis of St John's Wort essential oiled films with different ratios at 60 minutes' reaction time

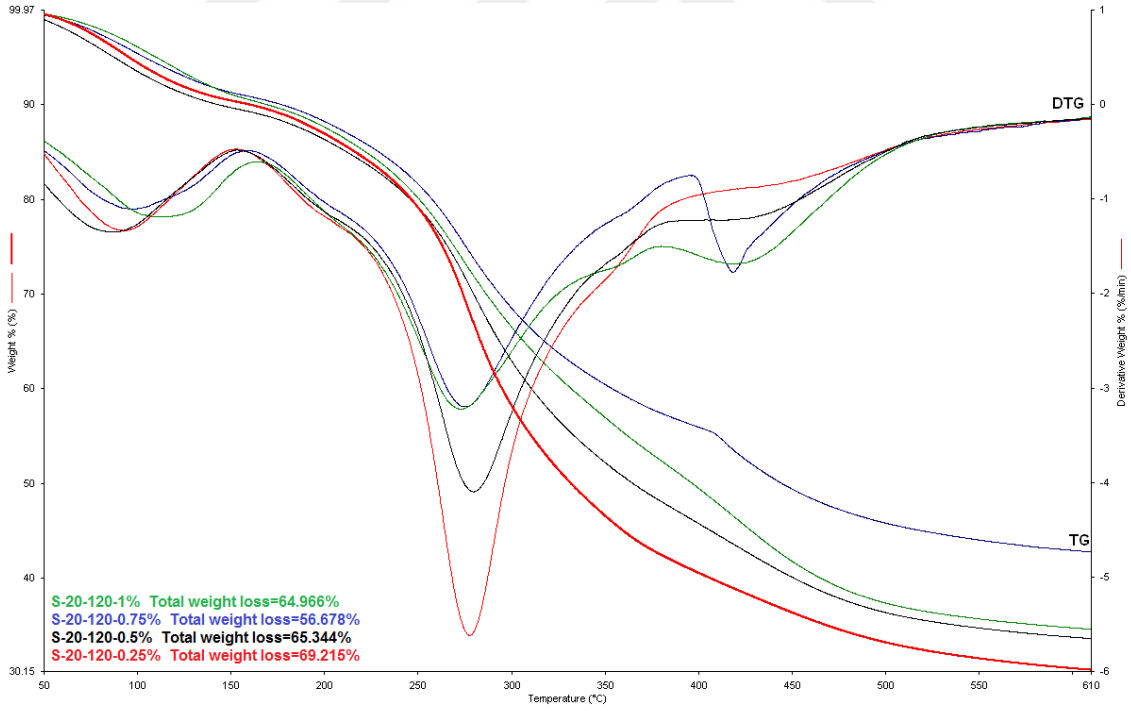


Figure 4.18 DTA-TG analysis of St John's Wort essential oiled films with different ratios at 120 minutes' reaction time

Table 4.9 St John's Wort essential oiled films with different ratios at 60 minutes' and 120 minutes' reaction time

Code	**1st step (°C)**	**2nd step (°C)**	**3rd step (°C)**
S-20-60-1	101.88	280.92	403.50
S-20-60-0.75	108.49	277.87	416.72
S-20-60-0.5	101.88	277.36	427.40
S-20-60-0.25	90.86	276.52	-
S-20-120-1	112.56	273.80	420.28
S-20-120-0.75	97.94	275.43	419.25
S-20-120-0.5	83.33	280.05	432.92
S-20-120-0.25	94.90	278.04	-

Table 4.10 Common Centaury essential oiled films with different ratios at 60 minutes' and 120 minutes' reaction time

Code	**1st step (°C)**	**2nd step (°C)**	**3rd step (°C)**
C-20-60-1	127.31	266.68	439.10
C-20-60-0.75	125.28	266.68	424.35
C-20-60-0.5	99.34	266.17	450.29
C-20-60-0.25	99.95	270.97	452.09
C-20-120-1	126.29	265.66	441.64
C-20-120-0.75	126.50	264.72	423.84
C-20-120-0.5	111.05	274.50	444.24
C-20-120-0.25	84.08	278.88	-

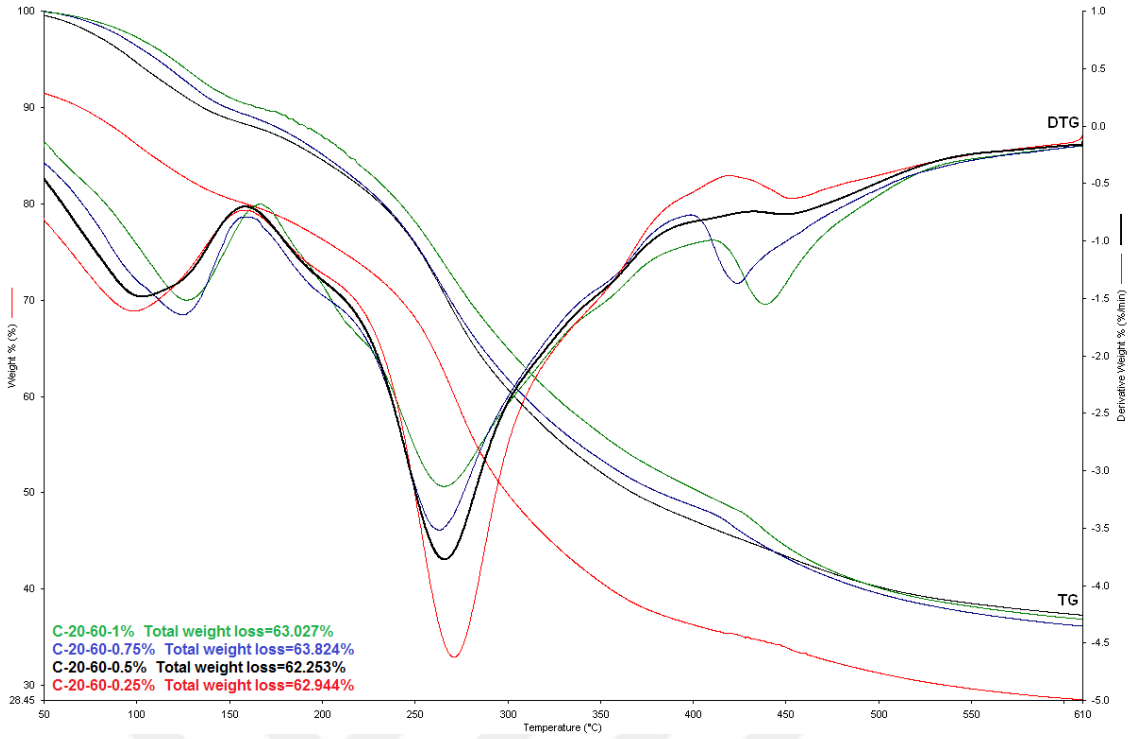


Figure 4.19 DTA-TG analysis of Common Centaury essential oiled films with different ratios at 60 minutes' reaction time

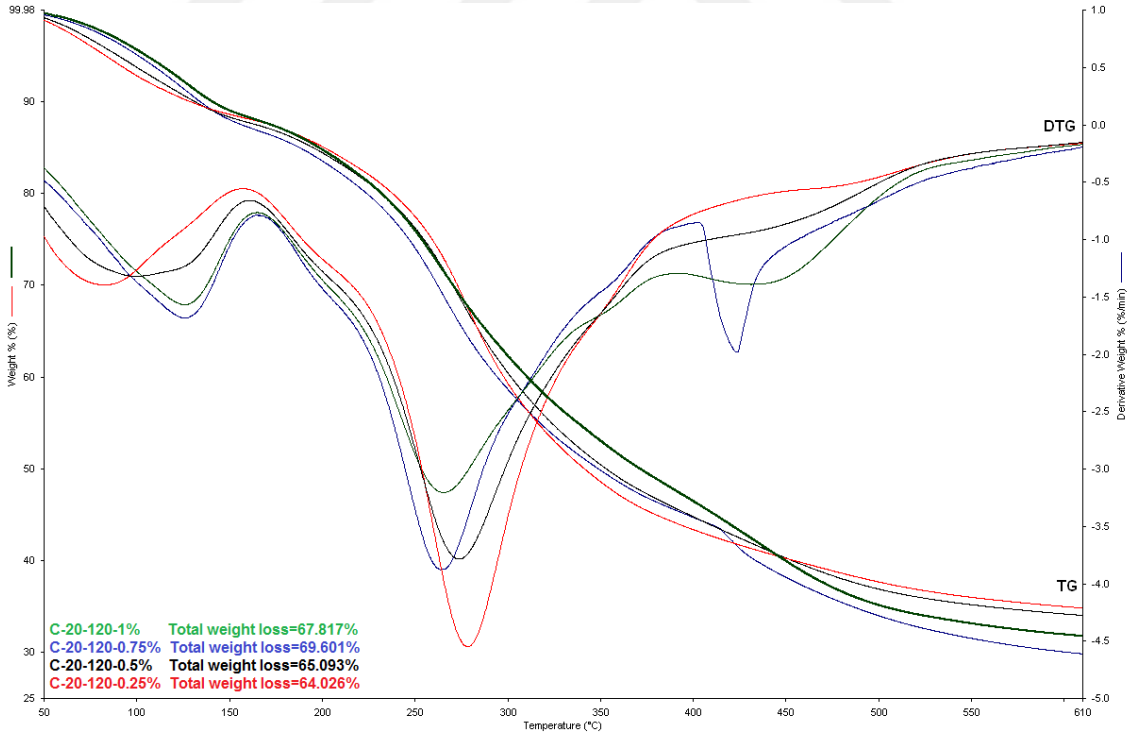


Figure 4.20 DTA-TG analysis of Common Centaury essential oiled films with different ratios at 120 minutes' reaction time

4.4 ICP-OES Results of Gentian Species

The amount of the mineral matter of plants have differences according to their genetics, the ability to get to the different elements within their structure, the soil in growing conditions, water minerals and climate conditions [50]. The results of elemental analysis of Gentian plants in mg/kg are shown in Table 4.11.

The major elements chosen as K, Mg, Ca, Fe in the essential oil which was obtained from the Gentian plants, the amounts of elements were determined with ICP-OES (Figure 4.21).

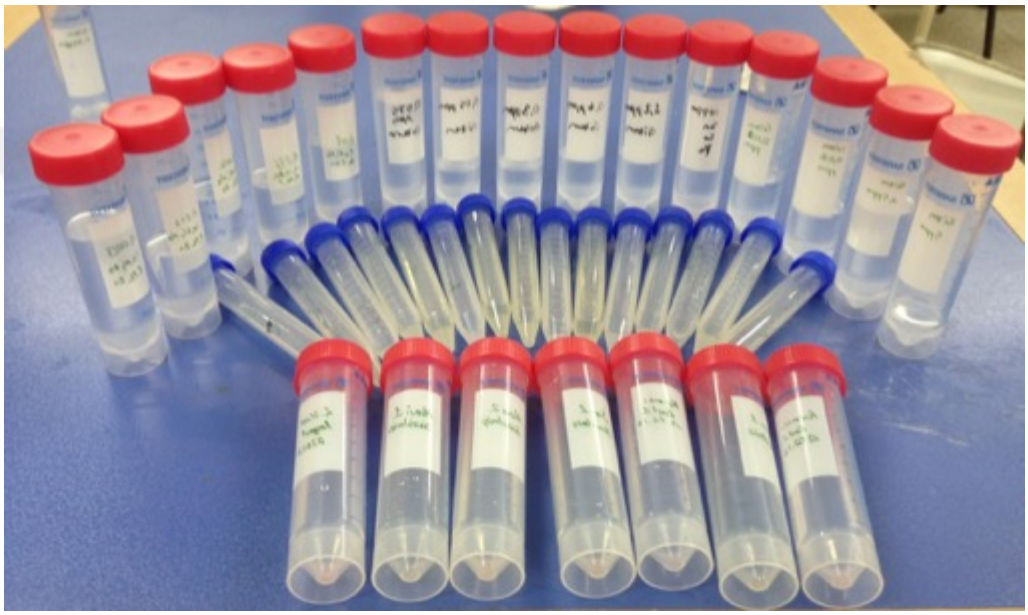


Figure 4.21 The ICP-OES standards and samples

Potassium (K) is substantially take part in chemistry of life functions, such as fluid balance, protein synthesis, nerve conduction, energy production, muscle contraction, synthesis of nucleic acids, and control of heart beat [80], [81].

Calcium (Ca) has important functions in human body such as regulating heartbeat, clotting blood, conducting nerve impulses and stimulating hormone secretions. The Ca mineral is necessary for developing and maintaining healthy bones and teeth [81], [82].

Magnesium (Mg) has many different effects on the human body processes. The existence of Mg element in human body has important role in enzyme activation. There are more than 300 enzymes which's cofactor is Mg and these are responsible for protein synthesis, muscle and nerve function, blood pressure regulation [80], [81], [83].

Iron (Fe) is an important mineral and lack of Fe mineral in human body causes the anemia, for that reason Fe is necessary in various body functions which's are the transport of oxygen and carbon dioxide in the blood. This is main mineral for providing energy in daily life of human [82], [83].

Table 4.11 ICP-OES results of gentian species

Element	**St John's Wort (mg/kg)**	**Bluebottle (mg/kg)**	**Common Centaury (mg/kg)**
Mg	1716.5	1521	1295
Cr	15.8	3.7	bdl
Cu	19.4	67	10.2
B	110.8	98.5	bdl
Na	2.4	1.2	bdl
Al	436.7	263.3	188.1
P	24.8	19.7	20
Ca	10530	5968	1749
Fe	332.4	204	103.5
Ba	8.8	3.2	3.2
Sr	24.8	11	7.7
Zn	bdl	bdl	bdl
Cd	bdl	bdl	bdl
Co	bdl	bdl	bdl
Ni	3.9	2.1	bdl
Mn	21.2	11.9	23.5
K	12370	10445	12280
Pb	bdl	bdl	bdl

The highest K element amount found in St John's Wort, then Common Centaury and Bluebottle with the amounts 12370 mg/kg, 12280 mg/kg and 10445 mg/kg, respectively.

Calcium element are different in each plant, there was no average amounts for it, the highest amount comes from St John's Wort as 10530 mg/kg, then Bluebottle as 5968 mg/kg and at least Common Centaury as 1749 mg/kg.

Magnesium element amounts were closely to each other in each plant, the element amounts found as 1716.5 mg/kg, 1521 mg/kg and 1295 mg/kg for St John's Wort, Bluebottle and Common Centaury, respectively.

The amount of Fe element determined lower than other elements but iron is an important element for human body, for that reason iron chosen as main element and the amounts of Fe element in gentian plants found as 332,4 mg/kg in St John's Wort, 204,05 in Bluebottle and 103.535 mg/kg in Common Centaury.

4.5 UV-Vis Analysis of Essential Oils

The UV-Vis of essential oil obtained from Bluebottle plant, St John's Wort plant and Common Centaury plant is presented in Figure 4.22, Figure 4.23 and Figure 4.24, respectively. According to UV result, the absorbance wavelengths (λ) in the range of 260 – 280 nm can be explained with polyphenol in structure. Flavonoids are seen between 320 – 370 nm [84]. The absorbance value at 590 nm indicates the red-orange colors characteristic peak in the Common Centaury plant's essential oil [85].

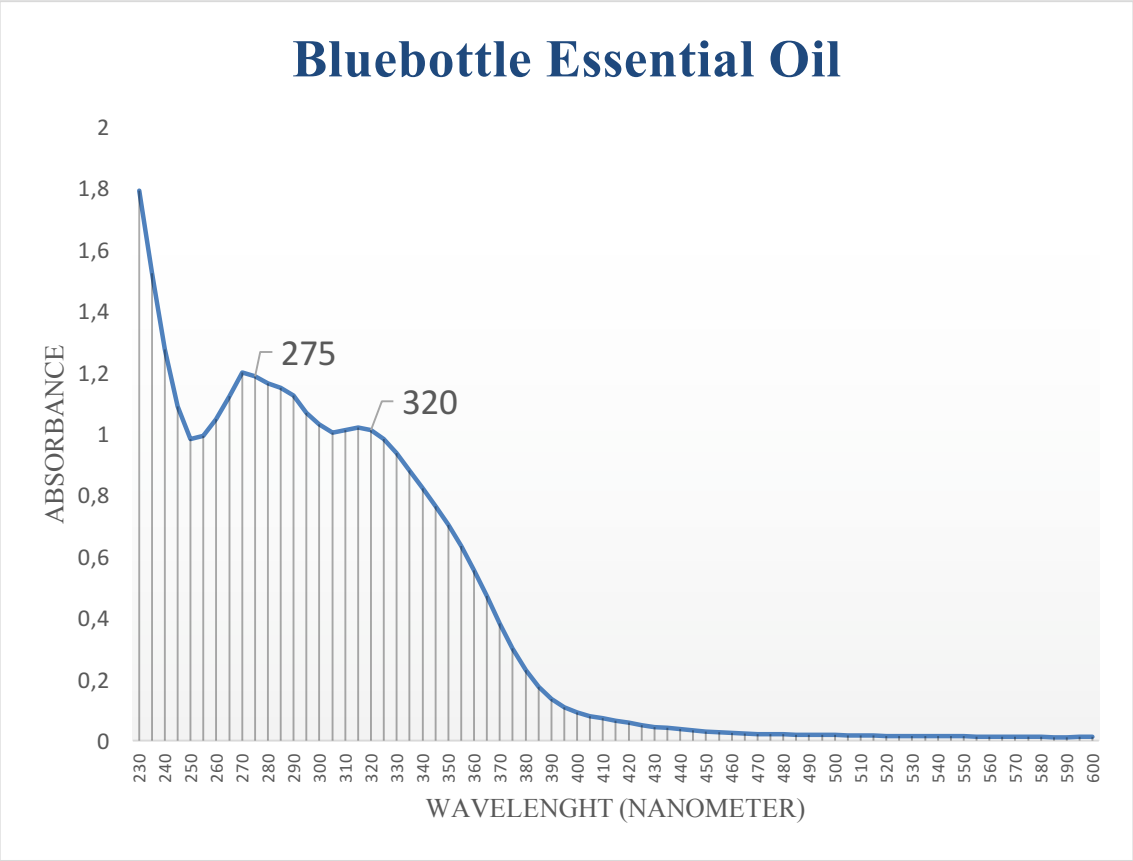


Figure 4.22 UV-Vis spectra of Bluebottle's essential oil

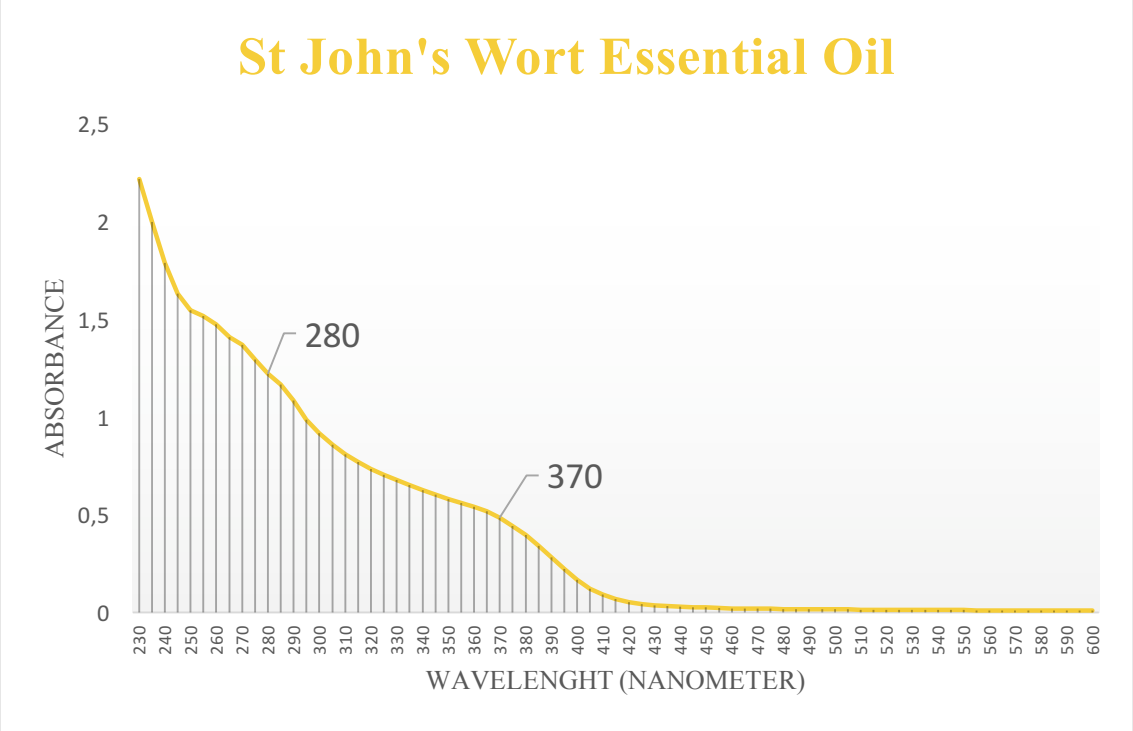


Figure 4.23 UV-Vis spectra of St John's Wort's essential oil

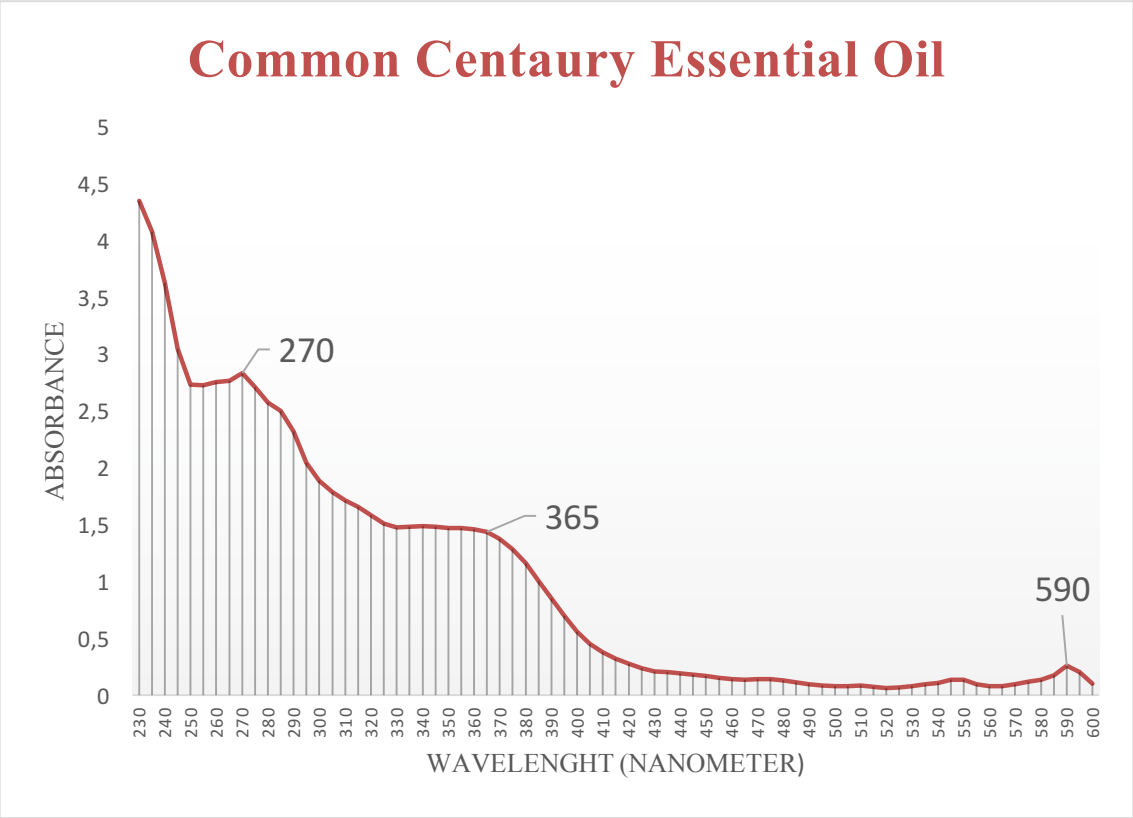


Figure 4.24 UV-Vis spectra of Common Centaury's essential oil

4.6 Swelling Test Results

The swelling ratios for pretesting experiments measured firstly to determine the optimum temperature of reactions. For that reason, specimens of pretests measured twice with PBS solution. The Figure 4.25 shows the swelling measurements hour by hour.

The specimen B-20-60-0.5 and B-20-120-0.5 showed higher swelling ratios than other temperatures with their reaction times. The swelling test helped us to find optimum reaction temperature. The swelling ratios of 60 minutes and 120 minutes was closed to each other, for that reason main experiments produced under 20°C by 60 minutes and 120 minutes of reaction time.

The swelling ratios of main films measured each singular hour during 9 hours, after that the films is left into PBS solutions for 3 days, after 3 days maximum swelling ratio of the films were measured.

But chitosan films cannot be measured after 5 hours because they swelled with highest ratio and lost their mechanical strength. As it seen from the Figure 4.26, highest swelling ratio composed with specimen C-20-60-0.25, then S-20-60-0.25, C-20-120-0.25

respectively. In the set of St John's Wort essential oil based films, S-20-120-0.5 showed higher swelling ratio then S-20-60-0.5. Swelling ratio decreases with increasing of essential oil amount but their strength in the PBS solution increases with it. In the set of Common Centaury essential oil based films, C-20-120-0.5 showed higher swelling ratio then C-20-60-0.5 too, but the highest swelling ratios hold by addition of 0.25% (w/w) essential oils.

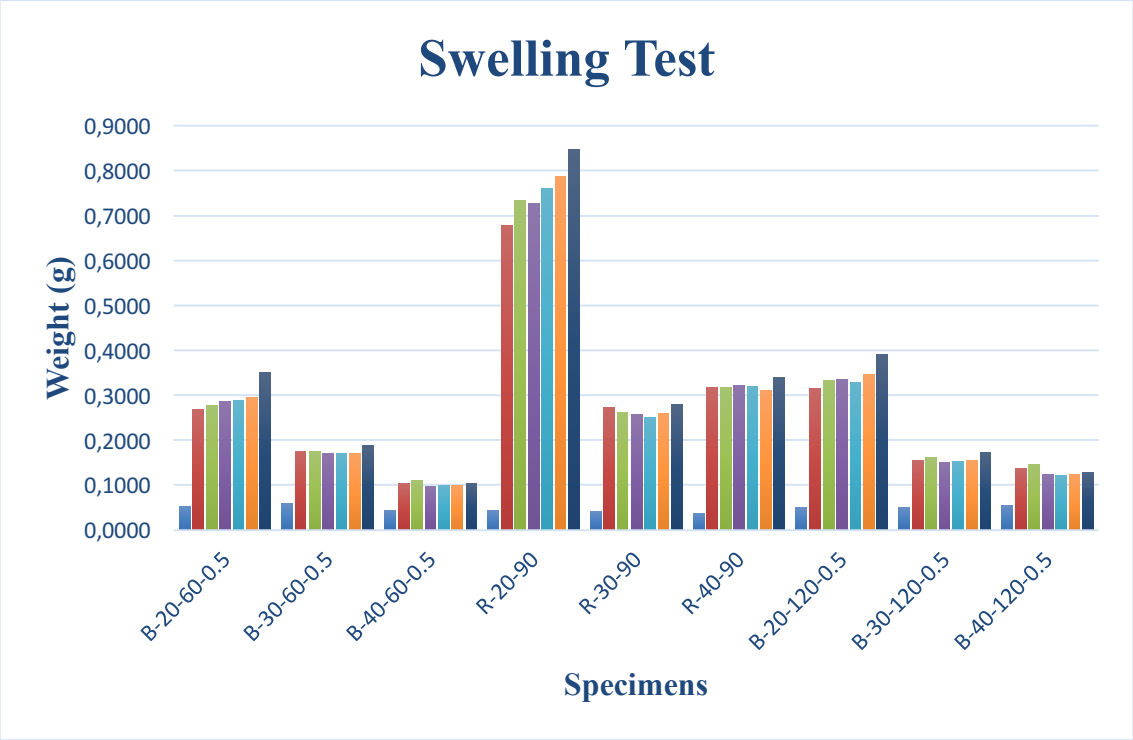


Figure 4.25 The swelling measurements of pretesting experiment 0h to 3 days

From the Figure 4.27, the swelling behaviors of films can be seen hour by hour. As it seen from the figure all specimens increased sharply after 1 hour, but after 9 hours to 3 days there no significant difference between weight measurements of specimens. There are no measurements of chitosan films after 5 hours because they swelled highly and their structure was defaced. The essential oil ratio of 0.25 had highest swelling ratios generally, because their structure is more similar with chitosan membranes.

The Table 4.12 shows the percentage of average of swelling test measurements.

According to table, swelling ratios of 0.25% (w/w) addition of essential oil percentage was changed after 9 hours to 3 days around 13% to 10%, for 0.5% (w/w) addition of essential oil changed around 9% to 7% after last measurement to 3day measurement. The other changes of 9 hours to 3 days calculated under the 5% for 0.75% and 1% ratios.

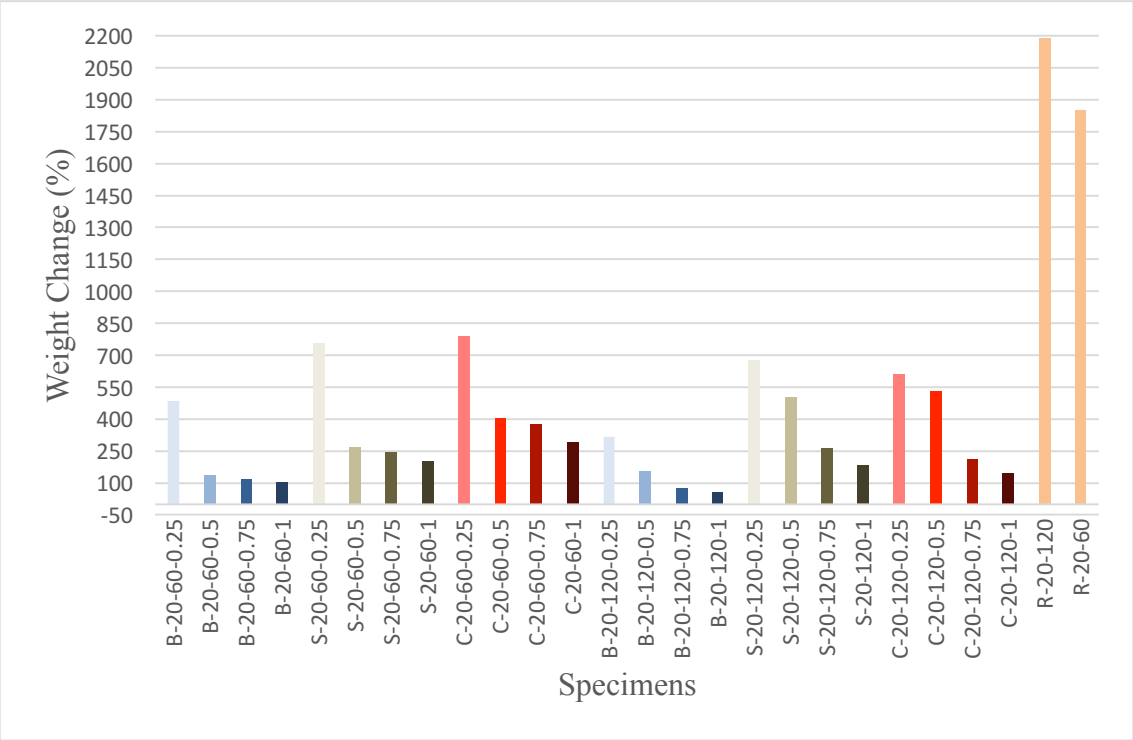


Figure 4.26 The graph of swelling test ratios after 3 days for each specimen

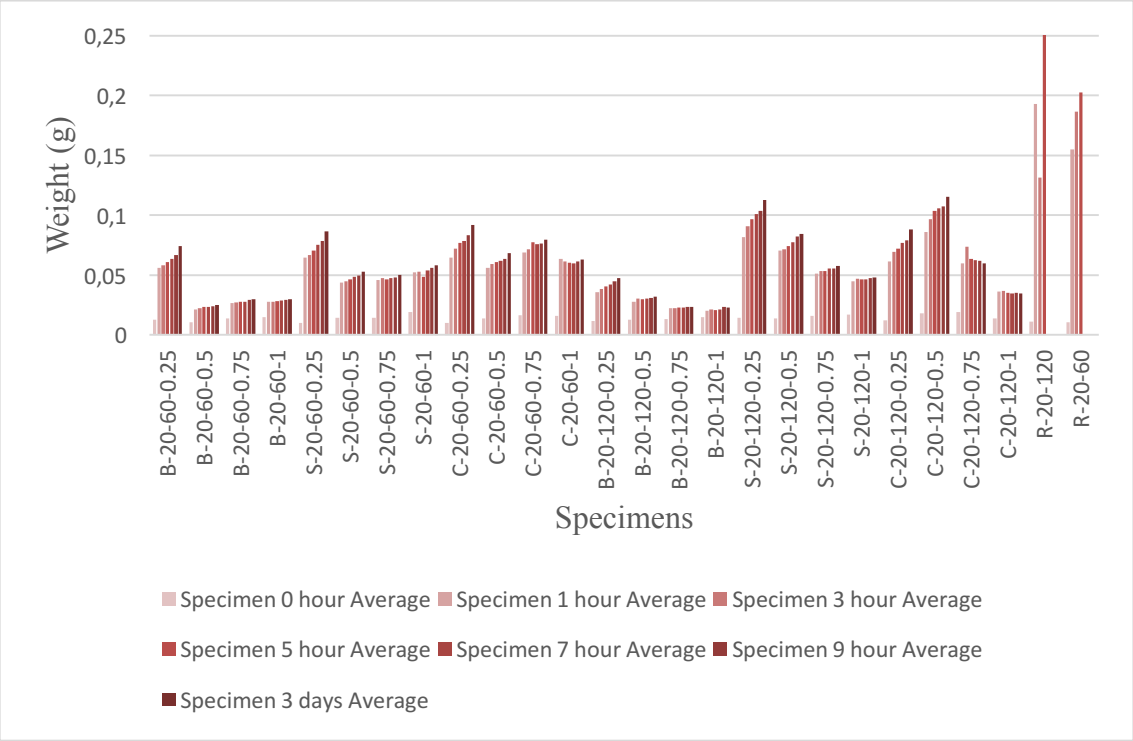


Figure 4.27 Swelling ratios hour by hour

Table 4.12 The percentages of average of swelling test measurements of main films

Specimen	% of 1h	% of 3h	% of 5h	% of 7h	% of 9h	% of 3 days
B-20-60-0.25	343	360	377	400	424	485
B-20-60-0.5	99	109	120	123	125	134
B-20-60-0.75	95	98	102	101	112	116
B-20-60-1	88	90	93	95	98	102
S-20-60-0.25	539	563	599	645	675	755
S-20-60-0.5	200	207	219	232	240	264
S-20-60-0.75	216	227	222	227	232	246
S-20-60-1	170	172	149	177	188	199
C-20-60-0.25	524	595	643	659	707	789
C-20-60-0.5	313	334	349	356	367	404
C-20-60-0.75	315	331	365	356	358	378
C-20-60-1	292	278	272	269	277	290
B-20-120-0.25	210	234	252	266	291	316
B-20-120-0.5	122	142	139	143	147	155
B-20-120-0.75	66	66	68	68	72	75
B-20-120-1	38	43	42	42	60	56
S-20-120-0.25	460	525	564	593	612	674
S-20-120-0.5	403	410	430	453	486	501
S-20-120-0.75	222	234	236	248	248	263
S-20-120-1	167	177	174	174	180	186
C-20-120-0.25	392	458	480	518	537	608
C-20-120-0.5	369	428	464	476	485	530
C-20-120-0.75	211	283	229	223	223	211
C-20-120-1	159	161	151	148	151	145
R-20-120	1625	1073	2190			
R-20-60	1388	1694	1850			

4.7 Antibacterial Tests

One day before planting bacteria on Nutrient agar petri dishes, bacteria were re-cultivated in shaker for 24 hours at 37°C. 100 ml of bacteria were taken from main bacteria culture which are obtained from Boğaziçi University, Molecular Biology and Genetic Department and was emptied to 15 ml sterilized falcon tube, then 900 ml of Nutrient

Broth, which was prepared before and stored in sun isolated cupboard at room temperature, added to falcon tubes, at the end of the liquid plantation falcon tube had 1000 ml of bacteria culture. This process done for E. Coli and S. Aureus, simultaneously. Bacteria culture taken from the shaker and dilution sets prepared just before of plantation. Dilution sets was prepared with addition of NaCl solution (8.5 g per mille). 100 µl of bacteria culture was taken from falcon tubes and poured into 1.5 ml of eppendorf tubes, tubes filled with addition of 900 ml of NaCl solution. This process repeated 6 times for standard dilution. Each dilution was made from the previous one.

The 100 µl of bacteria culture from 10^2 dilution sets planted on petri dishes for antibacterial tests. Also 100 µl of bacteria culture was taken from 10^4, 10^5 and 10^6 dilution sets for CFU calculation. Figure 4.28 and Figure 4.29 shows the colonies of E. Coli and S. Aureus bacteria with 10^4, 10^5 and 10^6 dilutions, respectively.

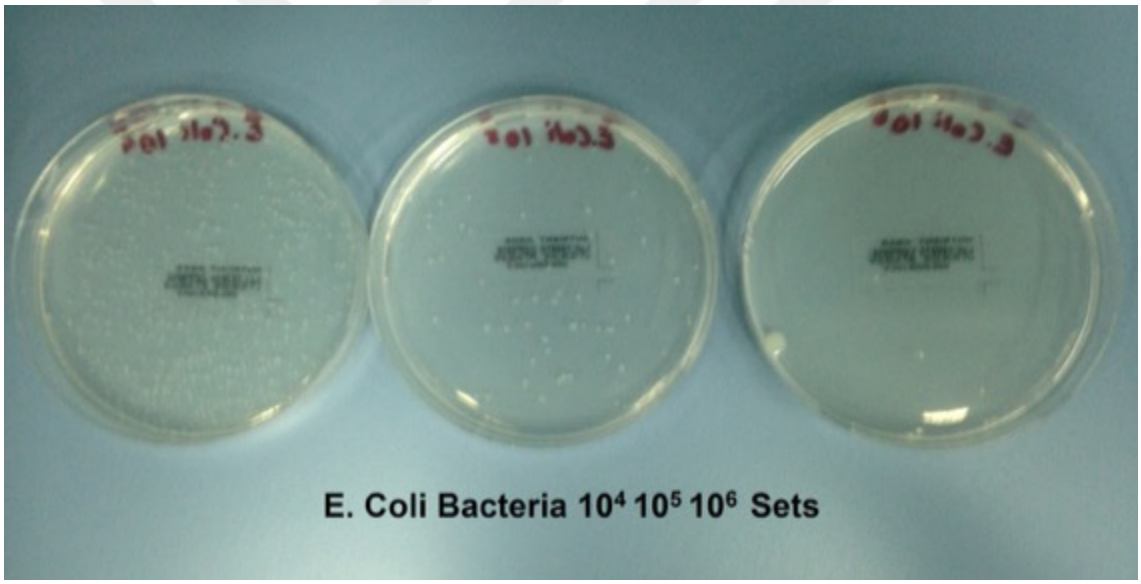


Figure 4.28 Bacteria E. Coli colonies for 10^4, 10^5 and 10^6 dilution sets, respectively

The colonies of 10^4 set was too much and 10^6 set was too few the colonies cannot be counted. But the colonies of 10^5 set was good enough to count the colonies of bacteria. The colonies counted as 55 on the petri dish.

The colony numbers of first two sets such as 10^5 set and 10^4 set was too much, the colonies cannot be counted. But the colonies of 10^6 set was clear enough to count the colonies of bacteria. The colony number counted as 32 on the petri dish.

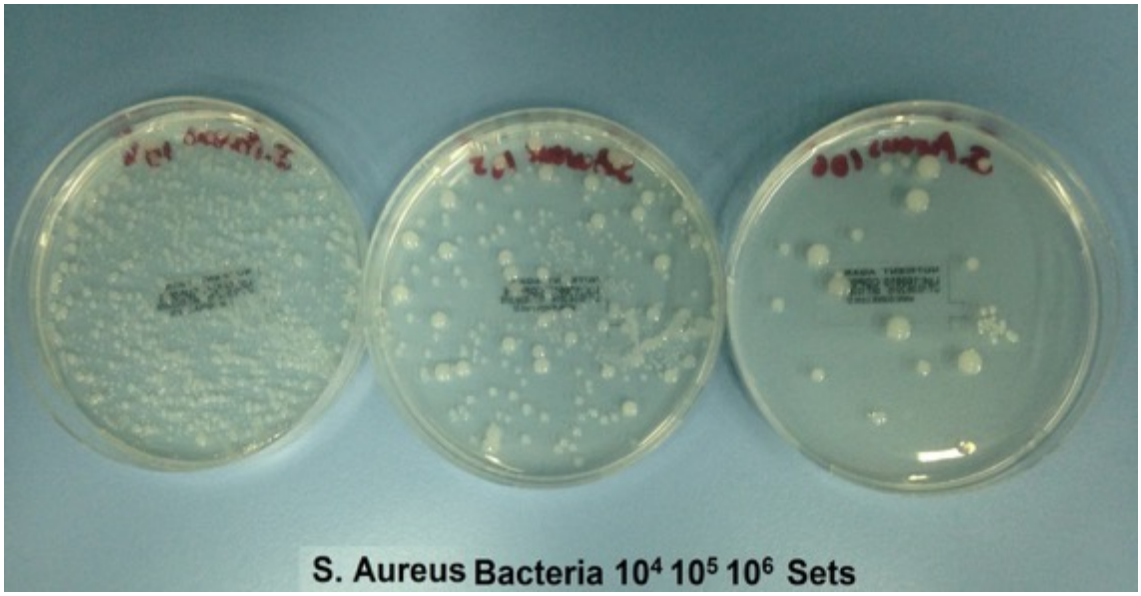


Figure 4.29 Bacteria S. Aureus colonies for 10^4, 10^5 and 10^6 dilution sets, respectively

The colony forming unit (CFU) was calculated from Equation 3.2. The bacteria E. Coli was found $0.55x10^8$ CFU and S. Aureus was found $3.2x10^8$ CFU.

Figure 4.30 and Figure 4.31 shows inhibition zones of Bluebottle essential oiled chitosan films with E. Coli bacteria for 60 minutes of reaction and 120 minutes of reaction respectively.

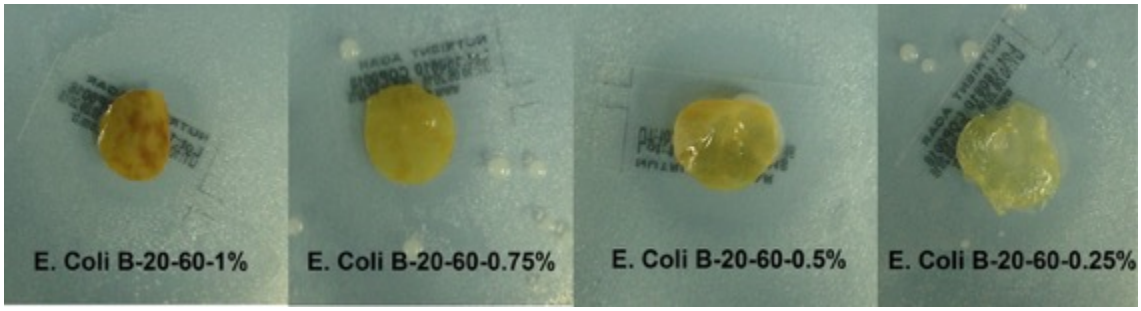


Figure 4.30 Inhibition zones of Bluebottle essential oiled chitosan films with 60 minutes of reaction time to E. Coli bacteria

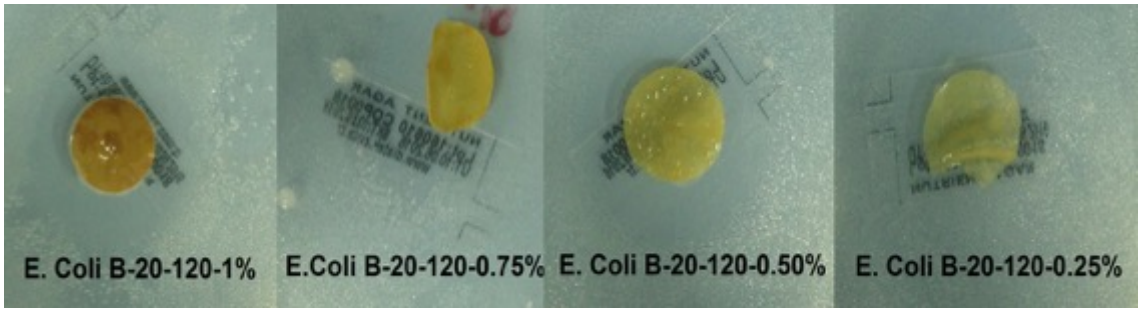


Figure 4.31 Inhibition zones of Bluebottle essential oiled chitosan films with 120 minutes of reaction time to E. Coli bacteria

Figure 4.32 and Figure 4.33 shows the inhibition zones of St John's Wort essential oil based chitosan films with 60 minutes and 120 minutes of reaction time, respectively.

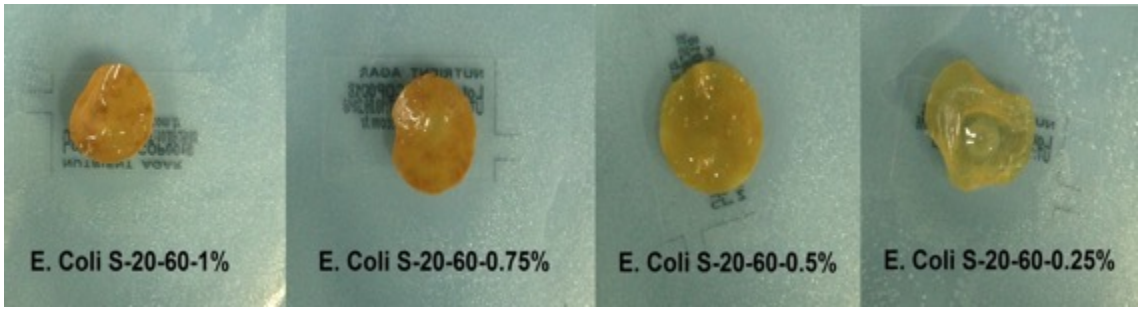


Figure 4.32 The E.Coli inhibition zones of St John's Wort essential oil based chitosan films (60 min reaction time)

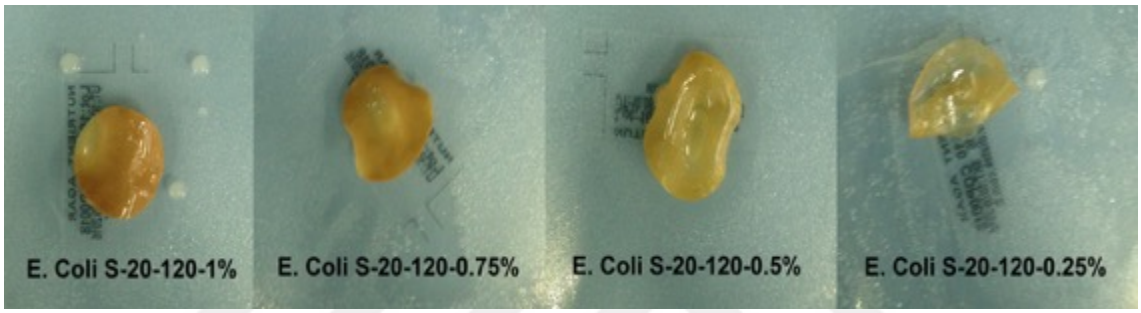


Figure 4.33 The E.Coli inhibition zones of St John's Wort essential oil based chitosan films (60 min reaction time)

Figure 4.34 and Figure 4.35 shows the inhibition zones of Common Centaury based chitosan films with 60 minutes and 120 minutes of reaction time, respectively.

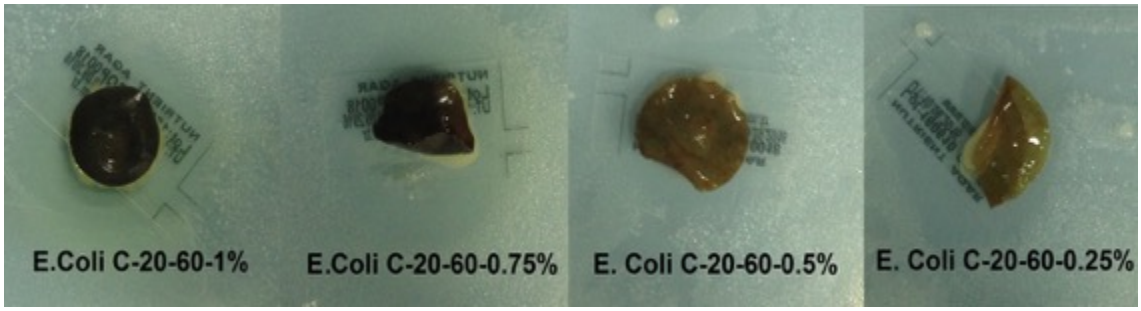


Figure 4.34 The inhibition zones of E. Coli bacteria with Common Centaury essential oil based chitosan films with 60 minutes of reaction time

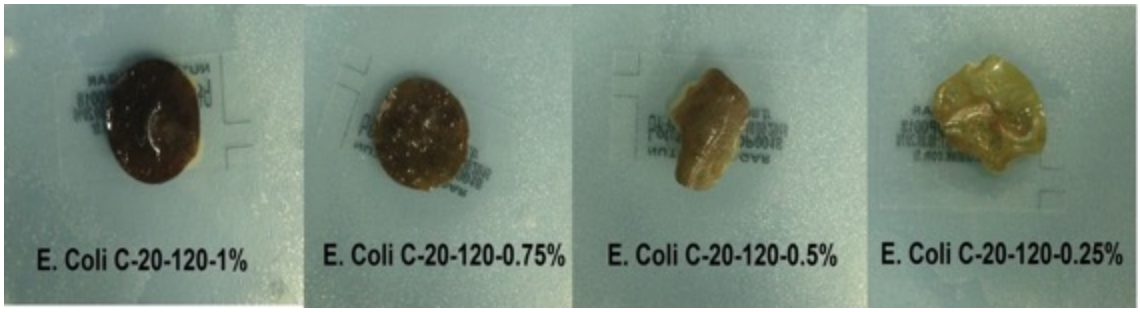


Figure 4.35 The inhibition zones of E. Coli bacteria with Common Centaury essential oil based chitosan films with 60 minutes of reaction time

The films were cut with round shape of 12 mm and after plantation they were put in middle of petri dishes and inhibition zone was measured after 24 hours at 37°C cultivation. Table 4.13 shows the measurements of inhibition zone diameters The measurements calculated with 12 mm of film diameter, in short the diameters includes films diameters.

Table 4.13 The radius of inhibition zones of E. Coli and S. Aureus

Specimen	**Inhibition Zone (cm)**	**Inhibition Zone (cm)**
	E. Coli	**S. Aureus**
R-20-60	2.3	0
R-20-120	2	0
B-20-60-0.25	2.45	0
B-20-60-0.5	3.15	0
B-20-60-0.75	2.45	1.2
B-20-60-1	2.45	1.25
B-20-120-0.25	2.25	0
B-20-120-0.5	3.2	0
B-20-120-0.75	2.5	1.1
B-20-120-1	3	1.3
S-20-60-0.25	2.4	0
S-20-60-0.5	2.7	1

Table 4.13 The radius of inhibition zones of E. Coli and S. Aureus (cont'd)

Specimen	**Inhibition Zone (cm)**	**Inhibition Zone (cm)**
	E. Coli	**S. Aureus**
S-20-60-0.75	2.45	1.2
S-20-60-1	2.5	1.2
S-20-120-0.25	2.1	0
S-20-120-0.5	2.55	1
S-20-120-0.75	2.25	1.2
S-20-120-1	2.3	1.2
C-20-60-0.25	2.05	0
C-20-60-0.5	2.25	0
C-20-60-0.75	2.4	1
C-20-60-1	3.05	1.35
C-20-120-0.25	2	0
C-20-120-0.5	2.15	0
C-20-120-0.75	2.35	1.2
C-20-120-1	2.8	1.3

Figure 4.36, Figure 4.37, Figure 4.38, Figure 4.39, Figure 4.40 and Figure 4.41 shows the inhibition zones of S. Aureus bacteria with Bluebottle essential oil based chitosan films with 60 minutes and 120 minutes' reaction time, St John's Wort essential oil based chitosan films with 60 minutes and 120 minutes' reaction time and Common Centaury essential oil based chitosan films with 60 minutes and 120 minutes' reaction time, respectively.

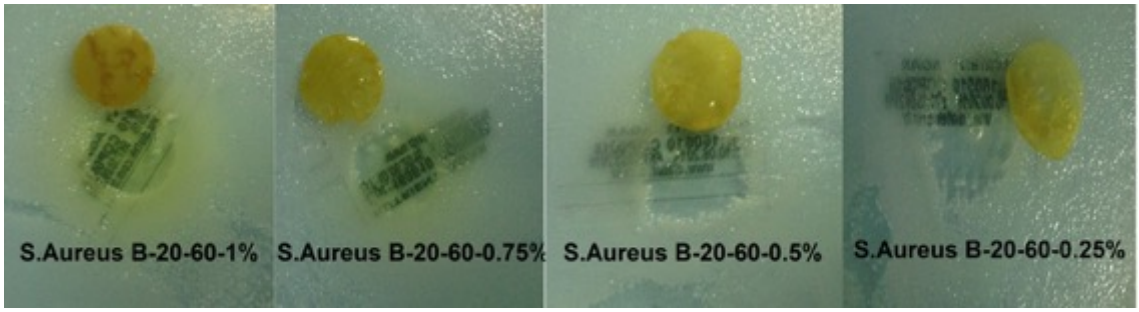


Figure 4.36 The inhibition zones of S. Aureus bacteria with Bluebottle essential oil based chitosan films with 60 minutes of reaction time

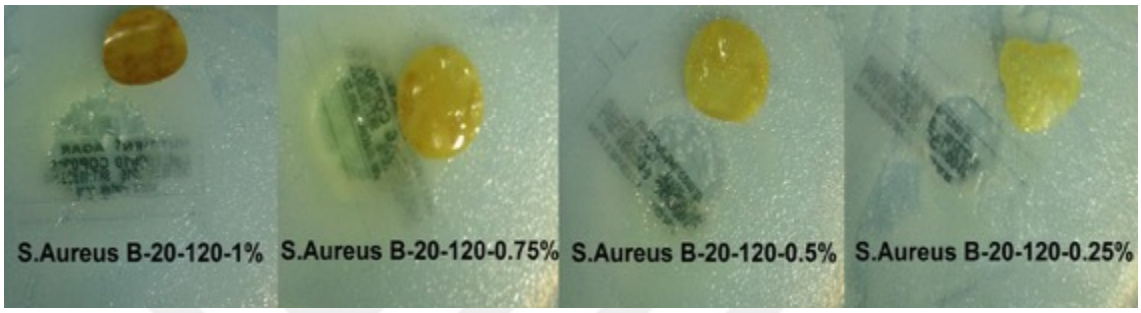


Figure 4.37 The inhibition zones of S. Aureus bacteria with Bluebottle essential oil based chitosan films with 120 minutes of reaction time

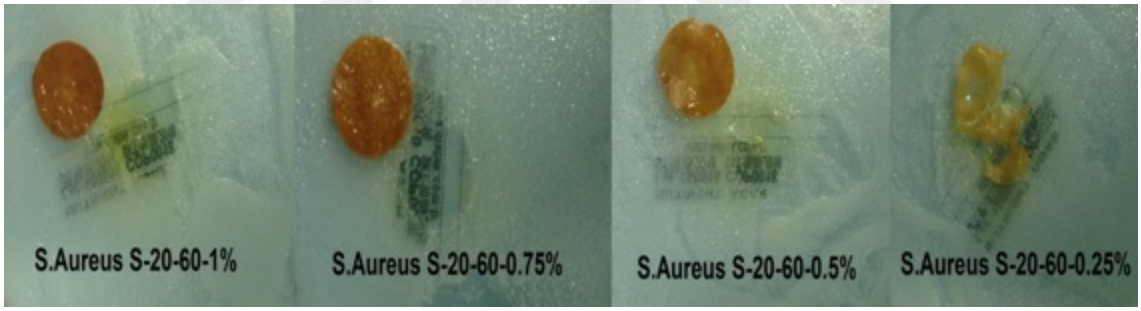


Figure 4.38 The inhibition zones of S. Aureus bacteria with St John's Wort essential oil based chitosan films with 60 minutes of reaction time

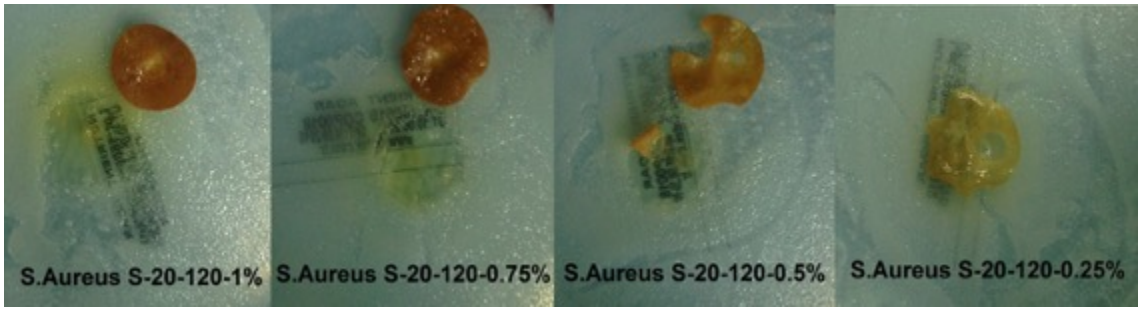


Figure 4.39 The inhibition zones of S. Aureus bacteria with St John's Wort essential oil based chitosan films with 60 minutes of reaction time

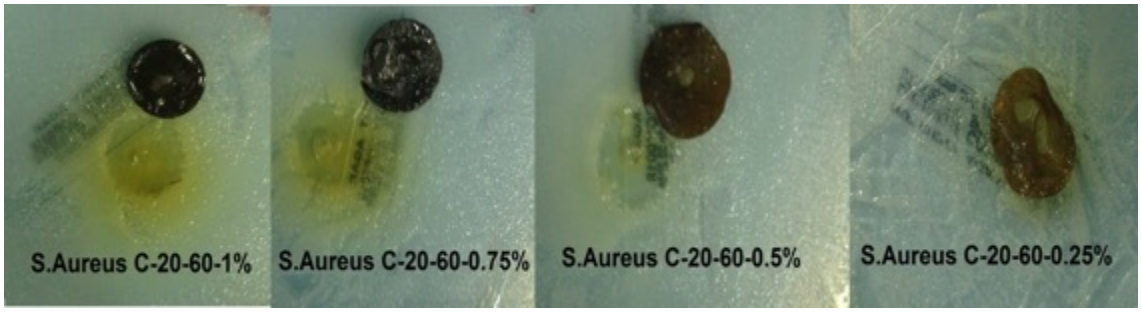


Figure 4.40 The inhibition zones of S. Aureus bacteria with Common Centaury essential oil based chitosan films with 60 minutes of reaction time

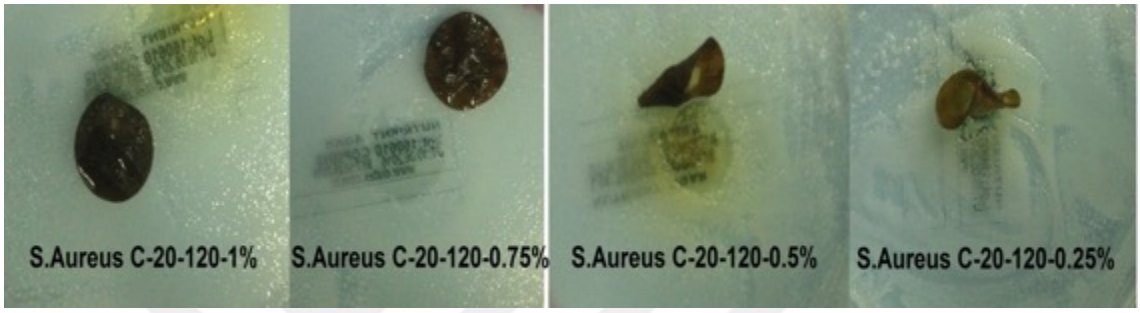


Figure 4.41 The inhibition zones of S. Aureus bacteria with Common Centaury essential oil based chitosan films with 120 minutes of reaction time

The highest inhibition zones were seen with E. Coli bacteria. The all essential oil based films which are obtained from different gentian plants such as Bluebottle, St John's Wort and Common Centaury has antibacterial effect on gram negative E. Coli bacteria. The highest result was measured as 3.2 cm of inhibition zone with B-20-120-0.5 specimen, also the specimen B-20-120-0.5 has 3.15 cm of inhibition zone. At this point the addition of 0.5% (w/w) of essential oil of Bluebottle is the optimum ratio for antibacterial test. Following this, St John's Wort essential oil based chitosan films had highest inhibition zone with addition of 0.5% (w/w) essential oil too, but their inhibition zone diameter is lower than Bluebottle. The inhibition zones of Common Centaury essential oil based chitosan films were increasing with addition of oil. The highest result seen with C-20-60-1 then C-20-120-1, as 3.05 cm and 2.8 cm, respectively. All these results were given for E. Coli bacteria.

The results for S. Aureus bacteria was not great as E. Coli bacteria. The films had inhibition zones but their measurements were lower than E. Coli. Only the St John's Wort essential oil based chitosan films had inhibition zone with 0.5% (w/w) ratio, the other had not. The antibacterial effect increased with increasing of essential ratio in all types of oil. The Common Centaury essential oil based chitosan films had the highest diameter of

inhibition zone such as 1.3 cm and 1.35 cm, with C-20-60-1 and C-20-120-1 specimens, respectively. All films with addition of 0.75% and 1%(w/w) ratio had antibacterial effect on S. Aureus bacteria but the diameter was low.

Pure chitosan films had not great antibacterial effects but they had effect around 2 cm to E. Coli bacteria, but not for S. Aureus. (Figure 4.42 and Figure 4.43)

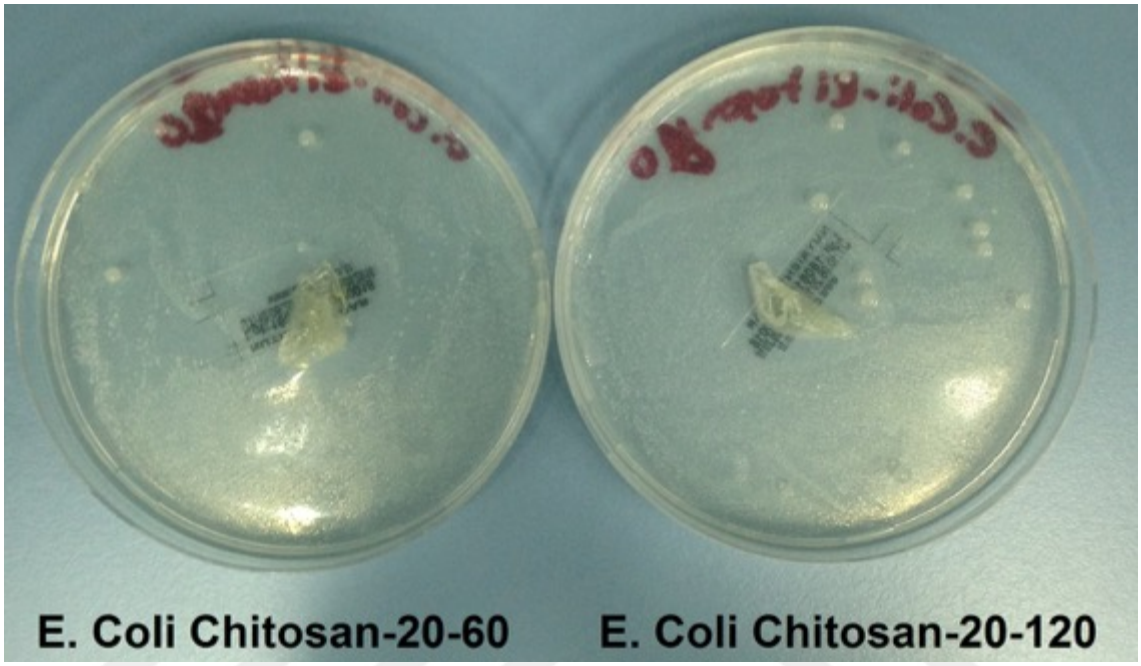


Figure 4.42 The inhibition zones of pure chitosan films with E. Coli

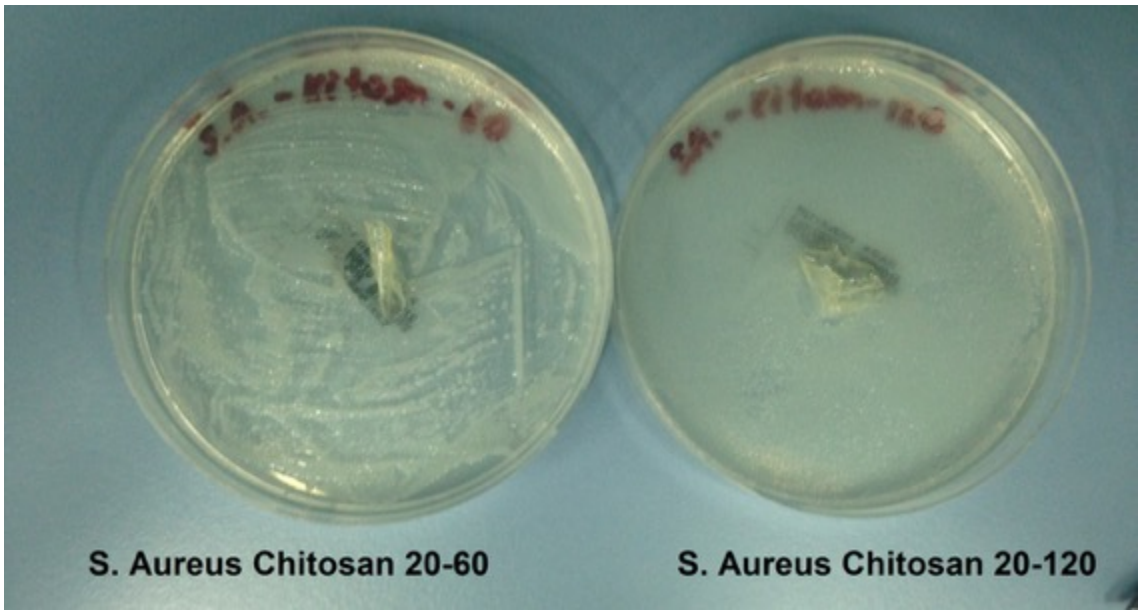


Figure 4.43 The inhibition zones of pure chitosan films with S. Aureus

4.8 Thickness Results of Films

The film's thickness was increased with increasing of essential oil ratio. Essential oils of gentian species such as Common Centaury, Bluebottle and St John's Wort gives more thickness to films. First pretesting experiments were measured. The results of this measurement can be seen from Table 4.14. As it seen from the table, 0.5% (w/w) addition of essential oil gave around 0.5 mm of more thickness. The average of thicknesses measured as 0.143 mm. The thicknesses didn't affect by difference of temperatures, neither reaction times.

Table 4.14 The thickness measurements of pretesting films

Specimen	**Thickness (mm)**
R-20-90	0.1
R-30-90	0.09
R-40-90	0.09
B-20-60-0.5	0.14
B-30-60-0.5	0.13
B-40-60-0.5	0.14
B-20-120-0.5	0.15
B-30-120-0.5	0.15
B-40-120-0.5	0.15

The main thicknesses of films were measured too. The Table 4.15 shows the result of measurements. As it indicated before, the addition of essential oils increases the thicknesses of the films, the 1% (w/w) addition gave the most thickness to films than 0.75%, 0.5% and 0.25% (w/w), respectively.

Table 4.15 The thicknesses of main films

Specimen	**Thickness (mm)**
B-20-60-1	0.16
B-20-60-0.75	0.15
B-20-60-0.5	0.14

Table 4.15 The thicknesses of main films (cont'd)

Specimen	**Thickness (mm)**
B-20-60-0.25	0.10
B-20-120-1	0.17
B-20-120-0.75	0.16
B-20-120-0.5	0.12
B-20-120-0.25	0.11
S-20-60-1	0.17
S-20-60-0.75	0.15
S-20-60-0.5	0.13
S-20-60-0.25	0.12
S-20-120-1	0.17
S-20-120-0.75	0.15
S-20-120-0.5	0.12
S-20-120-0.25	0.11
C-20-60-1	0.16
C-20-60-0.75	0.15
C-20-60-0.5	0.14
C-20-60-0.25	0.11
C-20-120-1	0.16
C-20-120-0.75	0.14
C-20-120-0.5	0.11
C-20-120-0.25	0.10
R-20-60	0.10
R-20-120	0.09

The essential oil difference didn't affect the thickness of films. The thicknesses only affected by essential oil ratio. The addition of 1% (w/w) essential oil to films, gave 0.07

mm more thickness to films, this is the average measurement for all specimens. Because temperature is fixed and the reaction time didn't affect the thicknesses. The addition of 0.75%, 0.5% and 0.25% (w/w) essential oil gave 0.055 mm, 0.031 mm and 0.013 mm more thicknesses to films, respectively. The average measurement of pure chitosan films measured as 0.095 mm. The average of thicknesses measured as 0.165 mm, 0.15 mm, 0.126 mm and 0.108 mm for addition of 1%, 0.75%, 0.5% and 0.25% (w/w) essential oils respectively.

4.9 Discussion

Chitosan was chosen as a raw material and the production of films made by addition of three different types of gentian plant's essential oils with different ratios such as 1%, 0.75%, 0.5% and 0.25% (w/w).

Gentian plants was analyzed with ICP-OES to determine major elements and harmful elements. The results showed that major element content of gentian plants closely to each other and harmful elements found below detection limit (bdl).

Essential oils were obtained with Soxhlet extraction method and characterization test was analyzed with UV-Vis, as it expected, gentian species had polyphenol and flavonoids in structure.

DTA-TG analysis showed that, addition of essential oil composed a new DTG peak around 400°C. The precision of DTG peak was increased with increasing of essential oil ratio. The addition of 0.25% (w/w) essential oil of St John's Wort and Bluebottle had not DTG peak around 400°C, only the Common Centaury essential oil addition had it. All degradation steps were slowed down with essential oil addition. The rapid degradations were occurred with addition of 1% (w/w) essential oils at 3rd step.

FT-IR and XRD results found compatible with literature researches. Pure chitosan powder and chitosan film was compared by FT-IR and polymerization characteristic peaks at 3354 and 1308 cm^{-1} reduced their volumes and new peaks occurs around 2290, 1540, 1400 and 1250 cm^{-1} .

The swelling ratio of films was decreased with increasing of essential oil addition but their structure got steadier. Because after 5 hours' pure chitosan films degrade in PBS solution. The highest swelling ratios were detected in C-20-60-0.25 specimen then C-20-

120-0.25 and S-20-120-0.25, respectively. The lowest swelling ratios were detected with addition of 1% (w/w) essential oil.

The results of antibacterial tests were more important than all other characterizations, because it provides inhibition zone around the wound. The films were selective to bacteria. All films had antibacterial effect to E. Coli bacteria but not to S. Aureus. Bluebottle essential oil addition gave the significant result with E. Coli bacteria, but Common Centaury essential oil addition gave normal results with E. Coli and S. Aureus, simultaneously. Also antimicrobial efficiency of Common Centaury essential oil based chitosan films was increased by increasing of essential oil ratio in both bacteria. The ratio of 0.5% (w/w) for Bluebottle and St John's Wort essential oil in the films were provided better antibacterial inhibition zone to E. Coli bacteria, but they were not antibacterial to S. Aureus. For S. Aureus bacteria, Common Centaury essential oil addition were provided better inhibition zones.

As a result of this thesis, Bluebottle essential oil based chitosan film with code B-20-120-0.5 is optimum wound dressing film for E. Coli bacteria and swelling ratio of the film is high enough. Common Centaury essential oil based chitosan film with code C-20-60-1 gives highest inhibition zone for S. Aureus and it is effective for E. Coli too.

Generally, essential oil addition gives antibacterial effect to all films, preserves film structure in PBS solution, increased film thickness, occurred new DTG peak area. Also it doesn't have any negative effects to polymerization structure which is found from FT-IR results.

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APPENDIX-A

XRD PATTERN FIGURES

Appendix A gives, other XRD patterns for all specimens, which are not given inside of the thesis.

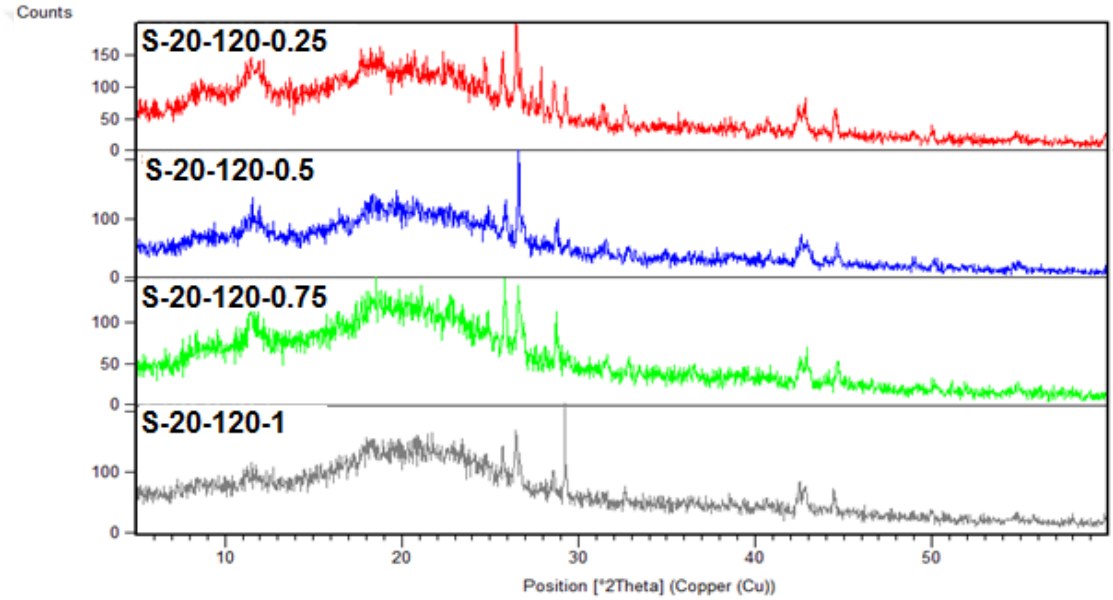


Figure A.1 XRD patterns of St John's Wort essential oil based chitosan films (120min)

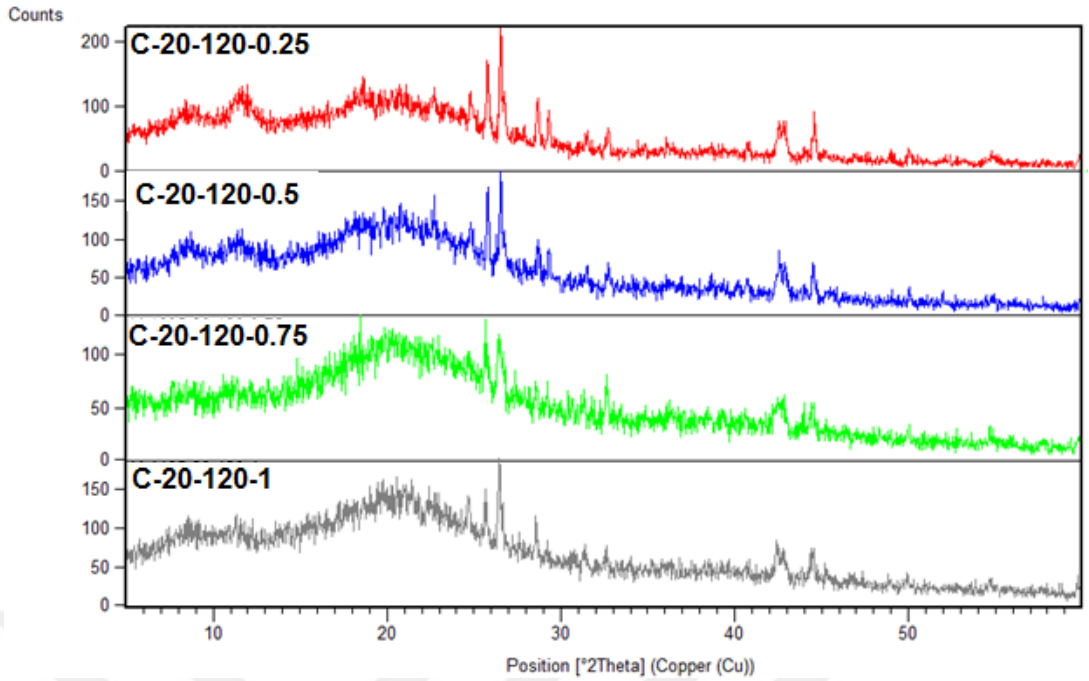


Figure A.2 XRD patterns of Common Centaury essential oil based chitosan films (120min)

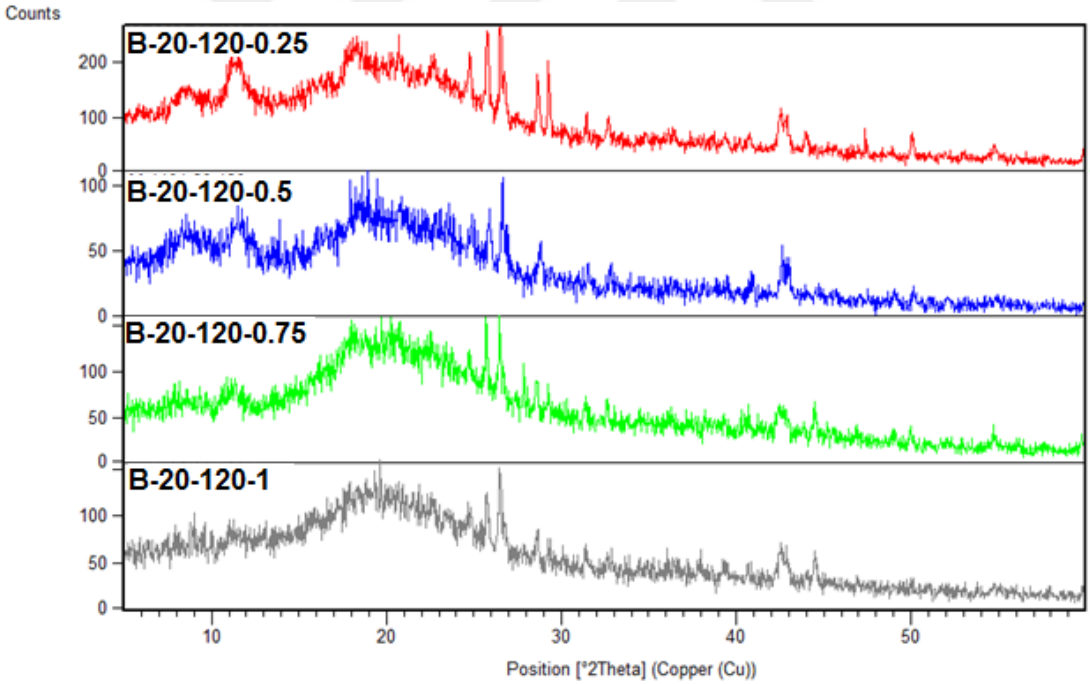


Figure A.3 XRD patterns of Bluebottle essential oil based chitosan films (120min)

APPENDIX-B

FT-IR SPECTRA FIGURES

The other specimen comparisons of temperature, reaction time, different essential oil effect, essential oil addition ratio effect, for FT-IR analysis are given below.

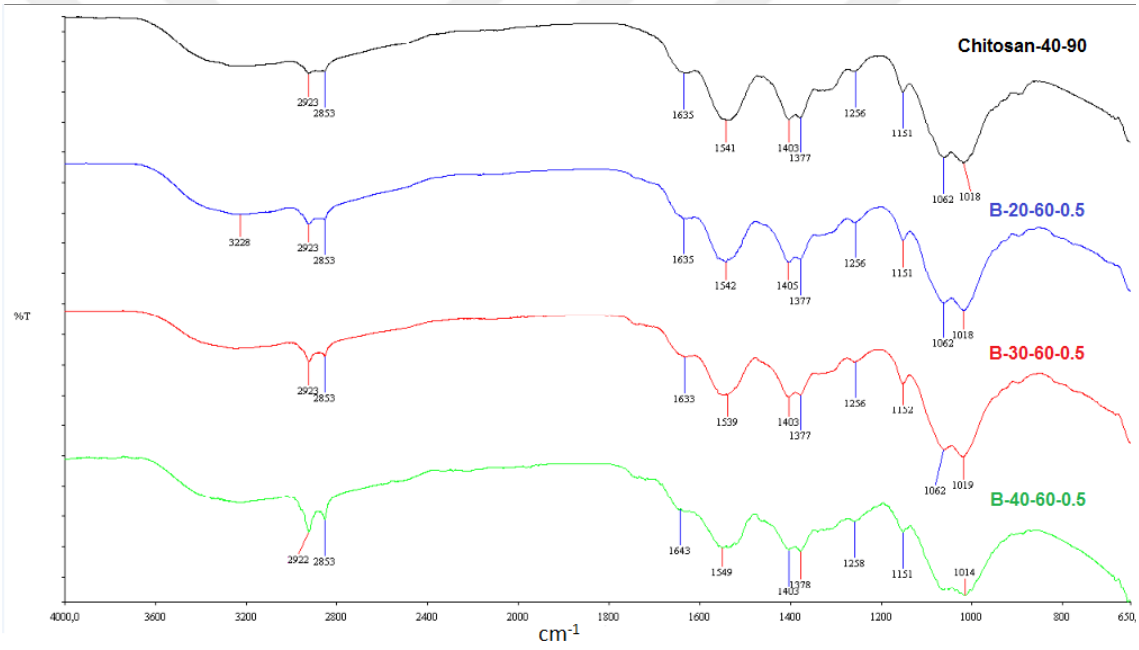


Figure B.1 Temperature scanning of pretesting specimens (60 min)

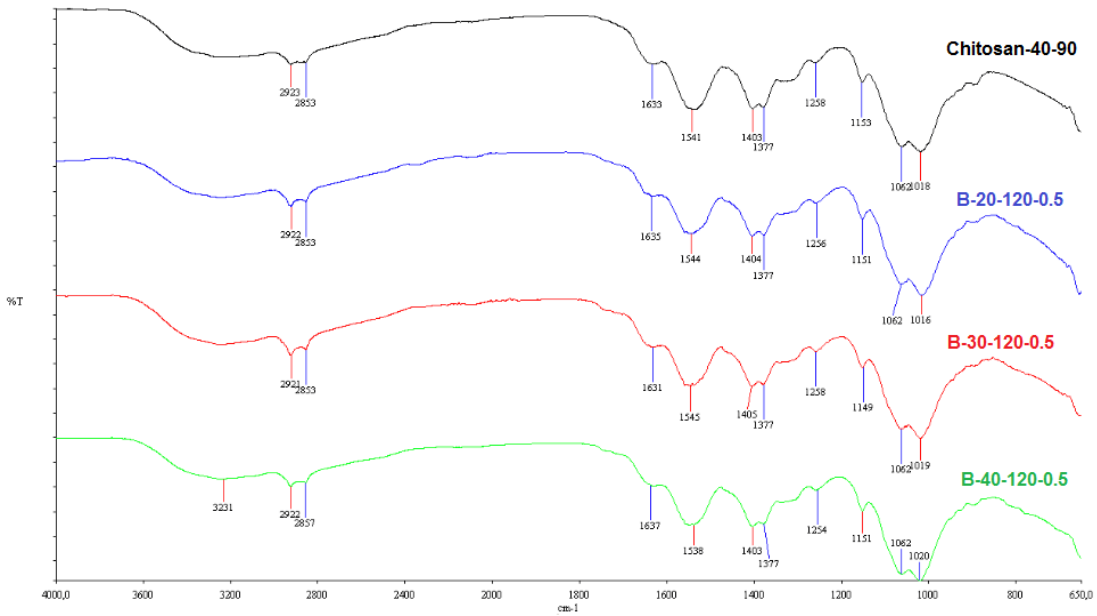


Figure B.2 Temperature scanning of pretesting specimens (120 min)

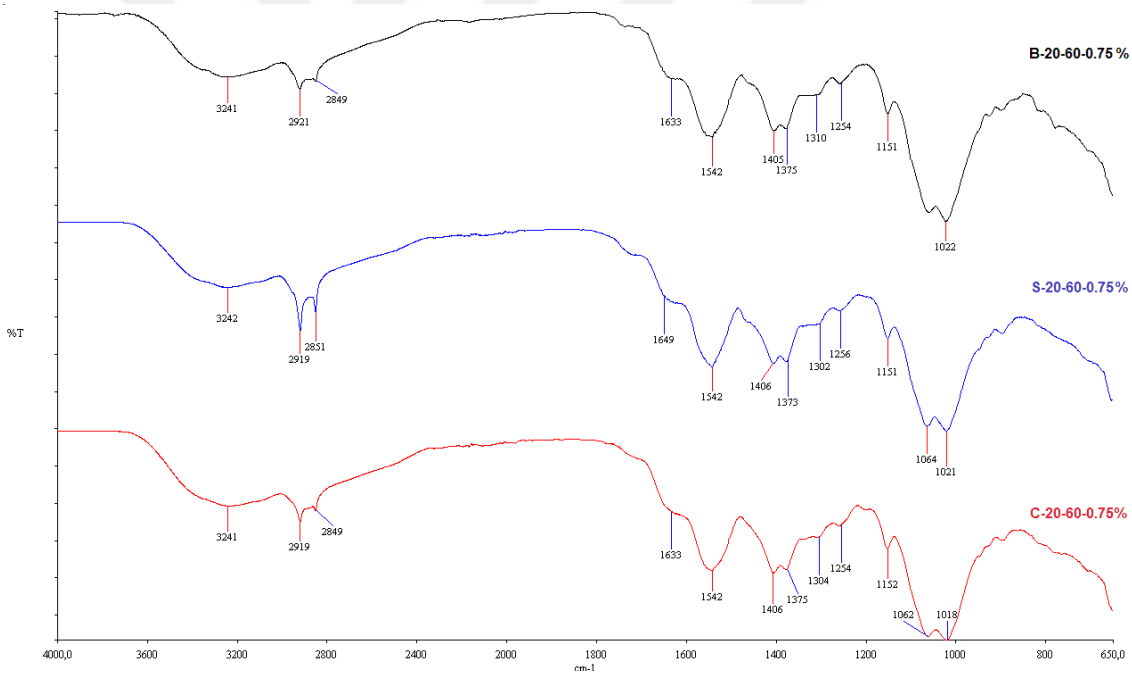


Figure B.3 FT-IR results of 0.75% (w/w) addition of essential oils (60 min)

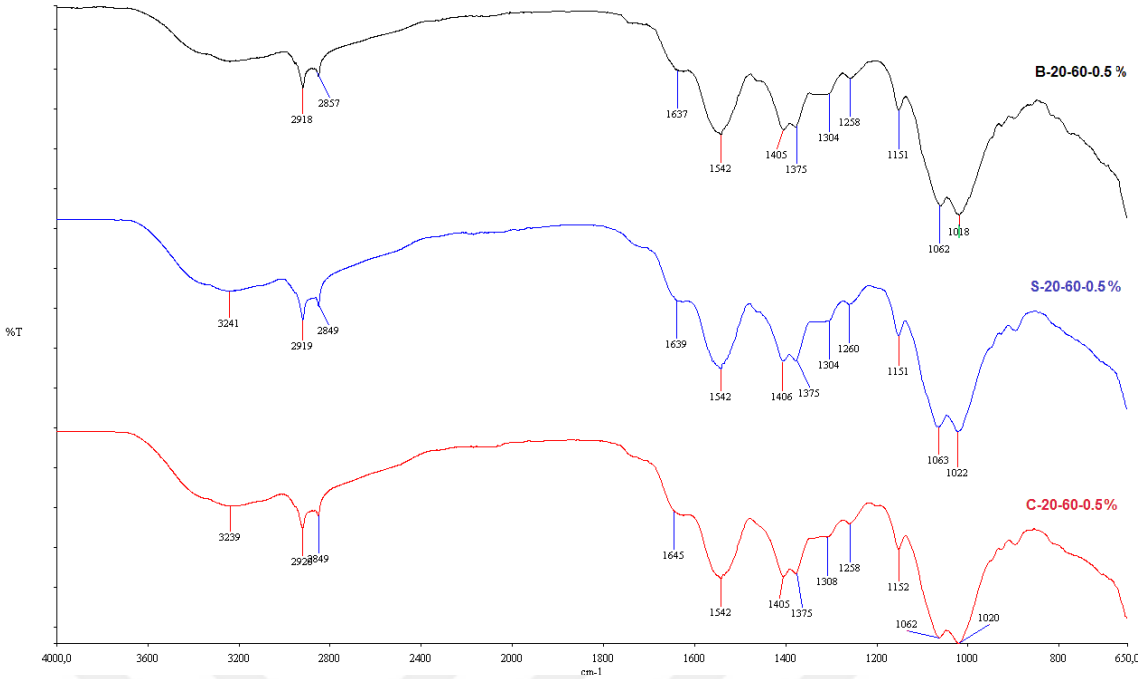


Figure B.4 FT-IR results of 0.5% (w/w) addition of essential oils (60 min)

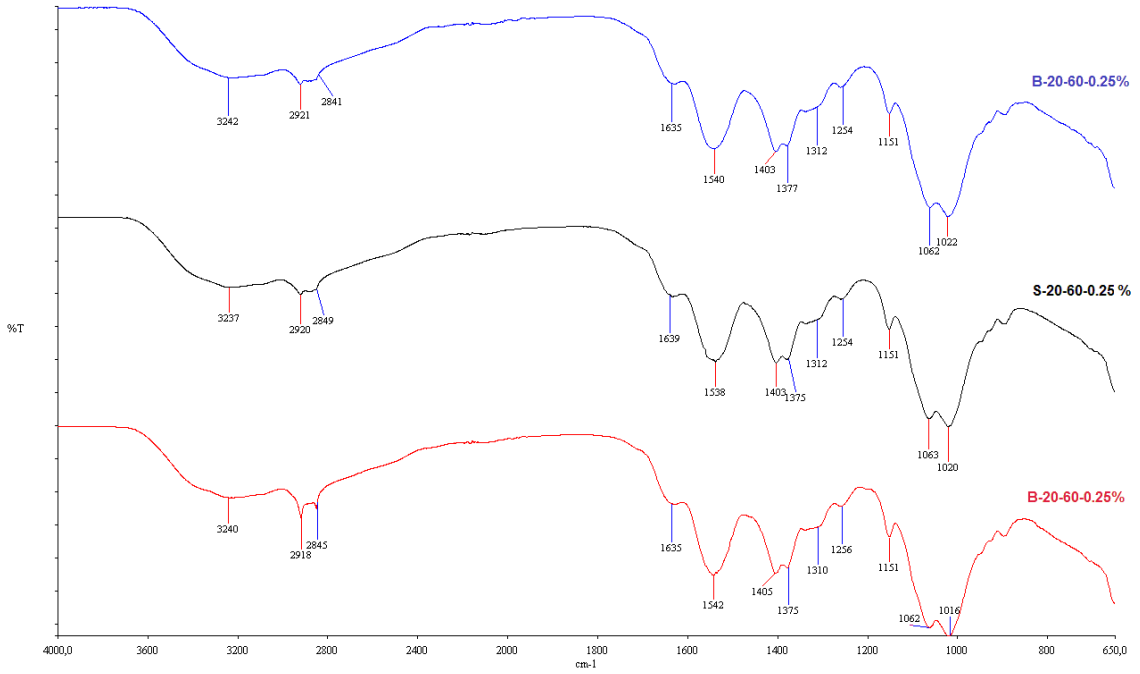


Figure B.5 FT-IR results of 0.25% (w/w) addition of essential oils (60 min)

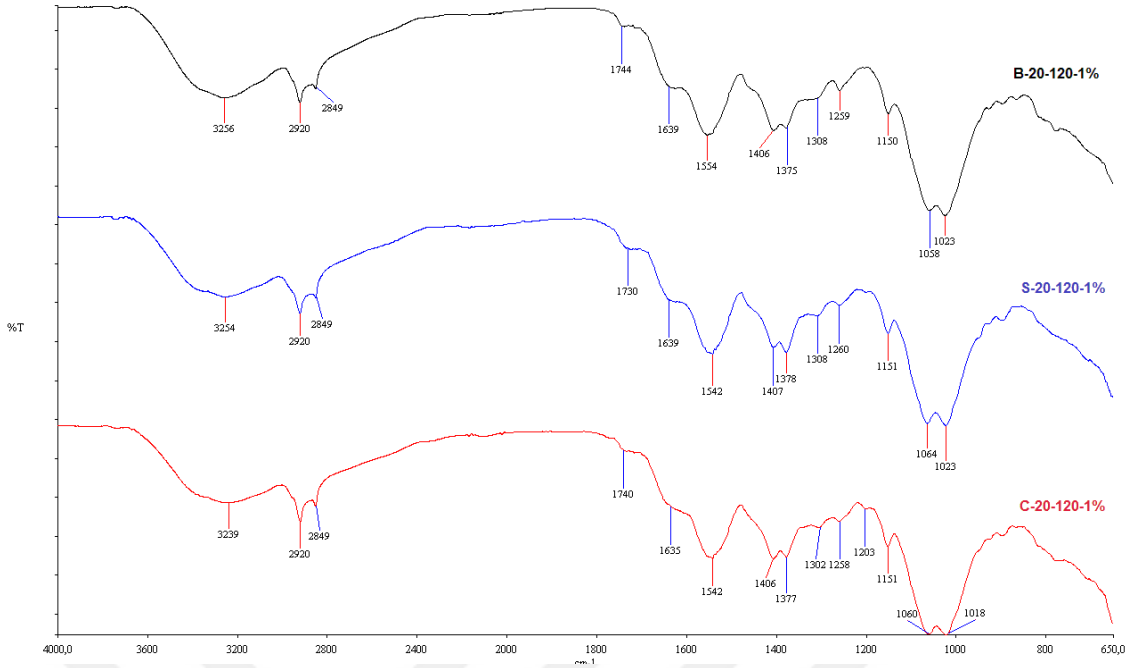


Figure B.6 FT-IR results of 1% (w/w) addition of essential oils (120 min)

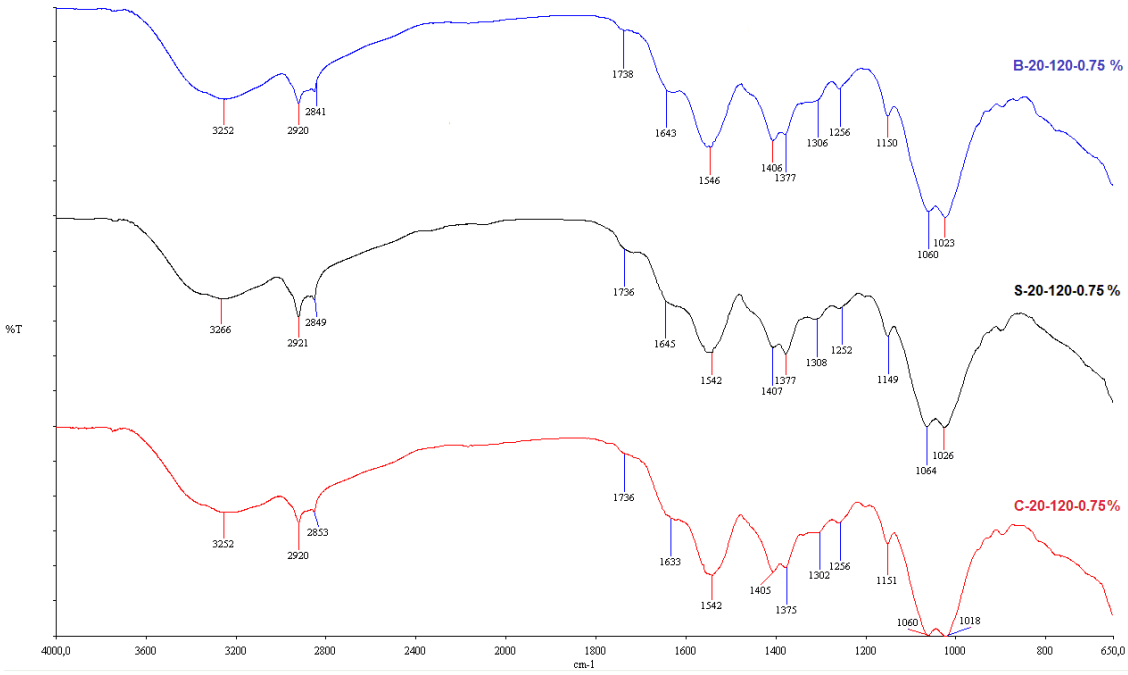


Figure B.7 FT-IR results of 0.75% (w/w) addition of essential oils (120 min)

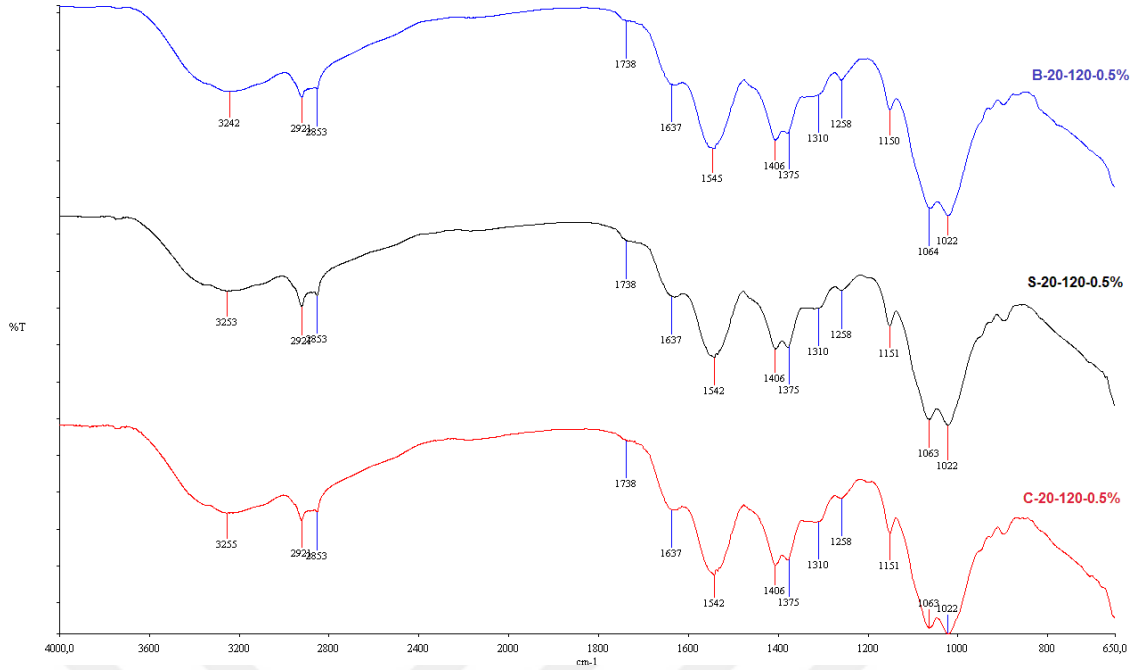


Figure B.8 FT-IR results of 0.5% (w/w) addition of essential oils (120 min)

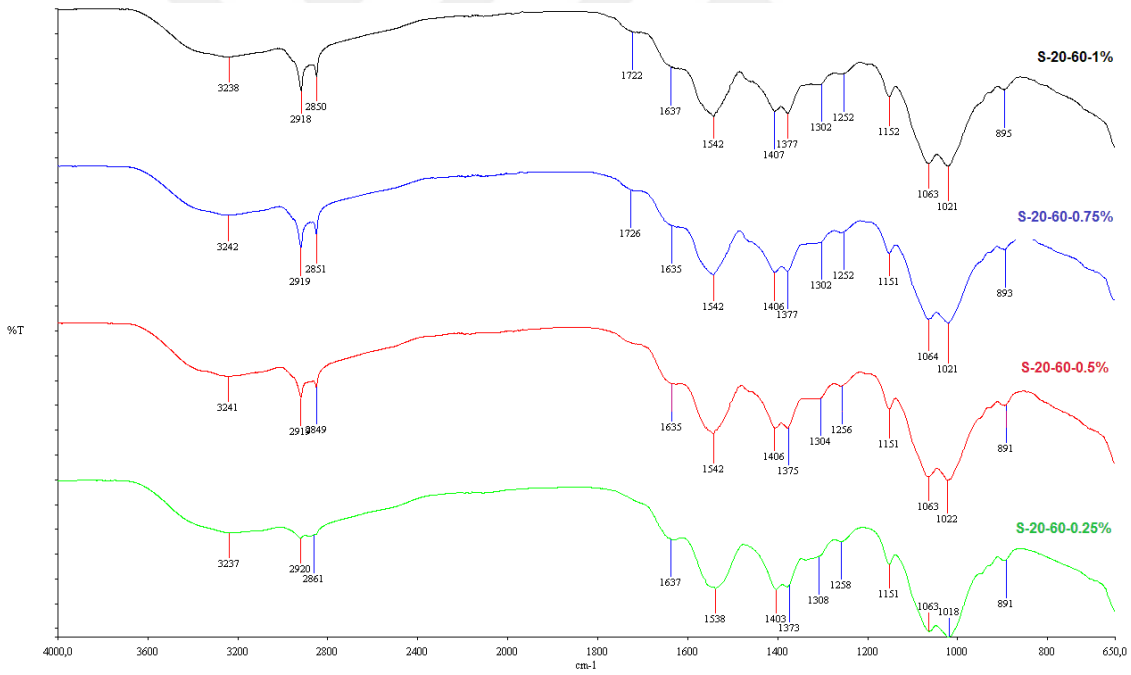


Figure B.9 FT-IR results for ratios of St John's Wort essential oil addition (60 min)

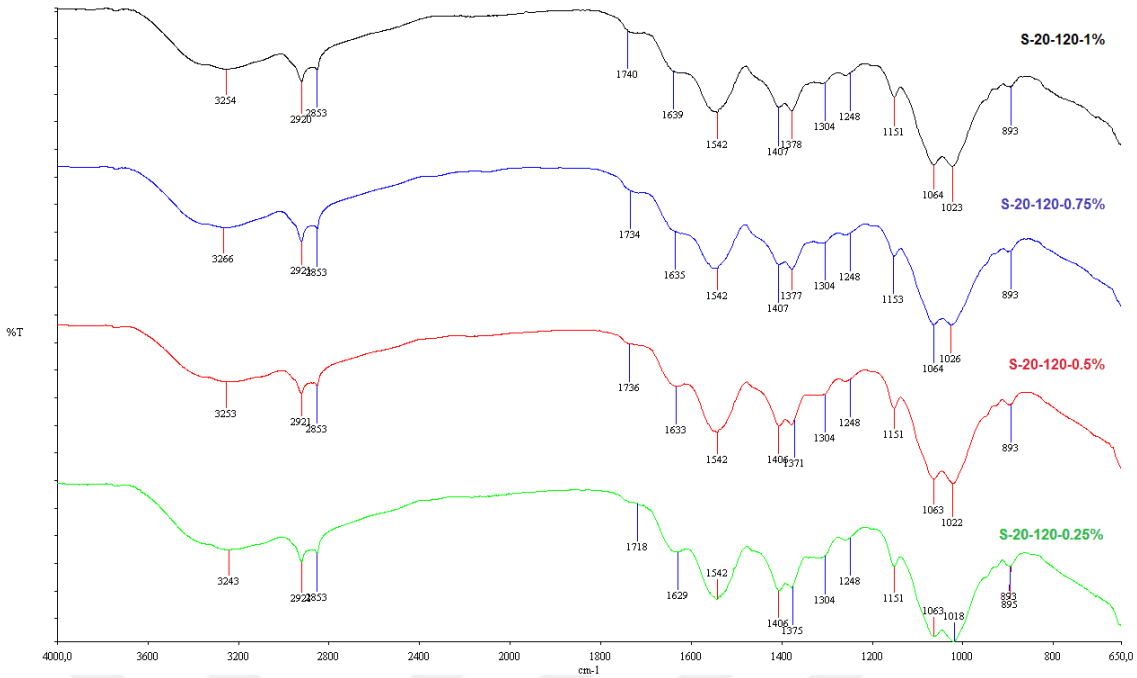


Figure B.10 FT-IR results for ratios of St John's Wort essential oil addition (120 min)

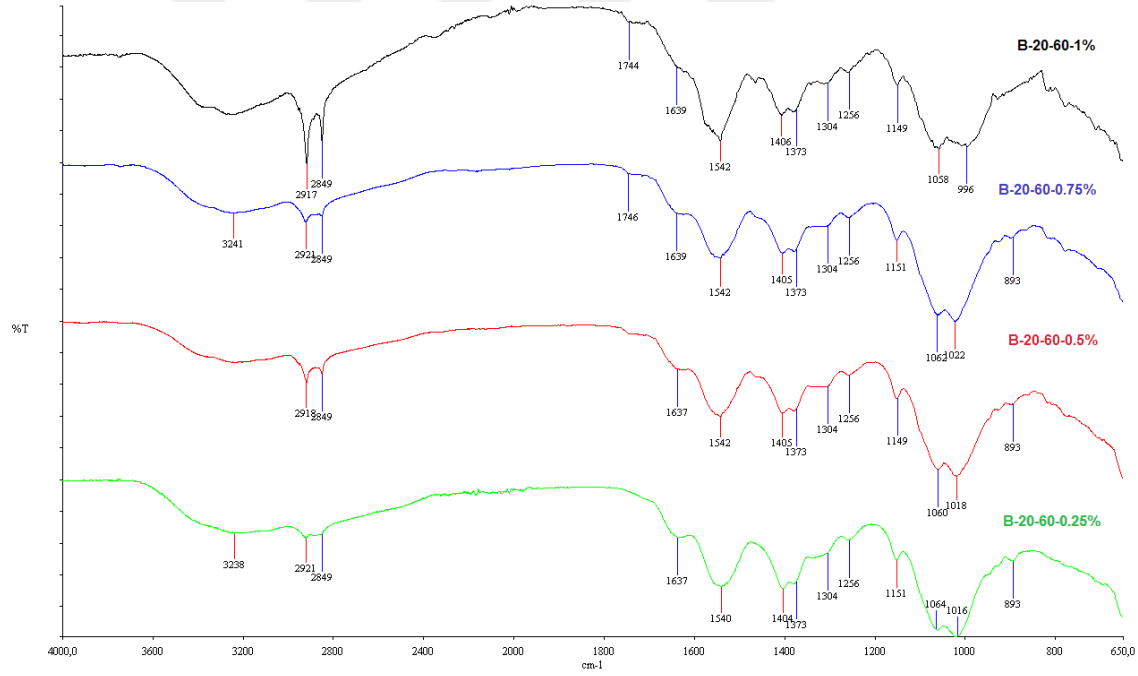


Figure B.11 FT-IR results for ratios of Bluebottle essential oil addition (60 min)

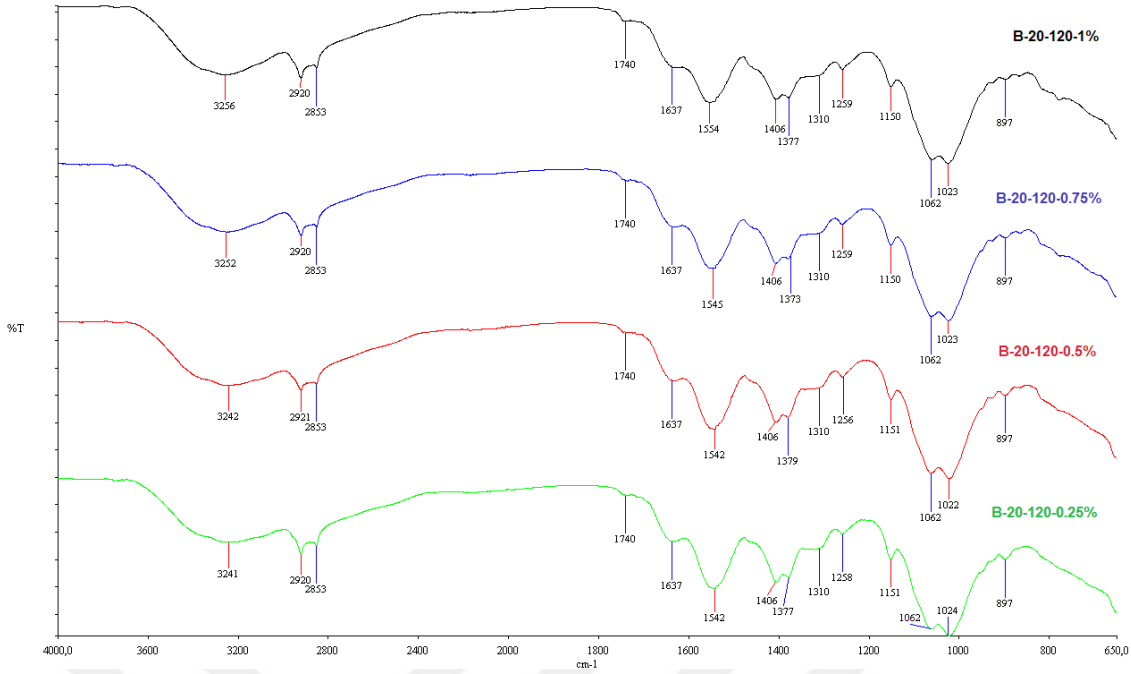


Figure B.12 FT-IR results for ratios of Bluebottle essential oil addition (120 min)

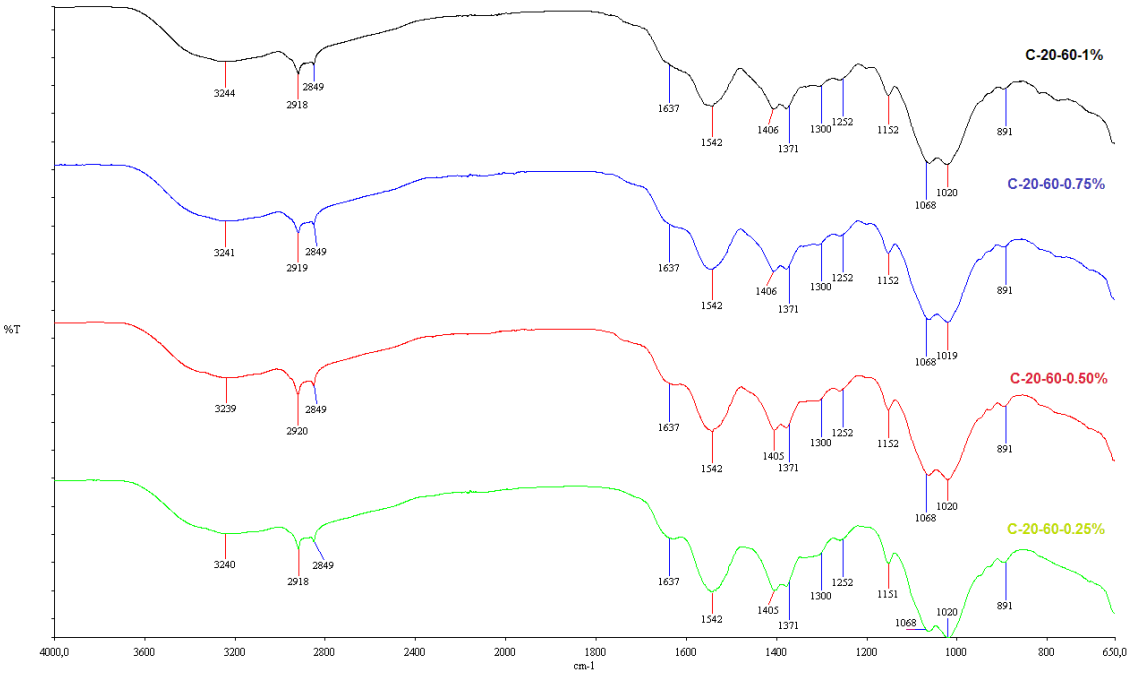


Figure B.13 FT-IR results for ratios of Common Centaury essential oil addition (60 min)

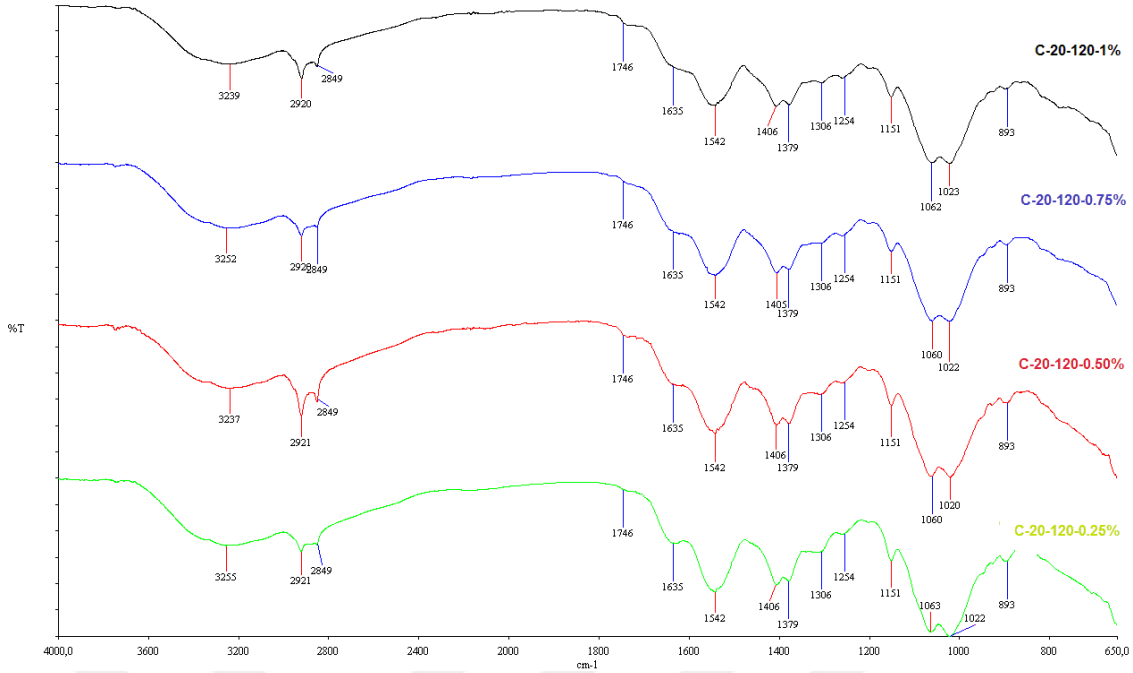


Figure B.14 FT-IR results for ratios of Common Centaury essential oil addition (120 min)

APPENDIX-C

DTA-TG GRAPHS

The other specimen comparisons of temperature, reaction time, different essential oil effect, essential oil addition ratio effect, for DTA-TG analysis are given below.

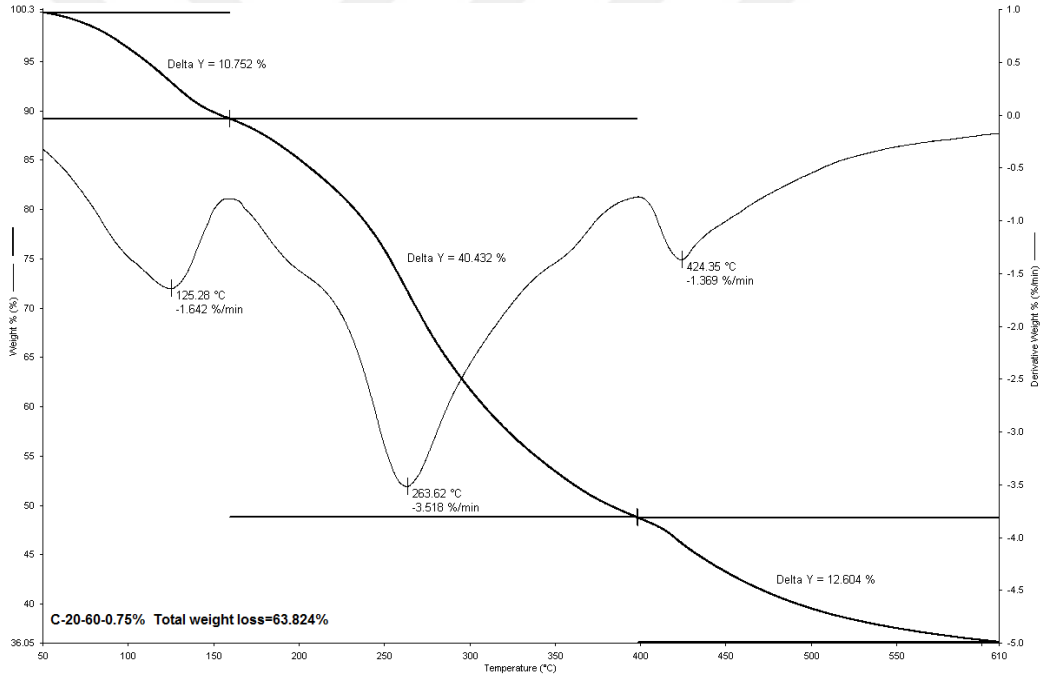


Figure C.1 DTA-TG graph of C-20-60-0.75

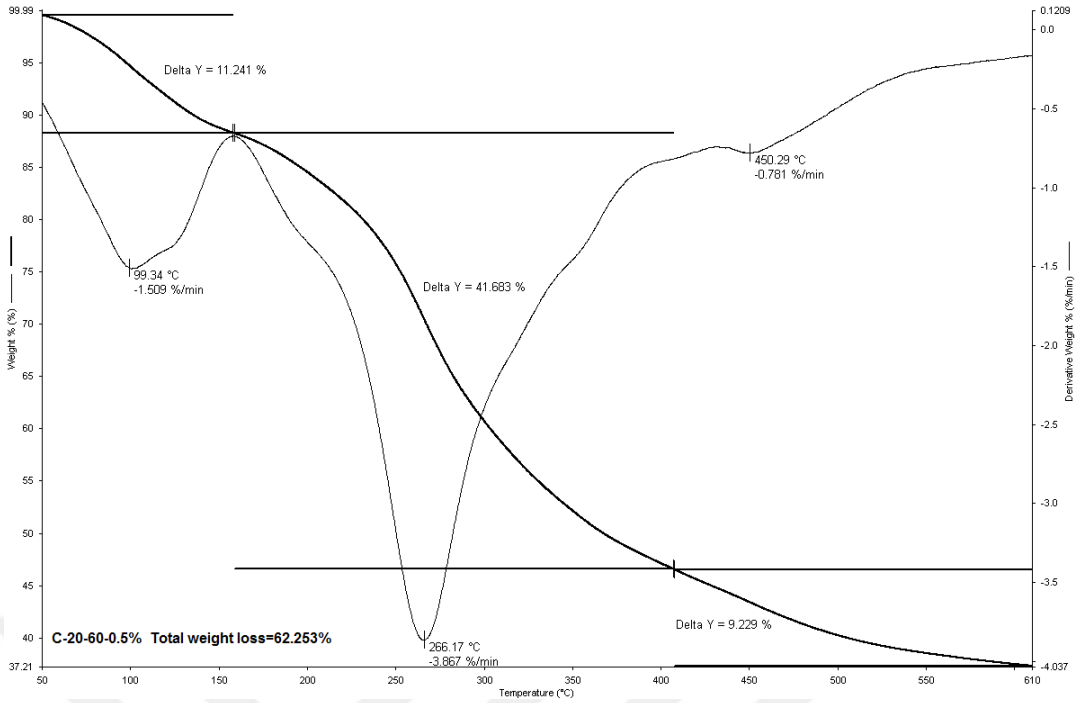


Figure C.2 DTA-TG graph of C-20-60-0.5

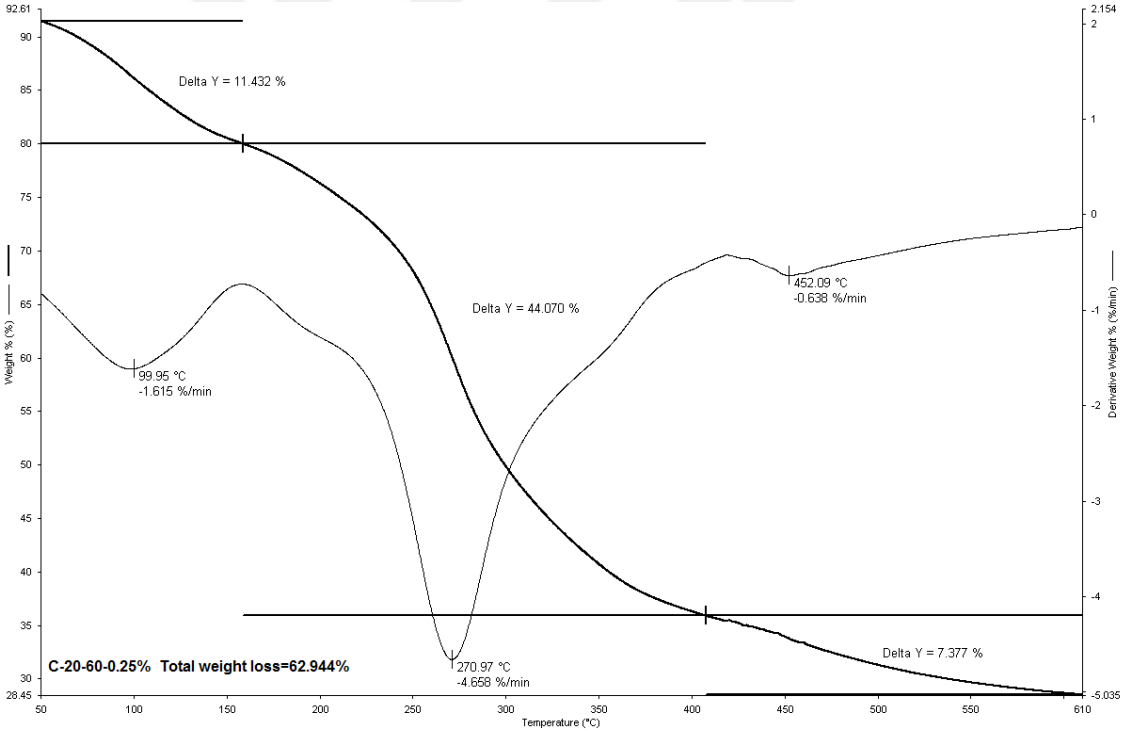


Figure C.3 DTA-TG graph of C-20-60-0.25

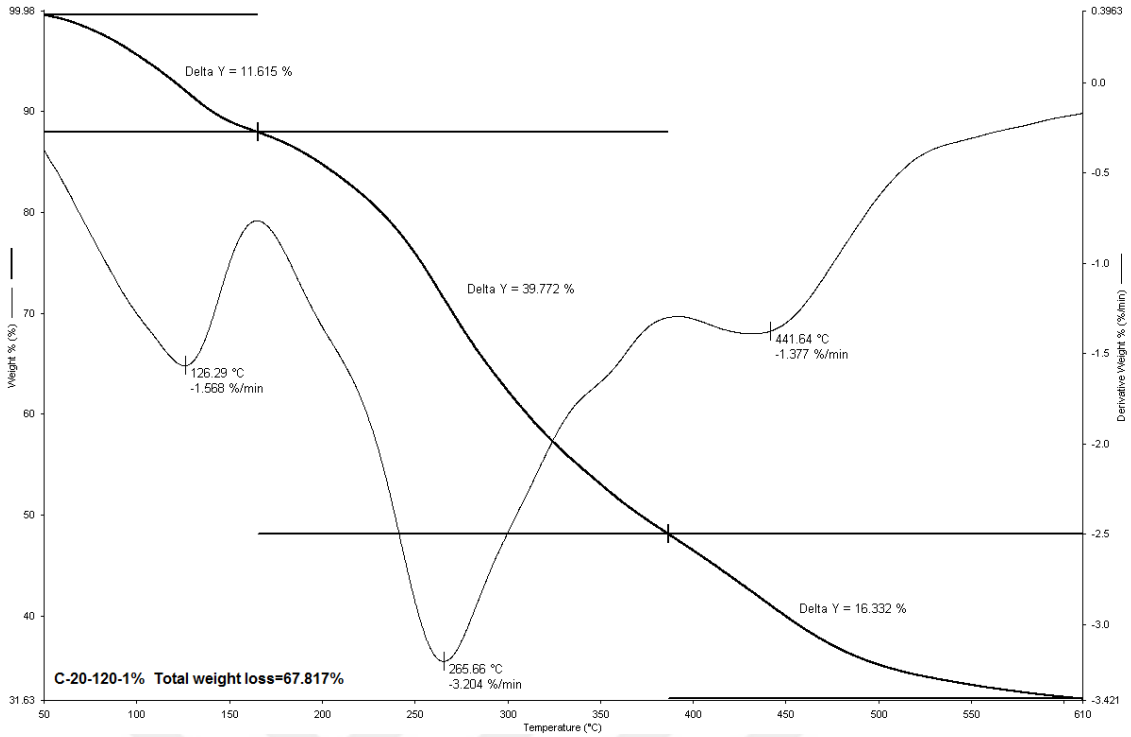


Figure C.4 DTA-TG graph of C-20-120-1

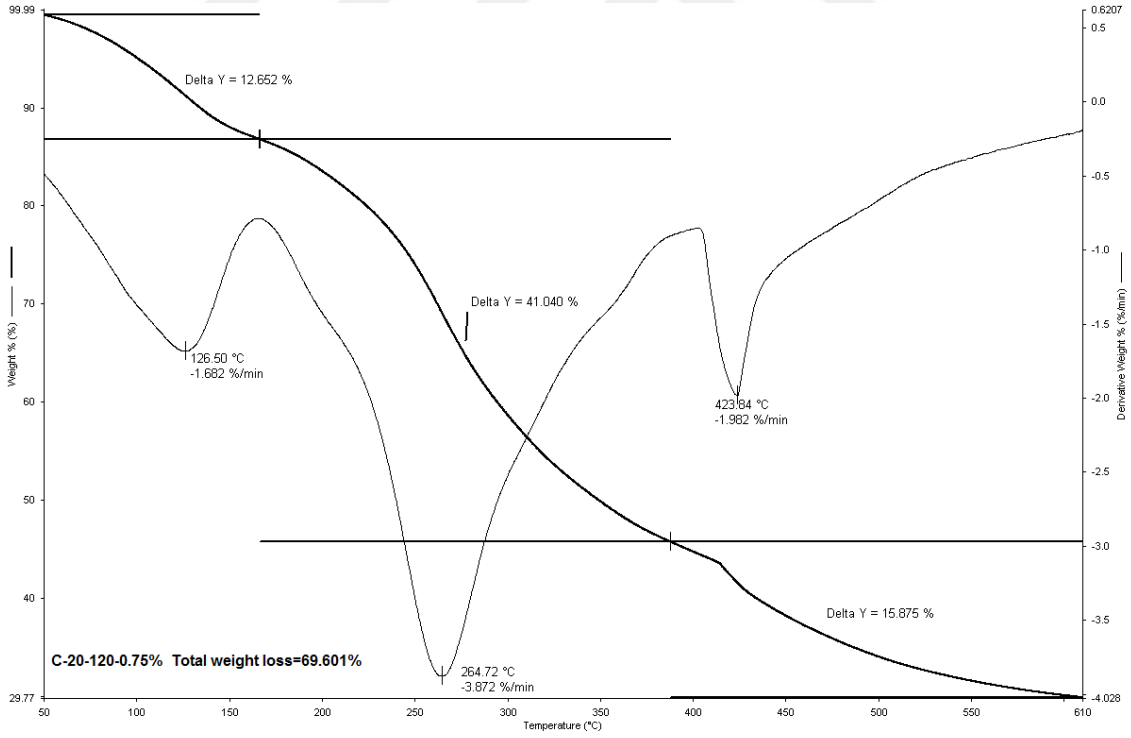


Figure C.5 DTA-TG graph of C-20-120-0.75

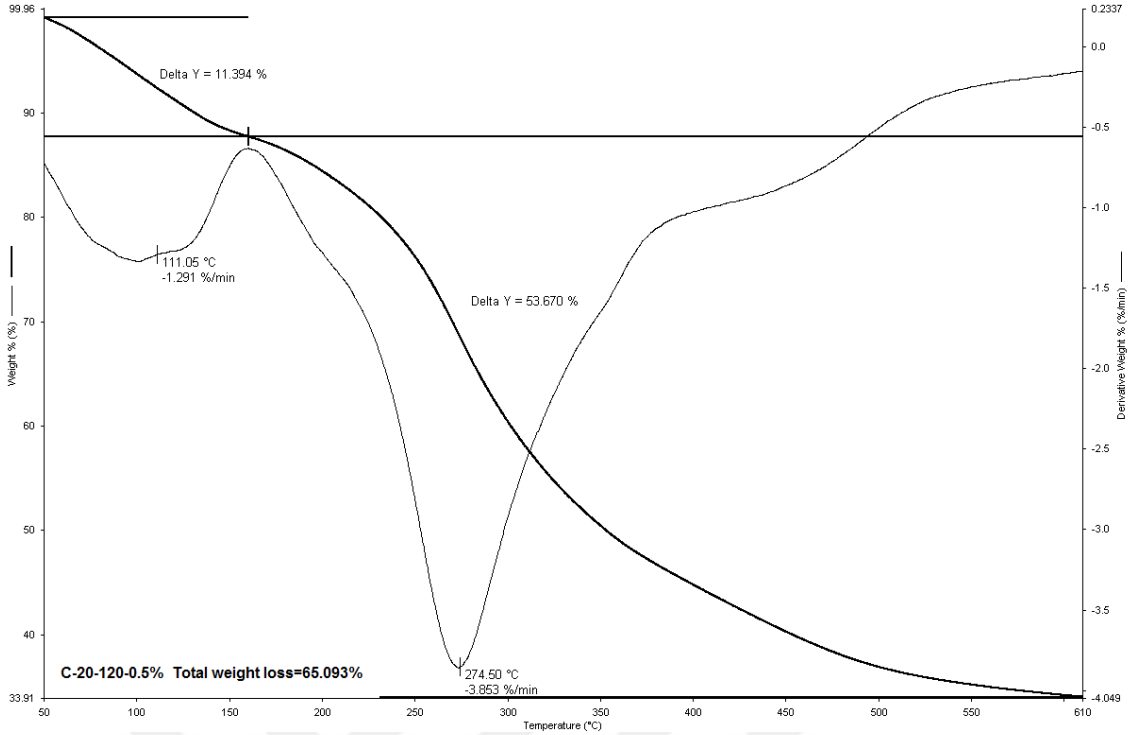


Figure C.6 DTA-TG graph of C-20-120-0.5

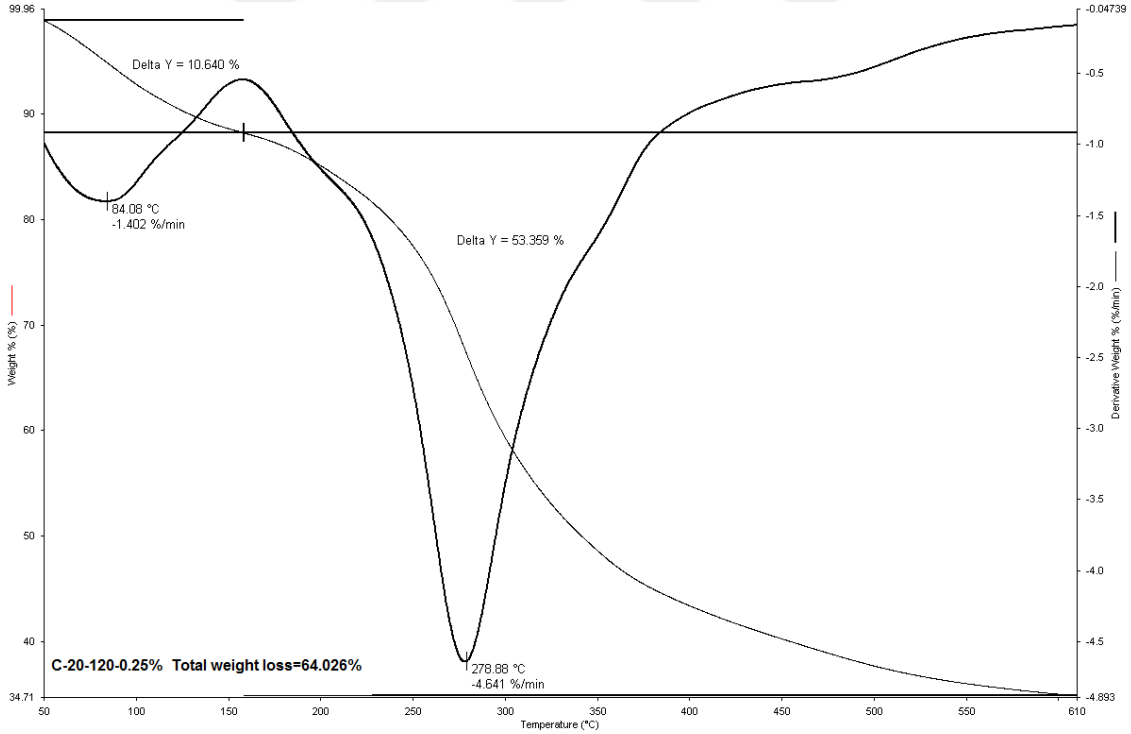


Figure C.7 DTA-TG graph of C-20-120-0.25

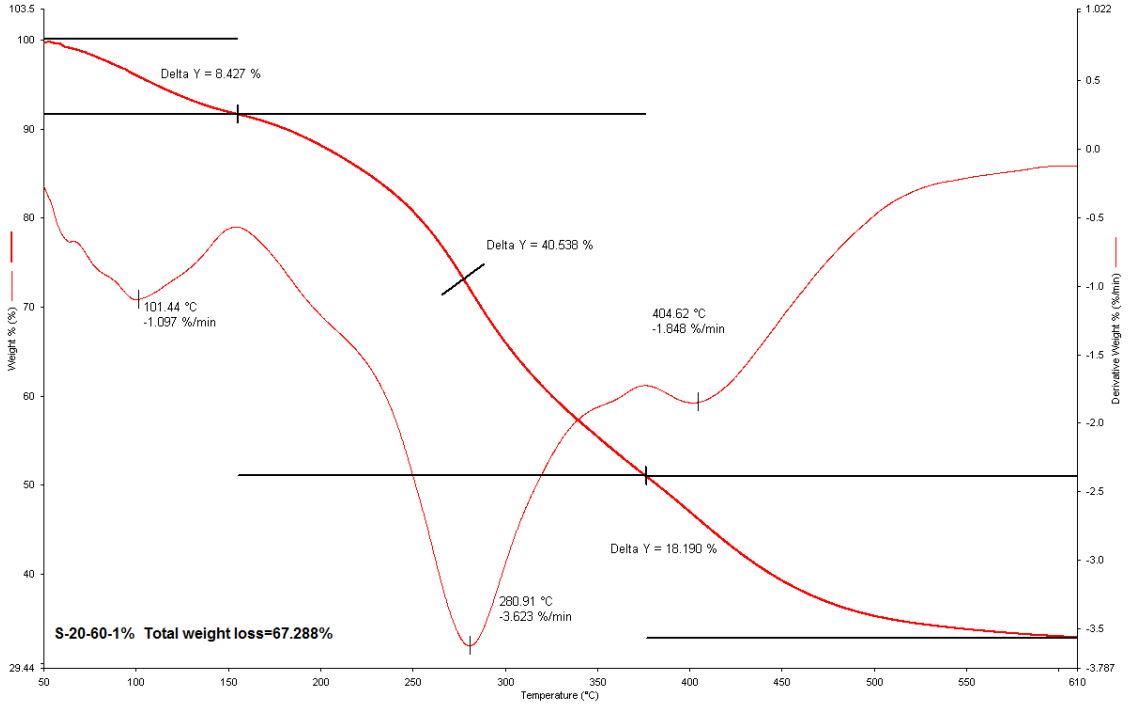


Figure C.8 DTA-TG graph of S-20-60-1

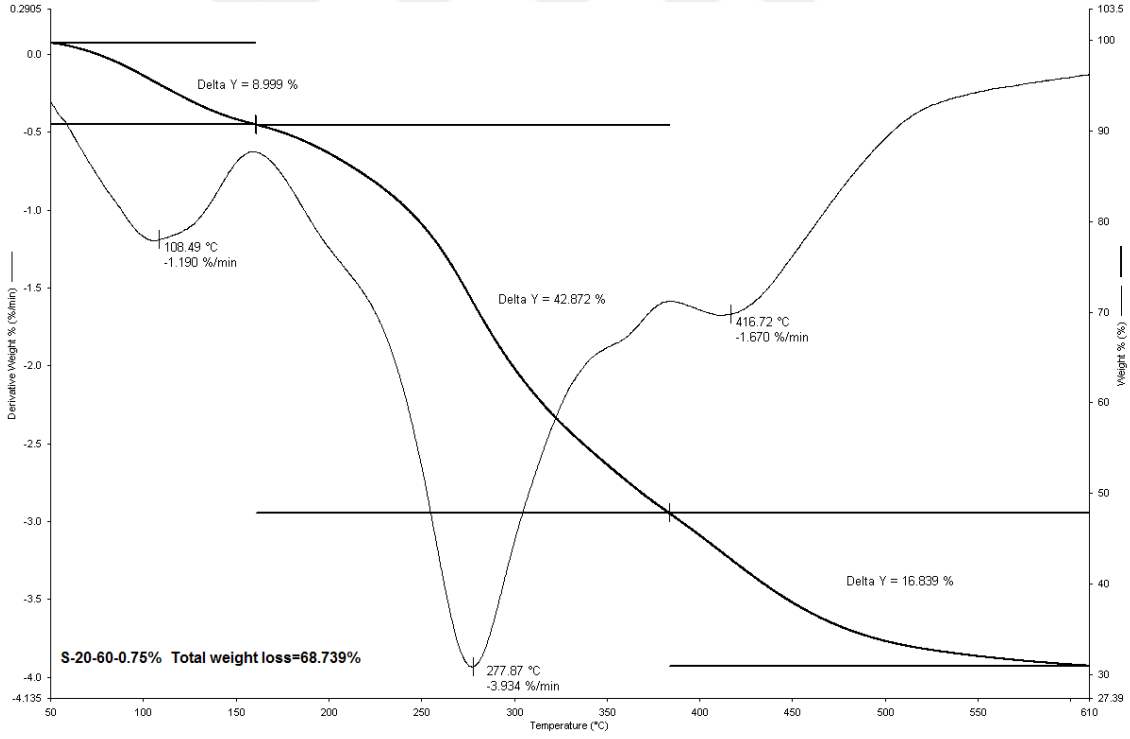


Figure C.9 DTA-TG graph of S-20-60-0.75

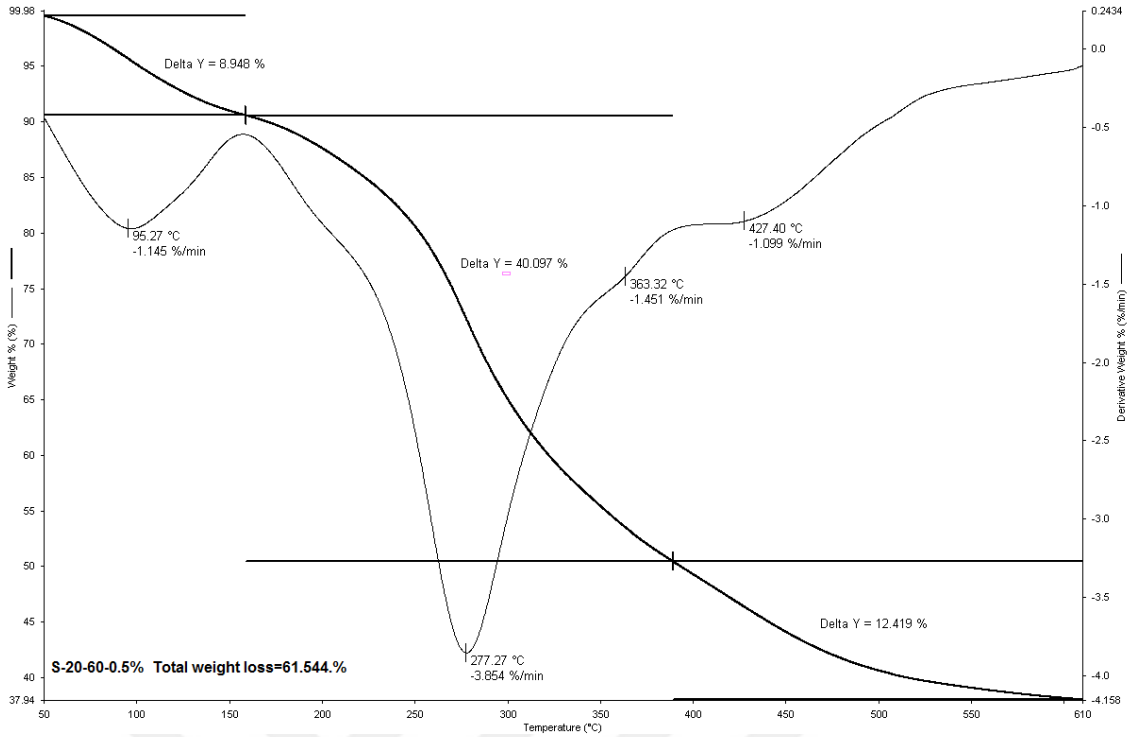


Figure C.10 DTA-TG graph of S-20-60-0.5

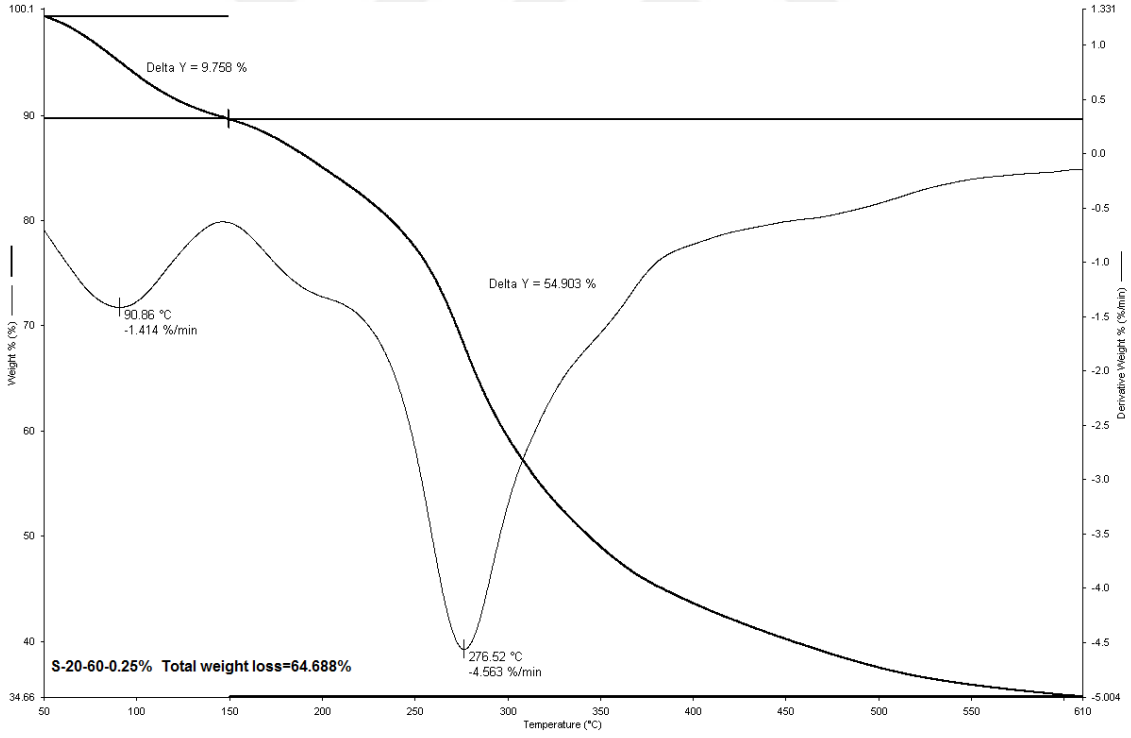


Figure C.11 DTA-TG graph of S-20-60-0.25

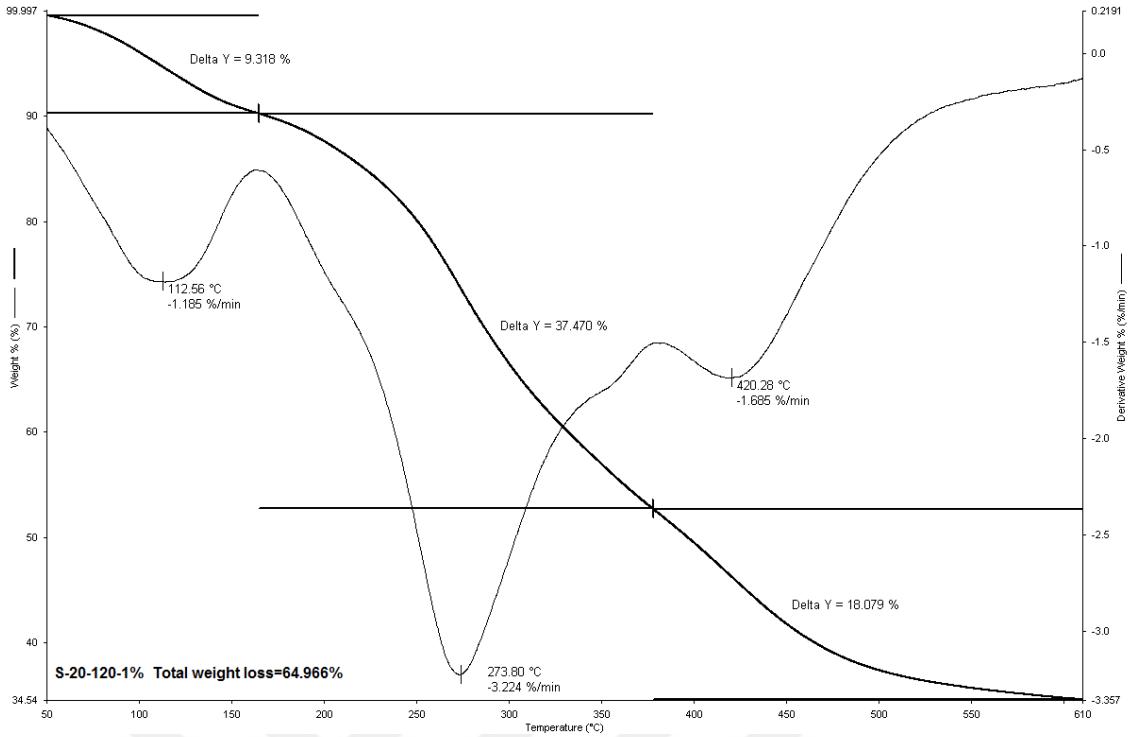


Figure C.12 DTA-TG graph of S-20-120-1

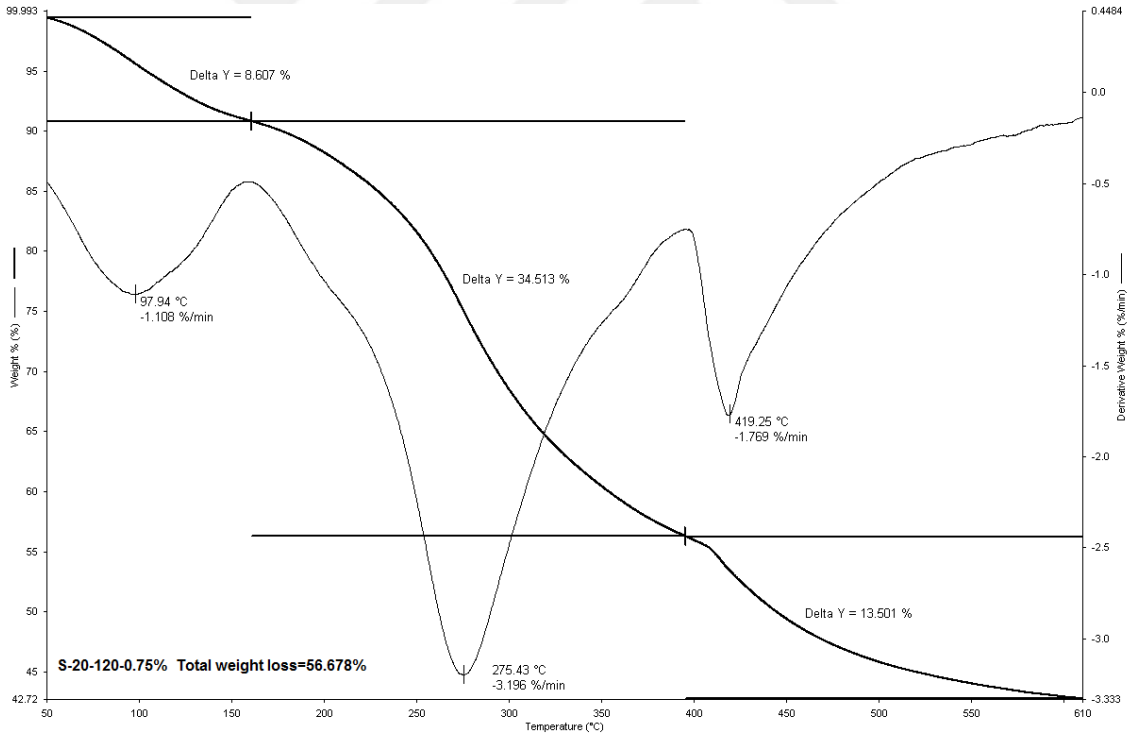


Figure C.13 DTA-TG graph of S-20-120-0.75

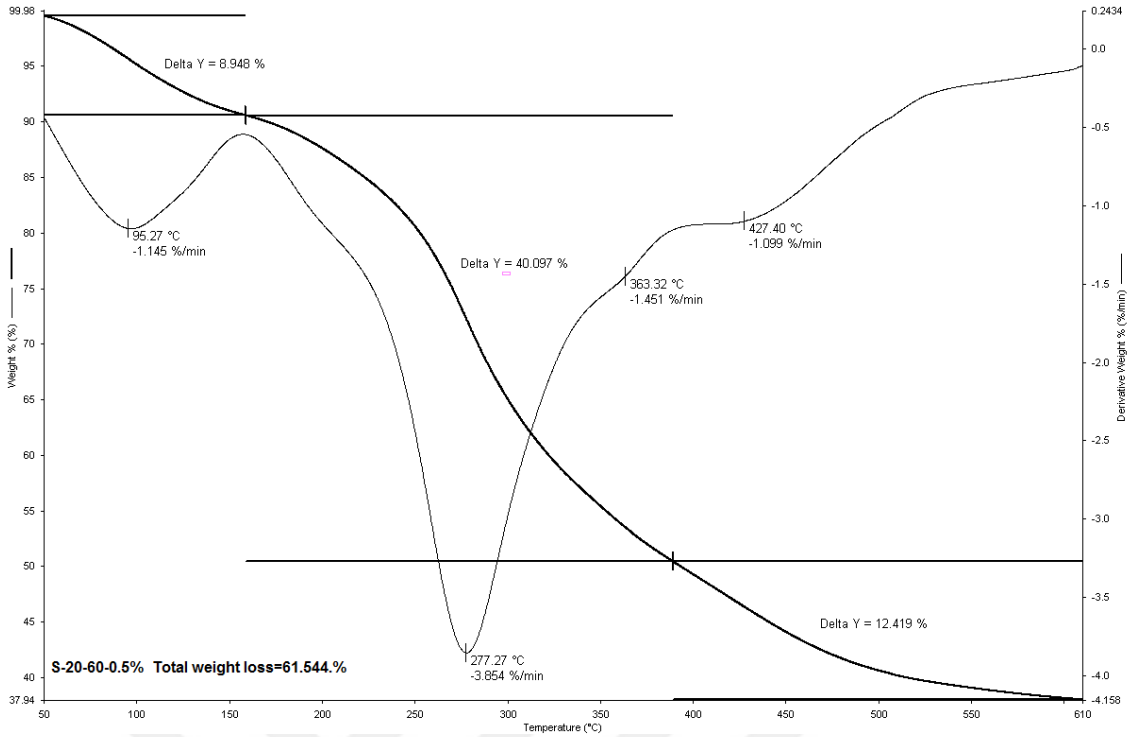


Figure C.14 DTA-TG graph of S-20-120-0.5

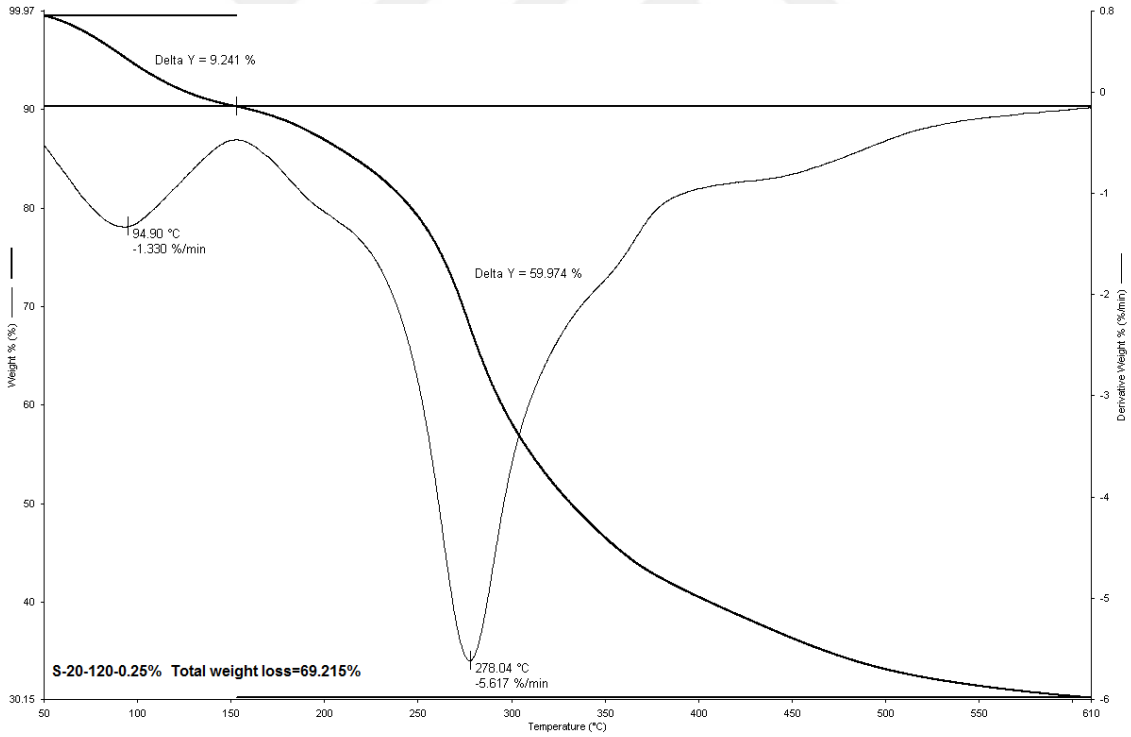


Figure C.15 DTA-TG graph of S-20-120-0.25

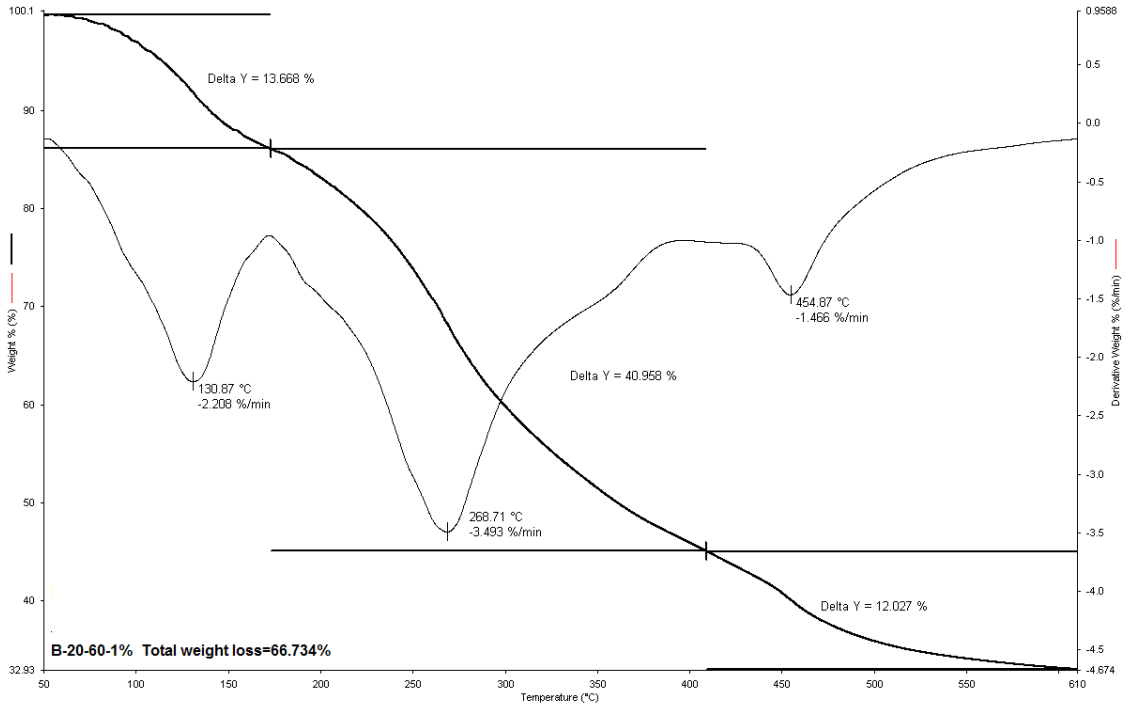


Figure C.16 DTA-TG graph of B-20-60-1

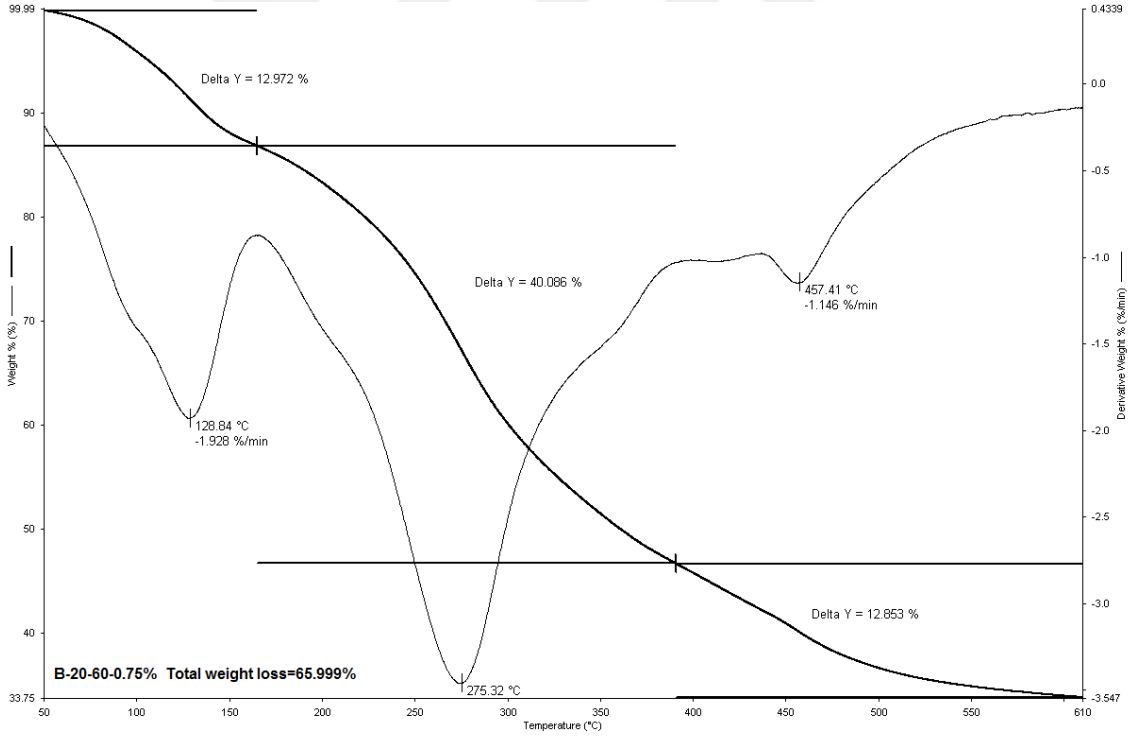


Figure C.17 DTA-TG graph of B-20-60-0.75

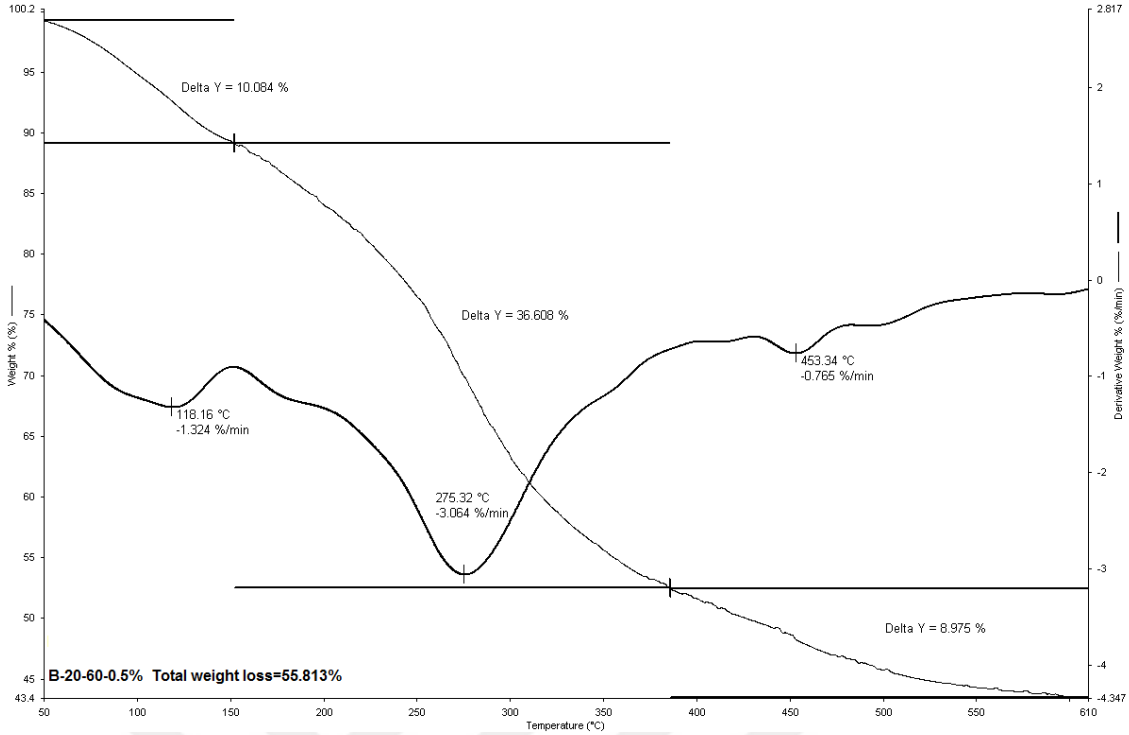


Figure C.18 DTA-TG graph of B-20-60-0.5

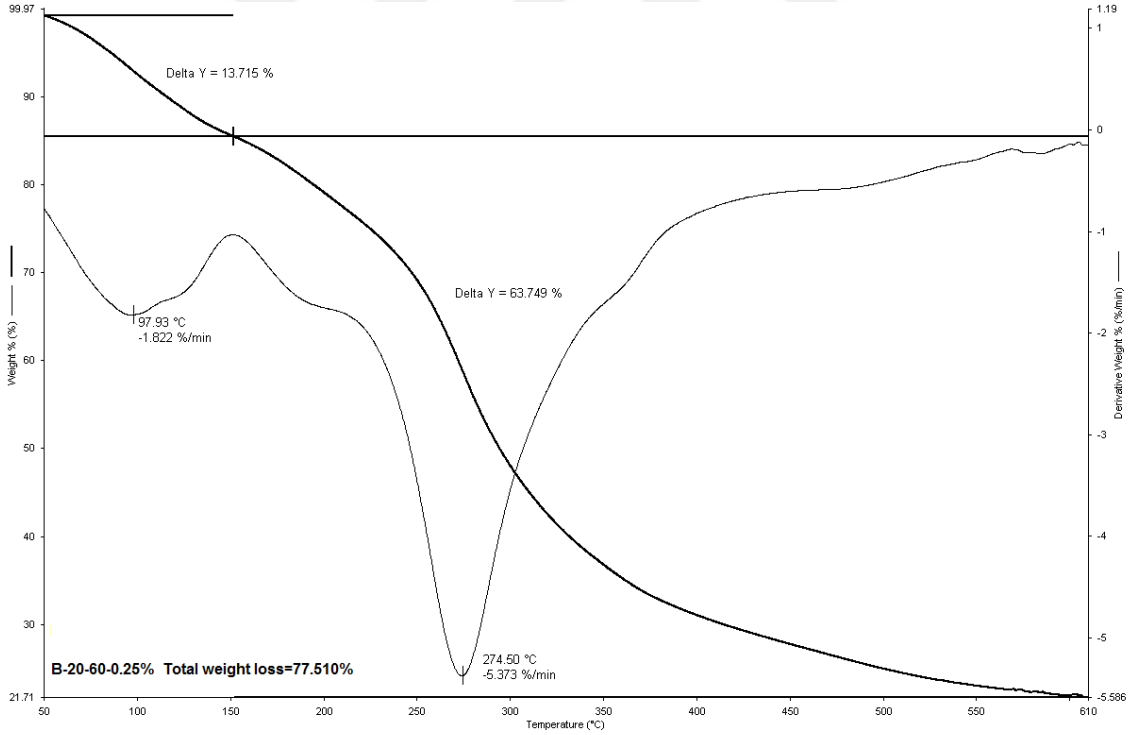


Figure C.19 DTA-TG graph of B-20-60-0.25

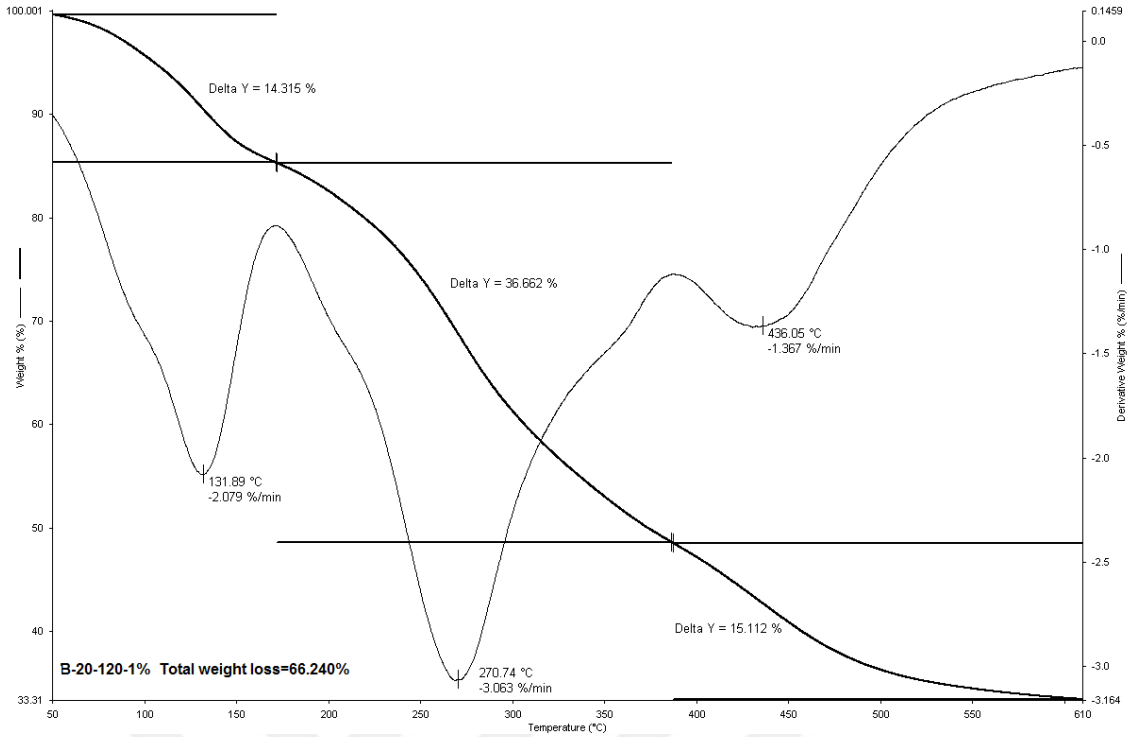


Figure C.20 DTA-TG graph of B-20-120-1

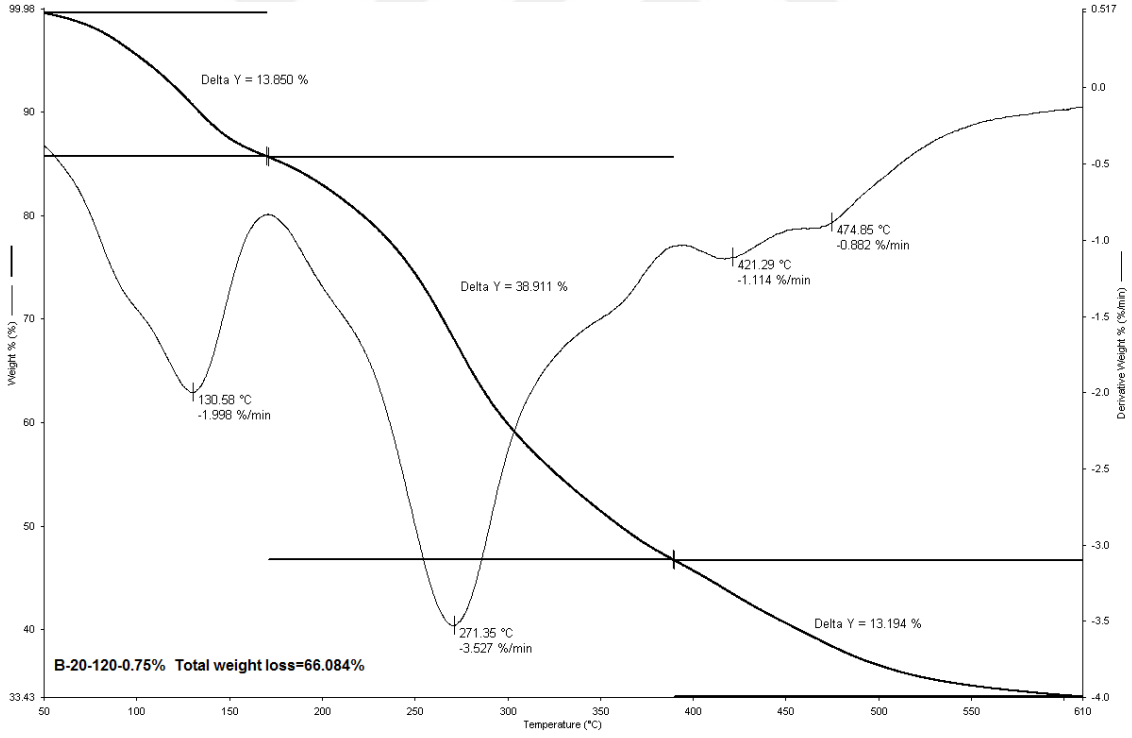


Figure C.21 DTA-TG graph of B-20-120-0.75

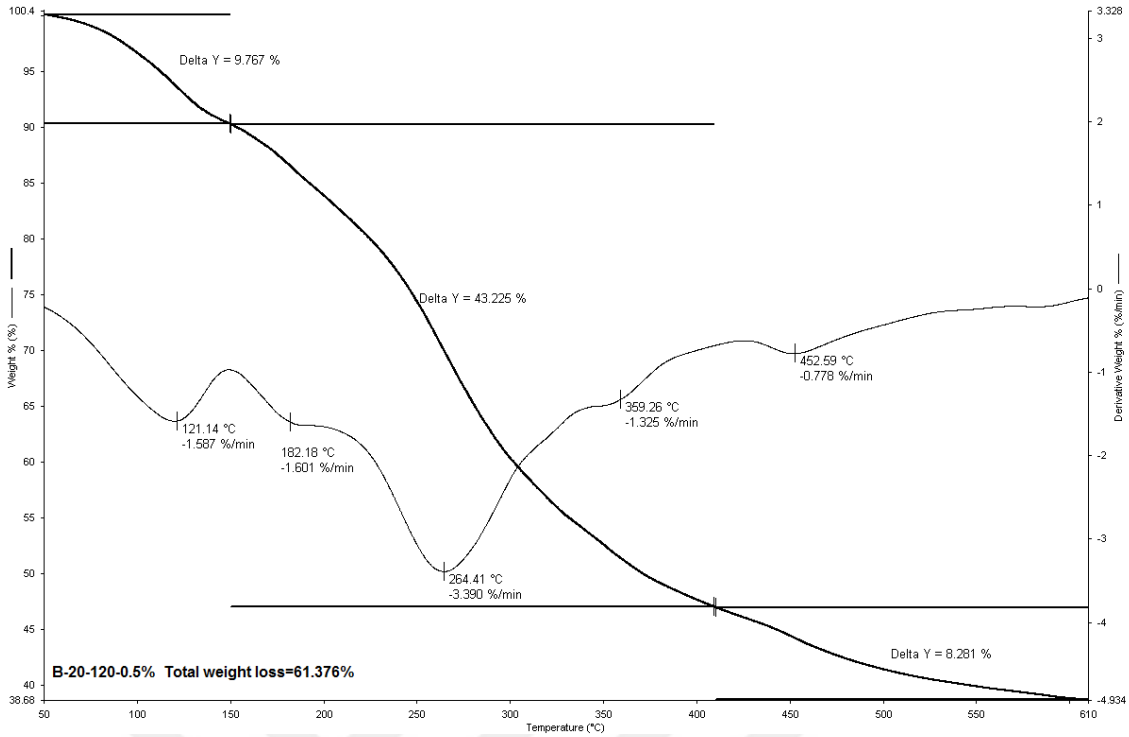


Figure C.22 DTA-TG graph of B-20-120-0.5

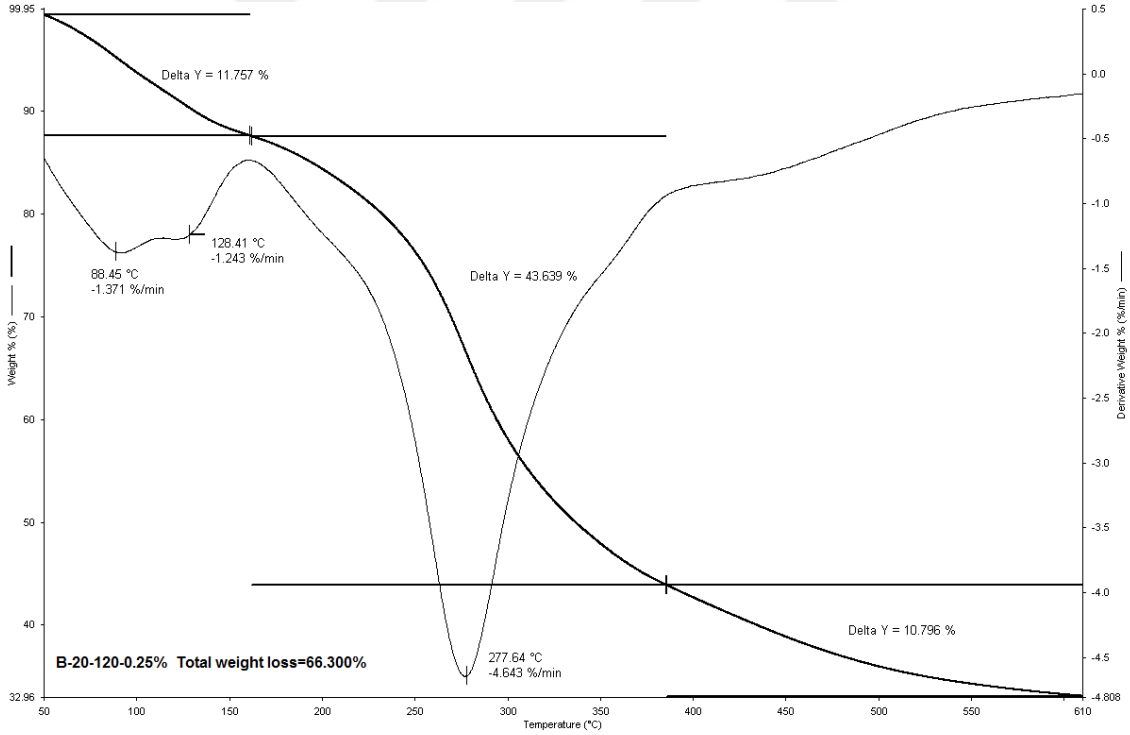


Figure C.23 DTA-TG graph of B-20-120-0.25

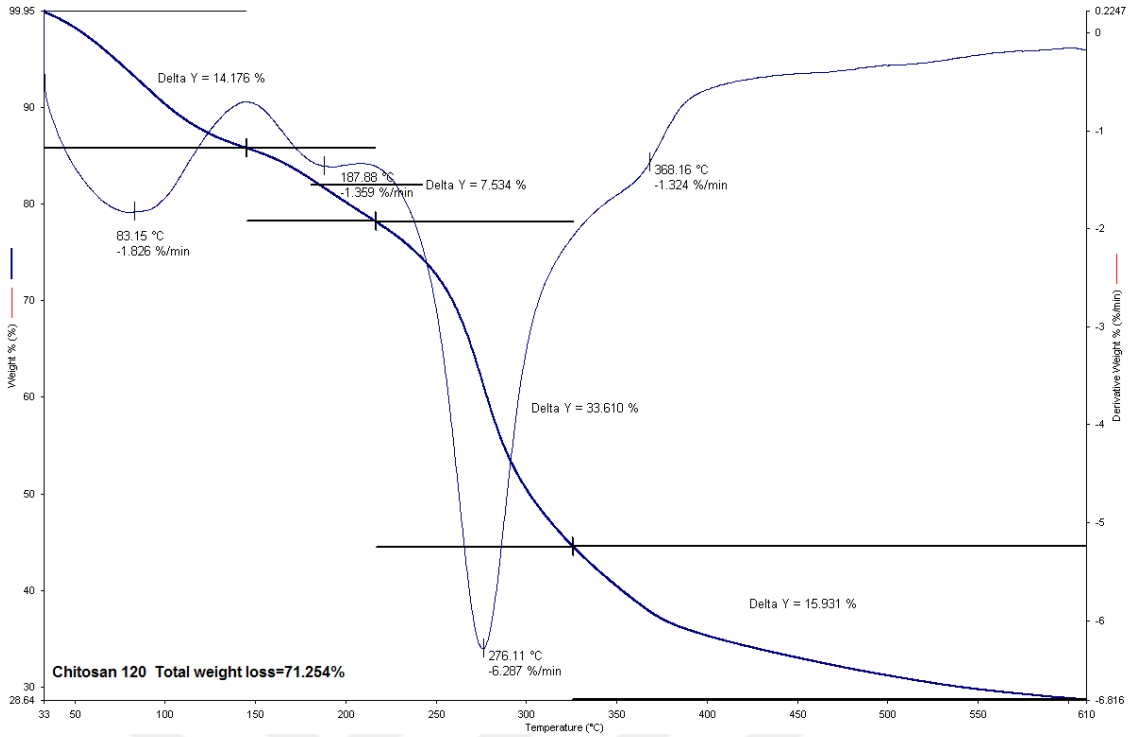


Figure C.24 DTA-TG graph of R-20-120

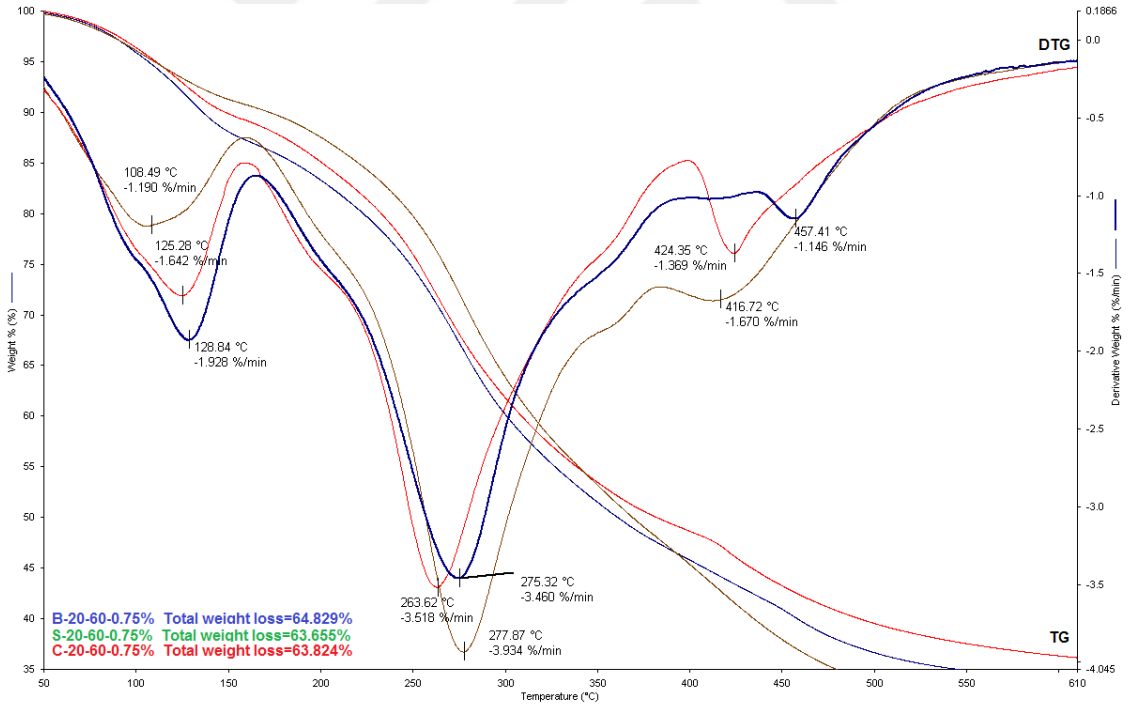


Figure C.25 Comparison of films with 0.75% (w/w) essential oil addition (60 min)

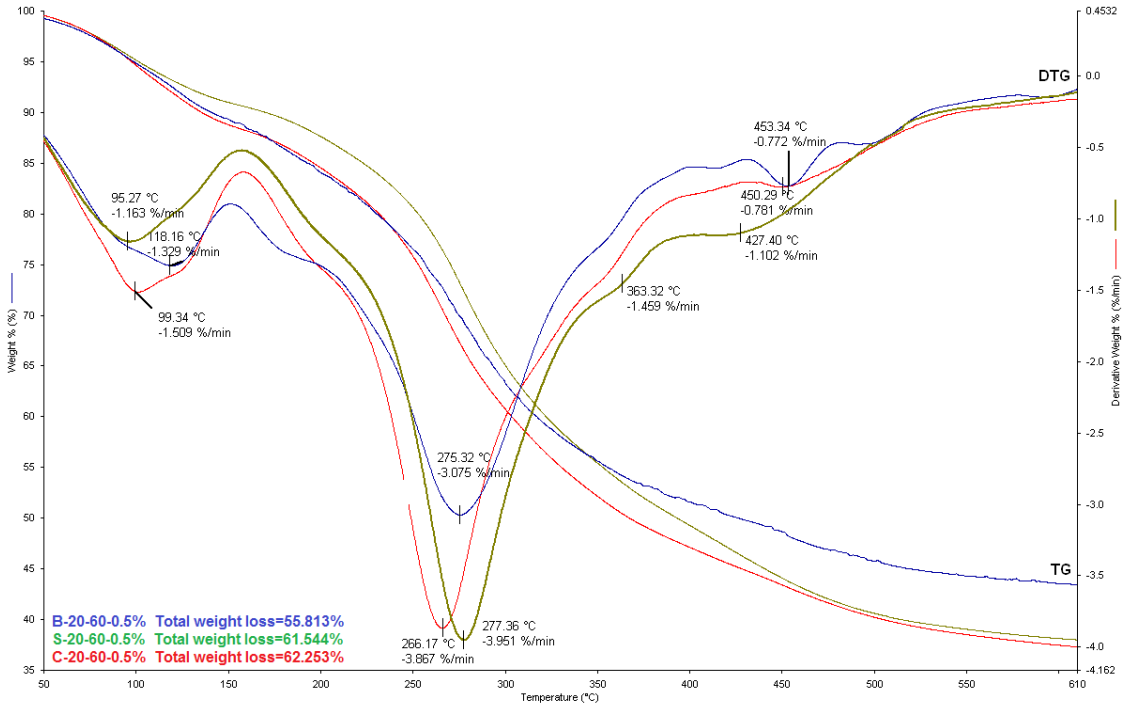


Figure C.26 Comparison of films with 0.5% (w/w) essential oil addition (60 min)

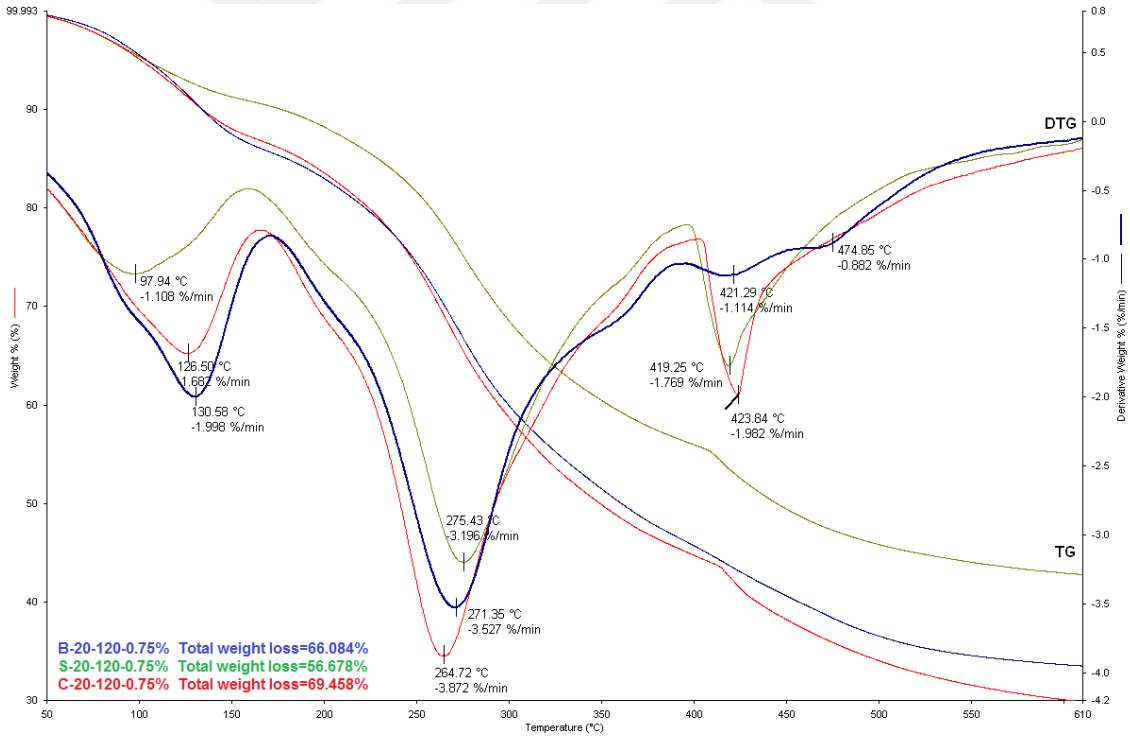


Figure C.27 Comparison of films with 0.75% (w/w) essential oil addition (120 min)

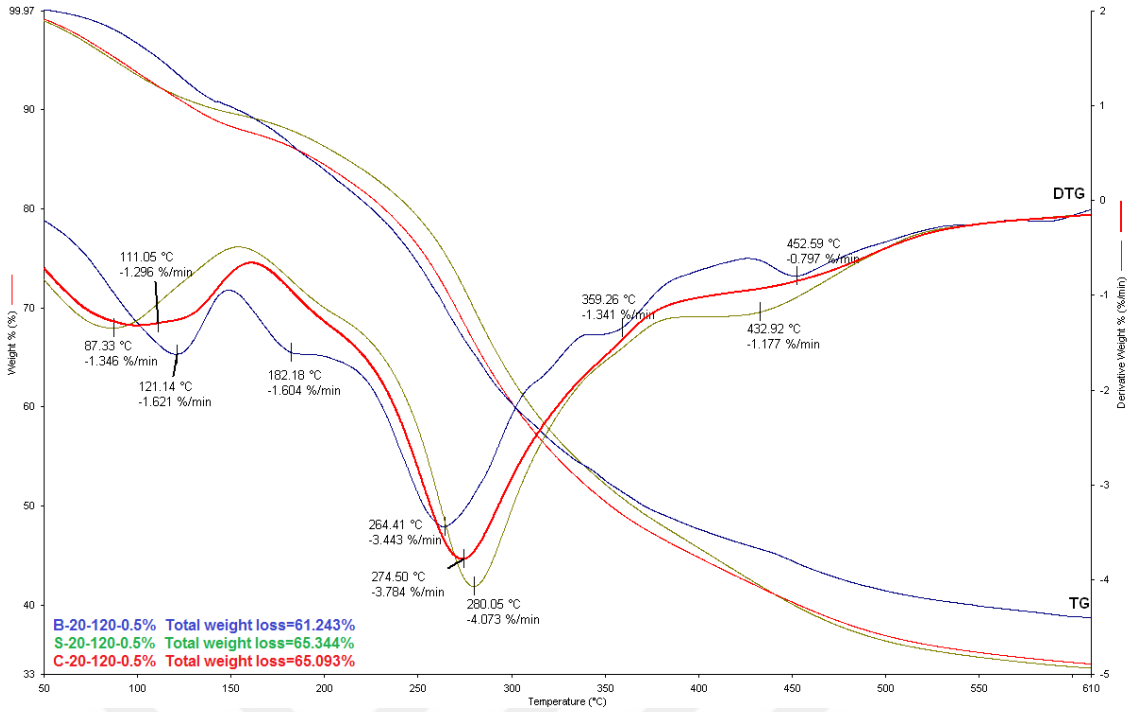


Figure C.28 Comparison of films with 0.5% (w/w) essential oil addition (120 min)

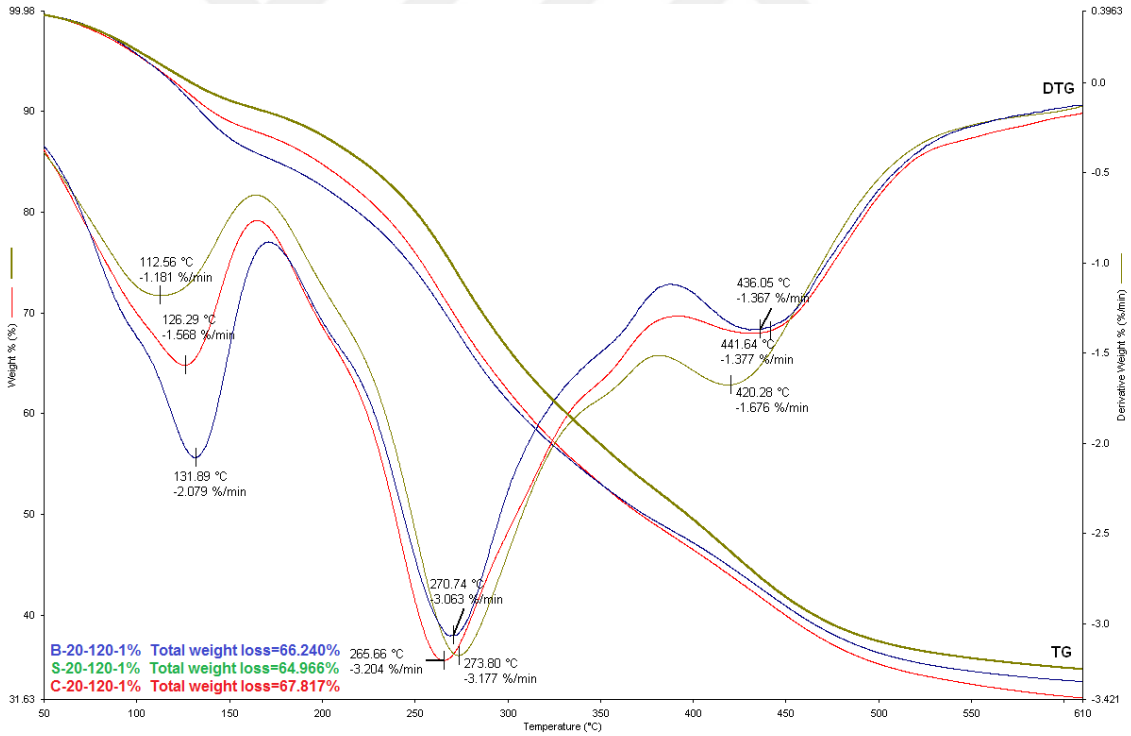


Figure C.29 Comparison of films with 1% (w/w) essential oil addition (120 min)

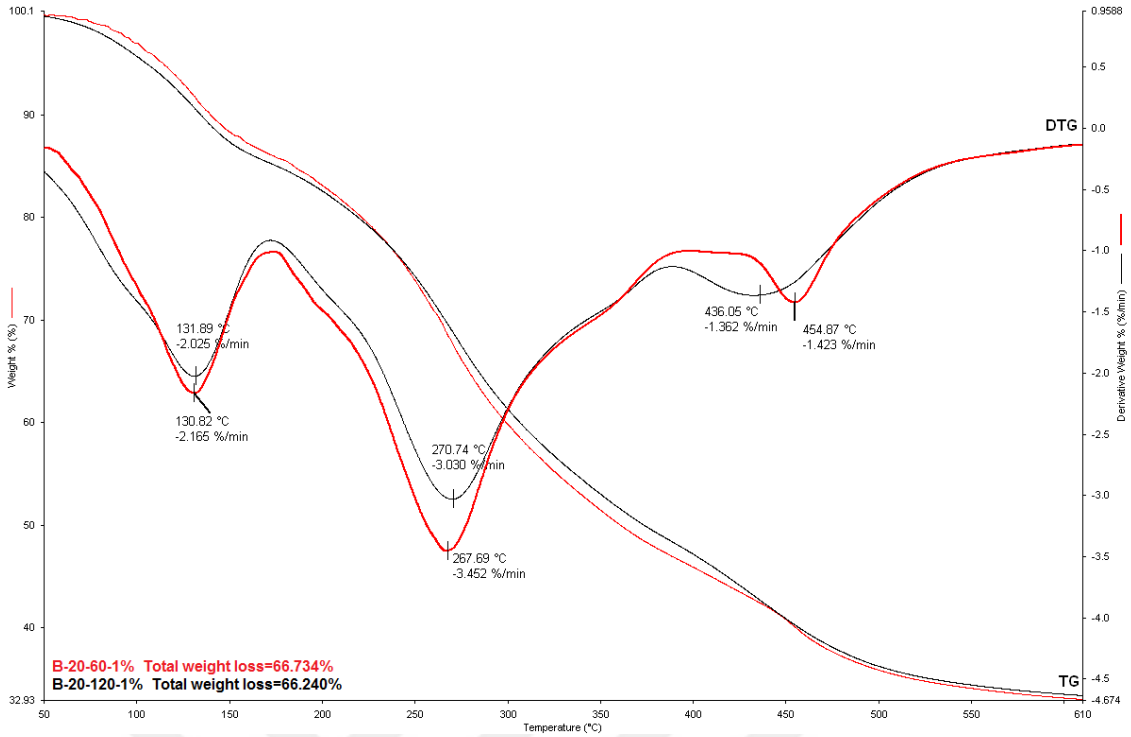


Figure C.30 Comparision of reaction time effect on Bluebottle based film (1%w/w)

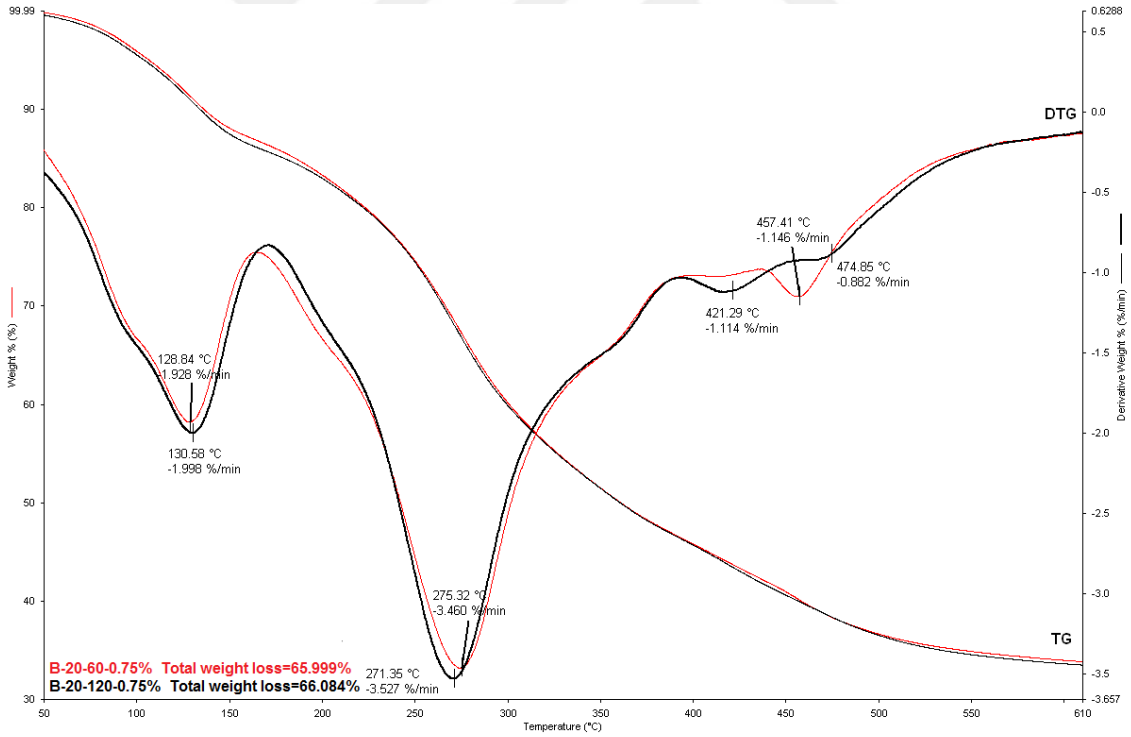


Figure C.31 Comparision of reaction time effect on Bluebottle based film (0.75%w/w)

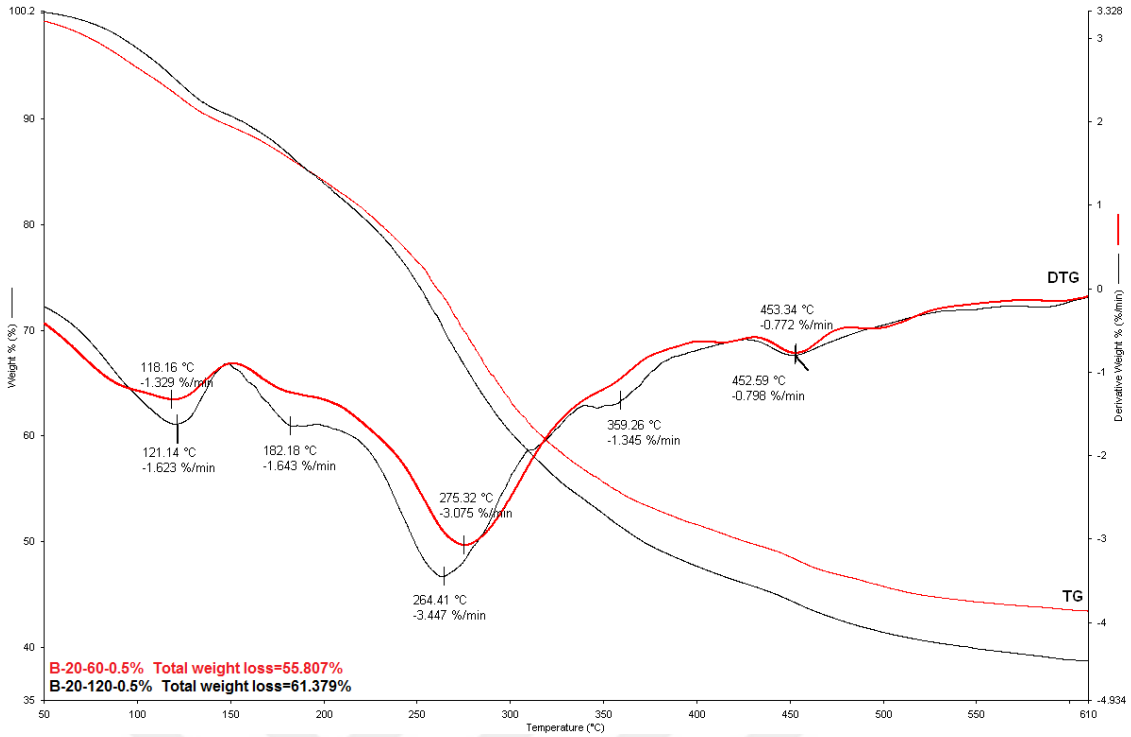


Figure C.32 Comparision of reaction time effect on Bluebottle based film (0.5%w/w)

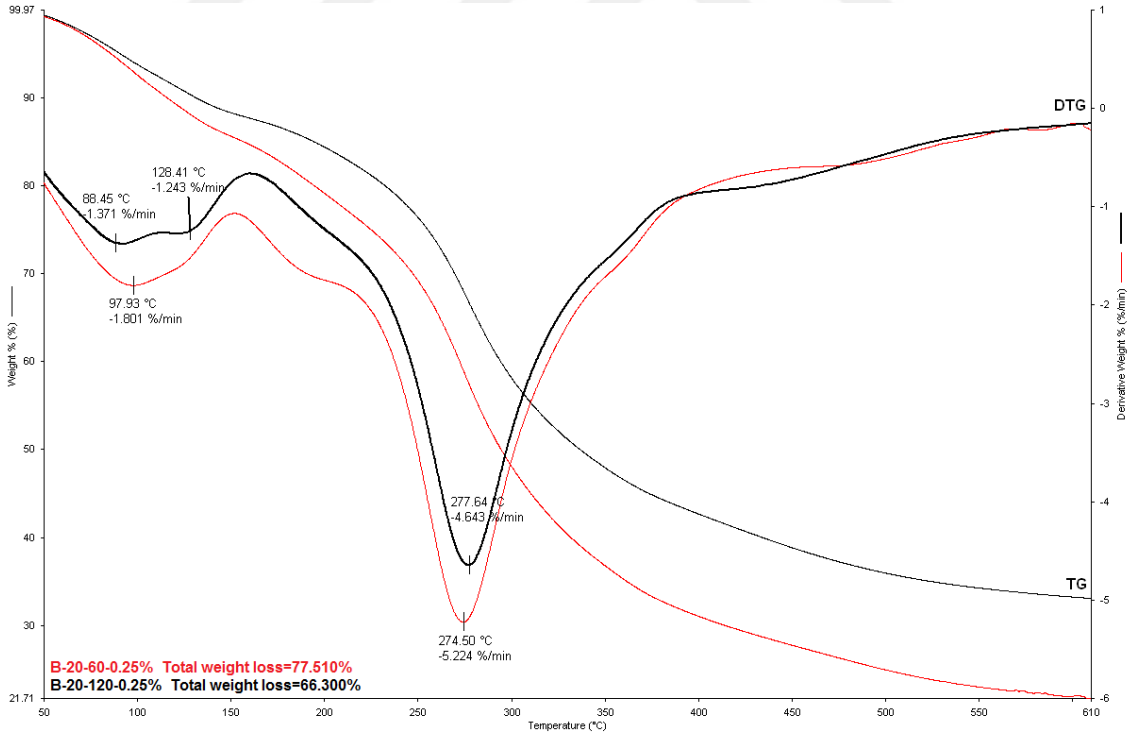


Figure C.33 Comparision of reaction time effect on Bluebottle based film (0.25%w/w)

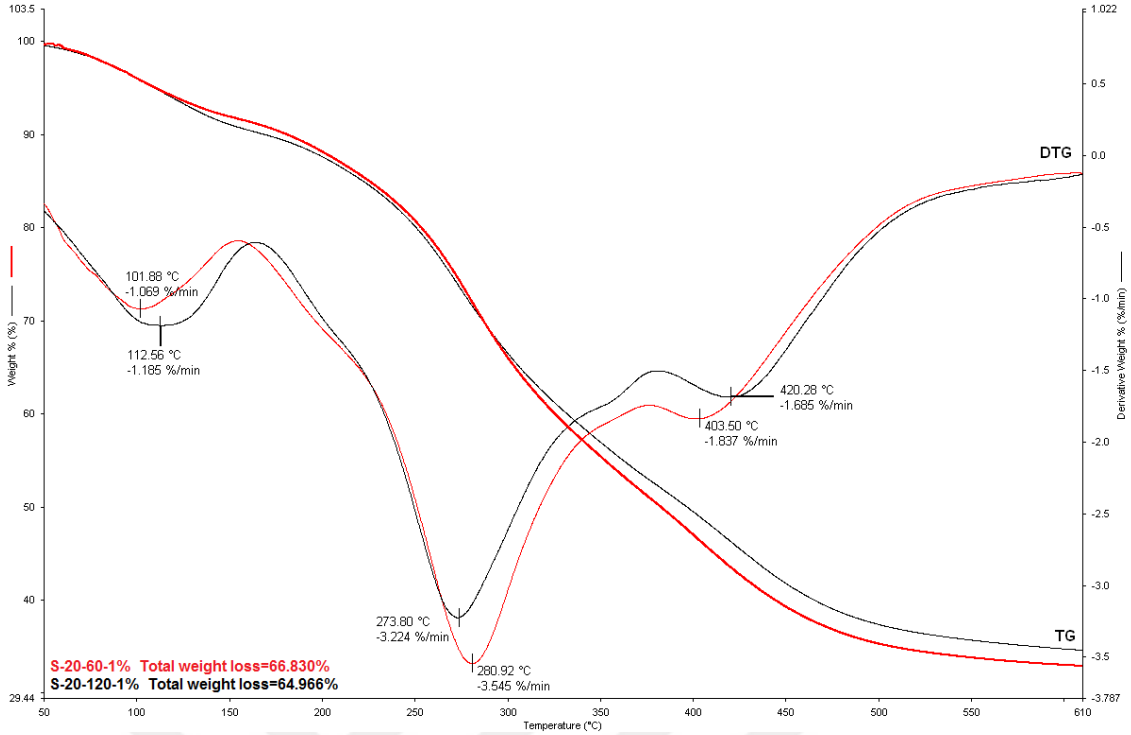


Figure C.34 Comparision of reaction time effect on St John's Wort based film (1%w/w)

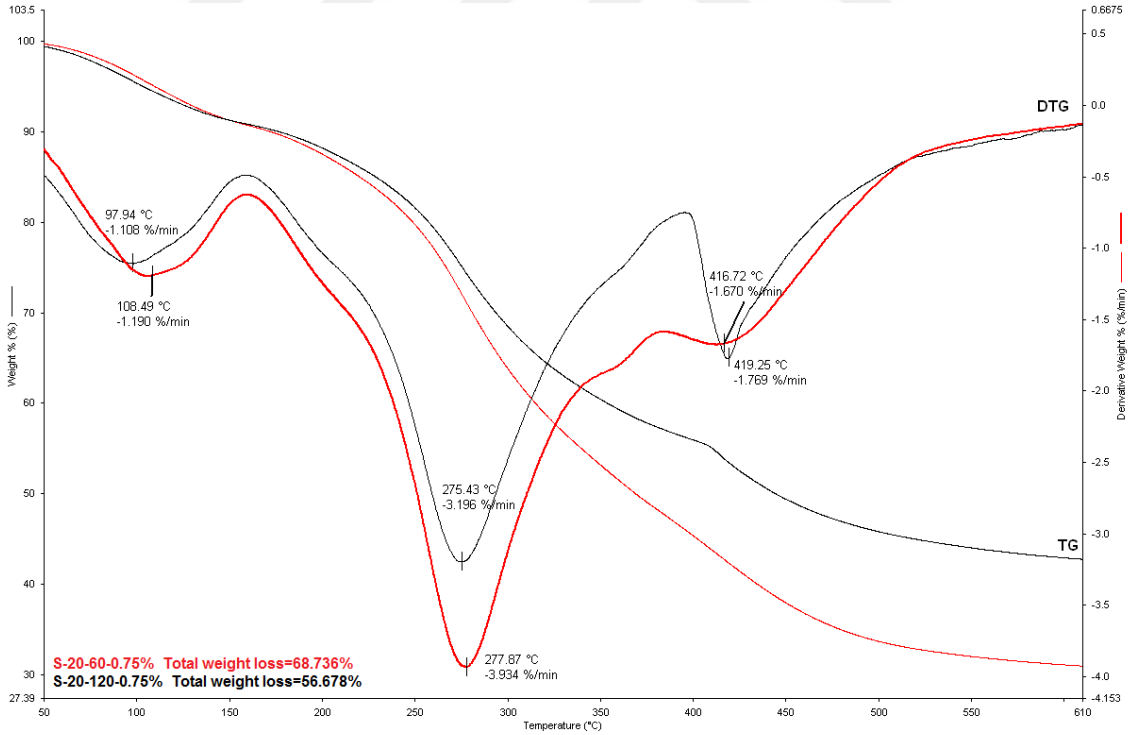


Figure C.35 Comparision of reaction time effect on St John's Wort based film (0.75%w/w)

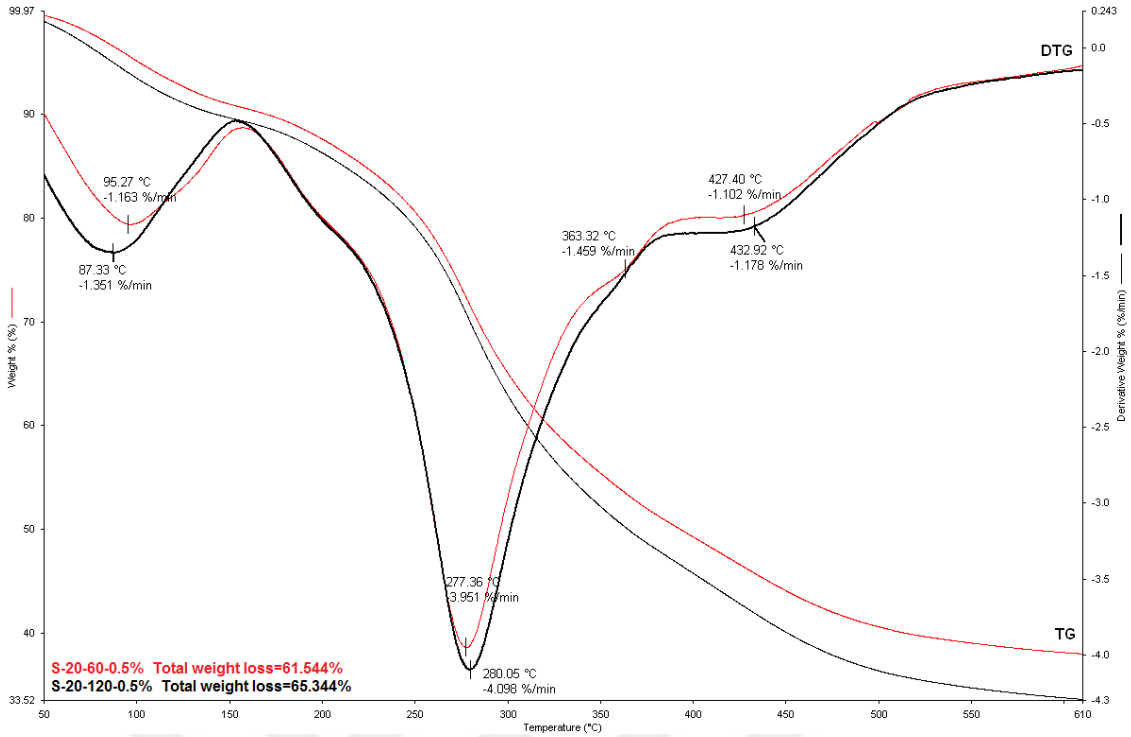


Figure C.36 Comparision of reaction time effect on St John's Wort based film (0.5%w/w)

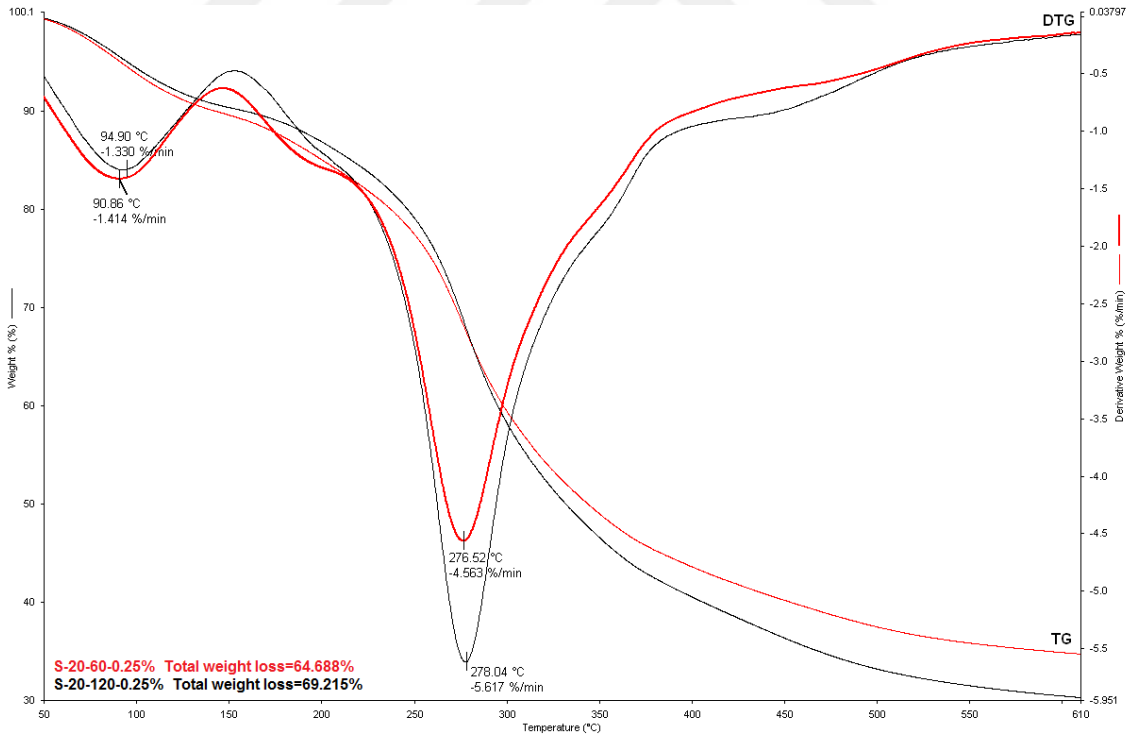


Figure C.37 Comparision of reaction time effect on St John's Wort based film (0.25%w/w)

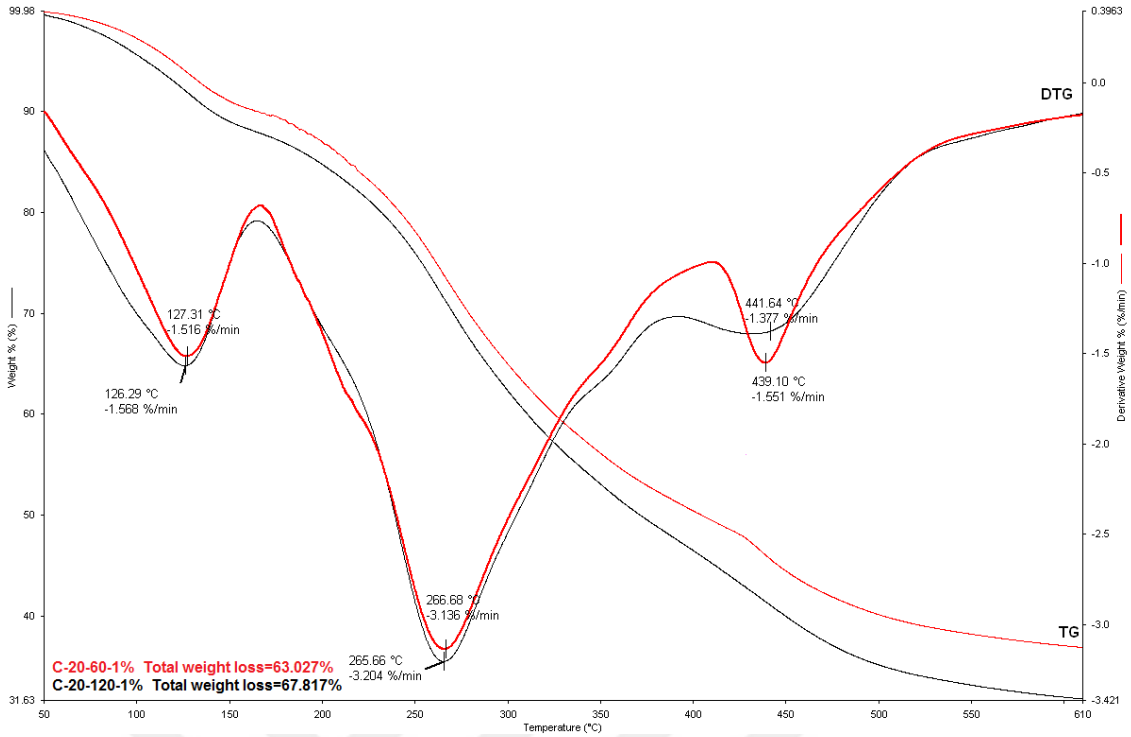


Figure C.38 Comparision of reaction time effect on Common Centaury based film (1%w/w)

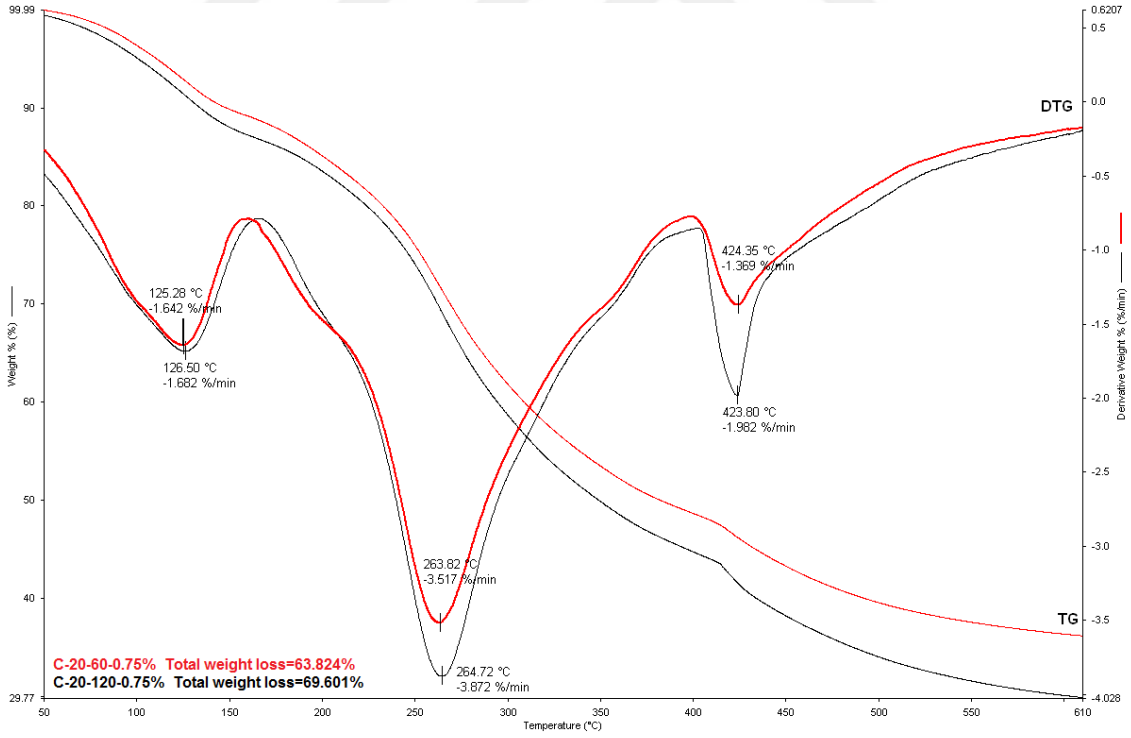


Figure C.39 Comparision of reaction time effect on Common Centaury based film (0.75%w/w)

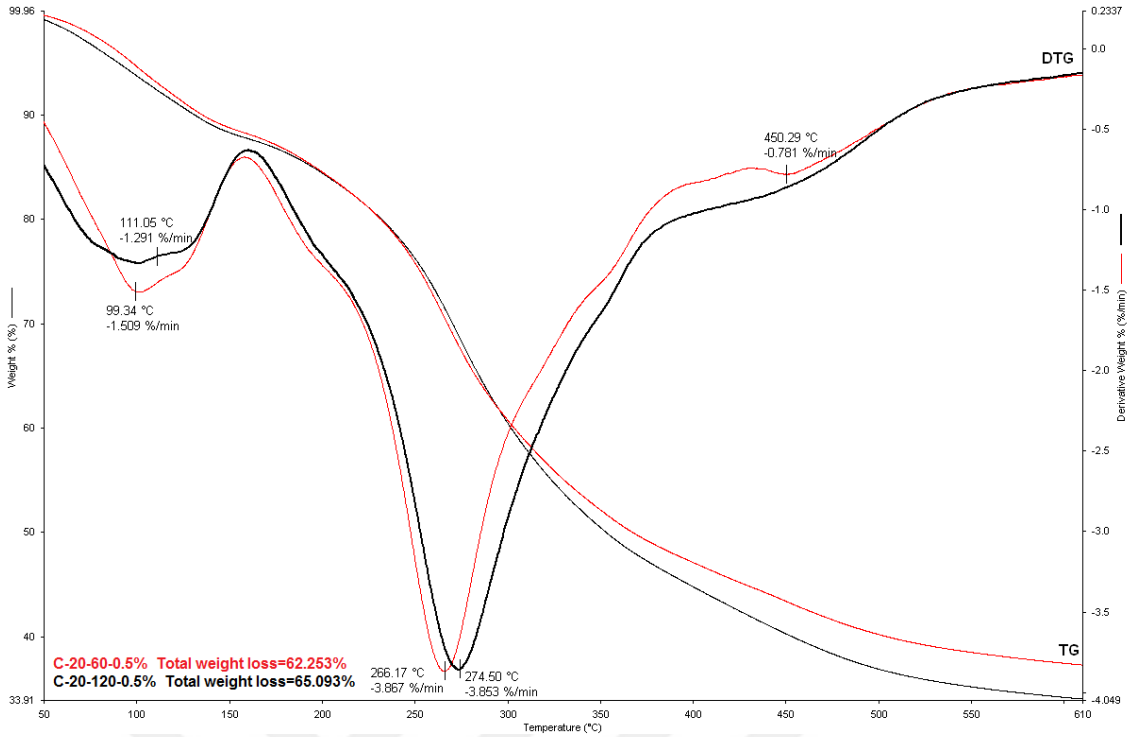


Figure C.40 Comparision of reaction time effect on Common Centaury based film (0.5%w/w)

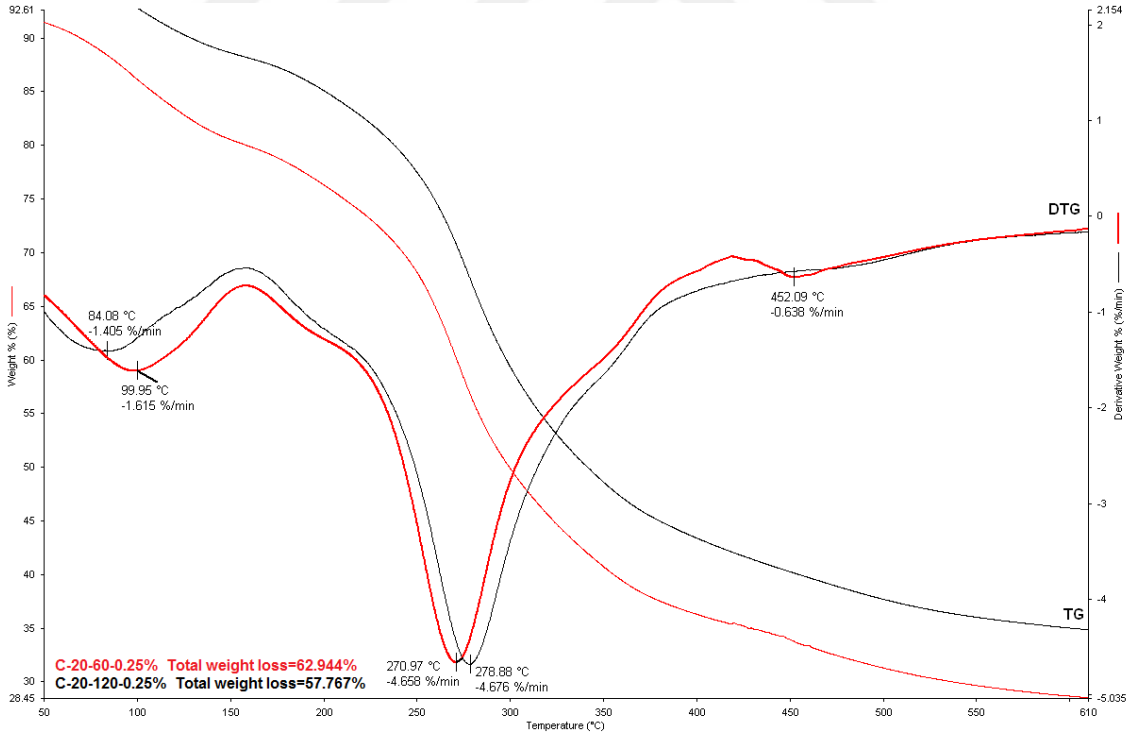


Figure C.41 Comparision of reaction time effect on Common Centaury based film (0.25%w/w)

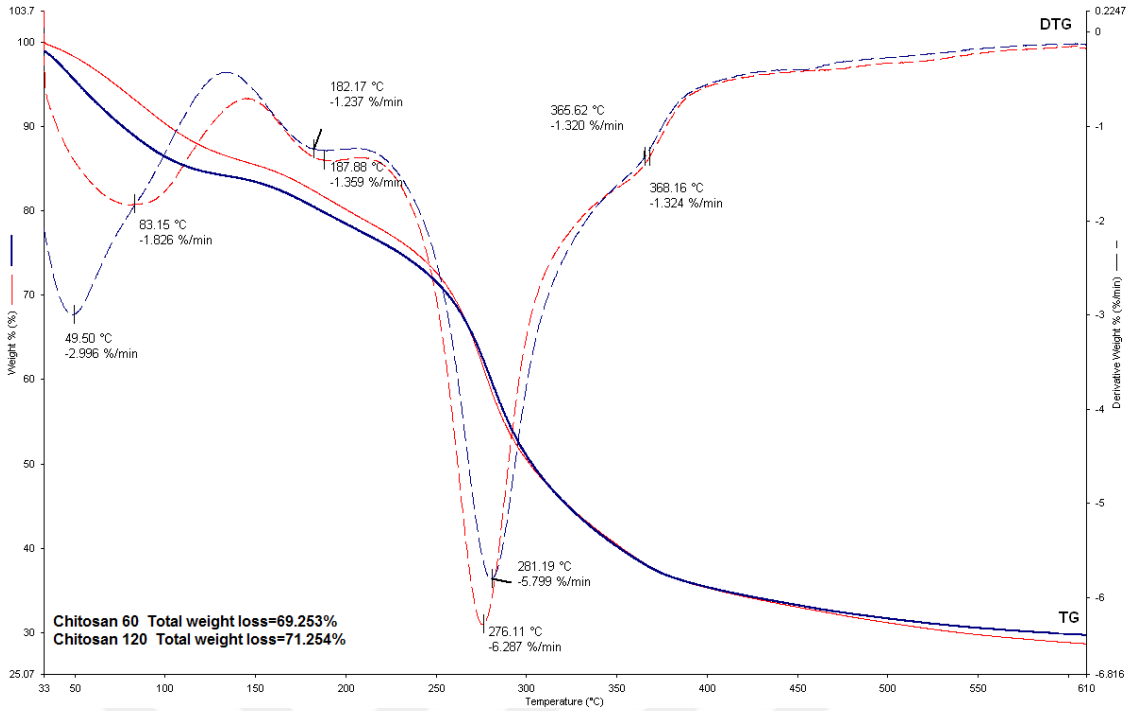


Figure C.42 Comparision of reaction time effect on pure chitosan films

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EDUCATION

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Master	Chemical Engineering	Yıldız Technical University	-
Undergraduate	Chemical Engineering	Yıldız Technical University	2014
High School	Science	Darüşşafaka Lisesi	2009

PUBLISHMENTS

Conference Papers

1. Balkan, G., Şenberber F.T., Pişkin, M.B., Derun, E., (2016). "Characterization Study of Centaurium Erythraea Using by UV-Vis and ICP-OES", 2nd International Conference on Advances in Mechanical Engineering, Yıldız Technical University, Istanbul

Projects

1.Wound Dressing Production with Essential Oils and Investigation of Antimicrobial Properties, study was supported by the Yildiz Technical University BAPK with project number of 2016-07-01-YL01.