

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**PREPARATION, CHARACTERIZATION AND
UTILIZATIONS OF CHITOSAN-SEPIOLITE
BIOCOMPOSITES**

by
Emel GÜR

October 2010
İZMİR

PREPARATION, CHARACTERIZATION AND UTILIZATIONS OF CHITOSAN-SEPIOLITE BIOCOMPOSITES

**A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylül University
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the Requirements for the Degree of Master of Science
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**by
Emel GÜR**

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M. Sc. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled “**PREPARATION, CHARACTERIZATION AND UTILIZATIONS OF CHITOSAN-SEPIOLITE BIOCOMPOSITES**” completed by **EMEL GÜR** under supervision of **PROF. DR. KADIR YURDAKOÇ** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

.....
Prof. Dr. Kadir YURDAKOÇ

Supervisor

.....

(Jury Member)

.....

(Jury Member)

Prof. Dr. Mustafa SABUNCU

Director

Graduate School of Natural and Applied Sciences

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ABSTRACT

Chitosan-clay biocomposites have been prepared in which Sepiolite (SEP) is used as nanofiller and diluted acetic acid is used as solvent for dissolving and dispersing chitosan and sepiolite respectively. The effect of SEP loadings in biocomposites and drug release has been investigated. Chitosan/SEP biocomposites were characterized with different methods (FTIR, TGA, XRD and SEM) of analysis. The FTIR and SEM results indicated the formation of a phase separated micro composite structure at low and high sepiolite content. Surface functional groups of biocomposites were also investigated by Boehm's titration technique. The point of zero charge was determined as neutral pH. The thermal behavior of the samples was examined by TGA/DTG. The dispersed clay improves the thermal stability of the matrix systematically with the increase of clay loading. The release of Tetracycline (TC) from CS/SEP biocomposites was studied in a batch system as function of pH conditions, clay content and contact time at thirty-seven degrees. The release of drug into the aqueous solution depends on the clay content. The maximum release at equilibrium was observed at a percent of clay content by plotting the amount of TC released by CS/SEP biocomposites (C_s : mmol.g⁻¹) versus time. Release of TC from biocomposites which have different clay contents showed that increase in the clay content of the composite resulted decrease in the release of TC. This result is due to the interaction of the silanol groups (-SiOH) of the clay and the -OH groups of the drug by the formation of the hydrogen bonding. Diffusion rate of TC to aqueous medium decreased by increasing clay content.

Keywords: chitosan, sepiolite, tetracycline, release, diffusion rate

KİTOSAN-SEPIYOLİT BİYOKOMPOZİT HAZIRLANMASI, KARAKTERİZASYONU, VE KULLANIMLARI

ÖZ

Kitosan-kil biyokompozitlerinin (CS/SEP) hazırlanmasında nanodoldurucu olarak Sepiyolit, seyreltik asetik asit ise Kitosan'ın çözülmesi ve kilin dağılmasında da çözücü olarak kullanılmıştır. Sepiyolit yüklemesinin biyokompozitler üzerindeki etkisi ve Tetrasiklin'in (TC) salınımı araştırılmıştır. Kitosan/SEP biyokompozit sistemleri farklı karakterizasyon yöntemleri (FTIR, TGA, XRD ve SEM) kullanılarak incelenmiştir. FTIR ve SEM sonuçları düşük ve yüksek SEP içerikli biyokompozitlerin faz ayrımlı mikrokompozit yapıda olduğunu göstermektedir. Biyokompozitlerin yüzey fonksiyonel grupları Boehm titrasyon tekniği ile araştırılmıştır. Yüzey net yükü, nötral olarak belirlenmiştir. Termal davranış TGA/DTG ile incelenmiştir. Kitosan matriksine dağılmış olan kil, kil miktarı arttıkça sistematik olarak kompozitlerin termal kararlılığını arttırmıştır. CS/SEP biyokompozitlerinden TC salınımı kesikli sistemde, otuz yedi derecede, pH, kil içeriği ve etki süresinin fonksiyonu olarak çalışılmıştır. İlacın sulu çözeltiye salınımı kil içeriğine bağlıdır. CS/SEP biyokompozitinden salınan TC miktarı (C_s : mmol.g⁻¹) zamana karşı grafiğe geçirildiğinde en fazla salınım yüzde bir kil içeriğinde gözlenmiştir. Farklı kil içeriklerinde biyokompozitlerden TC salınımı yüzdesi yapıdaki kil içeriği arttığında kilin silanol (-SiOH) ve grupları ve ilacın -OH grupları arasındaki hidrojen bağı nedeniyle salınım düşmektedir. Tetrasiklinin sulu ortama difüzyon hızı, artan kil miktarı ile azalmaktadır.

Anahtar sözcükler : kitosan, sepiyolit, tetrasiklin, salınım, difüzyon hızı

CONTENTS

	Page
THESIS EXAMINATION RESULT FORM	ii
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
ÖZ	v
CHAPTER ONE – INTRODUCTION	1
1.1. Chitosan.....	1
1.1.1 The properties of Chitosan	1
1.1.2 Applications of Chitosan	3
1.1.2.1 Usage of Chitosan as an Adsorbent in Wastewater Treatment.....	3
1.1.2.2 The Antimicrobial Activity and Food Applications of Chitosan.....	4
1.1.2.3 Pharmaceutical Uses of Chitosan.....	4
1.2 Clays.....	5
1.2.1 General definitions	5
1.2.2 Classification of Clay Minerals.....	7
1.2.3 Uses of Clay Minerals.....	7
1.2.3.1 Agricultural and Environmental Applications of Clay Minerals.....	8
1.2.3.2 Cosmetic and Aesthetic Applications of Clay Minerals.....	8
1.2.3.3 Pharmaceutical Applications of Clay Minerals	9
1.3 Sepiolite.....	10
1.3.1 Applications of Sepiolite.....	12
1.4 Polymer clay composite	14
1.4.1 Preparation Methods of Polymer/Clay Composite	14
1.4.2 Characterization of Polymer/Clay Composites	15
1.5 Purpose of the Study.....	16

CHAPTER TWO – MATERIALS AND METHODS..... 18

2.1 Materials.....	18
2.2 Preparation of Biocomposites	18
2.3 Characterization of Biocomposites	18
2.3.1 Fourier Transform Infrared (FTIR) spectra of the samples.....	18
2.3.2 Thermal Analysis.....	19
2.3.3 XRD Analysis.....	19
2.3.4 SEM Analysis.....	19
2.3.5 Determination of the Surface Functional Groups.....	19
2.3.6 Determination of the Point of Zero Charge	20

CHAPTER THREE – RESULTS AND DISCUSSION..... 21

3.1 Fourier Transform Infrared (FTIR) Spectra of the Samples	21
3.2 Thermal Analysis	23
3.3 XRD Analysis.....	25
3.4 SEM Analysis.....	27
3.5 Determination of the Surface Functional Groups.....	30
3.6 Determination of the Point of Zero Charge.....	30

CHAPTER FOUR – DRUG RELEASE FROM CS/SEP BIOCOMPOSITES..... 34

4.1 Tetracycline.....	34
4.2 Synthesis of Tetracycline Loaded Chitosan Films.....	36
4.3 Synthesis of Tetracycline Loaded Chitosan Sepiolite Films.....	37
4.4 Release Study from TC Loaded CS/SEP Films	37
4.5 Kinetic Study of the TC Release from CS/SEP/TC Biocomposites	40
4.6 Kinetic Study of the TC Diffusion from CS/SEP/TC Biocomposites.....	44

4.6.1 Case I or simple Fickian diffusion.....	44
4.6.2 Case II diffusion	44
4.6.3 Non-Fickian or anomalous diffusion	45
 CHAPTER FIVE – CONCLUSION	48
 REFERENCES.....	50

CHAPTER ONE

INTRODUCTION

1.1 Chitosan

1.1.1 The Properties of Chitosan

Chitosan is a linear polysaccharide of β -1,4-O-glycosyl-linked glucosamine residue, derived from deacetylation of chitin which is a major component of crustacean shells, exo-skeleton of insects, and the cell walls of fungal biomass. When its DA (degree of N-acetylation) is lower than 50%, the chitin becomes soluble in aqueous acidic solution and is named chitosan. Chitosan is insoluble in water, aqueous alkaline solutions, and common organic solvents, but it readily dissolves in aqueous inorganic and organic acid media.

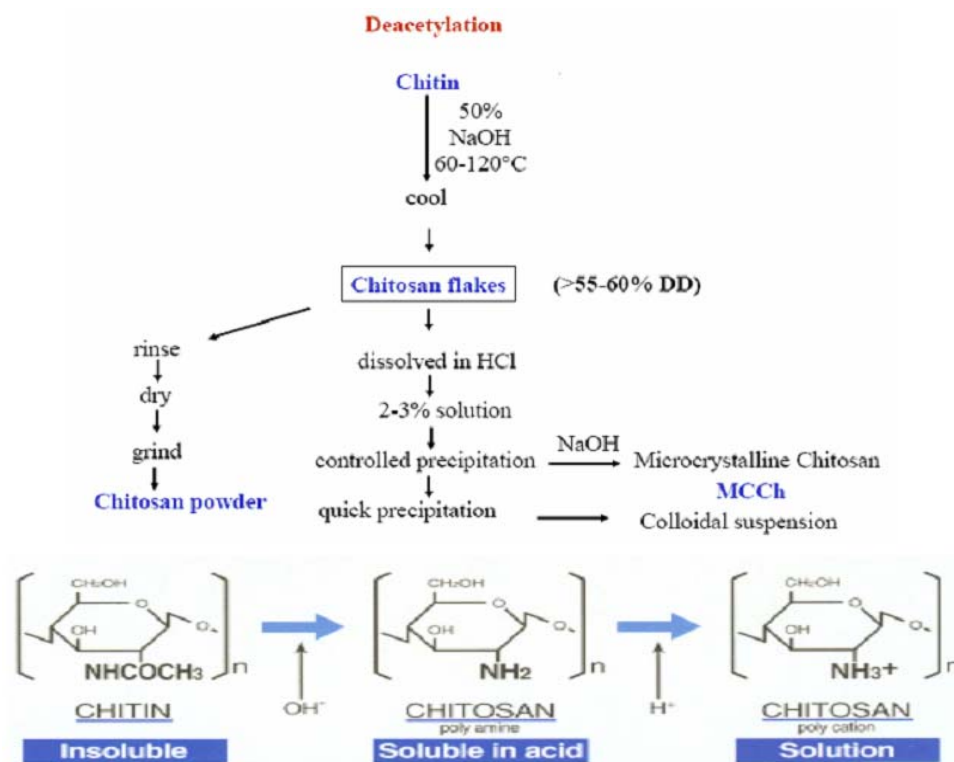


Figure 1.1 Deacetylation of chitin (www.kimica.jp/eng-chitosan-chemi-2.htm)

Chitosan, an unbranched cationic biopolymer, has three types of reactive functional groups that allow further chemical modification, i.e., amino or amido groups at C-2 positions as well as both primary and secondary hydroxyl groups at C-6 and C-3 positions, respectively. These active sites can give rise to hydrogen bonding and the linear molecule express sufficient chain flexibility. When chitosan is employed in aqueous media, the distribution of the surface functionalities is of fundamental importance. The gross conformation of chitosan in solution is manipulated by solution parameters, such as ionic strength, solvent, temperature, pH value of solution (Sorlier et al., 2002).

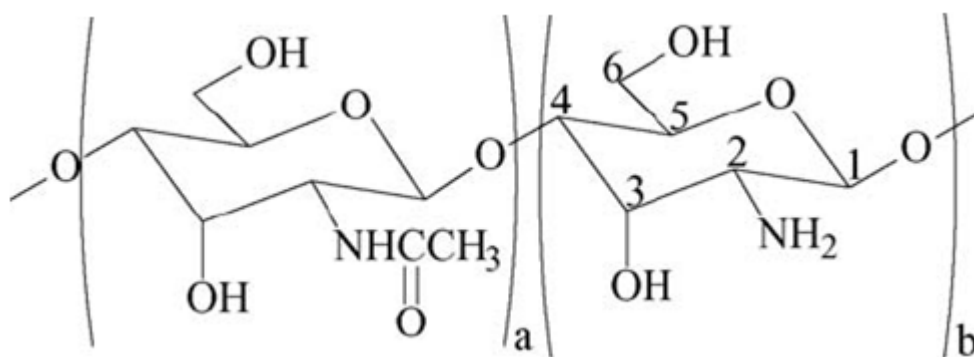


Figure1.2. Chemical structure of (a) 2-acetamido-2-deoxy-d-glucopyranose(Glc-Nac) and (b) 2-amino-2-deoxy-d-glucopyranose (GlcN) units joined by (1→4) glycosidic bond. The a and b contents distinguish chitin from chitosan (Cavalheiro & Guinesi, 2006).

Chitosan is a high molecular weight linear polyelectrolyte and a cationic polyamine. The physical properties of chitosan depend on the degree of N-acetylation and the distribution of N-acetyl groups and average molecular weight. If the pH values lower than 6.5, chitosan has high charged density. When protonated, adheres to negatively charged surfaces (bio and muco-adhesive) and forms gels with polyanions. Chitosan has reactive amino and hydroxyl groups and amiable to chemical modification, also forms salts with organic and inorganic acids and chelates certain transitional metals. Further it has a wide range of viscosity from high to low (Enescu et al., 2008).

The oral administration of chitosan with drugs enhanced their absorption from intestines into blood in animals. Chitosan is being evaluated in a number of

pharmaceutical and biomedical applications including wound healing and dressing, dialysis membranes, contact lenses, fibers for digestible sutures, liposome stabilization agents, enzyme supporting materials, antitumor uses and drug delivery uses and controlled-release systems (Altınışik, 2007).

Biological properties of chitosan are;

- Hydrophilicity
- Bioactivity
- Biocompatibility
- Biodegradability
- Osteoconductivity
- Anticholesteremicity
- Spermicidiality (having the ability to kill the sperm)
- Non-toxicity (its degradation products are known natural metabolites)
- Non-antigenicity (not induce an immune response)
- Antibacterial properties
- The ability to improve wound healing/or clot blood
- The ability to absorb liquids and to form protective films and coatings
- Selective binding of acidic liquids, there by lowering serum cholesterol levels

1.1.2 Applications of Chitosan

1.1.2.1 Usage of Chitosan as an adsorbent in wastewater treatment

Chitosan can be used as an effective coagulating agent for organic compounds, as a chelating polymer for binding toxic heavy metals, as well as an adsorption medium for dyes and small concentrations of phenols and PCBs present in various industrial wastewaters. In these specific applications, chitosan appears more affective than other polymers such as synthetic resins, activated charcoal, and even chitin itself. In addition,

the amino group in chitosan is an effective functional group that can be altered chemically for production of other chitinous derivatives with specific useful characteristics as effective absorptive agents.

1.1.2.2 The Antimicrobial Activity and Food Applications of Chitosan

Food quality and safety are major concerns in the food industry as consumers prefer fresher and minimally processed products. In particular, bacterial contamination of ready to eat products constitutes one of the most serious health hazards to human population. The antimicrobial activity of chitosan was observed against a wide variety of microorganisms including fungi, and some bacteria. The most feasible hypothesis is the leakage of cellular proteins and other intracellular constituents caused by the interaction between the positively charged chitosan and negatively charged microbial cell membranes. Other mechanisms proposed are the inhibition of microbial growth and toxin production by the chelation of essential metals and nutrients, spore components, as well as the penetration of the nuclei of the microorganisms, which leads to the interference of mRNA and protein synthesis (Tripathi, 2010).

1.1.2.3 Pharmaceutical Uses of Chitosan

Chitosan exhibits unique and most valuable properties that enhance its versatility in the biomedical and biotechnological fields, such as immuno stimulation, activation of macrophages, mucoadhesion, antimicrobial activity, and well assessed chemistry (Muzzarelli, 2009). Chitosan and its derivatives are suitable for tissue engineering applications because of their porous structure, gel forming properties, ease of chemical modification, biodegradability, biocompatibility, antibacterial activity, and high affinity to in vivo macro molecules. It is one of the most important biomaterials in tissue engineering and shows considerably very good physicochemical and biological properties (Honarkar and Barikani, 2009).

This material is also a good candidate for use as a carrier of drugs for controlled release and other pharmaceutical applications. Various types of chitosan derivatives have been used in skin, bone cartilage, liver, nerve, and blood vessel (Kim et al., 2008)

Polysaccharides based films have serious problems with their water barrier properties, due to the hydrophilic nature. A determinant factor in the mechanism of water transport through chitosan films is the polymeric matrix and the hydrophilic and electrolytic properties of film-forming solutions. The incorporation of smaller inorganic particles into larger polymers is desirable in order to combine parameters of both materials and improve the physicochemical properties of polymers.

1.2 Clays

1.2.1 General Definitions

Clay minerals are made up of layered silicates. They are crystalline minerals of very fine particle size ranging from 150 to less than 1 micron (colloidal form) (Varma, 2002). Clay has two definitions. According to the clay mineralogist, a clay mineral is a layer silicate mineral (also called a phyllosilicate) or other mineral which imparts plasticity and which hardens upon drying or firing (Guggenheim & Martin 1995). The word "clay" is also used to refer to a particle size in a soil or sediment. The term is used in the U.S. and by the International Society of Soil Science for a rock or mineral particle in the soil having a diameter less than 0.002 mm (2 microns), whereas sedimentologists classify particles smaller than 0.004 mm as clay (Seki, 2002).

Clay is used as a rock term and also as a particle – size term in the mechanical analysis of sedimentary rock, soils, etc. As a rock term it is difficult to define precisely, because of the wide variety of materials that have been called clays. In general the term clay implies a natural, earthy, fine-grained material which develops plasticity when mixed with a limited amount of water. Chemical analyses of clays show them to be

composed essentially of silica, alumina and water frequently with appreciable quantities of iron, alkali alkaline earths (Grim, 1968). In most cases clay finest as cheap filler have been used in polymer composites including rubbers, plastics, coatings and paints, etc (Jing Cao Dai et al. 1999).

In practice, "clay" is used to refer to the fine-grained, mineral fraction of earth material, and can include clay silicates and oxide-hydroxide minerals (and also to some extent of magnesium or iron), such as goethite, hematite, manganese oxides, and some zeolites. When examined under the scanning electron microscope, these minerals are seen to consist of readily identifiable particles, which can have a variety of geometric shapes. Despite this variety of morphology, clays are closely interrelated in terms of their basic crystal structures, and also in the characteristic physical and chemical properties resulting from their crystal chemistry (Wilson, 1987).

1.2.2 Classification of Clay Minerals

Clay minerals can be classified as indicated in scheme below (Rieder et al., 1998).

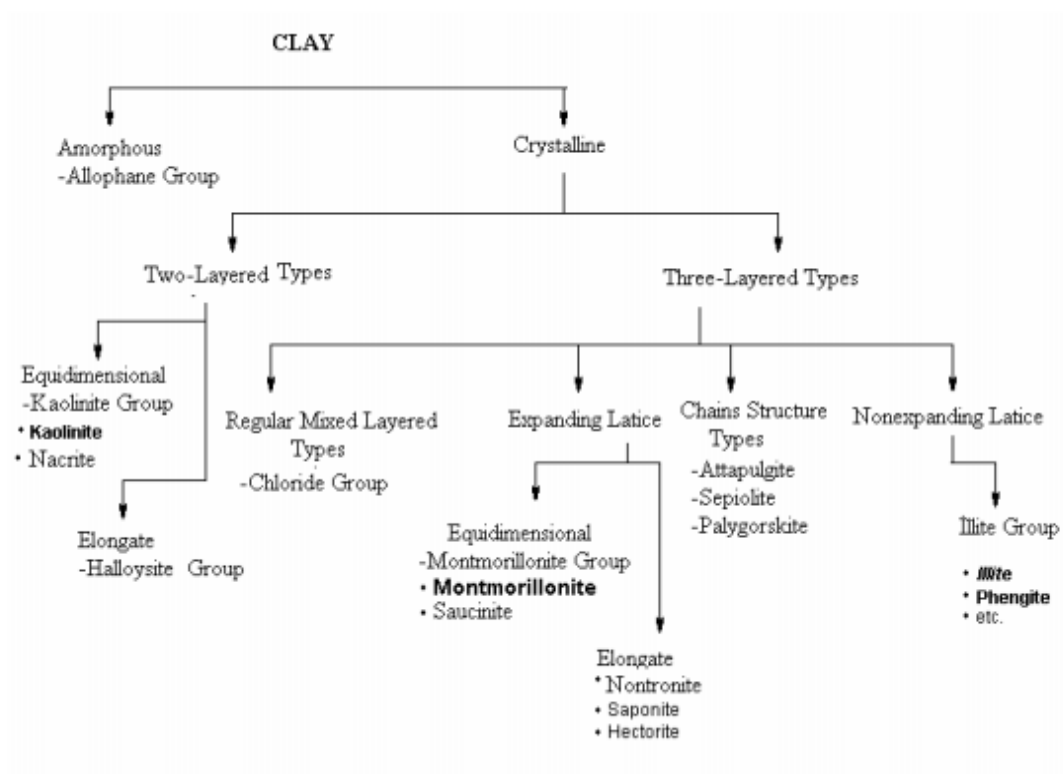


Figure 1.3 Classifications of Clay Minerals

1.2.3 Uses of Clay Minerals

Clay minerals occur abundantly in nature and their high surface area, sorptive and ion-exchange properties have been exploited for catalytic applications through decades. Solid clay catalysts (Pinnavia, 1983; Laszlo, 1987) have a broad range of functions including; use as catalytically active agents (usually as solid acids); as bifunctional or 'inert' supports; as fillers to give solid catalysts with required physical properties (e.g. attrition-resistance, density, specific heat capacity etc.) (Varma, 2002).

In those early days the clay was used for dolls, modeling and miniatures for doll houses.

Clay minerals have been used in;

- Agricultural and environmental applications
- Cosmetic applications and aesthetic medicine
- Pharmaceutical applications

1.2.3.1 Agricultural and Environmental Applications of Clay Minerals

Incorporation of clay minerals in animal feeds have been carried out for multiple purposes such as, anti-caking and palletizing aid in no medicated feeds and as a consolidating additive of feces (Ferrario et al., 2000).

Application of clays and clay minerals in pesticide formulations is currently attracting considerable interest (Choy et al., 2007). To increase pesticide efficiency and to reduce their leaching into the environment like air and water, it has been suggested that reversible binding of the pesticide on clay minerals would be one of the feasible solutions. Many studies have been focused on adsorption of pesticides by clay minerals for their removal from water and immobilization in soils.

1.2.3.2 Cosmetic and Aesthetic Applications of Clay Minerals

Clay minerals have been widely employed as thickeners and emulsion stabilizers in cosmetics. They are also used as active principles in cosmetics because of their high adsorptive capacity of substances such as greases, toxins, etc. They can be applied as antiperspirants to give the skin opacity, remove shine and cover blemishes. For the purpose of aesthetic medicine in cosmetic products, geotherapy, pelotherapy, and paramuds, the cationic clays are also used as active principles or excipients. These applications are mainly based on high cation exchange capacity, excellent swelling

property, remarkable hydration ability, and structural plasticity of the cationic clays (Choy et al., 2007).

1.2.3.3 Pharmaceutical Applications of Clay Minerals

Clays are common ingredients in pharmaceutical products both as excipients and active substances. In the 1960's it was observed that oral absorption of several drugs was reduced by co-administration of clay-based intestinal adsorbents or by the presence of clay stabilizers (e.g., suspending or emulsifying agents) in liquid formulations (Aguzzi et al., 2007).

In pharmaceutical formulations clays minerals are used as a active agent at oral applications (gastrointestinal) or additive in local applications because of high internal surface area, high sorption ability, high cation exchange capacity, rheological properties, chemical inertness and non-toxicity. When clay minerals are used as drug additive, the interaction between drug and mineral affects the biology of the active agent both during dissolution and dispersion process (Carretero et al., 2009).

Medicinal clays have been widely used in medicine and properly administered clays can purify the blood, reduce or even eliminate infection, heal ulcers, and even rid the body of certain allergies (Gorchakov et al., 2001). Cutaneous chemotherapy based on clay minerals for the treatments of seborrhoeic skin as antimicrobial and antifungal agents has also been developed. Nowadays, the importance of cellular delivery of various drugs and bio-active molecules in medicine leads to advanced development in novel area of chemistry, biology, material sciences.

Studies on both natural and synthetic clay minerals for biological applications are extensively carried out. Among them, researches on novel nano-bio hybrids that combine efficient and safe transport carriers and biological molecules provide a new paradigm. Clay minerals are used as excipients in pharmacological applications to

improve the organoleptic properties, for example, taste, smell, and color, or the physical and chemical ones, and to facilitate and promote the pharmacological formulations. They are used as lubricants and agents to disperse easily and effectively active principles based on their ability to swell in the presence of water and to buffer abrupt change of acidity. Also, their colloidal properties make them useful as emulsifying, gelling and thickening agents to avoid the segregation of the components of the pharmaceutical formulations. Contrary to these traditional applications of clay minerals, novel attempts have recently been explored to develop new potentials as drug carrier, protecting matrix, release controlling agents, and chemical modifiers (Choy et al., 2007).

1.3. Sepiolite

The name Sepiolite was first used in 1847 by Glöcker and takes its origins from the Greek word for cuttlefish. The bones of cuttlefish are as light and porous as the mineral. Sepiolite occurs only with a fibrous habit. Sepiolite, formerly known as Meerschaum (sea froth), is a non-swelling, lightweight, porous clay with a large specific surface area. Unlike other clays, the individual particles of Sepiolite have a needle-like morphology (Sepiolite, 2010, www.ima-eu.org).

The clay mineral Sepiolite is a microcrystalline hydrated magnesium silicate of theoretical unit cell formula $\text{Si}_{12}\text{O}_{30}\text{Mg}_8(\text{OH},\text{F})_4(\text{H}_2\text{O})_{4.8}\text{H}_2\text{O}$. (Figure 1.4) It exhibits a microfibrillar morphology and a particle size in the 2-10 μm length range. Sepiolite shows an alternation of blocks and tunnels that grow up in the fiber direction Figure 1. 5. The blocks are constituted by two layers of tetrahedral silica sandwiching a central magnesium oxide hydroxide layer, and the dimensions of the cross-section of tunnels are about $1.1 \times 0.4 \text{ nm}^2$.

The discontinuity of the silica sheets gives rise to the presence of silanol groups (Si-OH) at the edges of the channels, which are the tunnels opened to the external surface of the Sepiolite particles. Tunnels are filled with both the coordinated water molecules,

which are bonded to the Mg^{2+} ions located at the edges of octahedral sheets, and the zeolitic water, which is associated to the former by hydrogen bonding (Hitzky et al., 2006). These tunnels are accessible to water molecules (zeolitic water) and also a variety of species, the only restriction being related to the dimensions of the guest species, the Sepiolite acting as a molecular sieve (Saavedra et al., 2004).

When dispersed in water these elongate crystals are inert and non-swelling and form a random lattice capable of trapping liquid and providing excellent thickening, suspending, and gelling properties. These clays do not flocculate with electrolytes and are stable at high temperatures, which make them uniquely applicable for many uses (Murray, 2002).

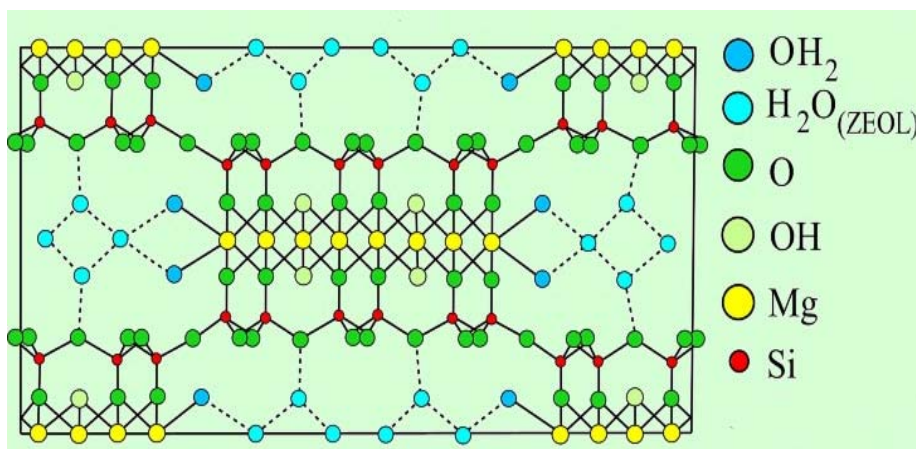


Figure 1.4 Structure of Sepiolite mineral (<http://pubs.usgs.gov>)

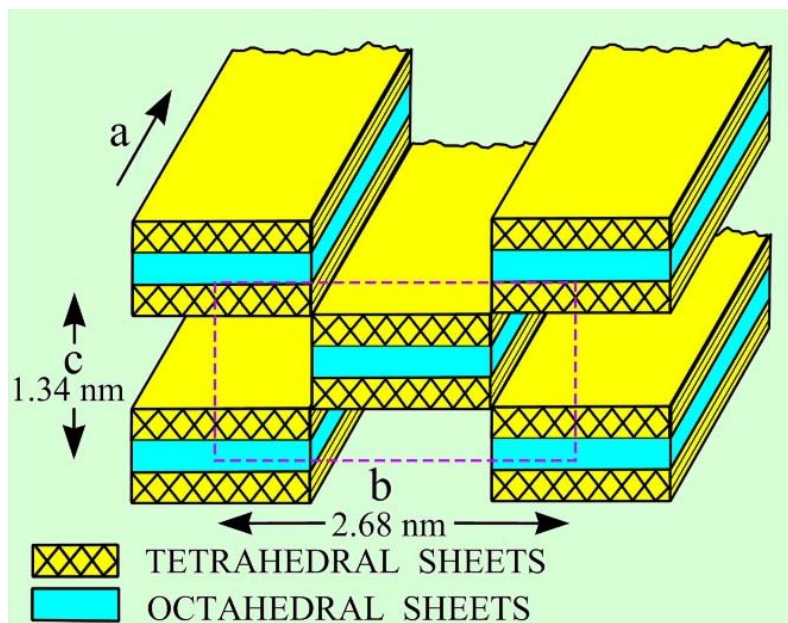


Figure 1.5 Tunnels and blocks of Sepiolite mineral (<http://pubs.usgs.gov>)

Sepiolite has been used for centuries because of their important colloidal and rheological properties and their capacity for sorption (Keith, 2000). The sorptive properties of Sepiolite classify it in a group of clays known collectively as fuller's earth. The term fuller's earth is a term used for highly absorbent and natural bleaching clays.

Sorptive properties of Sepiolite can be explained by three features:

- Oxygen ions associated with tetrahedral on ribbon edges may attract cations or molecules with dipoles to the mineral surface.
- H₂O molecules may be coordinated to magnesium ions at the edges of structural ribbons.
- Silanol (-SiOH) groups occur along the fiber axis of crystals.

1.3.1. Applications of Sepiolite

Sorptive grade Sepiolite is used as decolorizing and clarifying agents, filter aids, floor adsorbents, animal litter, and pesticide carriers, components of the No-carbon copy

papers, and catalysts and refining aids. Organically modified Sepiolite allows controlling the rheological behavior of different solvent-based systems as paints, greases, resins and inks enhancing their stability under a wide temperature range and making for easier application.

Sepiolite absorbs liquid spills and leaks keeping work and transit areas dry and safe. It is a non-flammable material with high liquid absorbing capacity, suitable mechanical strength of the granules even in wet conditions, and chemical inertness which avoids reaction with absorbed liquids. Sepiolite absorbs toxic and hazardous wastes in stabilization or inertisation treatments. Sepiolite absorbs active chemicals, as pesticides, remaining free-flowing and allowing an easy use and effective application of the product in the field.

The use of Sepiolite fillers improve processing, dimensional stability, mechanical strength and thermal resistance. Sepiolite allows controlling the rheological properties in heat application systems, improving fire resistance. It also improves binding of the components while increasing the fire resistance.

Sepiolite adsorbs excess humidity preventing condensation, corrosion, the proliferation of microorganisms and unpleasant odors. Then, Sepiolite can be used as cat and pet litters. The popularity of Sepiolite pet litters is due to its light weight, high liquid absorption and odor control characteristics. Sepiolite absorbs pet urine and has a dehydrating effect on solid faces which minimizes bad odors and inhibits bacteria proliferation. Sepiolite is registered in the EU as a technological additive for animal feed (E-562).

Sepiolite has numerous domestic applications such as moisture control, containment of accidental liquid spillages, and use in ashtrays to avoid smoke odor, control of liquid leakages and odors in dustbins, odor removal in refrigerators, etc (www.ima-eu.org).

1.4 Polymer Clay Composite

1.4.1 Preparation Methods of Polymer / Clay Composite

Several methods have been developed to produce clay/polymer nanocomposites. Three methods were developed in the early stages of this field and have been applied widely. These are:

- in situ polymerization
- solution induced intercalation
- melt processing

In situ polymerization involves inserting a polymer precursor between clay layers and then expanding and dispersing the clay layers into the matrix by polymerization. This method is capable of producing well-exfoliated nanocomposites and has been applied to a wide range of polymer systems. The solution-induced intercalation method applies solvents to swell and disperse clays into a polymer solution. However, solution-induced intercalation is applicable to water-soluble polymers, because of the low cost of using water as a solvent and its low health and safety risks, and can be used in the commercial production of nanocomposites. The melt processing method induces the intercalation of clays and polymers during melt. The efficiency of intercalation using this method may not be as high as that of in situ polymerization and often the composites produced contain a partially exfoliated layered structure (Gao, 2004).

Depending on the nature of components (polymer matrix, clay filler and organic surfactant) and processing conditions, clay particles can be present in three configurations when incorporated in the polymer matrix (Figure 1.6). If the polymer is unable to intercalate into the galleries, a phase separated composite is formed, whose properties are similar to that of traditional microcomposites; the poor interaction between the organic and the inorganic component results in relatively poor mechanical performance. On the other hand, an intercalated nanocomposite is obtained when

extended polymer macromolecules diffuse between unchanged clay sheets, leading to a well ordered multilayer structure of alternating polymeric and inorganic layers with a repeating distance of few nanometers between them. The properties of this type of nanocomposites typically resemble those of ceramic materials. The most significant changes in physical properties are observed in exfoliated hybrids, where clay layers are separated and uniformly dispersed, maximizing thus the polymer–clay interactions (Kiliaris, 2010).

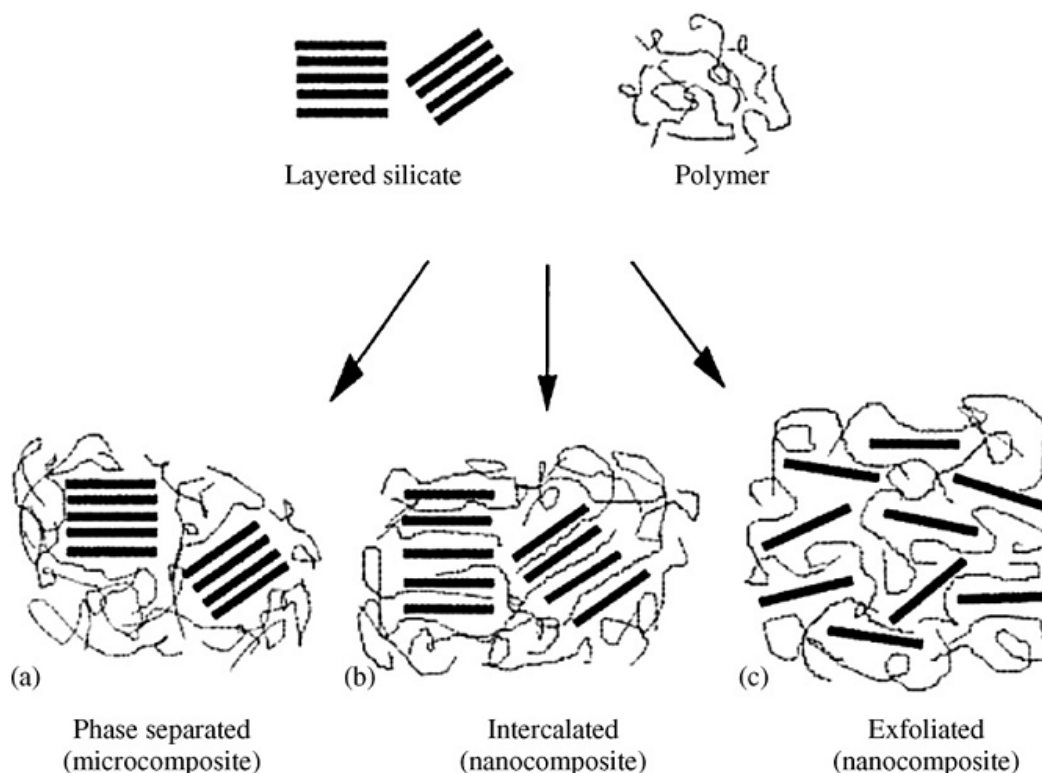


Figure 1.6 Different types of clay morphology in a polymer/clay nanocomposite: (a) phase separated microcomposite; (b) intercalated nanocomposite; (c) exfoliated nanocomposite.

1.4.2 Characterization of Polymer/Clay Composites

In order to characterize the morphology of nanocomposites, two complementary techniques are generally employed; X-ray diffraction (XRD) and transmission electron microscopy (TEM). XRD is used to probe alterations in the order of silicates by

monitoring the position, shape and intensity of their basal reflections. Increasing interlayer spacing is identified by a shift of the diffraction peak to lower angles, according to Bragg's law, leading eventually to feature less patterns (exfoliated structures). TEM is supplementary utilized as an effective means of developing in sights into the internal structure and spatial distribution of the various components, through direct visualization (Kiliaris, 2010).

1.5 Purpose of the Study

Chitosan has been extensively investigated for several decades for molecular separation, food packaging film, artificial skin, bone substitutes, water engineering and so on, owing to its good mechanical properties, biocompatibility, biodegradability, multiple functional groups as well as solubility in aqueous medium. Chitosan particles also can be used as a scaffold for drug delivery because of high swelling property. Chitosan-based materials swell considerably within a short period of time and a burst release of more than 50 % of the encapsulated molecule is often observed (Grech et al., 2003). To overcome this problem some inorganic filler such as clays can be added the hydrogel preparing process.

Sepiolite contain silanol groups (Si-OH) covering the external mineral surface. These groups can be effectively involved in hydrogen bonding by their association to OH, NH and other polar groups belonging to the biopolymers used. Chitosan can also be assembled with sepiolite to give the corresponding biopolymer-sepiolite composites, which exhibit good mechanical properties resulting from the combination of the fibrous inorganic substrate with the biopolymer. The interaction mechanisms governing the formation of sepiolite-based bio-composites are mainly ascribed to hydrogen bonding, but it must be taken into account that sepiolite exhibits cationic exchange capacity (CEC $\sim 15\text{meq}/100\text{g}$). Thus this silicate could also interact with positively charged polymers, such as chitosan, through electrostatic bonds.

The aim of this study is to prepare a chitosan scaffold for drug delivery by incorporating Sepiolite and to figure out the release profile. At first step the effect of Sepiolite on the rheological and colloidal properties of chitosan biocomposite dispersions at different Sepiolite concentrations will also be examined. The hydrogen bonding force between chitosan and Sepiolite and clay loading on the morphology, thermal stability and mechanical properties of the biocomposites will be investigated. The structural analysis of Chitosan/Sepiolite biocomposite materials will be studied using FTIR, SEM, XRD and mechanical testing. The thermal behavior of the biocomposite will be determined by using TGA and DTA. Surface functional groups and surface acidity of the composites will be examined. Furthermore; drug loaded biocomposites will be synthesized and drug release profile in different pH values for different Sepiolite contents will be examined.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

Chitosan (CS) (highly viscous) was purchased from Fluka (degree of deacetylation: 75-85%, average molecular weight: 500000-700000 g mol⁻¹) as a flaked material. Acetic acid (HAc) and sodium hydroxide (NaOH) were obtained from Riedel de Haen. Sepiolite (SEP) obtained from Eskişehir, Turkey. Tetracycline was supplied from Sigma (T3258-5G).

2.2 Preparation of Biocomposites

Chitosan solution was prepared by dissolving chitosan (CS) in a 2% (v/v) aqueous acetic acid solution at a concentration of 2 wt% followed by filtering to remove the insoluble material. SEP was first swelled by 50 mL distilled water and then added to 50 mL chitosan solution with SEP contents of 1wt%, 2 wt%, 5 wt%, followed by stirring at 60 °C for 6 h. After that, CS/SEP solutions were cast on a Petri dish and dried in vacuum oven at 60 °C for 48 h. The dry films still contained a small quantity of the solvent (HAc), which formed chitosonium acetate. After drying, the films were soaked in 1 M aqueous NaOH for 5 h to neutralize the acid followed by rinsing in distilled water to neutral and then dried at vacuum oven (0.5 atm) at 60 °C for 24 h. They were termed CS/SEP-X in which X is the content of SEP percentage.

2.3. Characterization of Biocomposites

2.3.1 Fourier Transform Infrared (FTIR) spectra of the samples

FTIR spectroscopy was used to obtain the information about the interactions between chitosan and clay sample. Chitosan film, Sepiolite and biocomposites were brought to constant weight in a drying oven at 60 °C for 24 h. Fourier transform infrared (FTIR)

spectra (transmission) were measured on a Perkin-Elmer FTIR spectrophotometer Spectrum BX-II in the range 4000-400 cm^{-1} at a resolution of 4 cm^{-1} .

2.3.2. Thermal Analysis

The thermal properties of biocomposites and pure chitosan were investigated by thermogravimetric analysis (TGA). TGA was performed under nitrogen flow and atmosphere air from room temperature to 800°C at a rate of 20°C/min with a Perkin Elmer Diamond TG/DTA instrument. The weights of samples varied from 2 to 3 mg.

2.3.3 XRD Analysis

The X-ray diffraction (XRD) patterns of the hydrogel films were recorded with oriented mounts, in a Philips X'Pert Pro X-Ray diffractometer using Cu K_{α} radiation at 45 kV and 40 mA in the 2θ range of 0-60°. X-ray diffraction measurement for natural Sepiolite and CS/SEP biocomposites were processed to powder and film form respectively.

2.3.4 SEM Analysis

The surface morphology of the CS/SEP samples was observed with an emission scanning electronic microscope. The samples were coated with a thin gold layer (two times, 40 mA, 60 s; approx. 30 nm) by a sputter coater unit (BALZER SCD 050 Sputter Coater, BAL-TEC) and surface topography was analyzed with a JEOL JSM 6300F Scanning Electron Microscope(SEM) operated at an acceleration voltage of 5 kV.

2.3.5 Determination of the Surface Functional Groups

Surface functional groups on CS/SEP samples were determined by Boehm's titration (Boehm 1994, Putra et al., 2009, Toles et al., 1999). In this method, certain titrable

surface functional groups on the samples can be determined and quantify the amount of various types of oxygenated groups.

According to this technique 0.2 g of CS/SEP biocomposites were immersed into 25 mL of 0.1 N, NaHCO_3 , Na_2CO_3 , NaOH solution for acidity analysis as well as HCl solutions for basicity analysis. All the solutions was waited for 24 h. 10 ml of each of supernatant liquid is titrated with standardized HCl for NaHCO_3 , Na_2CO_3 , NaOH solutions and standardized NaOH for HCl solutions with appropriate indicator (methyl red, methyl orange and phenolphthalein respectively). The number of the basic sites was calculated from the amount of HCl that reacted with the CS/SEP samples.

2.3.6 Determination of the Point of Zero Charge

Determination of the point of zero charge experiments was carried out in the NaCl solution. 25 mL of 0.01 M NaCl solution was put into several Erlenmeyer flask. The pH value of the NaCl solution within each flask was adjusted by 0.1 M HCl and 0.1 M NaOH solution in the range of between $\text{pH}=2$ and $\text{pH}=9$. Then, 0.5 g of CS/SEP biocomposites was added to each Erlenmeyer flask. The final pH value of the solution was measured after 48 h.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Fourier Transform Infrared (FTIR) Spectra of the Samples

The structure of the chitosan biocomposite films were analyzed by using FTIR spectroscopy. Figure 3.1 A–D shows FTIR spectra of CS/SEP biocomposites and Chitosan. Standard chitosan showed bands at $\sim 2914\text{ cm}^{-1}$ (aliphatic C–H stretching), 1379 cm^{-1} and 1414 cm^{-1} (C–H bending), 1651 cm^{-1} (N–H bending), 1152 and 1089 cm^{-1} (C–O stretching). The bands around 3540 cm^{-1} were assigned to OH stretching, indicating intramolecular hydrogen bonding of chitosan films and they also overlapped in the same region of an NH stretching at 3467 cm^{-1} . The C=O stretching (amide I) band near 1637 cm^{-1} and the NH bending (amide II) band near 1547 cm^{-1} are the characteristic bands of chitosan. Likewise, the band situated between 1200 and 1000 cm^{-1} , characteristic of sepiolite and attributed to Si–O–Si vibrations.

FTIR spectra of the Standard Sepiolite evaluated by Perraki and Orfanoudaki (2008), as follows;

- The band at $\sim 3689\text{ cm}^{-1}$ corresponds to stretching (ν_{OH}) vibrations of hydroxyl groups attached to octahedral Mg ions located in the interior blocks of natural sepiolite.
- The band at $\sim 3574\text{ cm}^{-1}$ is assigned to the Mg-coordinated water (edge Mg–OH stretching vibration).
- The bands at ~ 3419 and $\sim 1663\text{ cm}^{-1}$ are due to the OH stretching and bending vibrations of water molecules, respectively, located in the interior of the channels (zeolitic or interior or channel water).
- The band at $\sim 1449\text{ cm}^{-1}$ is attributed to carbonate (dolomite) impurities. The characteristic bands of silicate minerals are observed at the wavenumber ranges of 1400 and 400 cm^{-1} . These bands are assigned to Si–O bonds in the tetrahedral sheet and to Mg–O stretching vibrations, in the octahedral sheet. The band at $\sim 1209\text{ cm}^{-1}$ is

characteristic for minerals with tetrahedral sheet inversion and it is attributed to Si–O–Si bond.

- The bands at ~ 1023 and ~ 472 cm^{-1} are assigned to the Si–O–Si in plane vibrations.
- The band at ~ 785 and ~ 645 cm^{-1} may be attributed to Mg–OH inner bending vibrations.
- The band at ~ 440 cm^{-1} is attributed to Si–O–Mg bonds (between the tetrahedral and the octahedral sheets).

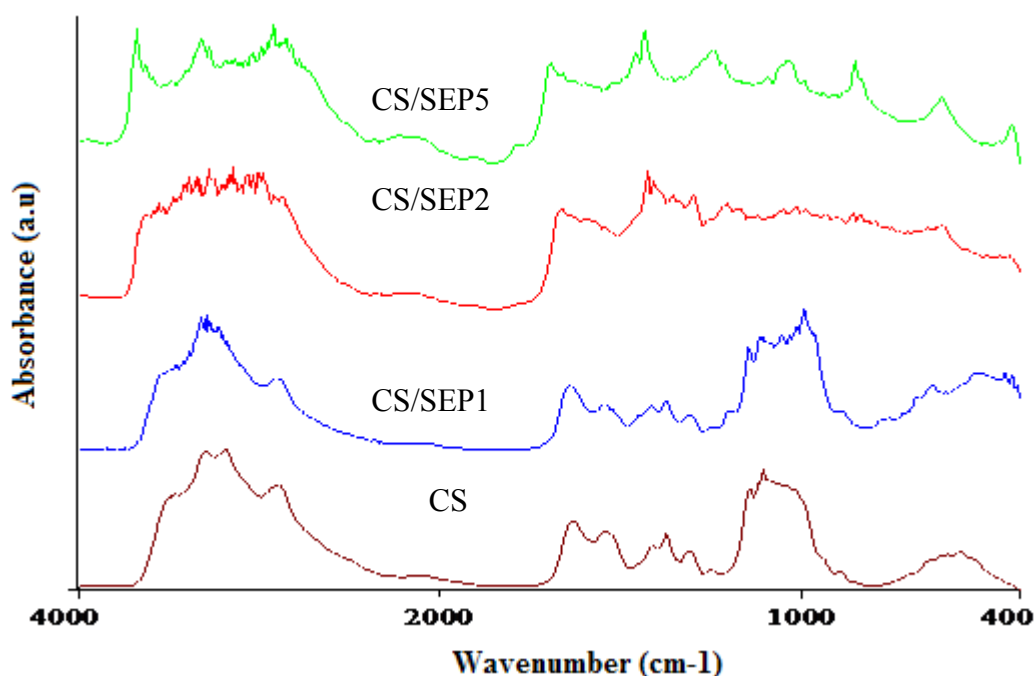


Figure 3.1. FTIR spectrum of the CS, CS/SEP1, CS/SEP2 and CS/SEP5 biocomposites.

O–H groups of primer alcohol in Chitosan give stretching vibration at ~ 3540 cm^{-1} . While the sepiolite content of the biocomposites increased, N–H bending and C–N vibrations at ~ 1651 cm^{-1} ~ 1547 cm^{-1} respectively disappeared and substituted by O–H bending vibrations ~ 1663 cm^{-1} . Aliphatic C–H stretching vibrations of Chitosan can be seen in clearly at 2914 cm^{-1} for CS and CS/SEP1 because of the lower clay content. As seen in Figure 3.1 amide I and amide II bands (at 1637 cm^{-1} and 1547 cm^{-1}) were

decreased by the increasing of the clay content. This result indicated that chitosan interacted with sepiolite.

Table 3.1. Results of FTIR spectra of the samples.

		CS	CS/SEP1	CS/SEP2	CS/SEP5
$\nu_{\text{OH}} (\text{cm}^{-1})$	Stretching	3540	3535	3532	3529
	Bending	Not observed	1653	1669	1663
$\nu_{\text{CH}} (\text{cm}^{-1})$	Stretching	2914	2918	2924	2924
	Bending	1415-1379	1420-1376	1412	Not observed
$\nu_{\text{NH}} (\text{cm}^{-1})$	Stretching	3467	3535	3530	Not observed
	Bending	1547	1558	1594	Not observed
$\nu_{\text{CN}} (\text{cm}^{-1})$	Stretching	1148-1101	1149-1160	Not observed	Not observed
$\nu_{\text{CO}} (\text{cm}^{-1})$	Stretching	1637	1653	1669	1697
$\nu_{\text{SiO}} (\text{cm}^{-1})$	Stretching	Not observed	1205	1211	1215
$\nu_{\text{MgO}} (\text{cm}^{-1})$	Stretching	Not observed	Not observed	3680	3686
	Bending	Not observed	897-640	864-618	858-618

3.2 Thermal Analysis

The thermal stability of the chitosan and its biocomposites has been investigated by TGA under both nitrogen flow (Figure 3.2). On the TGA curve, a non-oxidative degradation occurs. There are two steps of degradation. The first range (25-200°C) is associated with the loss of water about 4-5 wt%, whereas the second range (210-410°C)

corresponds to the degradation and deacetylation of chitosan and left about 60-80 wt% solid residue.

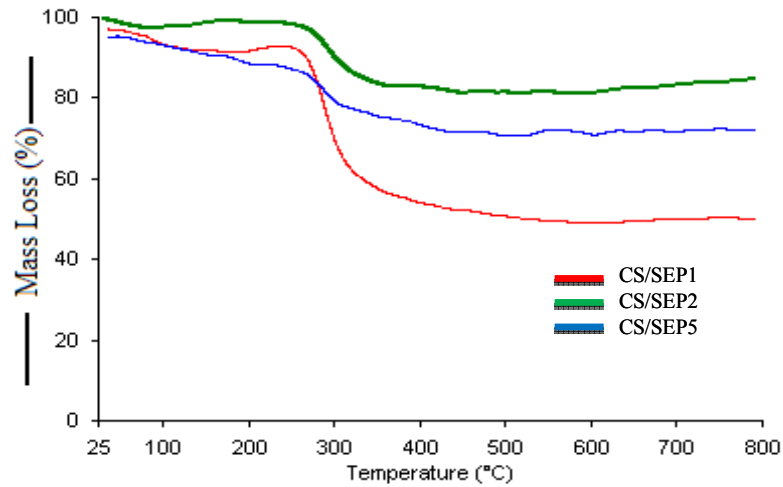


Figure 3.2 Thermogravimetric curves of CS/SEP1, CS/SEP2 and CS/SEP5 in nitrogen flow.

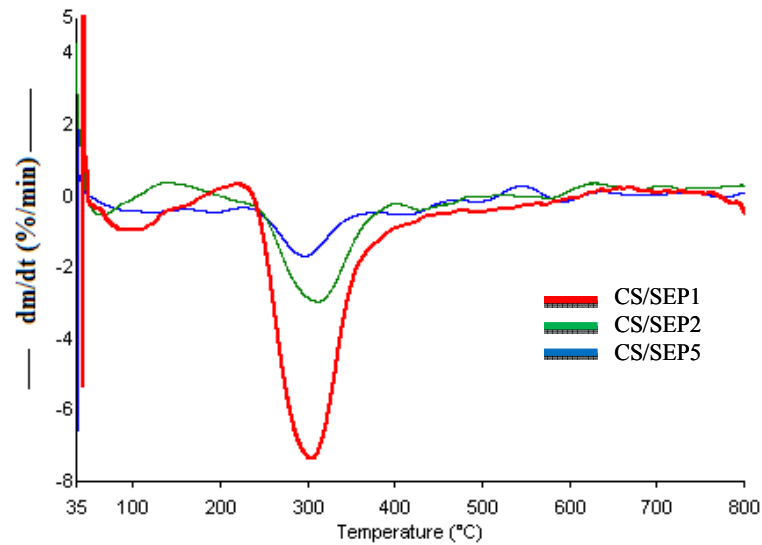


Figure 3.3 DTG curves of CS/SEP1, CS/SEP2 and CS/SEP5 in nitrogen flow;

The DTA curve corresponding to the chitosan shows an endothermic process at 250°C (Altınışık, 2007). The DTA peaks corresponding to chitosan decomposition have

been shifted toward higher temperature values, appearing at 300 °C. This result indicates that Sepiolite mineral reduces the thermal decomposition of the Chitosan.

Table 3.2 Results of termogravimetric analysis of CS/SEP biocomposites in nitrogen flow.

Sample	First Stage		Second Stage		Mass % remaining after 600°C
	T (°C)	Mass Loss %	T (°C)	Mass Loss %	
CS	56	13.4	250	37.9	42.6
CS/SEP1	102	4.8	299	41.9	58.2
CS/SEP2	55	4.9	305	17.8	83.2
CS/SEP5	86	5.3	296	16.0	81.7

As can be seen from Table 3.2 that second stage thermal decomposition temperatures of the composites increased as compared with pure Chitosan.

3.3 XRD Analysis

X-ray diffraction (XRD) reveals the basal spacing of the clays before and after in-situ incorporation indicating the morphology of the biocomposites (exfoliated, intercalated or only dispersed). Sepiolite mineral is characterized by of X-ray diffraction peaks at 2θ degree $\sim 6-7$ which is commonly assigned to the basal 001 reflection (d_{001}). The intensities of the peaks have been decreased by decreasing Sepiolite content of the biocomposites. There is no shift of the 2θ values of the all biocomposites this indicates that internal spaces of the Sepiolite channels have not been changed. These results have shown that polymer matrix cannot be introduced into the tunnels of Sepiolite mineral. Basal spaces have been determined at around 12Å and given in Table 3.3. X-ray diffractograms demonstrates that intercalation process have been possessed that the

formation of the biocomposites. X-ray diffractograms of the each biocomposites have been given as below.

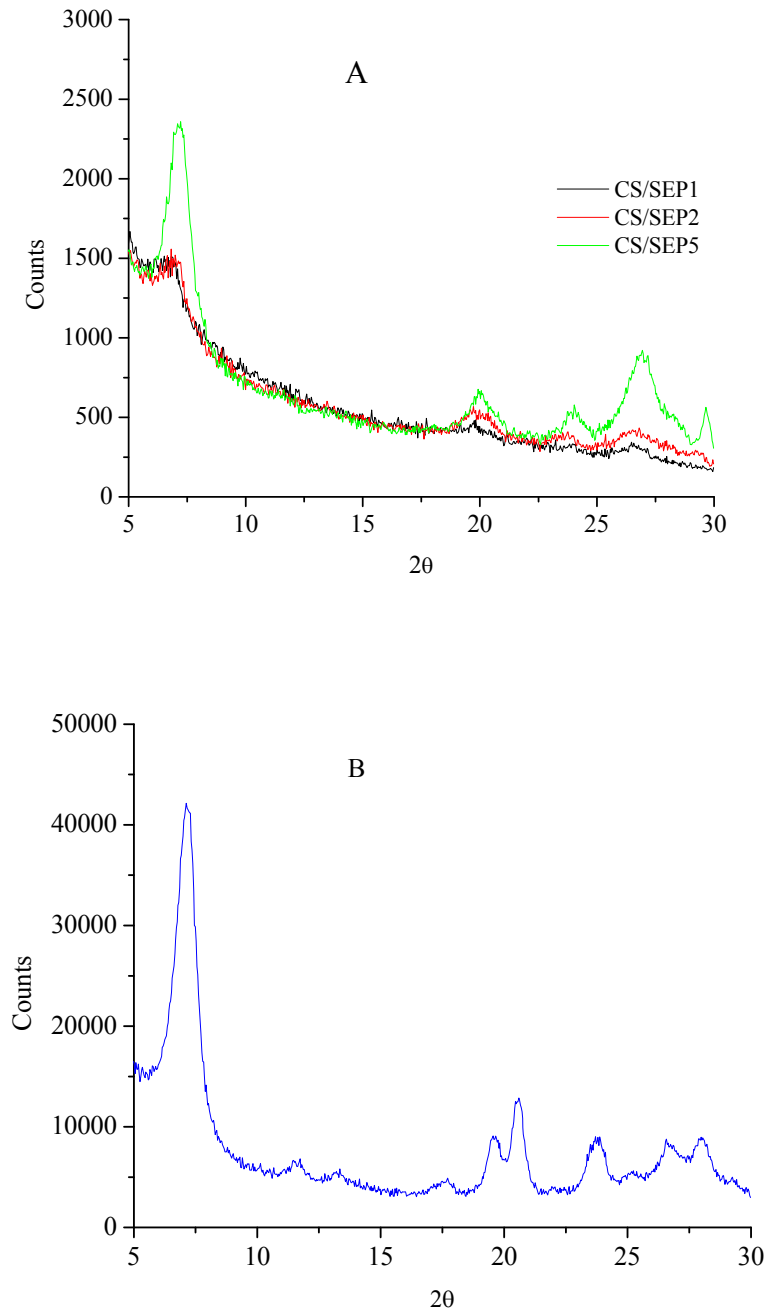


Figure 3.4 XRD patterns of the A) CS/SEP1, CS/SEP2, CS/SEP5 biocomposites and B) Sepiolite.

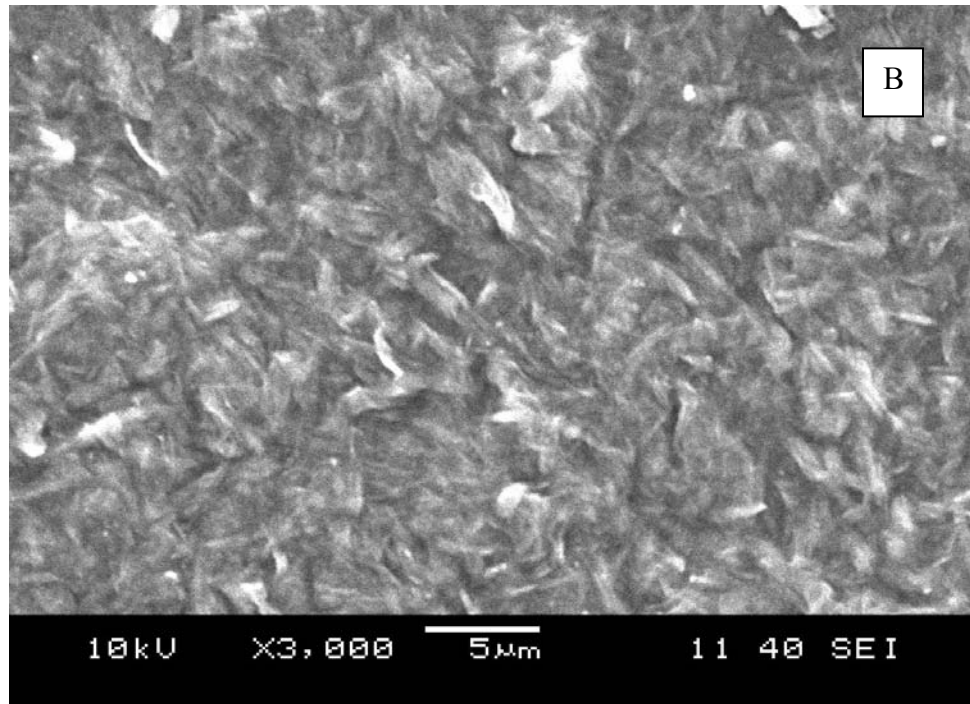
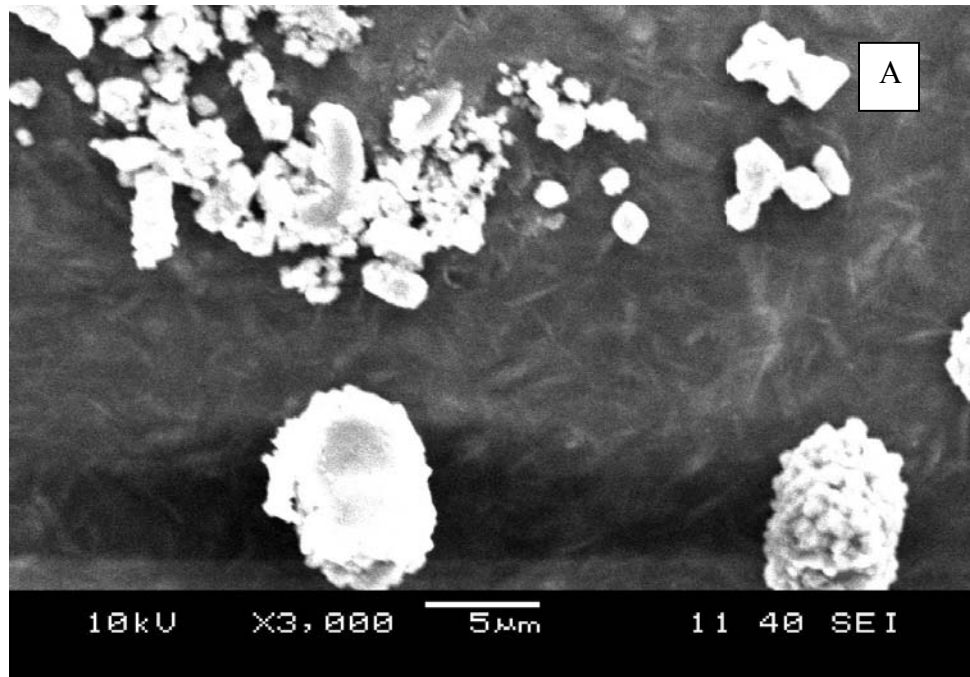
Table 3.3 XRD patterns of natural Sepiolite and CS/SEP biocomposites

Sample	CS/SEP1	CS/SEP2	CS/SEP5	SEPIOLITE
2 θ (degree)	6.86	7.02	6.88	7.34
d ₀₀₁ (Å)	12.88	12.59	12.85	12.04

3.4 Scanning Electron Microscopy Analysis (SEM)

Scanning electron microscopy (SEM) studies were carried out by observation of the topography of the fracture surface. Small pieces of samples were broken from a hand specimen to expose a fresh and undamaged surface. CS/SEP samples (CS/SEP1, CS/SEP2 and CS/SEP5) were mounted on a standard aluminum SEM stub and coated with a micro thin-layer of gold in a vacuum chamber before examination.

SEM micrographs showed that Sepiolite mineral have been covered by the polymer matrix. The fibrous like morphology of the clay cannot allow the polymer to penetrate the inside of the tunnels, because of the larger molecular structure of the Chitosan. Silanol groups on the surface of the Sepiolite interacted by the hydroxyl groups of the Chitosan. Coating of the clay mineral by the polymer mixture caused the agglomeration in biocomposite films. SEM images of the CS/SEP1, CS/SEP2 and CS/SEP5 have showed as below respectively. Some agglomerates of the clay content can be seen in Figure 3.5 (A and C). Polymer clay interaction in the mixture was higher at the low clay content (1%), this raised the agglomeration in the mixture. Similarly in Figure 3, high clay content in the mixture (5%) was influenced the homogeneity. When dispersed clay particles added to the polymer mixture, polymeric structure coated the clay particles before homogen diffusion of the clay, which caused the agglomeration. To have a homogen polymer-clay mixture and to have fewer agglomerates in the biocomposite structure, polymer-clay proportion must be arranged accordingly. Figure 3.5, picture B showed that homogen film surface of the CS/SEP biocomposites.



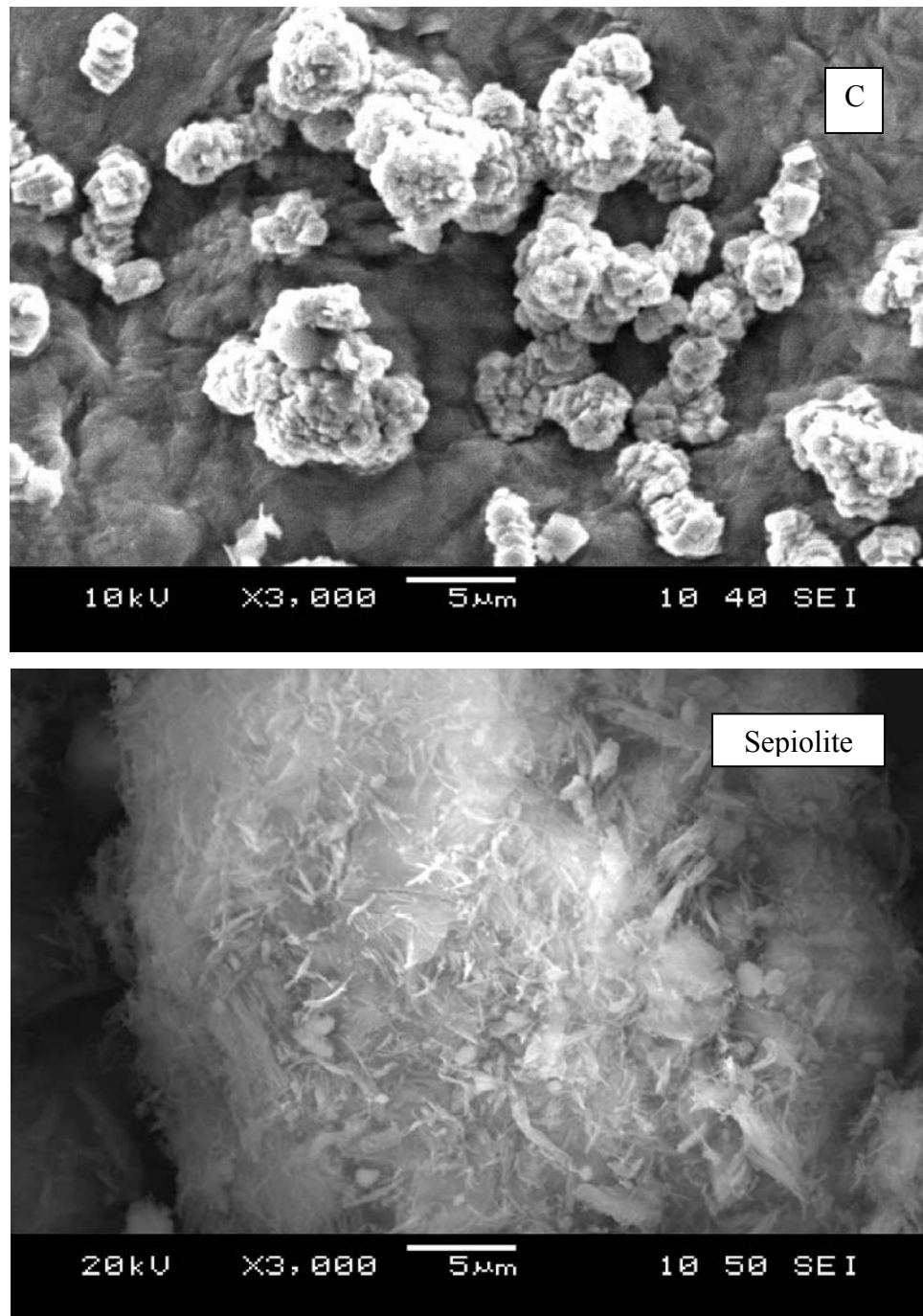


Figure 3.5. SEM micrographs showing the morphologies of the samples (A) CS/SEP1, (B) CS/SEP2, (C) CS/SEP5 and Sepiolite

3.5 Determination of the Surface Functional Groups

Surface functional groups on CS/SEP samples were determined by Boehm's titration (Boehm 1994, Putra et al., 2009, Toles et al., 1999). In this method certain titrable surface functional groups on the samples can be determined and quantify the amount of various types of oxygenated groups. The various free acidic groups were derived using the assumption that; NaOH neutralizes carboxyl, lactone and phenolic groups, Na₂CO₃ neutralizes carboxyl and lactone groups and NaHCO₃ neutralizes only carboxyl groups.

Boehm titration results were calculated as meq/g by the Equation 1.

$$\text{meq/g} = \{(V_{\text{base}} \times N_{\text{base}}) - [S_{\text{acid}} \times (V_{\text{base}}/V_{\text{titrant}}) \times N_{\text{acid}}]\} / m \quad (1)$$

V is the volume of the solution, S is the volume of the titrant and N is the concentrations of the solutions, m is mass of the sample in g. Results have been given for each biocomposites at Table 3.4.

Table 3.4. Boehm's titration results for CS/SEP biocomposites for determination of the surface functionalities.

	NaOH (meq/g)	Na ₂ CO ₃ (meq/g)	NaHCO ₃ (meq/g)	HCl (meq/g)
CS/SEP1	1.27	12.69	0.74	2.06
CS/SEP2	1.29	11.62	0.21	1.82
CS/SEP5	-0.18	12.64	0.46	2.61

Results have been showed that any acidic and basic group have not been found on the surface of the biocomposites.

3.6 Determination of the Point of Zero Charge

The point of zero charges of adsorbents (pH_{pzc}) is a point where an adsorbent have zero potential charge on its surface. At this point on the surface of the adsorbent there is

no positive or negative charge density. The presence of H^+ or OH^- ions in solutions may change the potential surface charges of adsorbents. If the pH of solution is above its pH_{pzc} the surface functional groups on adsorbents will be protonated by the excess H^+ ions; on the contrary if it is below its pH_{pzc} , the surface functional groups will be deprotonated by the OH^- ions presence in the solution (Putra E. K. et al., 2009). If the pH value of the solution is lower than the pH_{pzc} adsorbent surface cannot interact with acidic groups (positively charged), because there is a competition from H^+ ions for surface complexation sites of adsorbent and the surface have net positive charged (Kubilay et al., 2007). Similarly if the pH value of the solution is higher than pH_{pzc} the adsorbent surface cannot interact with basic groups (negatively charged) because of the repulsion between the same charged groups.

Determination of the point of zero charge experiments was carried out in the NaCl solution. 25 mL of 0.01 M NaCl solution was put into several Erlenmayer flasks. The pH value of the NaCl solution within each flask was adjusted by 0.1 M HCl and 0.1 M NaOH solution in the range of between pH=2 and pH=9. Then, 0.5 g of CS/SEP biocomposites was added to each Erlenmayer flasks. The final pH value of the solution was measured after 48 h. The pH_{pzc} is defined as the point where the curve pH_{final} vs $pH_{initial}$ crosses the line $pH_{final} = pH_{initial}$.

Figure 3.6, 3.7 and 3.8 indicates that the point of zero charge of the CS/SEP1, CS/SEP2 and CS/SEP5 respectively. Results have been shown in Table 3.5.

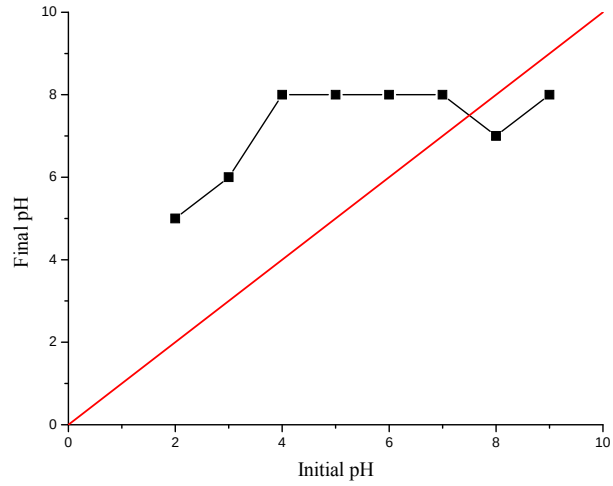


Figure 3.6 Point of zero charge of CS/SEP1 biocomposites, m=0.5g, V=25mL.

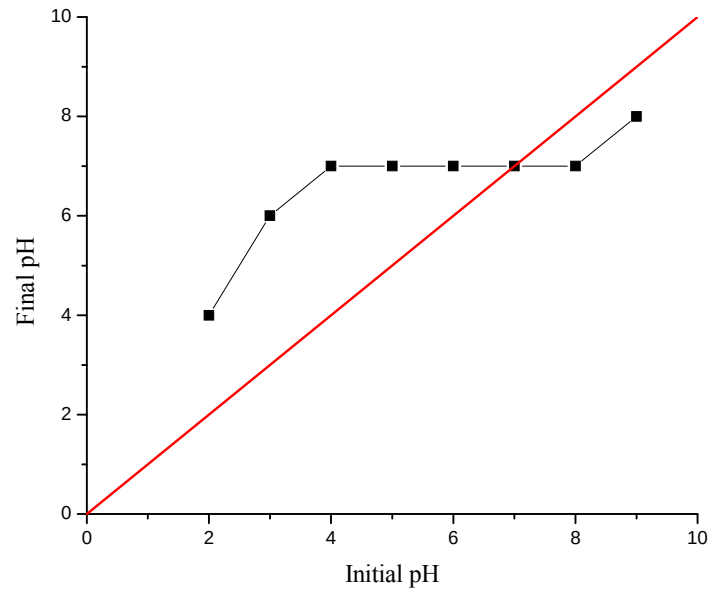


Figure 3.7 Point of zero charge of CS/SEP2 biocomposites, m=0.5g, V=25mL.

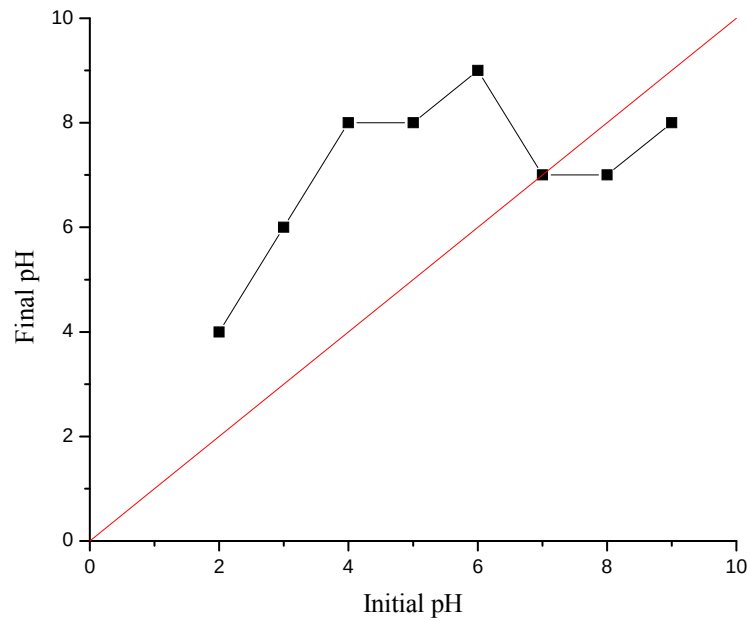


Figure 3.8 Point of zero charge of CS/SEP5 biocomposites, $m=0.5\text{g}$, $V=25\text{mL}$.

Table 3.5. point of zero charge of the CS/SEP biocomposites.

Sample	Point of Zero Charge pH
CS/SEP1	7.5
CS/SEP2	7
CS/SEP5	7

Point of the zero charge of biocomposites explain that the surface of the biocomposites have not been included any positive or negative charge. The surface acidity or basicity gives the surface charge to any structure. The results of the surface functional groups, and point of zero charge analysis have been supported each other.

CHAPTER FOUR

DRUG RELEASE FROM CS/SEP BIOCOMPOSITES

4.1 Tetracycline

Robert Burns Woodward determined the structure of Oxytetracycline enabling Lloyd H. Conover to successfully produce tetracycline itself as a synthetic product (www.wikipedia.org, 08/2010).

The chemical name for tetracycline hydrochloride is 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl 1,11-dioxo-2-naphthacenecarboxamide mono-hydrochloride. Figure 4.1 and 4.2 shows the molecular structures of tetracycline. Tetracycline produced by a bacterium, *Streptomyces Rimosus* and broad-spectrum antibiotics, which interfere in the protein synthesis of bacteria. Drug molecule enters the bacteria cell and links the 30S unit of the ribosome, so blocking the aminoacyl transfer RNA to acceptor point of 50S sub-unit and preventing amino acid binding to peptide sequence (Kayaalp, 2008). Tetracyclines have bacteriostatic activity against a wide variety of pathogens that are responsible for many common and some exotic infections. They are particularly valuable in the treatment of typical pneumonia syndromes, chlamydial genital infections, rickettsial infection (Rocky Mountain spotted fever, typhus, Q fever), Lyme disease, and ehrlichiosis (Smilack, 1999).

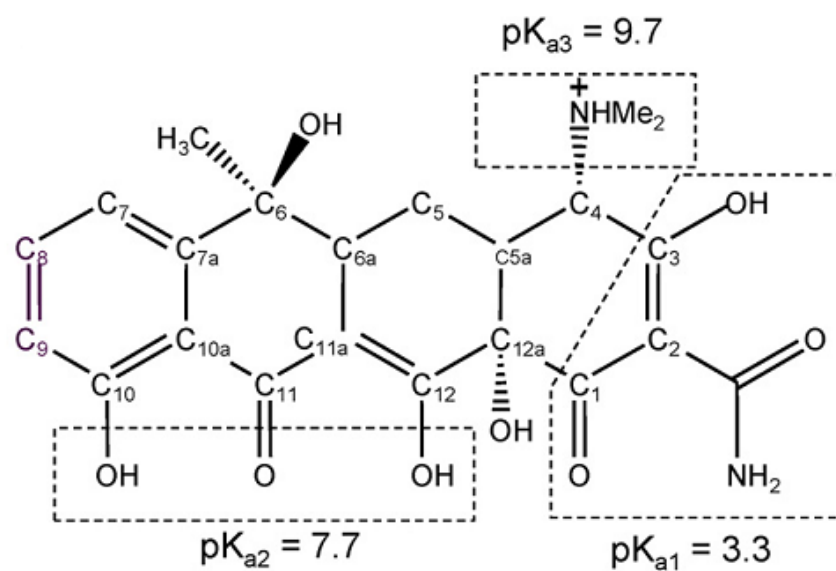


Figure 4.1 Molecular structure of TC on planar view..

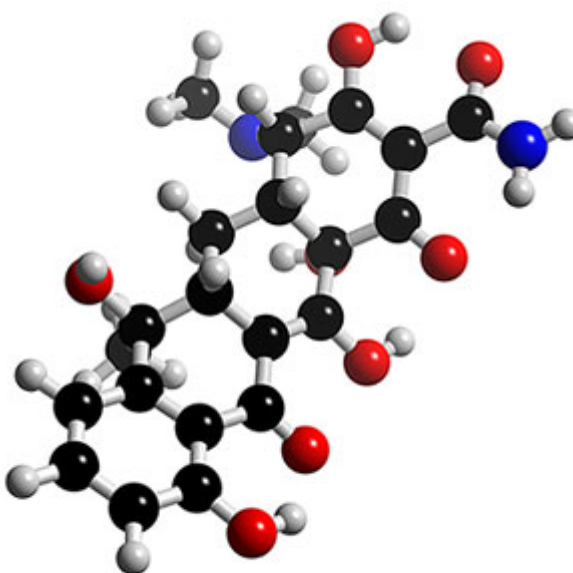


Figure 4.2 The molecular structure of Tetracycline in space

(<http://www.3dchem.com/molecules.asp?ID=145#>)

Tetracycline is known by its ability to bind with hard tissues in body and its administration is not recommended during the odontogenesis for this reason (can cause discoloration of teeth) (Medvecky et al, 2007). The tetracyclines are associated with dental staining and interference with bone growth (Smilack, 1999). It took the bloodstream by the liver, concentrated and sends to intestine through gall bladder. Tetracycline absorbed from intestine and passes the blood, and throws away by kidney. Tetracycline absorbs from jejunum %77 percent. Even after a single dose Tetracycline spread to bone tissue and can be seen in under ultraviolet light (Kayaalp, 2008).

Tetracycline could have different charges on different site depending on solution pHs (Figure 4.1). When solution pH is below 3.3, Tetracycline exists as a cation, $+00$, due to the protonation of dimethylammonium group. At pH between pH 3.3 and 7.7, Tetracycline exists as a zwitterion, $+ - 0$, due to the loss of a proton from the phenolic diketone moiety. At solution pH greater than 7.7, a monovalent anion, $+ - -$ or a divalent anion $0 - -$, from the loss of protons from the tricarbonyl system and phenolic diketone moiety will prevail (Changa et al., 2009). This situation allow drug to interact with different surfaces.

4.2 Synthesis of Tetracycline Loaded Chitosan Films

%2 Chitosan (CS) solution was prepared by dissolving 1 g of CS for one night, in acetic acid solution % 2 w/v, 50 mL. CS solution has filtered cheesecloth to remove insoluble part. 0.1 g of Tetracycline (TC) was put into CS solution and dissolved for one night. The mixture was covered with aluminum foil to keep from light. CS/TC solution was poured into Petri dish, and then put in to oven at 60°C for 2 h. Later put in to vacuum oven at 60°C for 48 h.

4.3 Synthesis of Tetracycline Loaded Chitosan Sepiolite Films

% 2 Chitosan (CS) solution was prepared by dissolving 1 g of CS for one night, in 50 mL, % 2 acetic acid solutions. CS solution has filtered cheese cloth to remove insoluble part. 0.1 g of Tetracycline (TC) has put into CS solution and dissolved for one night. The mixture covered with aluminum foil to keep from light. Different amounts of Sepiolite (1%, 2%, 5% SEP denoted as %X) swelled by 50 mL water for 2 h. SEP mixture has pour into CS/TC solution. Mixture has stirred at room temperature, for 6 h. Final mixture has poured into Petri dish to form a film, and then put in oven for 2 h., later dried in vacuum oven for 48 h. The final film was named as CS/XSEP/TC because of %X SEP content.

4.4 Release Study from TC Loaded CS/SEP Films

The release study was done in aqueous medium. Buffer solutions were prepared to examine the release behavior in different pH values. TC release was observed in saliva (pH=6.5), and intestine (pH=7.4) pH values in PBS buffer solutions. Release percentage was calculated from the calibration curves of TC as shown in Figure 4.3 and Figure 4.4 below.

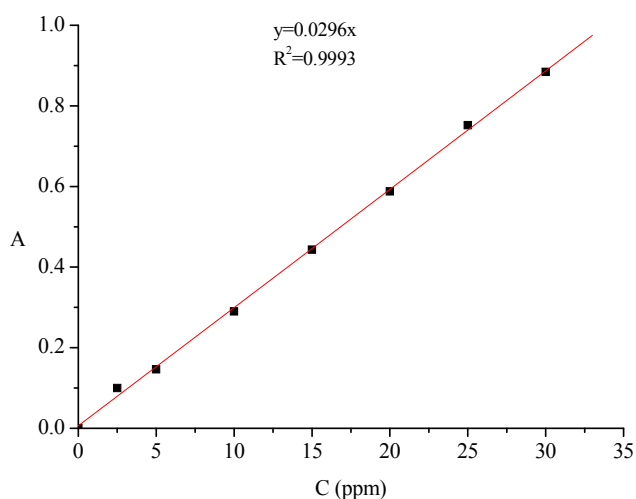


Figure 4.3 Calibration curve of TC at pH=6

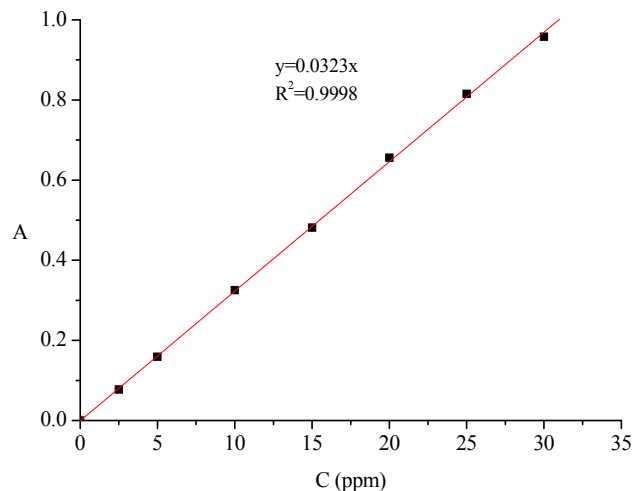


Figure 4.4 Calibration curve of TC at pH=7.4

0.1 g of adsorbent has cut with scissors and put into Erlenmeyer flask. The experimental study was carried out in buffer solution at pH=6.5 to simulate saliva and pH=7.5 to simulate intestine. The buffer solution at pH=6.5 was prepared by dissolving 1.1348g of KH_2PO_4 and 0.5233g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 250 mL of water. The buffer solution at pH=7.5 was prepared by dissolving 1.1600g of KH_2PO_4 and 1.1866g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 100 mL of water. 10 mL of buffer solution has put into Erlenmeyer flask then shake at 150 rpm at 37.5°C by GFL model thermostatic shaker. Buffer solution was refreshed in every ten minutes. The results were shown in Figure 4.5 and Figure 4.6 as below.

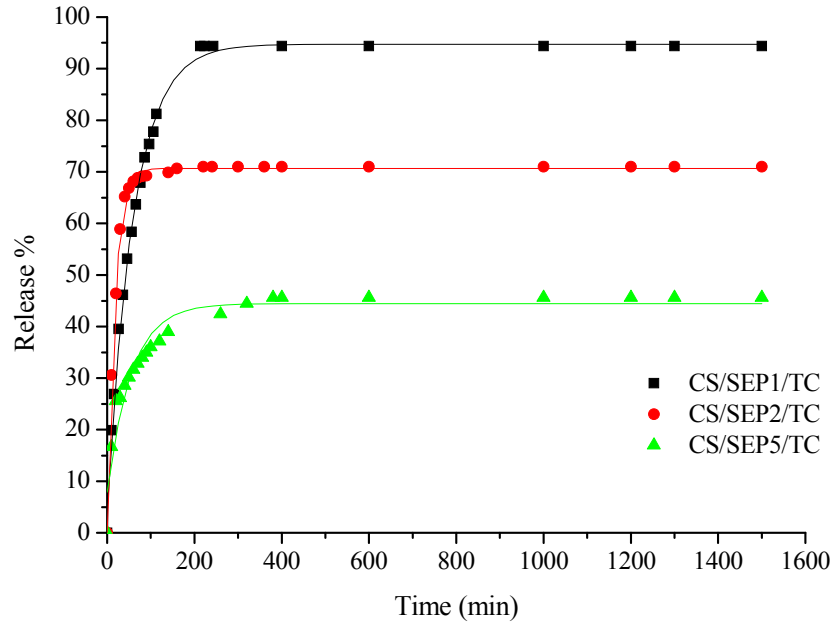


Figure 4.5 TC release from CS/SEP1/TC, CS/SEP2/TC and CS/SEP5/TC biocomposites at 37.5°C and pH=6.5

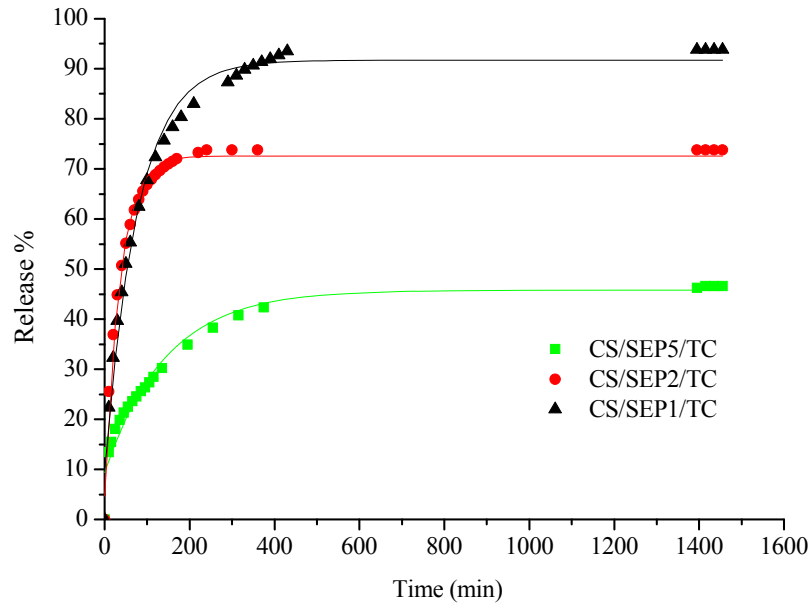


Figure 4.6. TC release from CS/SEP1/TC, CS/SEP2/TC and CS/SEP5/TC biocomposites at 37.5°C and pH=7.4.

Table 4.1 Release percentage of the CS/SEP/TC biocomposites at 37.5°C, pH= 6.5 and 7.4

Biocomposites	% Release	
	pH=6.5	pH=7.4
CS/SEP1/TC	94.39	93.53
CS/SEP2/TC	70.97	73.83
CS/SEP5/TC	45.61	46.58

CS/TC hydrogel which does not contain Sepiolite, swelled considerably and broke into pieces by itself so; have not been studied in this release medium.

4.5 Kinetic Study of the TC Release from CS/SEP/TC Biocomposites

Sustained or controlled drug delivery occurs while embedded within a polymer that may be natural or semi-synthetic or synthetic in nature. The polymer is judiciously combined with the drug or other active ingredients in such a way that the active agent is released from the material in a predetermined fashion and released the drug at constant rate for desired time period.

Controlled release should follow three steps. First step is the penetration of the dissolution medium in the polymer (hydration). Second step is the swelling with concomitant or subsequent dissolution or erosion of the matrix and third step is the transport of the dissolved drug, either through the hydrated matrix or from the parts of the eroded tablet, to the surrounding dissolution medium (Shoaib et al, 2006). The release of drug from the matrix depends on the nature of polymer. Hydrophilic polymers that become hydrated, swollen and facilitates to diffuse the drug (Biswas et al, 2008).

To analyze the in vitro release data various kinetic models were used to describe the release kinetics. The percent cumulative release (%R) of TC from CS/SEP/TC biocomposites was calculated by;

$$R\% = (C_t / C_0) \times 100 \quad (\text{Eq. 4.1})$$

Where C_t is the amount of drug released at time t and C_0 is the amount of drug in dry biocomposites. To investigate the parameters of release kinetics, Equation [b] was used and the plots of t/C_t versus t are presented in Figure 4.7.

$$t/C_t = \alpha + \beta t \quad (\text{Eq. 4.2})$$

Here, C_t is the amount of drug released at time t , β is the inverse of the maximum amount of released drug.

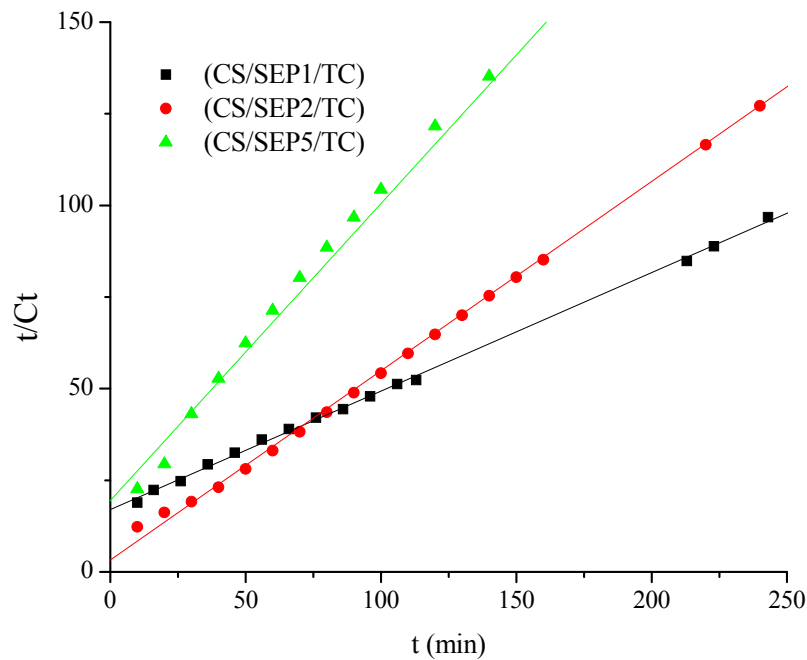


Figure 4.7 Release kinetic curves of TC from CS/SEP1/TC, CS/SEP2/TC, CS/SEP5/TC at pH=6.5

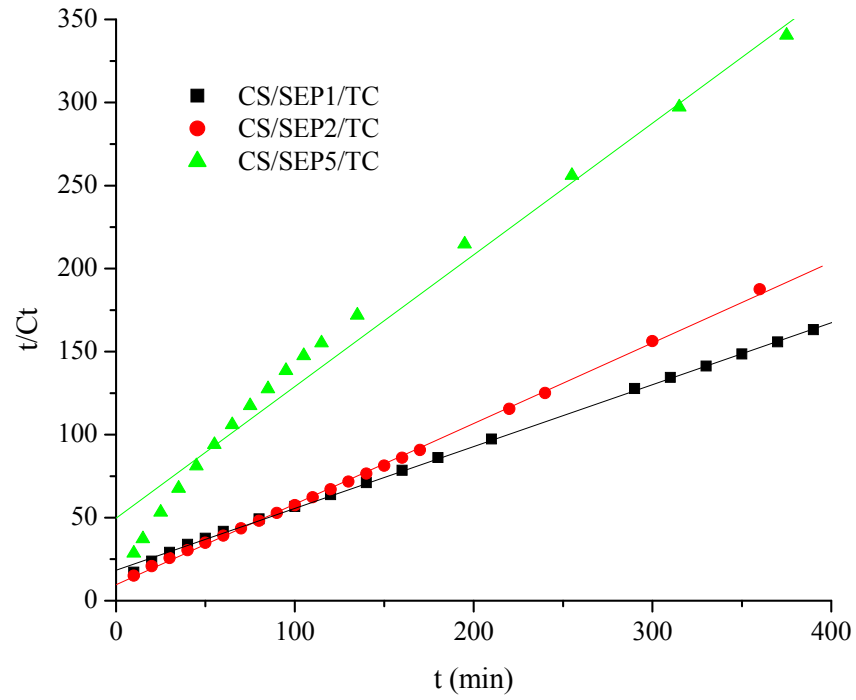


Figure 4.8 Release kinetics of TC from CS/SEP1/TC, CS/SEP2/TC, CS/SEP5/TC biocomposites at pH=7.4

In this study release kinetic of the biocomposites has been calculated from the equations; where C is the degree of release at time t , is the inverse of the maximum release as following;

$$\beta = 1/C_{\max} \quad (\text{Eq. 4.3})$$

is the inverse of the maximum amount of released drug.

$$\alpha = 1/C_{\max}^2 \cdot k_{\text{rel}} \quad (\text{Eq. 4.4})$$

$$\alpha = 1/r_0 \quad (\text{Eq. 4.5})$$

r_0 is the initial release rate, α is the inverse of the initial release rate (r_0), and k_{rel} is the constant of the kinetic of release. The relation represents second-order kinetics.

The kinetic parameters calculated from the lines in Figure 4.7 and 4.8 are presented in Table 4.2.

Figure 4.7 show the linear regression of the release curves at pH=6.5 and Figure 4.8 show the linear regression of the release curves at pH=7.4 obtained by means of equation [b]. Maximum release value, C_{max} are calculated from the slope, the value of initial release rate, r_0 and release rate constant, k_{rel} values are calculated from the intersection of the lines.

Because chitosan drug solution to be around pH=5 is connected to the -NH group. This increases the interaction between the clay and the drug release is reduced. It showed that increase in the clay content of the composite resulted decrease in the release of TC. This result is due to the interaction of the silanol groups (-SiOH) of the clay and the -OH groups of the drug by the formation of the hydrogen bonding. Also, the addition of clay to chitosan builds a strong cross-linking structure because of the negatively charged clay and positively charged NH_3^+ groups of chitosan. This influences the swelling behavior of the composite and consequently influences diffusion of the drug through the bulk entity.

The initial burst release was attributed to diffusion of the drug due to rapid swelling and was also partially related to drug adsorbed on the surface. However, the release rates were affected by pH and the weight ratio of chitosan to clay. The drug release behavior is influenced by pH and the chitosan/clay ratio.

Table 4.2 Kinetic parameters for CS/SEP/TC biocomposites at pH 6.5 and 7.4 at 37°C.

pH 6.5				
	R (%)	C_{max} mg.L⁻¹	k_{rel}10⁻³ s⁻ⁿ	r₀10⁻³
CS/SEP1/TC	93.39	3.09	6.14	58.9
CS/SEP2/TC	70.98	1.94	83.3	312.2
CS/SEP5/TC	45.61	1.23	33.7	51.4
pH 7.4				
	R (%)	C_{max} mg.L⁻¹	k_{rel}10⁻³ s⁻ⁿ	r₀10⁻³
CS/SEP1/TC	93.53	2.68	7.59	54.66
CS/SEP2/TC	73.83	2.06	24.61	104.35
CS/SEP5/TC	46.58	1.26	12.67	20.15

4.6 Kinetic Study of the TC Diffusion from CS/SEP/TC Biocomposites

4.6.1. Case I or simple Fickian diffusion

Case I or Fickian diffusion occurs when the rate of diffusion is much less than that of relaxation. When the drug is loaded into the hydrogels by equilibrium swelling in the drug solution, drug release from the swollen gel follows Fick's law. Drug release from Case I systems is dependent on $t^{1/2}$ (Ritger and Peppas, 1987a,b).

4.6.2. Case II diffusion

Case II diffusion (relaxation – controlled transport) occurs when diffusion is very rapid compared with the relaxation process. In Case II systems, diffusion of water through the previously swollen shell is rapid compared with the swelling – induced relaxation of polymer chains. Thus, the rate of water penetration is controlled by the

polymer relaxation. For film specimens, the swelling zone moves into the membrane at a uniform rate and the weight gain increases indirect proportion to time. If the hydrogels contain a water-soluble drug, the drug is essentially immobile in a glassy polymer, but being a diffuse out as the polymer swells by absorbing water (Alfreyet al., 1966; Peppas and Korsmeyer, 1987).

4.6.3. Non-Fickian or anomalous diffusion

Non-Fickian or anomalous diffusion occurs when the diffusion and relaxation rates are comparable. Drug release depends on two simultaneous rate processes, water migration into the device and drug diffusion through continuously swelling hydrogels is highly complicated (Ritger and Peppas, 1987 a,b). The generalized empirical equations have been widely used to describe both the water uptake through the swellable glassy polymers and the drug release from these devices.

$$\ln C_0 / C_t = \ln k + n \ln t \quad (\text{Eq.4.6})$$

where k is the diffusion constant and n is the diffusion coefficient.

C_0/C_t is the fractional release of drug in time t, 'k' is the constant characteristic of the drug-polymer system, and 'n' is the diffusion exponent characteristic of the release mechanism. When the plot is drawn between $\ln C_0 / C_t$ and $\ln t$, the slope of the plot gives the value of 'n' and intercept will tell about 'k'. This equation applies until 60% of the total amount of drug is released. It predicts that the fractional release of drug is exponentially related to the release time and it adequately describes the release of drug from slabs, spheres, cylinders and discs from both swellable and non-swellable matrices (Figs.8 and 9). Normal Fickian diffusion is characterized by 'n' = 0.5, while Case II diffusion by 'n' = 1.0. A value of 'n' between 0.5 and 1.0 indicates a mixture of Fickian and Case II diffusion, which is usually called non-Fickian or anomalous diffusion

(Alfrey et al., 1966). The values of the 'n' and 'k' have been evaluated from the release studies and the results are presented in Table 4.3.

Kinetic study of the drug release showed that the results were not fit to any of the Fickian Case's. pH of the release media and the clay content of the biocomposites caused to deviation from the Fickian Case's.

Table 4.3 Diffusion parameters for CS/SEP/TC biocomposites at 37°C, pH=6.5 and 7.4

Biocomposites	<u>pH=6.5</u>		<u>pH=7.4</u>	
	k(%/min)	n	k(%/min)	n
CS/SEP1/TC	21.32	0.64	14.23	0.51
CS/SEP2/TC	12.93	0.60	11.34	0.47
CS/SEP5/TC	6.07	0.15	11.88	0.24

The n value of the diffusion parameters indicates Non-Fickian diffusion has been seen at pH=6.5 for CS/SEP1/TC and CS/SEP2/TC biocomposites. When clay content increased, n value which calculated from the slope of the plot decreased. These results explain that, polymeric matrix expanded in aqueous medium and drug molecules diffuse easily the outer media. When clay content is increase in biocomposites, the swelling properties of polymeric matrix decreased, so it caused to drift from the Fickian's law.

At higher pH value (pH=7.4) Case I diffusion have been seen. Polymer relaxation rate greater than diffusion rate it implies slow release rate from the biocomposites. Release percentages at pH= 6.5 and 7.4 have supported the current case. While equilibrium time for each biocomposites at pH 6.5 approximately 200 min, at pH=7.4 it

can be reached 400 min. Diffusion kinetics of the biocomposites have been affected by clay content of the biocomposites and release media.

CHAPTER FIVE

CONCLUSIONS

The aim of the study was to prepare Chitosan-Sepiolite biocomposites, their characterization and usage as a drug support material for the controlled release.

As can be seen in Figure 3.1, and from the FTIR data of the samples in Table 3.1, amide I and amide II bands (at 1637 cm^{-1} and 1547 cm^{-1}) were decreased by the increasing of the clay content. This result indicated that Chitosan interacted with Sepiolite. On the other hand, Chitosan cannot diffuse the tunnels of the clay. The basal peak of Sepiolite related d_{001} reflection, $12.04\text{\AA}$ was conserved in each sample and shifted to $12.88\text{\AA}$ for CS/SEP1. This result was confirmed by XRD data.

In the evaluation of TG/DTG data, it was observed that the second stage losses in the temperature range of $200\text{--}400^{\circ}\text{C}$, corresponding to the degradation and deacetylation of chitosan temperatures increased at about 50°C for the composites and left about 60-80 wt% solid residue. This means that thermal stabilities of the samples were increased compared with pure Chitosan. It was also observed that sepiolite particles improved the thermal properties of Chitosan.

SEM micrographs showed that Sepiolite mineral have been covered by the polymer matrix. The fibrous like morphology of the clay cannot allow the polymer to penetrate the inside of the tunnels, because of the larger molecular structure of the Chitosan. Coating of the clay mineral by the Chitosan caused the agglomeration in biocomposite films. Some agglomerates of the clay content can also be seen in Figure 3.5 (A and C).

Surface functional groups on CS/SEP samples were determined by Boehm's titration. Results have been showed that any acidic and basic group have not been found on the surface of the biocomposites. Point of the zero charge of biocomposites explain that the surface of the biocomposites have not been included any positive or negative charge.

The surface acidity or basicity gives the surface charge to any structure which is very important for the drug release studies.

For the usage of the prepared biocomposites, release studies of Tetracycline were carried out. Release of the drug molecules from the biocomposites depended on the clay content of each structure. The release percentage of the CS/SEP1/TC, CS/SEP2/TC and CS/SEP5/TC at pH=6.5 was found similar for each biocomposites at pH=7.4. Drug release was not pH-dependent. The percent cumulative release of TC from biocomposites was not higher in pH=6.5 or pH=7.4. The diffusion of the drug molecules out of the biocomposite is based on the amount of the clay particle. The release of drug is decreased when the quantity of SEP increased. When the pH value of the release media decreased, the drug diffusion rate to the aqueous medium increased in some respect. According to this result, drug diffusion rate can be determined for intended use of drug.

On the other hand, in diffusion kinetics studies of TC from the biocomposites, at higher pH value (pH=7.4) Case I diffusion have been seen. Polymer relaxation rate greater than diffusion rate it implies slow release rate from the biocomposites. Release percentages at pH= 6.5 and 7.4 have supported the current case. While equilibrium time for each biocomposites at pH=6.5 approximately 200 min, at pH=7.4 it can be reached 400 min. Diffusion kinetics of the biocomposites have been affected by clay content of the biocomposites and release media.

As a final sentence, sepiolite mineral can be used as a support material for the preparation of the biocomposites. Further studies should be done for the drug release.

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