KADIR HAS UNIVERSITY
SCHOOL OF GRADUATE STUDIES
PROGRAM OF MATERIALS SCIENCE AND NANOTECHNOLOGY

THE SYNTHESIS AND CHARACTERIZATION OF 3D-PRINTED CHITOSAN BASED COMPOSITES FOR BIOMEDICAL APPLICATIONS

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THE SYNTHESIS AND CHARACTERIZATION OF 3D-PRINTED CHITOSAN BASED COMPOSITES FOR BIOMEDICAL APPLICATIONS

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the School of Graduate Studies of Kadir Has University
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APPROVAL

This thesis/project titled **THE SYNTHESIS AND CHARACTERIZATION OF 3D-PRINTED CHITOSAN BASED COMPOSITES FOR BIOMEDICAL APPLICATIONS** submitted by OUBADAH ALAYOUBI, in partial fulfillment of the requirements for the degree of Master of Science in Program of Materials Science and Nanotechnology is approved by

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- this Master of Science Thesis does not contain any material from any research submitted or accepted to obtain a degree or diploma at another educational institution;
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In addition, I acknowledge that any claim of irregularity that may arise in relation to this work will result in a disciplinary action in accordance with the university legislation.

OUBADAH ALAYOUBI

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Date (30/11/23)

To my dearest parents whose love and support inspired me to pursue my dreams...

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THE SYNTHESIS AND CHARACTERIZATION OF 3D-PRINTED CHITOSAN BASED COMPOSITES FOR BIOMEDICAL APPLICATIONS

ABSTRACT

The present study is aimed to develop two-dimensional (2D) tungsten disulfide (WS2)-doped Chitosan biocompatible composites containing poly (vinyl alcohol) (PVA), alginic acid and poly (lactic acid) (PLA), which can be an alternative to existing three-dimensional structures for use in biomedical applications. Chitosan is a linear polysaccharide and deacetylated derivative of Chitin. The unique structural and chemical properties of Chitosan make it a widely used component in various biomedical industries that benefit from its versatile biological properties. WS_2 is a biocompatible material with antimicrobial effect and enhancing the mechanical properties of the final product. Three types of composites were produced: PLA/Chitosan, Alginic acid/ Chitosan, and PVA/Chitosan. A variety of WS_2 amounts (0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg) was added to the composite blends. The effect of the WS_2 amount on the optical and swelling studies of the composites by comparing the results of running transmittance and temperature tests was investigated. The transmittance experiments were conducted using UV-vis spectrophotometer to detect patterns of gelation and calculate the time each type of composite takes to be gel. The results showed that during the gelation process the temperature decreases as well as the transmittance ability of the composites. Moreover, it was observed that the addition of WS_2 improves elasticity of the polymer composite and affects the swelling property. As a result, CS/PVA/WS_2, CS/Alginic Acid/WS_2, and CS/PLA/WS_2 composites could have superior mechanical characteristics and biocompatibility, making them highly applicable in the tissue engineering field.

Keywords: Chitosan, PLA, PVA, Alginic acid, Tungsten Disulfide.

BIYOMEDIKAL UYGULAMALARA YÖNELIK 3D BASKILI KITOSAN ESASLI KOMPOZITLERIN SENTEZI VE KARAKTERIZASYONU

ÖZET

Bu çalışmanın amacı, biyomedikal uygulamalarda kullanılmak üzere mevcut üç boyutlu yapılara alternatif olabilecek poli (vinil alkol) (PVA), aljinik asit ve poli (laktik asit) (PLA) içerikli İki boyutlu (2D) tungsten disülfür (WS_2) ile katkılandırılmış Kitosan biyouyumlu kompozitlerin oluşturulmasıdır. Kitosan, Chitin'in lineer bir polisakkarit ve deasetillenmiş türevidir. Kitosanın benzersiz yapısal ve kimyasal özellikleri, onu çok yönlü biyolojik özelliklerinden yararlanan çeşitli biyomedikal endüstrilerde yaygın olarak kullanılan bir bileşen haline getirmektedir. WS2, antimikrobiyal etkiye sahip ve nihai ürünün mekanik özelliklerini artıran biyouyumlu bir malzemedir. Üç tip kompozit üretilmiştir: PLA/Kitosan, Aljinik asit/Kitosan ve PVA/Kitosan. Kompozit karışımlara çeşitli WS_2 miktarları (0,5 mg, 1,0 mg, 1,5 mg, 2,0 mg) eklenmiştir. Ölçülen geçirgenlik ve sıcaklık testlerinin sonuçları karşılaştırılarak WS_2 konsantrasyonunun kompozitlerin optik geçirgenlik ve şişme çalışmalarına etkisi araştırıldı. Geçirgenlik deneyleri, jelleşme modellerini tespit etmek ve her bir kompozit tipinin jel haline gelmesi için gereken süreyi hesaplamak için UV-vis spektrofotometresi kullanılarak gerçekleştirildi. Sonuçlar, jelleşme işlemi sırasında kompozitlerin geçirgenlik yeteneğinin yanı sıra sıcaklığın da düştüğünü gösterdi. Ayrıca, WS_2'nin eklenmesi polimer kompozitin elastikiyetini arttırdığı ve şişme özelliğini etkilediği gözlendi. Sonuç olarak CS/PVA/WS_2, CS/Aljinik Asit/WS_2 ve CS/PLA/WS_2 kompozitleri üstün mekanik özelliklere ve biyouyumluluğa sahip olabilir ve bu da onları doku mühendisliği alanında oldukça uygulanabilir hale getirebilir.

Anahtar Sözcükler: Kitosan, PLA, PVA, Aljinik asit, Tungsten Disülfür.

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LIST OF ACRONMYMS AND ABBREVIATIONS

PHA: Polyhydroxyalkanoate

PLA: Poly (lactic acid)

PVA: Poly (Vinyl alcohol)

WS_2: Tungsten disulfide

1. INTRODUCTION

The overconsumption and accumulation of plastic products is one of the most pressing contemporary issues. Globally, there has been around 370 million Mt of plastics produced in 2019with a very small percentage (9%) of it recycled. Most of the produced plastic (79% in 2015) is accumulating in landfills. The microplastic resulting from the degradation of plastic littered in the sea jeopardizes marine life as well as human life through the consumption of the affected animals (Alberto Di Bartolo, 2021).

These dreadful statistics shed light on the immensity of the issue and its far-reaching influence on the environment and our lives. Bioplastics may serve as a viable remedy, this special type of plastics are either derived from natural resources and/or biodegradable (Alberto Di Bartolo, 2021). Thus, they eschew much of the harm caused by petroleum-based plastics. Depending on their source and biodegradability, we can classify bioplastics into three maincategories:

a. Bio-based and biodegradable bioplastics: e.g. Polylactic acid (PLA), Polyhydroxyalkanoates (PHAs).

b. Bio-based but not biodegradable bioplastics: e.g. Bio-polyethylene (Bio-PE), Bio-polyamides (Bio-PP).

c. Fossil-based but biodegradable: e.g. Polycaprolactone (PCL), Polyvinyl alcohol(PVA) (Alberto Di Bartolo, 2021).

Among these, polylactic acid (PLA) and polyvinyl alcohol (PVA) are notable as important participants in the field of biodegradable polymers (Alberto Di Bartolo, 2021). PLA, which is made from renewable resources like sugarcane or corn starch, has remarkable mechanical strength and transparency, making it a competitive alternative to petroleum-based plastics in the packaging and textile industries (Garlotta, 2001). PVA is also used in industries like adhesives, films, and packaging materials. It is recognized for being biodegradable in specific situations (Gaurav Saxena, 2020).

On the other hand, for the biomedical applications, usage of these bioplastic is crucial for leaving safe environment to new generations. One of the most preferable areas for using bioplastics is tissue engineering. Within the field of tissue engineering, chitosan

composites and usage of bioplastics have attracted a lot of attention due to their critical function in the creation of three-dimensional scaffolds. These scaffolds act as supporting frameworks that imitate the extracellular matrix, creating an environment that is favorable for cell proliferation and tissue regeneration. With its exceptional biocompatibility, biodegradability, and antimicrobial qualities, chitosan—a derivative of chitin that is widely distributed in crustacean shells—becomes a desirable option for tissue engineering applications (Ali Sadeghianmaryan, 2022) (Inmaculada Aranaz, 2021). Chitosan forms composite materials with improved mechanical strength, flexibility, and biodegradability when combined with different bioplastics, such as polylactic acid (PLA) (Sunpreet Singh, 2020) (Lihua Li, 2003) or polyhydroxyalkanoates (PHA). Moreover, by adding two-dimensional (2D) materials to the structure of these biopolymers, researchers are looking into ways to improve their characteristics. Graphene is an example of 2D materials that can be integrated with bioplastics to improve their mechanical strength and flexibility (Abdul Mukheem, 2022). After the graphene, many 2D materials such as metal dichalcogenides have been investigated since they exhibit tunable properties like bandgap, electrical conductivity, and optical behavior, offering flexibility for various applications including biomedical applications. This combination of 2D materials and bioplastics offers a breakthrough in the development of high-performance, sustainable materials that have the potential to completely transform a number of industries while having a major positive impact on the environment (Abdul Mukheem, 2022).

These composites made of chitosan provide an adaptable framework for building three-dimensional scaffolds that are customized for different tissue types. These scaffolds can be precisely engineered with intricate pore structures through techniques such as electrospinning or 3D printing, which enables optimal cell adhesion, proliferation, and nutrient exchange (Ali Sadeghianmaryan, 2022). Furthermore, these composites' regenerative qualities are further enhanced by the addition of bioactive agents, growth factors, or nanoparticles, which promote tissue regeneration and functional recovery. A new era in regenerative medicine is being ushered in by the special synergy between chitosan and bioplastics in the scaffold fabrication process for tissue engineering.

2. LITERATURE REVIEW

2.1. Chitosan

Is a linear polysaccharide and deacetylated derivative of Chitin. It's composed of (β1→4) linked residues of N-acetyl-2 amino-2-deoxy-D-glucose (glucosamine, GlcN, i.e., D monomers) and 2-amino-2-deoxy-D-glucose (N-acetyl-glucosamine, GlcNAc, i.e. A monomers) residues (Fig.1) (Inmaculada Aranaz, 2021). Chitosan is widely used in a variety of medical and industrial applications. Table.1 shows some properties of Chitosan.

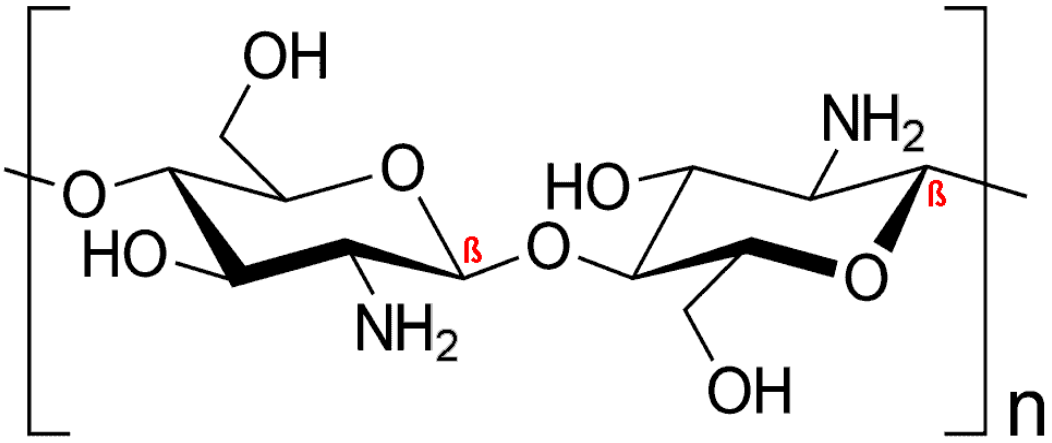

Fig.1: Chitosan chemical structure (Wikipedia, the free encyclopedia, 2023).

Table.1: Properties of Chitosan (National Center for Biotechnology Information, 2023)

Molecular Formula	$C_{56}H_{103}N_9O_{39}$
Molecular Weight	1526.5
Hydrogen Bond Donor	29
Hydrogen Bond Acceptor	47
Rotatable Bonds	27

Although Chitin is highly common in nature, Chitosan is less abundant naturally and can mainly be extracted from the fungi (*Mucoraceae*). Commercially, Chitosan is synthesized by the deacetylation of Chitin extracted from Crustaceans, where some *N*-acetylate glucosamine residues are transformed into glucosamine unit (Inmaculada Aranaz, 2021).

The degree of deacetylation of Chitosan and the pH of the media dictates its charge density. While, its solubility is related to various factors, like:

- Molecular weight.
- The degree of deacetylation.
- Temperature.
- Degree of Crystallinity.

The protonation of 50% of the NH_2 groups causes the solubility of Chitosan in acidic solutions (pKa = 6.5).

The viscosity of Chitosan is proportional to its molecular weight, and it can be determined by the Mark-Houwink-Sakurada equation (Eq 2.1):

$$\eta = KM_v^{\alpha} \tag{2.1}$$

Where K, α are experimental constants related to the solvent composition, pH, and its ionic strength.

In Chitosan, the functional groups of its structure are:

- Primary $–NH_2$ groups (C_2).
- Primary and secondary –OH groups.
- Glycosidic bonds (-D-D-, -A-A-, -D-A-, -A-D- bonds).
- The acetamide group.

Chemical modification of these functional groups allows researchers to synthesize a myriad of Chitosan derivatives with improved solubility and biodegradation for various applications. For example, Chitosan derivative 6-O-sulphated Chitosan is an efficient neural differentiation promoter (Inmaculada Aranaz, 2021).

The degradation of Chitosan targets the glycosidic bonds of its structure. There are multiple methods for the degradation of Chitosan:

a. Ultrasonic degradation: For moderate degradation of Chitosan where the degree of deacetylation and polydispersity is preserved.
b. Oxidative-reductive degradation: This method uses H_2O_2 for the random degradation of Chitosan to generate monomers and Chitooligosacchrides.
c. Nitrous Acid degradation: HNO_2 targets only glycosidic bonds that follow D-monomers where it specifically interacts with the primary amine of glucosamine.

d. Acid Hydrolysis: The degradation of Chitosan using HCl hydrolyzes *O*-glycosidic bonds as well as *N*-acetyl bonds at a lower rate.
e. Non-Specific enzymatic degradation: This type of degradation encompasses a wide range of enzymes families which are efficient in the degradation of Chitosan, like: Protease, lipase, cellulose, hemicelluloses families.
f. Specific enzymatic degradation: There are two main categories of Chitosan degradation enzymes:
    - ❖ Chitosanases Enzymes: glycosyl hydrolases enzymes which target β-1,4-glycosidic bonds of partially acetylated Chitosan for endo hydrolysis.
    - ❖ Chitinases: Are active in the degradation of A-A or A-D glycosidic bonds only but don't show any efficiency in the degradation of D-D glycosidic bonds (Inmaculada Aranaz, 2021).

Chitosan is a nontoxic to mammals and biocompatible substance that can be hydrolyzed by enzymes (e.g. lysozymes in mucus, tears, or saliva) into glucosamine (Mariana Adina Matica, 2019).

The unique structural and chemical properties of Chitosan make it a widely used component in various biomedical industries that benefit from its versatile biological properties, such as:

a. Antimicrobial Activity:

Chitosan, along with its derivatives and Chitooligosacchride, is an excellent antimicrobial agent against a wide variety of bacterial species. As the only polycation in nature (Inmaculada Aranaz, 2021), it sabotages the negatively charged bacterial cell wall. It can also target bacterial DNA and inhibit the activity of mRNA of protein synthesis.

Another antimicrobial approach that Chitosan executes is the chelation of metal ions (Ni^{+2}, Zn^{+2}, Co^{+2}, Fe^{+2}, Cu^{+2}) which are beneficial for synthesis of bacterial cell walls. Chitosan inhibits bacterial cell walls growth by chelating these bivalent metal ions when its pH value is higher than its pK_a value.

Including Chitosan in wound dressing products helps precipitate healing of the wound and halts its infection (Mariana Adina Matica, 2019).

b. Antifungal Activity:

Chitosan antifungal activity follows a similar mechanism to its antimicrobial activity as it disrupts the negatively charged phospholipids of the fungi cellular membrane thus

causing a leakage of interior cellular components. However, Chitosan can't apply this effect on Chitosan-resistant fungi (Mariana Adina Matica, 2019).

c. Antioxidant Activity:

Chitosan can act as a scavenger for free radicals with its amino and hydroxyl groups. Some Chitosan derivatives like Chitosan Sulphates or N-2 carboxethyl Chirosan have superior antioxidant efficiency (Inmaculada Aranaz, 2021).

d. Anti-Inflammatory Activity:

Chitosan with reduced molecular weight and Chitooligosacchrides show improved anti-inflammatory activity (Inmaculada Aranaz, 2021).

Still, as a pure material, Chitosan doesn't have the mechanical strength required for certain biomedical structures like scaffolds. This is why researchers use PLA to provide the necessary mechanical support (Sunpreet Singh, 2020).

The following section illustrates several examples of the biomedical application of the PLA/Chitosan composites.

2.2. Polylactic Acid (PLA)

Biodegradable aliphatic polyester with superior physical and mechanical properties that makesit a valuable resource in various industrial and medical applications. PLA is derived from the polymerization of lactide, the cyclic dimer of Lactic acid. Lactic acid is the simplest hydroxy acid and extracted from plant crops like corn or sugar cane. The poly (L-lactide) (PLLA) enantiomer is the most important type of PLA (Fig.1) (Garlotta, 2001) (Xuan Pang, 2010).

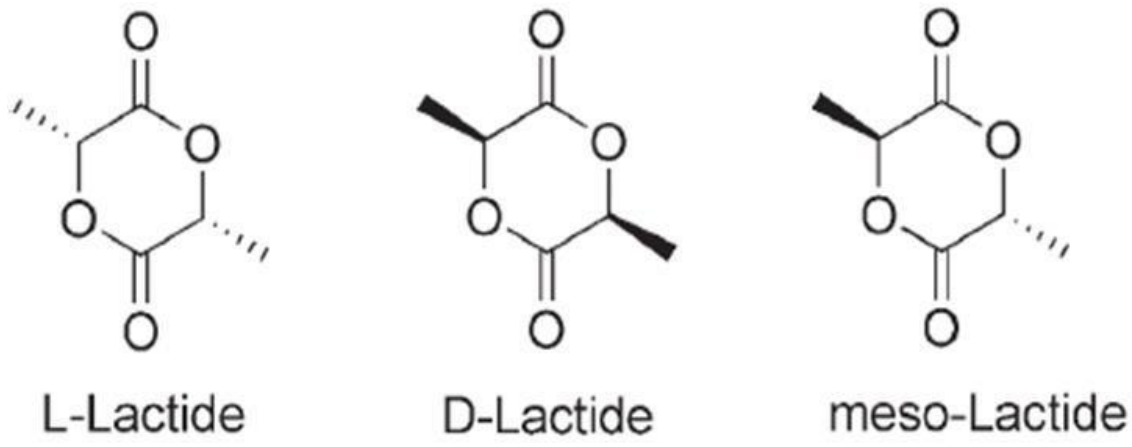

Fig.2: Stereoisomers of lactide (Xuan Pang, 2010).

As the above figure illustrates, lactide molecules have two chiral C atoms, therefore, the polymerization of lactide renders three stereoisomers (L-lactide, D-lactide, and meso-lactide). Lactic acid is extracted from a plant resource by microorganism-aided fermentation (Typically Lactobacilli strains) which convert carbohydrates into lactic acid (Alberto Di Bartolo, 2021).

The polymerization of PLA takes the following route (Fig.2):

1. Condensation Polymerization of Lactic acid:

This step produces fragile low molecular weight PLA which is unuseful for industrial applications. The assembly of higher molecular weight PLA requires the application of externalcoupling agents, including:

a. Esterification-promoting adjuvant: (Dicyclohexylcarbodiimide (DCC), carbonyldiimidazole, or bis (trichloromethyl) carbonate).
b. Chain-extending agents.

2. Ring opening Polymerization (ROP) of lactide:

This step is classified according to the mechanism to:

a. Cationic ROP
b. Anionic ROP
c. Coordination-insertion ROP

Due to the ease of controllability of the polymerization process, the ring-opening polymerization of lactide is preferred over direct polycondensation of lactic acid which is an inefficient process for high molecular weight PLA synthesis. This is because each step of the process produces a molecule of water, which requires additional steps to remove the water molecules.

Another method of polymerization is the one-step azeotropic condensation polymerization of lactic acid which obviates the use of external chain extenders or adjuvant in the synthesis of high molecular weight PLA (Xuan Pang, 2010).

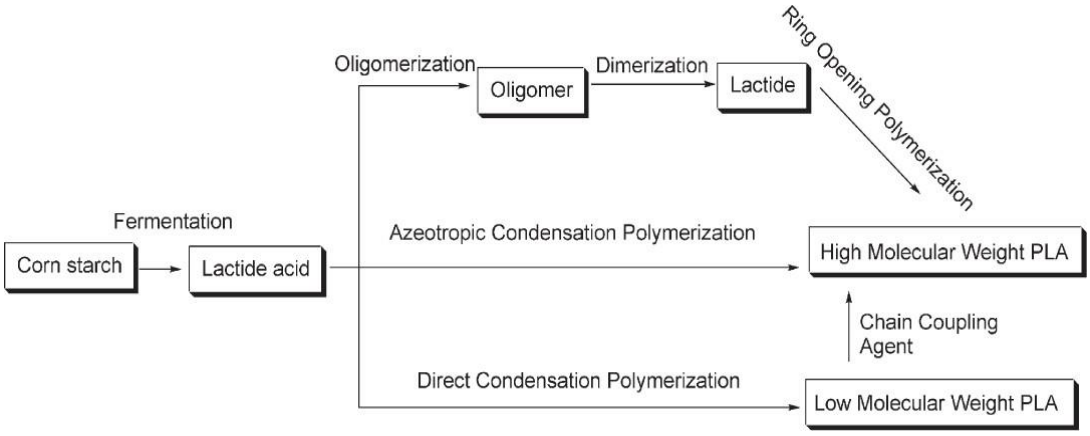

Fig.3: PLA synthesis methods
(Xuan Pang, 2010).

The hydrolysis of the ester bond of the PLA causes its degradation. This degradation process can be initiated without the application of enzymes and its rate is related to the size and shape of the object, the temperature of the hydrolysis, and the isomer ratio.

Compared to other synthetic polyester, PLA has a lower degradation rate due to the poor waterpermeability of semicrystalline PLA (Table.1) (Xuan Pang, 2010).

Temperatures above 200 C cause the thermal degradation of PLA. However, we can decrease the degradation temperature and increase its rate by the application of catalysts and oligomers (Garlotta, 2001). The composition of PLA degrades it into CO_2 and water and thus safely reintroduces it into nature (Xuan Pang, 2010).

The stereochemistry of the lactide monomers (L-lactide/ D-lactide) affects the stereochemical structure of the PLA (PLLA, PDLA respectively). Thus, we can tune the physical properties of the PLA by managing the polymerization of the optical monomers (Xuan Pang, 2010) (Garlotta, 2001). Table.2 shows some physical properties for various PLA types.

Table.2: Physical properties for various PLA types (Xuan Pang, 2010).

	Tg (C)	Tm (C)	Mol.mass/kDa	Density (g/cm^3)	Tensile Strength (GPa)	Elongation at break (%)	Degradation rate
PLLA/PDLA	58	170-190	100-300	1.25-1.29	0.12-2.3	4-7	50 % in 1-2 years
Stereocomplex PLA	65-72	220-230	-	-	0.88	30	-
PGA	40	225-230	-	1.50-1.69	0.08-1	30-40	100% in 2-3 months
PCL	-60	60	40-80	1.07-1.20	0.004-0.78	20-1000	50% in 4 years

PLA is a malleable polymer which can be modified to form molded objects, fibers, or films for various industrial applications. The methods of large-scale thermoplastic polymers processing,like extrusion, injection molding, blow molding, thermoforming, or fiber spinning, can be applied for the processing of PLA when it maintains thermal stability to avoid thermal degradation and the loss of its molecular weight and properties (Garlotta, 2001).

PLA has a modulus and tensile strength higher than other polyesters. Still, it doesn't exhibit the sufficient toughness necessary for various industries.

Researchers have developed various methods to enhance the mechanical toughness of the PLA.Including:

a. Stereocomplex formation between PLLA and PDLA: The solid interaction between L-lactyl unit sequence and D-lactyl unit sequence improves the mechanicalproperties and thermal resistance of the PLA and enhances its resistance to hydrolysis.

b. Copolymerization: This method is considered the most effective as it allows the tuning of the physical properties of the produced copolymers to make soft and elasticmaterials as well as stiff products. The addition of a polyester backbone (e.g.Poly (glycolide), poly(ε-caprolactone), etc..) to the PLA reduces its crystallinity and melting point.

c. Polymer blending: This method depends on the incorporation of materials like plasticizers, olefin polymer/ fibers, or inorganic materials, etc... to adjust the properties of PLA. The physical properties of the produced blend are dependent on the nature of the composing materials and the composition of the blend (Xuan Pang, 2010).

Products manufactured with PLA can be recycled by melting and reprocessing or by hydrolysis.It has been proven that PLA is safe for contact with food and possesses a good shelf-life; Therefore, it has been widely used for single-use packaging industries. One of the earliest examples of this usage is the Dannon and McDonald's PLA-made yogurt cups and cutlery.

PLA is a highly biocompatible polymer. The degradation of PLA produces lactic acid which is acomponent of the tricarboxylic acid cycle and excreted without any toxicity. These qualities of the PLA made it a favored polymer in various biomedical applications. To improve its biocompatibility and its interaction with the cell, PLA has been attached with biomaterials like biomimetic apatite, extracellular proteins (fibronectin, collagen, vitornectin, and entacin), and RGD peptides with covalent or non-covalent bonds. PLA is also widely used as a fiber in many industries like its usage in filament yarns and spun yarns for clothes industry (Xuan Pang, 2010).

However, as a pure material, PLA accumulates acidic products during degradation which restricts its use in medical industries. This issue can be controlled with the addition of Chitosan (Lihua Li, 2003).

2.3. Chitosan/PLA Composites

One of the complications related to the use of PLA in medical applications is its ability to cause inflammation. In vivo, PLA starts degrading slowly into low molecular weight acidic products that are expelled by metabolism. However, as the degradation proceeds, the degradation reaction rate accelerates which leads to the accumulation of acidic products. This phenomenon is linked to nonbacterial inflammations common after the transplant of PLA materials.

A common approach to circumvent this effect is the synthesis of PLA/Chitosan composites.

As a rare alkaline saccharide, its alkalescence can act as a neutralizing agent for PLA acidic products and can decelerate its degradation rate (Lihua Li, 2003).

PLA/Chitosan composite integrates the qualities of both of its components. PLA enhances the mechanical properties of the fragile Chitosan. On the other hand, Chitosan can fix the hydrophobicity of PLA which creates a robust and biocompatible composite that is suitable for cell adhesion and proliferation (Sunpreet Singh, 2020) (Lihua Li, 2003). As well as improving compressive strength proportionately with the weight fraction of Chitosan. However, both tensile strength and flexural strength are inversely proportional to the weight fraction of Chitosan as was shown by Singh et al. on the PLA/Chitosan scaffold manufactured with fused filament fabrication (FFF) 3D printing technique (Sunpreet Singh, 2020).

PLA/chitosan composite has a malleable structure that can be molded into various shapes. The flexibility of the composite allows researchers to design numerous 3D structures (like membrane, filaments, or fluke, etc...) according to the required application (Lihua Li, 2003).

Also, doping the composite with metal nanoparticles influences its durability and bioactivity (Julia Radwan-Pragłowska, 2020).

The treatment of burning wounds is one area where researchers benefit from the superior qualities of doped PLA/Chitosan composites as demonstrated by Praglowska et al. study. Using electrospinning technique, they successfully synthesized 3D nanofibrous bilayer scaffold from PLA/Chitosan composite that mimic the fibrous components of extracellular matrix (Collagen, elastin, and laminin) and doped it with (ZnO, Fe_3O_4, and Ag) metal nanoparticles.

The use of PLA in the scaffold enabled the researchers to synthesize homogenous and continuous nanofibers, while the use of Chitosan -which has a high similarity to the ECM components proteoglycan- helped evading the problem of accumulated PLA-degradation acidic products. With metal nanoparticles, the researchers applied electrical stimulation to boost cell proliferation. The formed hybrid biocompatible scaffold proved to be an efficient biomaterial in burning- wounds treatment especially with injuries that require grafting (Julia Radwan-Pragłowska, 2020).

Nazeer et al. merged Hydroxyapatite (HA) [$Ca_{10}(PO4)_3OH$] with PLA/Chitosan composite as an osteoconductive material for a bone regeneration treatment. With a desktop 3D printer, they designed a macroporous scaffold of PLA/Chitosan/HA composite. This type of scaffold provided an improved medium for cell adhesion and

proliferation compared to scaffold made solely from PLA (Muhammad Anwaar Nazeer, 2020).

Another successful application for the PLA/ Chitosan composite is in the treatment of the inflammation of tooth supporting tissue diseases (Periodontitis). As Shen et al. showed, nanofibrous PLA/chitosan scaffolds, with their superior mechanical properties, served as an excellent enhancer for the osteogenic differentiation of bone marrow stem cells (BMSCs) through the promoting of osteogenic marker genes (like BSP, Ocn, Collagen I, and OPN) and improved ECM mineralization. However, test results demonstrated that it may result in the over expression of inflammatory mediators and TLR4 of human periodontal ligament cells (hPDLCs). An issue that can be managed through TLR4 pathway (Renze Shen, 2018).

These are merely a few examples of the wide medical use of PLA/Chitosan composite. Our research project aims to benefit from the powerful qualities of this type of biomaterials to explore novel biomedical applications for them.

2.4. Poly (Vinyl Alcohol) PVA

Is a synthetic polymer extracted from fossil fuel resources (Gaurav Saxena, 2020). It's a water-soluble polymer with low toxicity which is widely used in various biomedical applications (Fig.3) (Gaurav Saxena, 2020) (C.C. DeMerlis, 2003). Table.3 shows some physical and chemical properties of PVA.

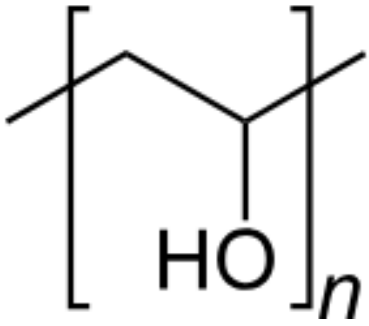

Fig.4: Poly (vinyl alcohol) structural formula (Polyvinyl alcohol, 2023)

Table.3: Physical and chemical properties of PVA (ChemSrc, 2023)

Molecular Formula	C_2H_4O
Molecular Weight	44.053
Density	0.8 ± 0.1 g/cm^3
Boiling Point	23.5 ± 13.0 C at 760 mmHG
Melting Point	230-240 C

PVA is synthesized by the hydrolysis of Poly (Vinyl acetate) (PVAc) a homopolymer which is synthesized via free-radical mechanism in an alcoholic medium (Emo Chiellini, 2003) (Fig.4).

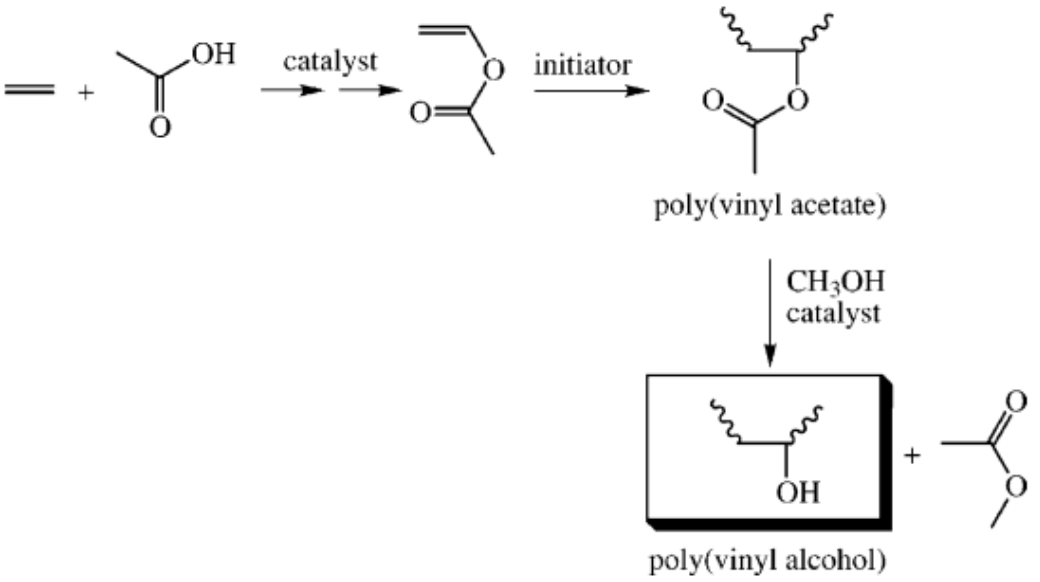

Fig.5: The synthesis of Poly (vinyl alcohol) from Poly (vinyl acetate) (Emo Chiellini, 2003).

The length of the parent PVAc polymer chain as well as the degree of hydrolysis influences the properties (the molecular weight, tensile strength, solubility, etc....) of the product PVA polymer chain (C.C. DeMerlis, 2003).

Although it isn't synthesized from natural resources, PVA is a biodegradable polymer where certain strains of bacteria like *Pseudomonas* (Gaurav Saxena, 2020) (Emo Chiellini, 2003), *Alcaligenes, Bacillus* species (Emo Chiellini, 2003) as well as fungal species like *Penicilium* sp. and *Geotrichum fermentans* WF 9101 (Gaurav Saxena, 2020) can degrade PVA.

The low toxicity and solubility of PVA enabled its use in various medical and industrial applications. For example, PVA is an important component in the paper industry where it's safe to be used in the packaging of meat and poultry products. Also, PVA is used in the production of medical products such as synthetic tears and oral tablets (C.C. DeMerlis, 2003).

2.5. Chitosan/PVA Composites

One of the most beneficial characteristics of PVA polymers is its ability to form cross-linking hydrogels due to the hydroxyl groups held by its side chains (Hitesh Chopra, 2022). This ability of PVA, besides its biocompatibility and mechanical stiffness, made it a common ingredient in composite with chitosan in many biomedical products. PVA provides the necessary firmness for chitosan which exhibits poor mechanical properties. Thus, PVA/ Chitosan composites encompass the biological benefits of chitosan (Like its antimicrobial activity) along with the mechanical strength and biocompatibility of PVA (Saif El-Din Al-Mofty, 2022).

One example of biomedical application of PVA/Chitosan composites is in wound healing. Chopra et al. synthesized PVA/ chitosan- based hydrogel films loaded with honey via solvent casting method. The use of PVA provided the hydrogel film with the ability to carry aqueous material to secure the drug release from the hydrogel films. Also, the flavonoid and phenolic ingredients of honey provided wound-healing activity. Researchers found this type of hydrogel films showed excellent properties for wound healing materials such as high swelling and moisture uptake abilities as well as superior mechanical traits. They were also able to examine an in vitro release of honey from the hydrogel film as a way of making an efficient wound- healing drug delivery system (Hitesh Chopra, 2022).

Kanani et al. developed chitosan-based nanofibrous scaffolds for the treatment of burn wounds. The researchers chose to synthesize nanofibrous scaffolds because of the similarity of its structure to ECM structure because of its microporous 3D structure and its high surface area. The use of chitosan provides an antimicrobial and cell-viability effect. PVA was added to allow for the electrospinning of the nanofibrous scaffolds because electrospinning process can't be completed without the addition of hydrophilic polymer because the high solution viscosity, the strong intermolecular forces, and the poly-cationic nature of the pure chitosan-based scaffold. Poly(ε-caprolactone) (PCL) was added to the composite to reduce the hydrophilicity of the PVA/Chitosan composites. The PCL/PVA/Chitosan nanofibrous scaffolds were applied to excision and burning wounds on a dog's trunk. The tests that the researchers conducted on the nanofibrous scaffolds showed high efficiency in accelerating the treatment of excision and burning ulcers due to their superior biological characteristics (Adeleh Gholipour-Kanani, 2018). Another example of PVA/Chitosan composite application is the composite formulated by Kouchak et al. for the loading of the wound-treating antibiotic Nitrofurazone which has a high skin permeability which limits its effectiveness. So, the researchers aimed to improve the release of the antibiotic.

The PVA/Chitosan composite films showed excellent vapor and oxygen penetration, and high water swelling rate as well as superior mechanical properties due to the addition of PVA. The Nitrofurazone-loaded composites significantly inhibited the growth of the microorganism *P.aeruginosa* despite the inefficiency of the Nitrofurazone alone against the microorganism (Maryam Kouchak, 2014).

Lin et al. team studied the effect of the addition of dextran to the PVA/ Chitosan composite. Dextran is a complex glucan with α-1,6 linkages that can initiate angiogenic response as well as promoting skin regeneration for burning wounds. The researchers chose to use PVA in the wound dressing composition for its ability to preserve moisture due to its high solubility and polarity. Keeping the wound dressing wet promotes the proliferation of fibroblast cells, prevents scar formation, as well as reducing pain. PVA also provides an optimal 3D microenvironment for cell proliferation. The crosslinking of Dextran/ PVA/ Chitosan hydrogel was achieved by using glutaraldehyde (GA).

The assays that the team conducted showed that the antimicrobial activity of chitosan was effective against both (+) and (-) gram bacteria while dextran was solely effective against

(-) gram bacteria. Also, the tests demonstrated that the increase of the dextran concentration is related to the improvement of the cell proliferation because of the positive charges and the large pore size of the hydrogel. The research concluded that Dextran/ PVA/ Chitosan composite has improved thermostability, wettability, mechanical properties and antimicrobial effect which makes it an ideal component for burning wounds dressings products (Shin-Ping Lin, 2019).

2.6. Alginic Acid

Is a linear hydrophilic polysaccharide that is composed of α-L-glucuronic (G residues) acid linked with β- D-mannuronic acid (M residues) with 1,4-glycosidic bonds (Fig.5) (Farhad Abasalizadeh, 2020) (Drug Bank, 2017).

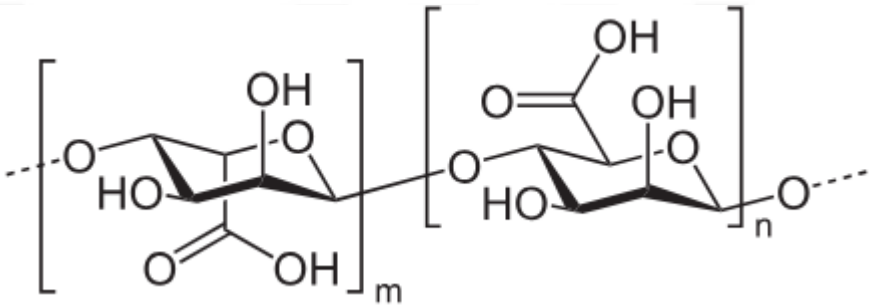

Fig.6: Alginic Acid structural formula (Wikipedia, 2023).

Alginic acid is found in the cell walls of specific brown seaweed (*Phacophycae*) species (including *Ascophyllum nodosum, Laminaria Hyperborean, Macrocystis pyrifera*) as well as some bacterial species (like *Azotobacter vinelandii, and Pseudomonas spp*.) (Farhad Abasalizadeh, 2020) and can be extracted via alkaline extraction (Drug Bank, 2017) using diluted HCL then the addition of NaCl or $CaCl_2$ causes the precipitation of sodium or calcium alginate (Farhad Abasalizadeh, 2020). Alginates that are extracted from seaweed resources have 0 acetylation unlike the alginates which are extracted from bacterial resources which have a high degree of acetylation (Farhad Abasalizadeh, 2020). It is an anionic copolymer that is insoluble in acidic solutions but soluble in alkaline and neutral solutions (H.M. Fahmy, 2008). Its monomer residues can arrange into homogenously (Poly M, Poly G) or heterogeneously (MG) (Fig.6) (Farhad Abasalizadeh, 2020). Table.4 shows some physical and chemical properties of Alginic acid.

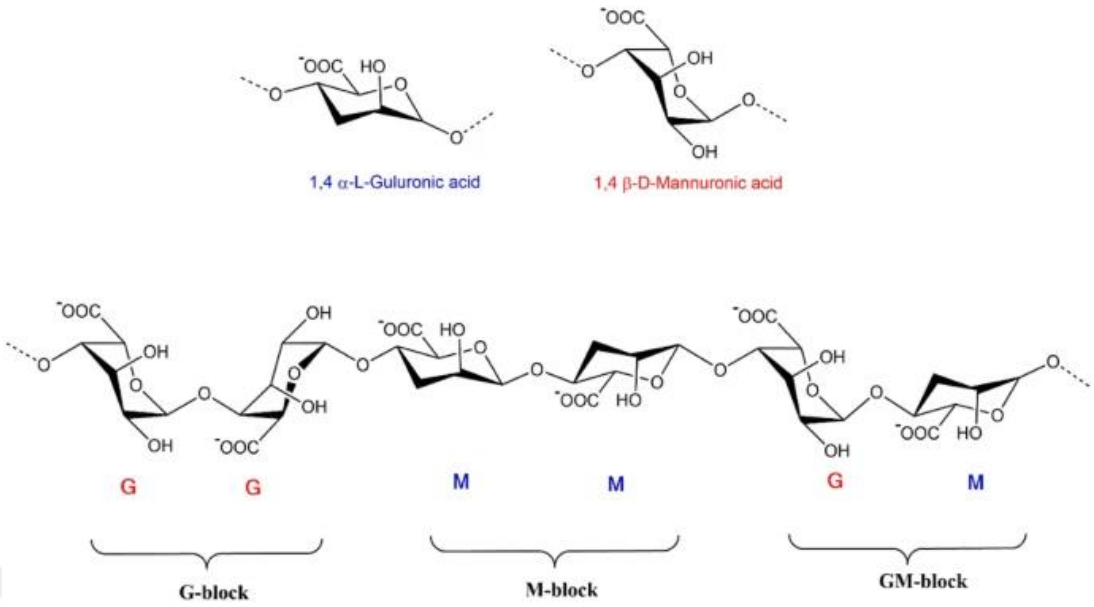

Fig.7: The conformations of alginate monomers residues (Farhad Abasalizadeh, 2020).

Table.4: Physical and chemical properties of Alginic Acid (PubChem, 2023)

Molecular Formula	$C_{12}H_{20}O_{12}P_2$
Molecular Weight	418.23
Boiling Point	716 - 718 C at 760 mm HG
Melting Point	230-240 C

The decrease in pH value causes the protonation of the carboxylate groups which results in the formation of hydrogen bonds and ultimately to the increase in viscosity. However, the molecular weight of the alginate is directly proportional to its viscosity where an increase in molecular weight leads to high viscosity (Farhad Abasalizadeh, 2020). The partial oxidation of alginate using Sodium Periodate renders the alginate susceptible to biodegradation. Sodium Periodate severs the cis-diol groups' carbon-carbon bonds (Ankur Kumar, 2023). Alginic acid is available as an ingredient in a lot of food products such as an emulsifier, thickener, or stabilizer in soup mixes (Drug Bank, 2017). In biomedical applications, it can be used in cancer drug delivery vehicles, bioink for 3D bioprinting, and in wound dressings (Farhad Abasalizadeh, 2020). It also can be available in antacid drugs (e.g. Gaviscon) where its contact with gastric acid causes it to precipitate in a barrier expelling acid pocket following a meal (Drug Bank, 2017).

2.7. Alginic Acid/Chitosan Composites

Researchers benefited from the excellent characteristics of acidic acid/ chitosan composite in the manufacturing of various biomedical products. One example of such innovation is the use of alginic acid and chitosan blend as a bioink for the 3D printing of complex hydrogels for tissue engineering purposes. This project was conducted by Liu et al. where they found that the addition of chitosan improved the viscosity of the hydrogel. They could successfully print a 3D model of a human nose as the resultant hydrogel was strong enough to construct complex structures with 3D printing (Qiongqiong Liu, 2018). Fahmy et al. examined a new technique to crosslink alginic acid and chitosan to synthesize drug carrier matrices for sodium diclofenac using citric acid/ sodium hypophosphite as crosslinking agents. They modified certain variables of the crosslinking agents (like the concentration of citric acid and the citric acid/ sodium hypophosphite molecular ratio, reaction time, temperature) to examine the effect of such changes on the crosslinking process and on the drug delivery ability. Their experiments showed that the selected crosslinking agents were successful in the synthesis of drug carrying matrices of alginic acid / chitosan. They noticed that the increase in the ratio of alginic acid / chitosan also increases the swellability of the composite matrix. They also showed the direct proportionality between the concentration of alginic acid and the sodium diclofenac drug release ratio (H.M. Fahmy, 2008). Jabeen et al. tried bis phenol-F-diglycidyl ether as a crosslinking agent for the synthesis of alginic acid/ chitosan films using solution casting method. They diversified the concentration of bis phenol-F-diglycidyl ether to study the effect of its concentration on the crosslinking process.

The FTIR test that they conducted proved the successful integration of the functional groups of the components of the composite. However, the researchers also noticed a dwindling pattern in the mechanical properties of the composite (Saira Jabeen, 2016).

Alginic acid / chitosan blend can be successfully applied in tissue engineering field as was proven by You et al. experiments. For the synthesis of injectable and biocompatible hydrogel, the researchers first initiated an oxidation reaction between alginic acid and sodium periodate to produce a biocompatible polysaccharide dialdehyde alginic acid (DAA) crosslinker via the partial oxidation of alginic acid backbone's hydroxyl groups into aldehyde groups. Then, they applied a Schiff reaction between chitosan's amine groups and the aldehyde groups of the backbone of alginic acid. The reaction took place

at a physiological ph.

The produced biodegradable hydrogel exhibited superior mechanical qualities and provided an excellent microenvironment for the proliferation of rat myocardial cells (H9c2) for two weeks without losing their original state (Yujing You, 2019).

2.8. Tungsten Disulfide (WS_2)

WS_2 is a biocompatible 2D nanomaterial with a lamellar structure that resembles MoS_2. It can be used as a solid lubricant, but it is also available in various other industrial products like integrated circuits, transistors, or sensors, etc. as well as in biomedical applications like drug and gene delivery and the coating of many medical supplies such as stents and catheters (Fig.7) (Abdul Mukheem, 2022) (Martin, 2007). Table.5 shows some physical and chemical properties of Tungsten disulfide.

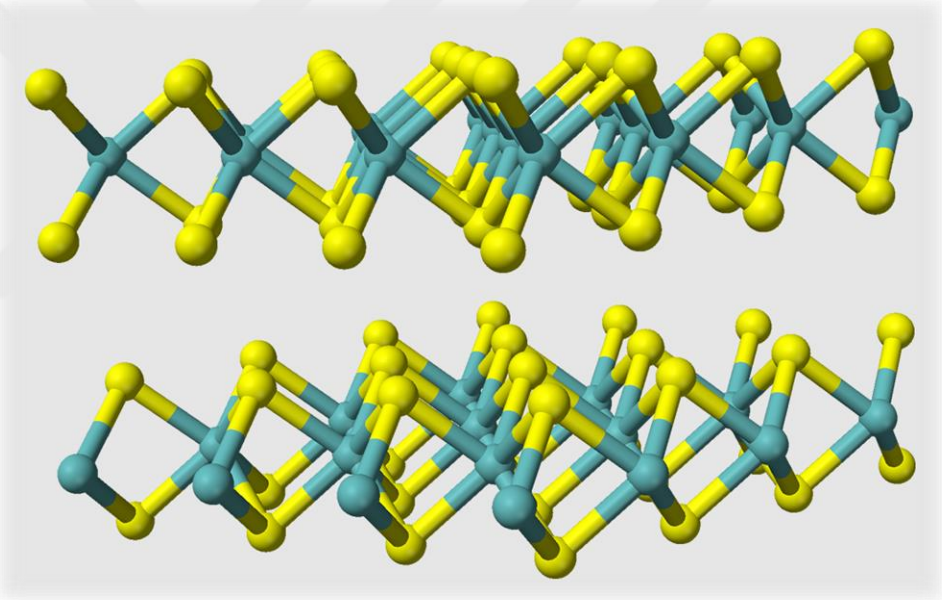

Fig.8: Tungsten Disulfate structure (Wikipedia, 2023).

Table.5: Physical and chemical properties of Tungsten disulfide *(Azonano, 2013)*

Molecular Formula	WS_2
Molar Mass	247.98 g/mol
Density	7.5 g/cm^3
Melting Point	1250 C

Tungsten disulfide is only partially soluble in water and acidic solutions. It's naturally available as tungstenite metal (Azonano, 2013).

Tungsten disulfide's acute edges rupture the cell membrane of bacteria which induces oxidation stress and results in the demise of the bacterial cell (Fig.8).

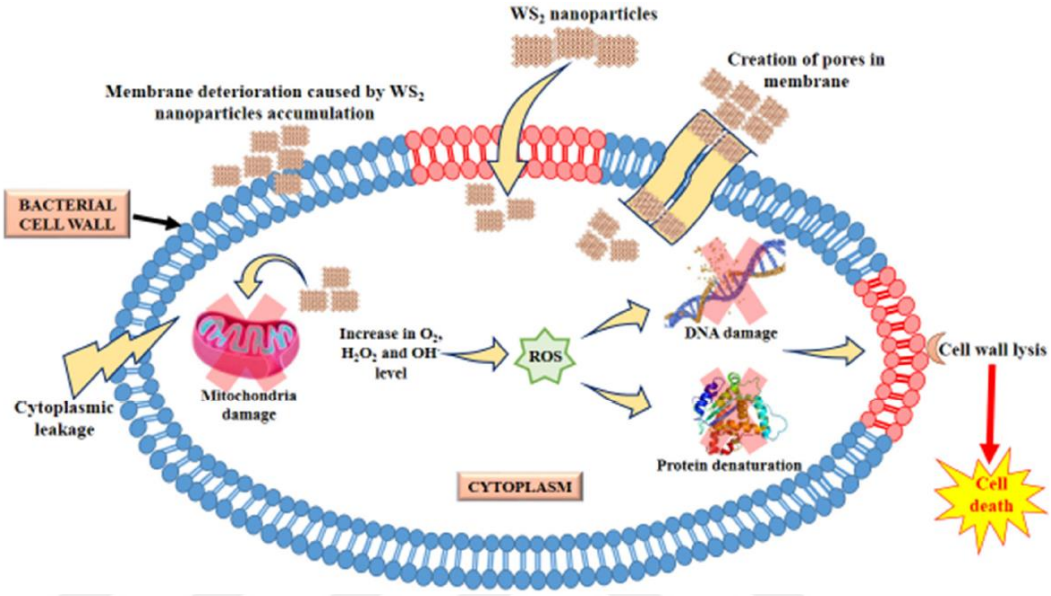

Fig.9: A representation of the antibacterial mechanism of Tungsten disulfide (Abdul Mukheem, 2022).

This well-established antibacterial effect of Tungsten disulfide made a widely available component in many therapeutic and sanitizing products.

One example of such research that benefits from the biocompatible properties of WS_2 is Mukheem et al.'s synthesis of biocompatible Polyhydroxyalkanoate (PHA)/Tungsten disulfide/ Chitosan nanocomposite with solvent casting technique. Polyhydroxyalkanoate is a biodegradable biopolymer that serves as an Energy storage and intracellular carbon for more than 300 species of (+) and (-) gram bacteria. The tests that the team conducted showed the high antibacterial efficiency of the nanocomposites against *E.coli* K1 and MRSA for up to 24 hours. Thus, confirming the ability of the nanocomposite to serve as an antibacterial scaffold (Abdul Mukheem, 2022).

3. MATERIALS AND METHODS

3.1. Materials:

- Sigma-Aldrich Chitosan.
- Aldrich Tungsten (IV) sulfide nano powder 2μm
- PLA polymer.
- Sigma-Aldrich Polyvinyl alcohol 20-98.
- 100 % glacial Acetic Acid (CH_3COOH).
- Sigma-Aldrich Alginic acid from seaweed.
- Honey.

3.2. Synthesis of PLA/Chitosan/ WS_2 Composite

0.15 g Chitosan is dissolved in 5 ml of acetic acid to make 3% solution of Chitosan. The 3% chitosan/ acetic acid solution is mixed with 0.05 g of PLA. Tungsten disulfate is added to each of the samples in variable amounts (0.5 mg,1 mg,1.5 mg, 2.0 mg). The mixture of PLA and Chitosan is then heated at 65C and stirred constantly until a homogeneous mixture is formed (Suryani H. A., 2018).

3.3. Synthesis of PVA/Chitosan/WS_2 Composite

0.1 g of Chitosan is dissolved in Acetic Acid (3%v/v) with constant stirring for 2 h. 0.025 g of PVA is dissolved in distilled water for 4h at 50 C (5%w/v). Chitosan and PVA solutions are mixed with 0.05 g of honey with mechanical blending. Tungsten disulfate is added to each of the samples in variable amounts (0.5 mg,1 mg,1.5 mg, 2.0 mg). The mixture is transferred to a chocolate mould and is left to dry at room temperature for 48 h (Hitesh Chopra, 2022).

3.4. Synthesis of Alginic Acid/Chitosan/WS_2 Composite

0.1 g of chitosan is dissolved in 5 ml 0.1M acetic acid (2.87 ml of acetic acid in double distilled water) to make 2% solution of chitosan. 10 ml of 1M alginic acid solution (0.088 g of alginic acid in 1 ml distilled water) is added to the 2% chitosan solution with constant stirring. 5% solution of ammonium persulfate (0.025 g of ammonium persulfate in 1 ml of distilled water) is added

to the mixture as an initiator and 3% solution of N, N'-Methylene bis acrylamide (0.015 g of N, N'-Methylene bis acrylamide in 1 ml of distilled water) as a crosslinker drop by a drop with constant stirring. The prepared mixture constantly stirred on a hot plate for 3-4 h at 65 C (Gaurav Sharmaa, 2017).

3.5. Gelation Experiment

Using UV-spectrophotometry, the transmittance of each composite is measured while in liquid form to estimate the time it takes each sample to turn into gel. During the experiment the temperature of the sample is measured using a thermometer.

3.6. Swelling Experiment

Following the synthesis of each sample, the sample is poured into a chocolate mould to make two small squares.

The 1st square is put into a petri dish filled with distilled water and its weight is measured using a sensitive scale in 10 minutes intervals. The swelling ratio is calculated according to the following formula (Eq 3.6) (Hitesh Chopra, 2022):

$$\text{Swelling Ratio} = (W_d - W_s)/W_d * 100 \qquad (3.6)$$

W_d: Initial weight.

W_s: Weight of the sample.

The 2nd square is put into a cuvette filled with distilled water and its transmittance is measured using UV-spectrophotometer.

4. RESULTS AND DISCUSSION

4.1. Gelation Experiment Results

4.1.1. For PLA Samples:

The following graphs represent the changes of the transmittance and temperature of PLA samples over time.

4.1.1.1. PLA1 (with 0.5 mg of WS_2)

The results show a decrease in the transmittance (from 1.8% to 1.1%) of PLA1 sample during gelation process.

Initial conditions of the experiment: Humidity:43%, Room temperature: 23 C, initial sample temperature before experiment: 33 C.

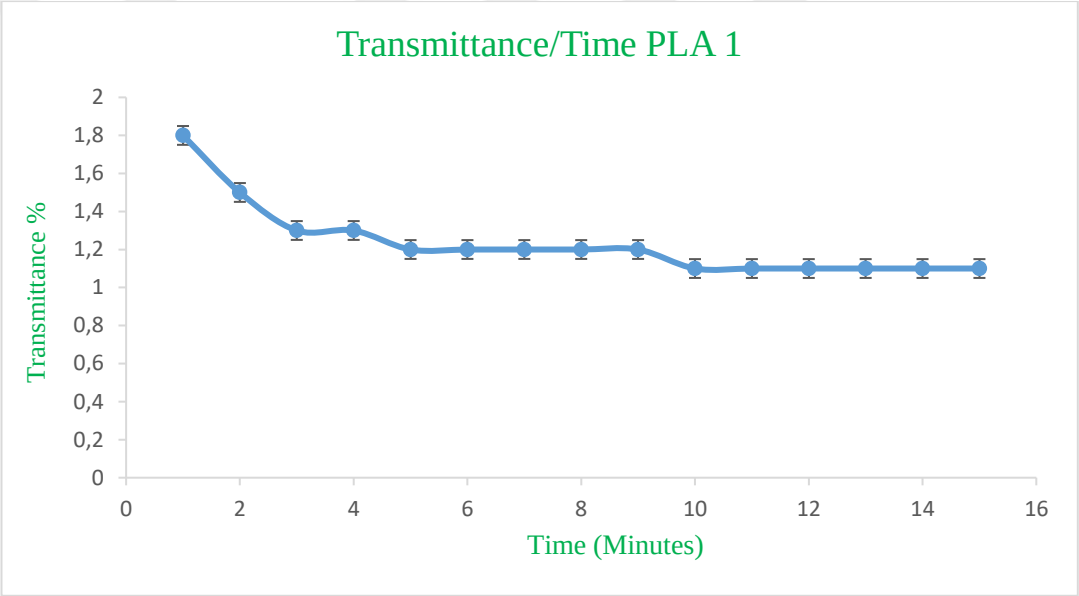

Fig.10: A graph representing the change of transmittance of PLA 1 sample.

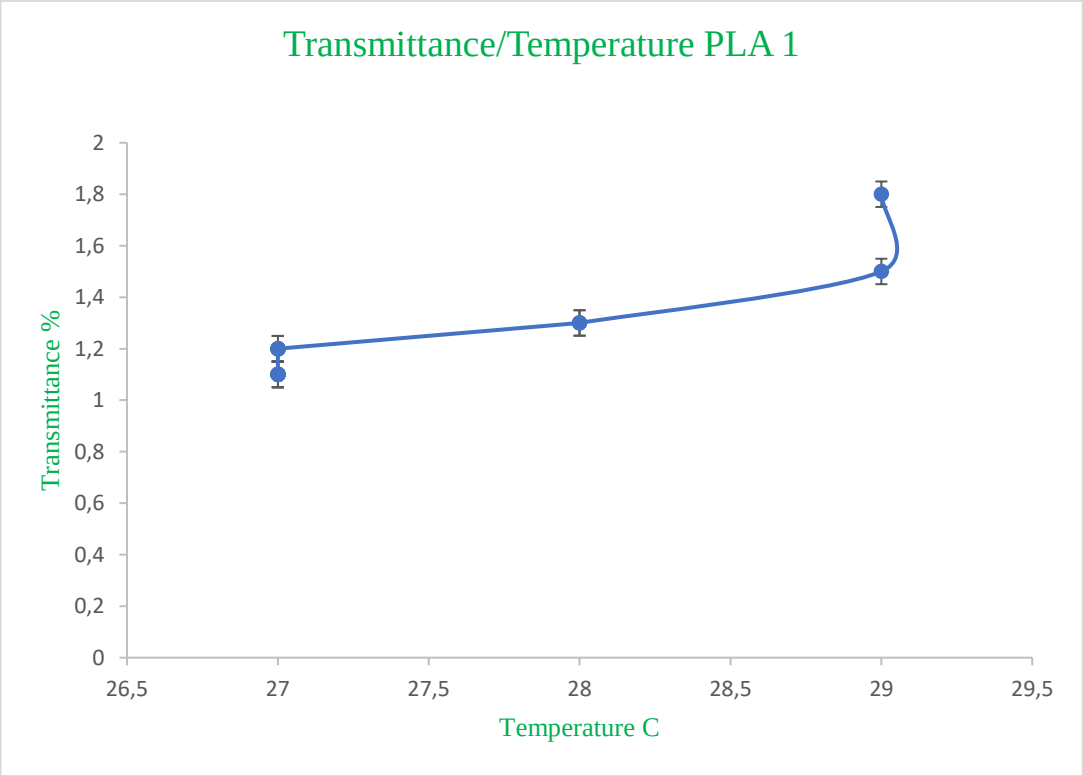

Fig.11: A graph representing the change of transmittance/temperature of PLA 1 sample.

4.1.1.2. PLA2 (with 1.0 mg of WS_2)

The results show a decrease in the transmittance (from 31% to 29.7%) of PLA2 sample during gelation process.

Initial conditions of the experiment: Humidity:43%, Room temperature: 22.7 C, initial sample temperature before experiment: 31 C.

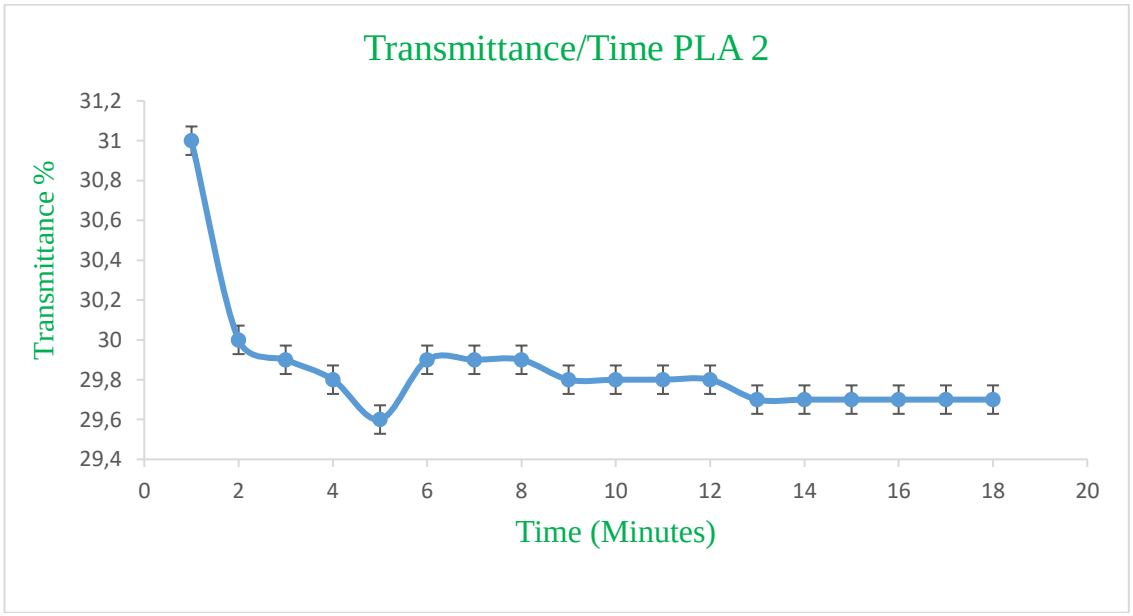

Fig.12: A graph representing the change of transmittance of PLA 2 sample.

4.1.1.3. PLA3 (with 1.5 mg of WS_2)

The results show a decrease in the transmittance (from 30.4% to 27.2%) of PLA3 sample during gelation process.

Initial conditions of the experiment: Humidity:45%, Room temperature: 21.7 C, initial sample temperature before experiment: 29 C.

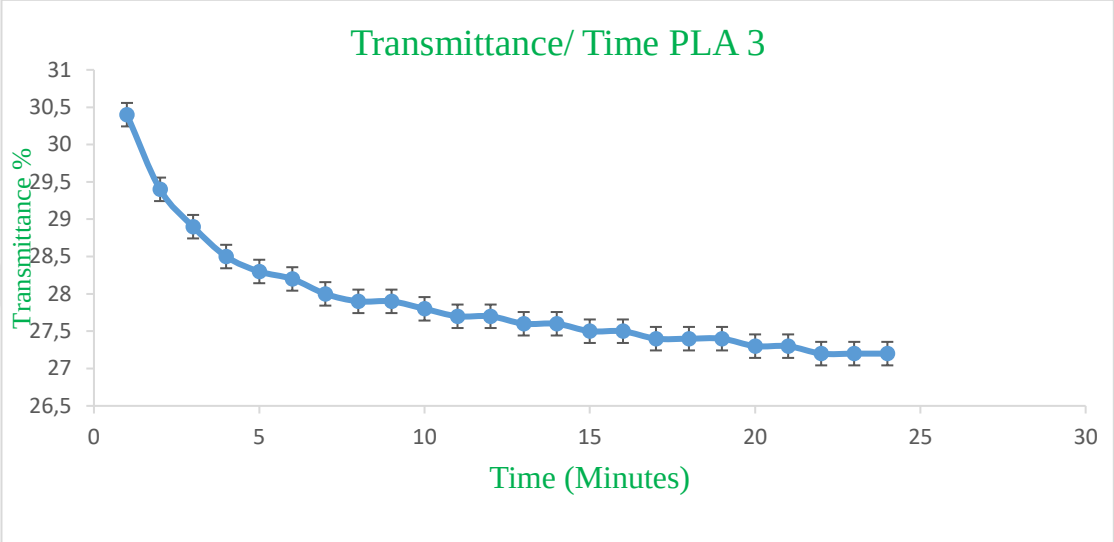

Fig.13: A graph representing the change of transmittance of PLA 3 sample.

4.1.1.4. PLA4 (with 2.0 mg of WS_2)

The results show a decrease in the transmittance (from 30.4% to 27.2%) of PLA4 sample during gelation process.

Initial conditions of the experiment: Humidity:39%, Room temperature: 22.2 C, initial sample temperature before experiment: 39 C.

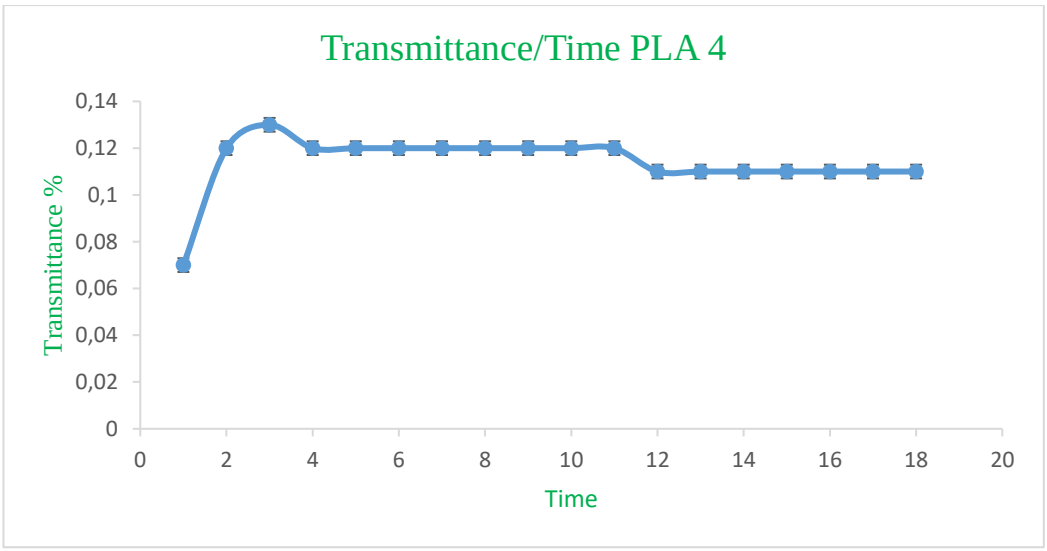

Fig.14: A graph representing the change of transmittance of PLA 4 sample.

4.1.2. For PVA Samples:

The following graphs represent the changes of the transmittance and temperature of PVA samples over time.

4.1.2.1. PVA1 (with 0.5 mg of WS_2)

The results show a decrease in the transmittance (from 60.9% to 57.1%) and of PVA1 sample during gelation process. Initial conditions of the experiment: Humidity:39%, Room temperature: 20.9 C, initial sample temperature before experiment: 38 C.

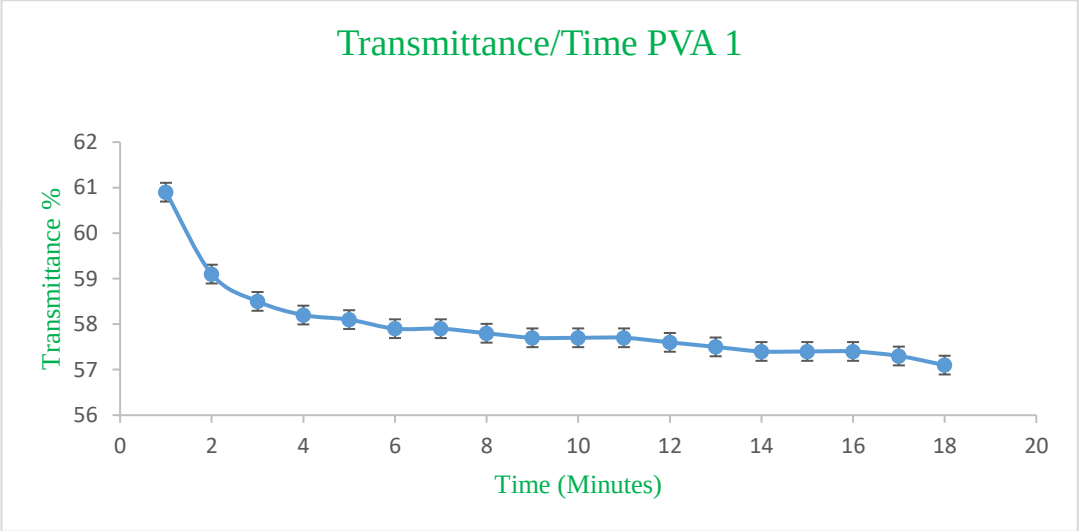

Fig.15: A graph representing the change of transmittance of PVA 1 sample.

4.1.2.2. PVA2 (with 1.0 mg of WS_2)

The results show a decrease in the transmittance (from 36.5% to 3.4%) of PVA2 sample during gelation process. Initial conditions of the experiment: Humidity:39%, Room temperature: 21.1 C, initial sample temperature before experiment: 50 C.

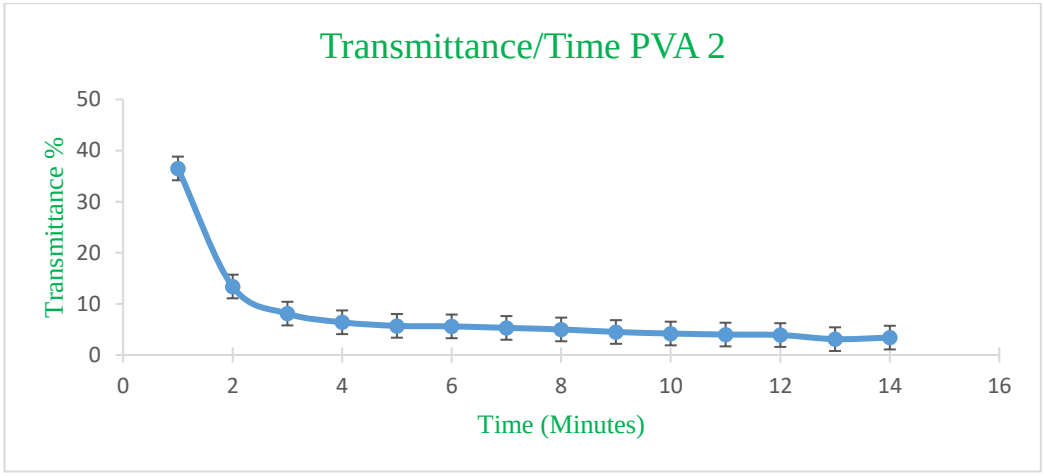

Fig.16: A graph representing the change of transmittance of PVA 2 sample.

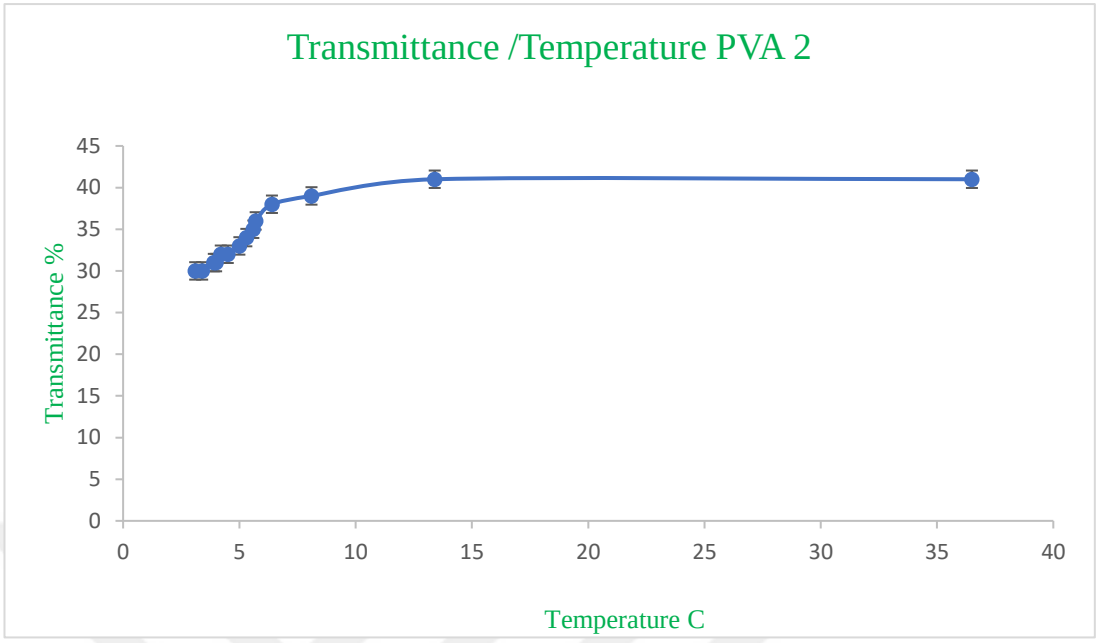

Fig.17: A graph representing the change of transmittance/temperature of PVA 2 sample.

4.1.2.3. PVA3 (with 1.5mg of WS_2)

The results show a decrease in the transmittance (from 73.9% to 71.6%) of PVA3 sample during gelation process. Initial conditions of the experiment: Humidity:37%, Room temperature: 20.4 C, initial sample temperature before experiment: 34 C.

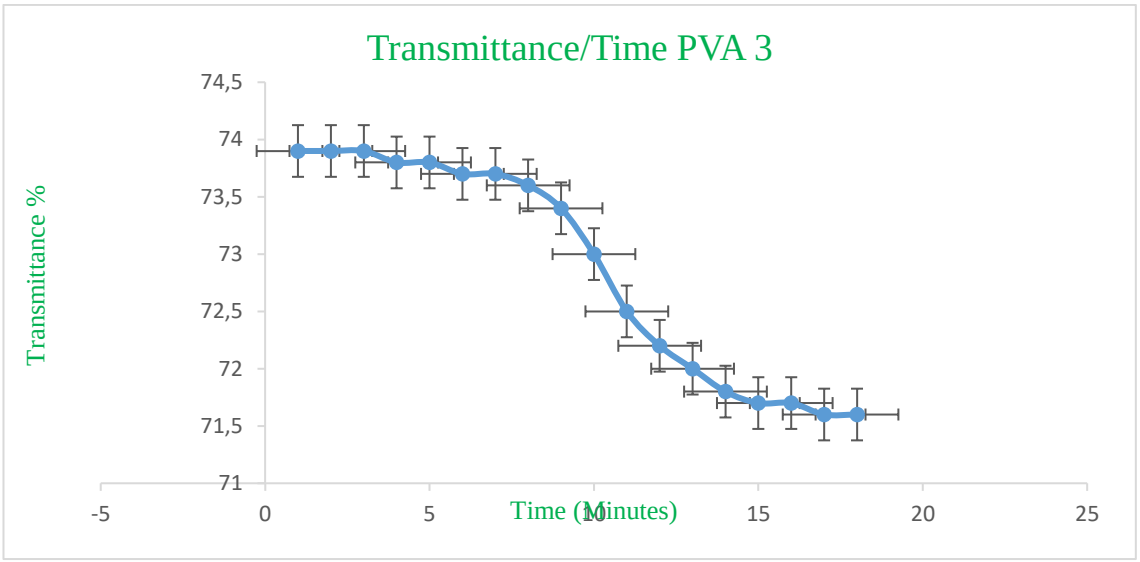

Fig.18: A graph representing the change of transmittance of PVA 3 sample.

4.1.2.4. PVA4 (with 2.0 mg of WS2)

The results show a decrease in the transmittance (from 61.1% to 50.4%) of PVA4 sample during gelation process.

Initial conditions of the experiment: Humidity:46%, Room temperature: 21.3 C, initial sample temperature before experiment: 45 C.

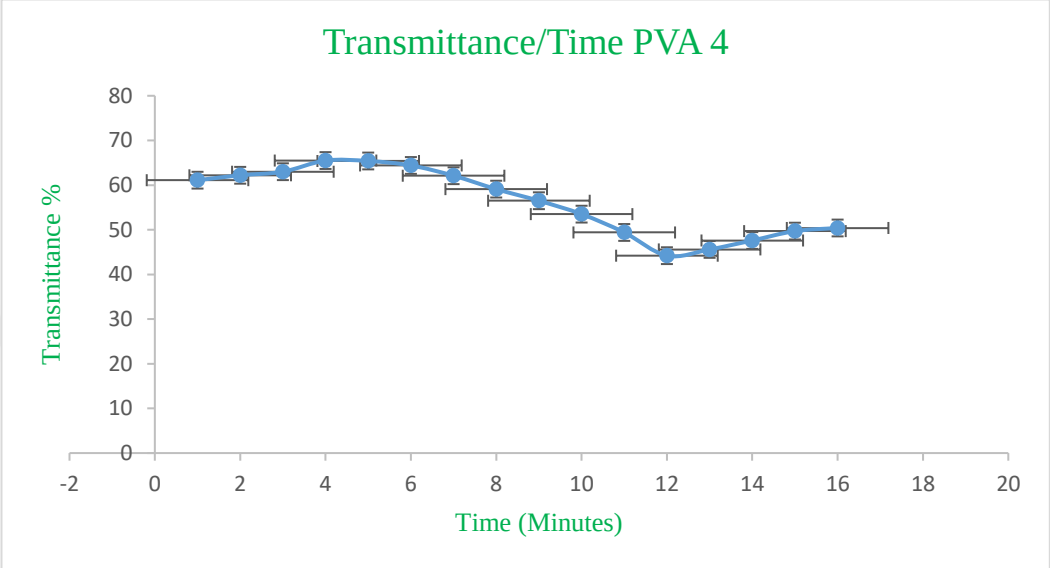

Fig.19: A graph representing the change of transmittance of PVA4 sample.

4.1.3. For Alginic Acid Samples:

The following graphs represent the changes of the transmittance and temperature of Alginic acid samples over time.

4.1.3.1. Alginic acid 1 (with 0.5 mg of WS_2)

The results show the change in the transmittance (from 35.4% to 34.8%) and of Alginic Acid 1 sample during the gelation process. Initial conditions of the experiment: Humidity:37%, Room temperature: 20.6 C, initial sample temperature before experiment: 38 C.

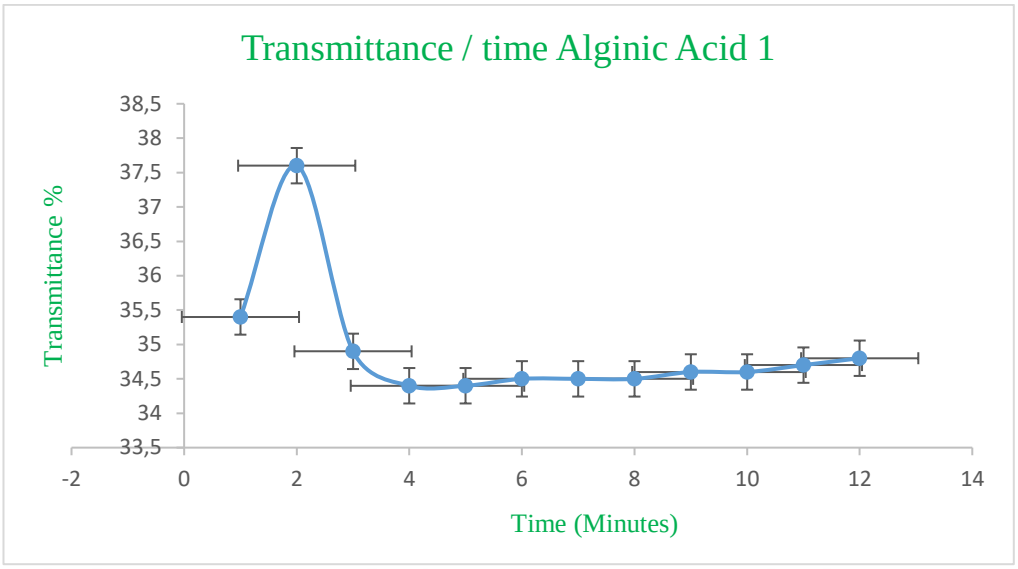

Fig. 20: A graph representing the change of transmittance of Alginic acid 1 sample.

4.1.3.2. Alginic Acid 2 (with 1.0 mg of WS_2)

The results show a decrease in the transmittance (from 0.18% to 0.17%) Alginic Acid 2 sample during the gelation process. Initial conditions of the experiment: Humidity:49%, Room temperature: 21.7 C, initial sample temperature before experiment: 29 C.

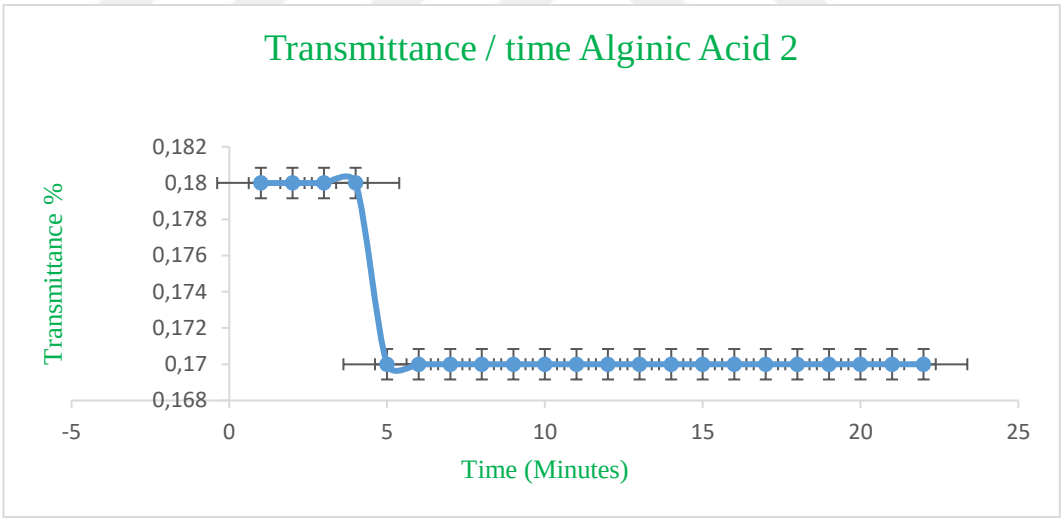

Fig. 21: A graph representing the change of transmittance of Alginic acid 2 sample.

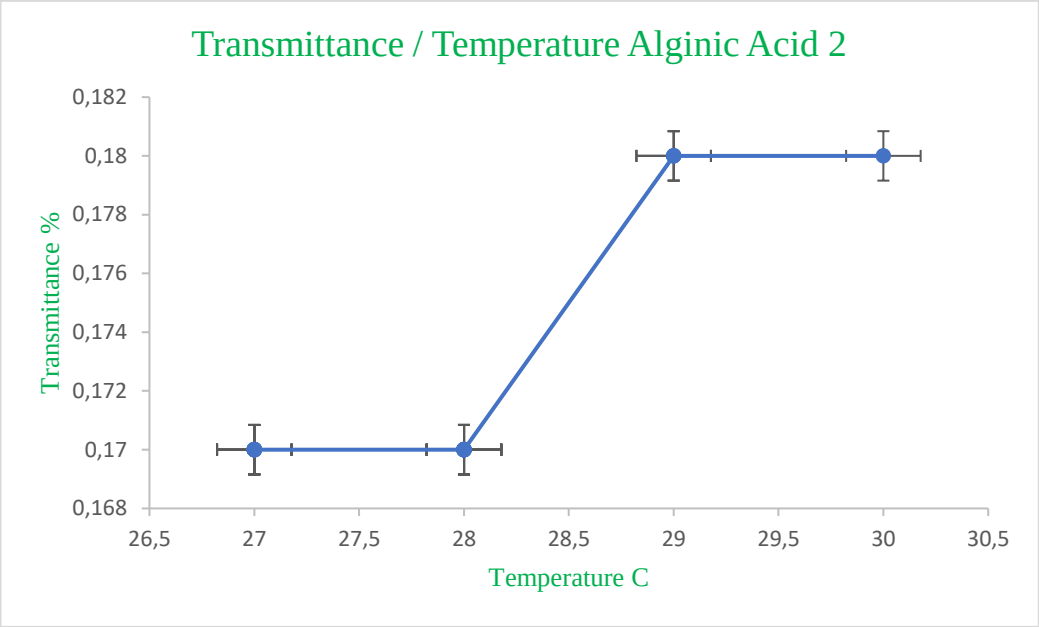

Fig. 22: A graph representing the change of transmittance/temperature of Alginic acid 2 sample.

4.1.3.3. Alginic Acid 3 (with 1.5 mg of WS_2)

The results show the change in the transmittance (from 0.11% to 0.14%) and the temperature (30 C – 27 C) of Alginic Acid 3 sample during gelation process.

Initial conditions of the experiment: Humidity:50%, Room temperature: 21.9 C, initial sample temperature before experiment: 31 C.

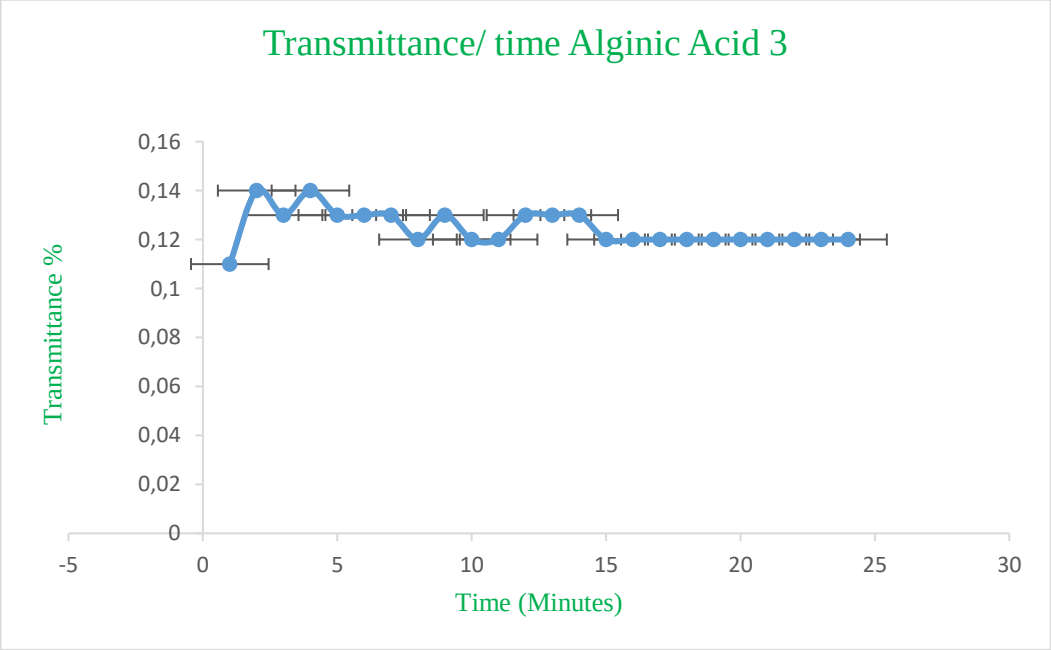

Fig.23: A graph representing the change of transmittance of Alginic acid 3 sample.

4.1.3.4. Alginic Acid 4 (with 2.0 mg of WS_2)

The results show the change in the transmittance (from 19.7% to 18.2%) and the temperature (31 C – 28 C) of Alginic Acid 4 sample during gelation process.

Initial conditions of the experiment: Humidity:37 %, Room temperature: 20.6 C, initial sample temperature before experiment: 30 C.

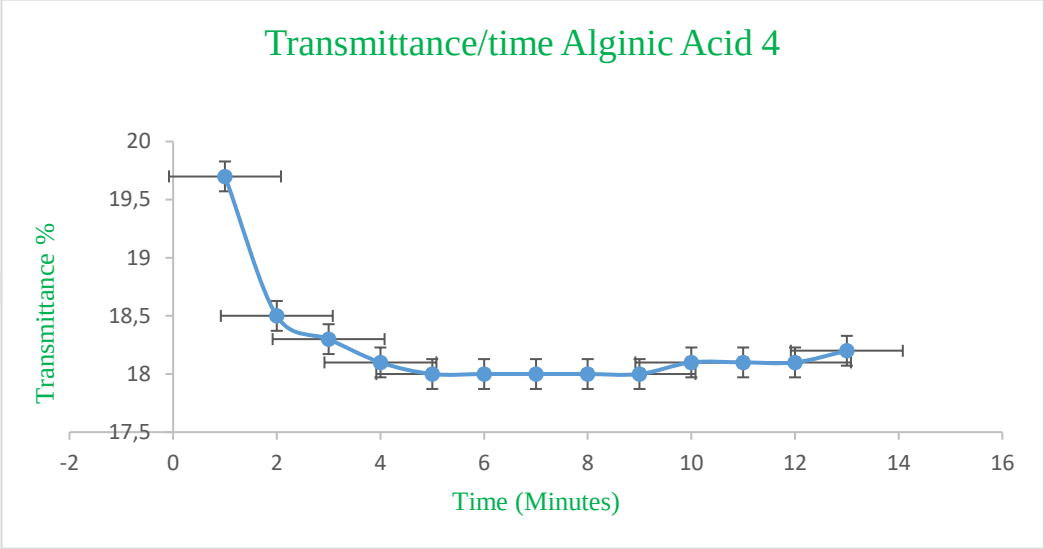

Fig. 24: A graph representing the change of transmittance of Alginic acid 4 sample.

4.2. Swelling Experiment Results:

The following graphs and tables represent the results of the swelling experiments. The results show the remarkable ability of the composites samples to absorb distilled water up well and the significant increase in its weight before starting to break down due to fragility.

4.2.1. PLA Samples

The results show that Chitosan/PLA composites' ability to absorb distilled water up to 30 minutes where its weight increase significantly before degrading (Table.6).

Table.6: The results of the swelling experiments of PLA samples.

Time (minutes)	PLA 1 (mg)	Swelling Ratio PLA 1(%)	PLA 4 (mg)	Swelling Ratio PLA 4 (%)
0	2.90	-	9.50	-
10	58.60	1920.69	242.60	2453.68
20	72.10	2386.21	295.20	3007.37
30	86.30	2875.86	224.45	2262.63

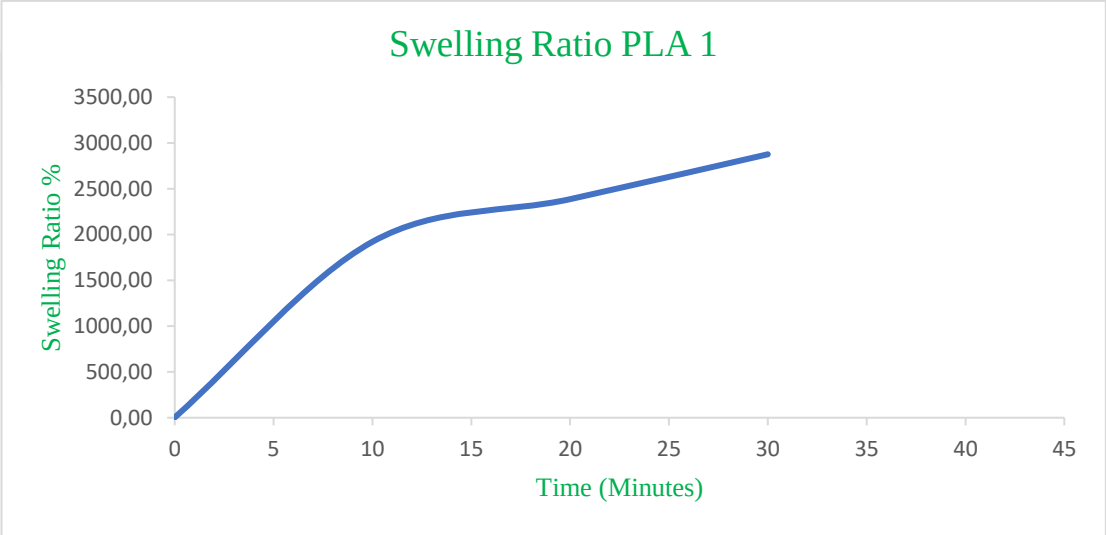

Fig.25: Swelling ratio graph for PLA 1 sample.

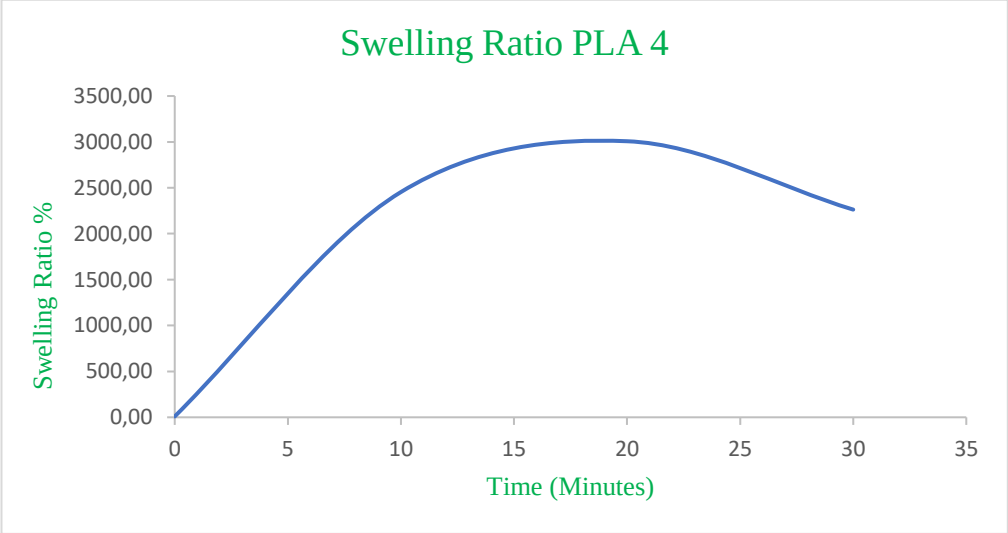

Fig.26: Swelling ratio graph for PLA 4 sample.

4.2.2. PVA Samples

The results show that Chitosan/PVA composites' ability to absorb distilled water up to 20 minutes where its weight increase significantly before its swelling ability decrease and then it breaks down.

Table.7: The results of the swelling experiments of PVA samples.

Time (minutes)	PVA 1 (mg)	Swelling Ratio PVA 1 (%)	PVA 4 (mg)	Swelling Ratio PVA 4 (%)
0	7.10	7.10	3.24	3.24
10	18.10	154.93	15.00	362.96
20	32.10	352.11	16.00	393.83
30	17.30	143.66	13.40	313.58

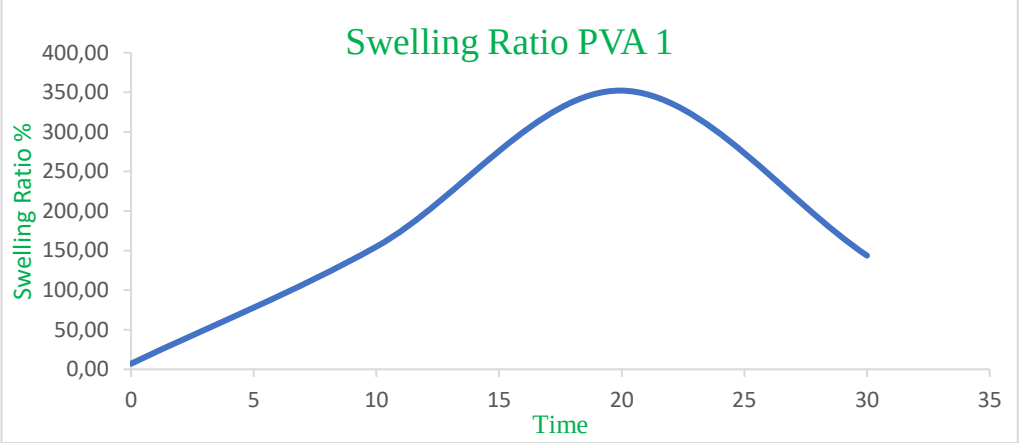

Fig.27: Swelling ratio graph for PVA 1 sample.

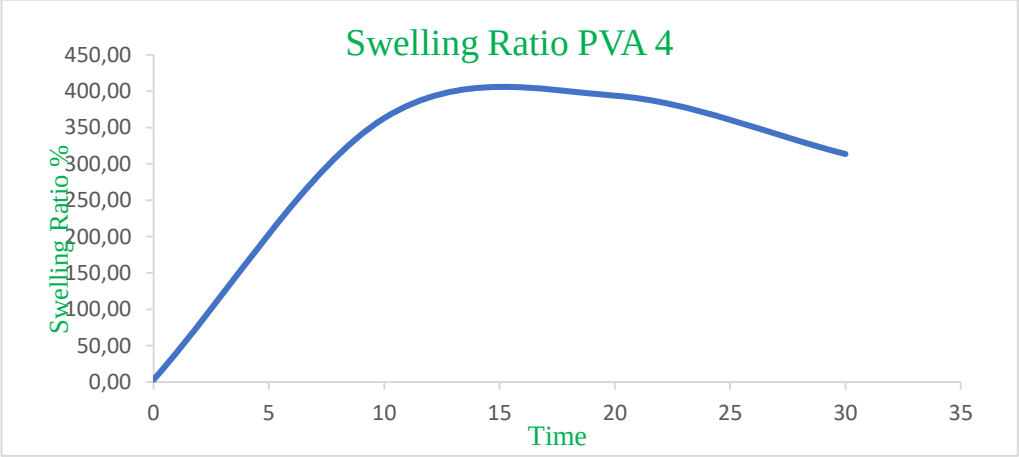

Fig.28: Swelling ratio graph for PVA 4 sample.

4.2.3. Alginic Acid Samples

The results show that Chitosan/Alginic acid composites' ability to absorb distilled water up to 30 minutes where its weight increase significantly before degradation.

Table.8: The results of the swelling experiments of Alginic Acid samples.

Time (minutes)	Alginic Acid 1(mg)	Swelling Ratio Alginic Acid 1 (%)	Alginic Acid 4 (mg)	Swelling Ratio Alginic Acid 4
0	3.49	-	3.50	-
10	5.23	49.86	5.50	57.14
20	10.15	190.83	16.10	360.00
30	13.40	283.95	17.50	400.00

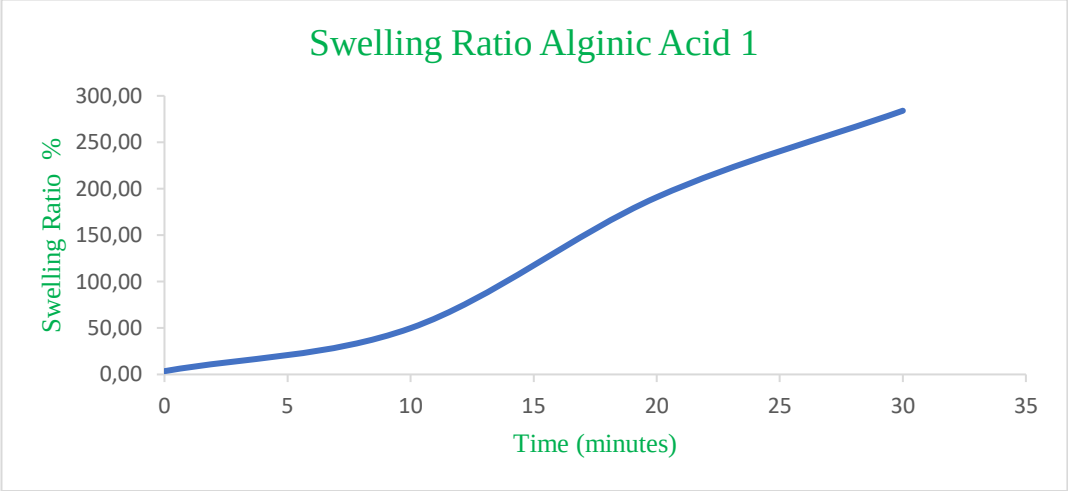

Fig.29: Swelling ratio graph for Alginic acid 1 sample.

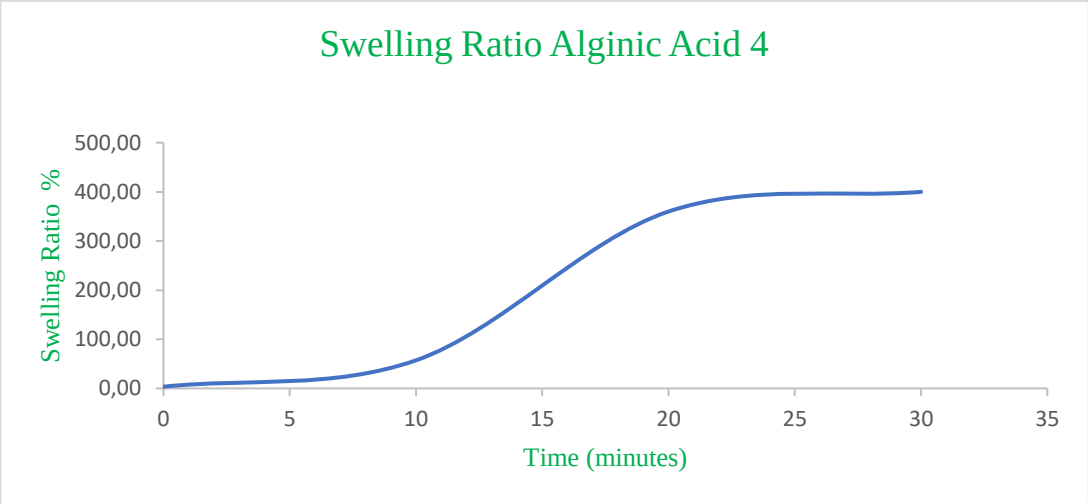

Fig.30: Swelling ratio graph for Alginic acid 1 sample.

4.3. Swelling Transmittance Experiment Results

The following graphs show the effect of swelling of distilled water of the chitosan-based composites on the transmittance ability of the samples.

4.3.1. PVA1 Samples

The results show a decrease in the transmittance (from 34% to 20%) of PVA1 sample during swelling process.

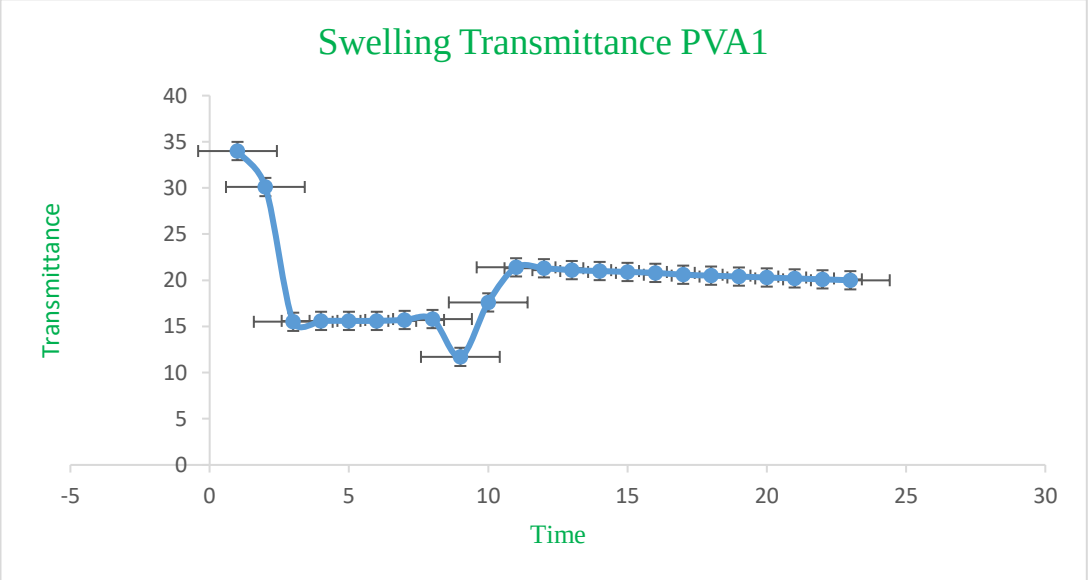

Fig.31: Swelling transmittance graph for PVA1 sample.

4.3.2. PVA4 Samples

The results show a decrease in the transmittance (from 79.7% to 76.6%) of PVA4 sample during swelling process.

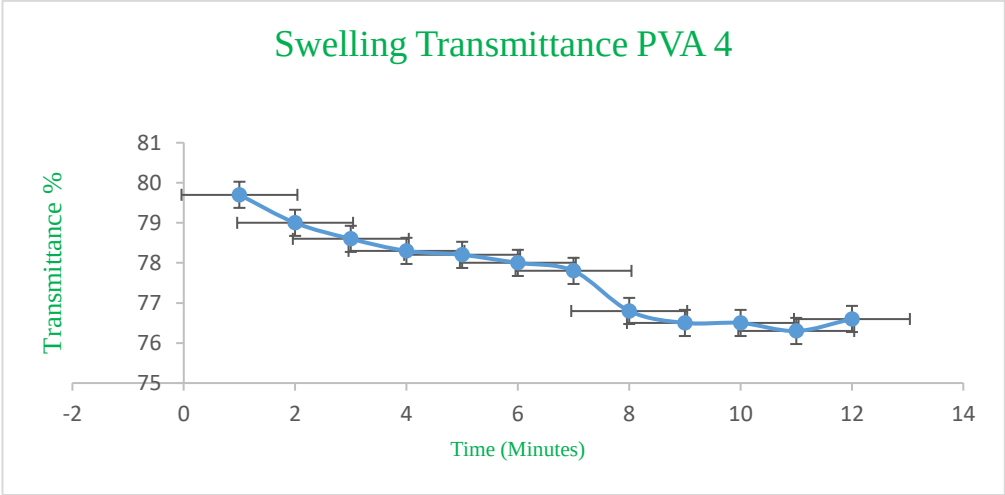

Fig.32: Swelling transmittance graph for PVA4 sample.

4.3.3. PLA1 Samples

The results show a decrease in the transmittance (from 35.8% to 6%) of PLA1 sample during Swelling process.

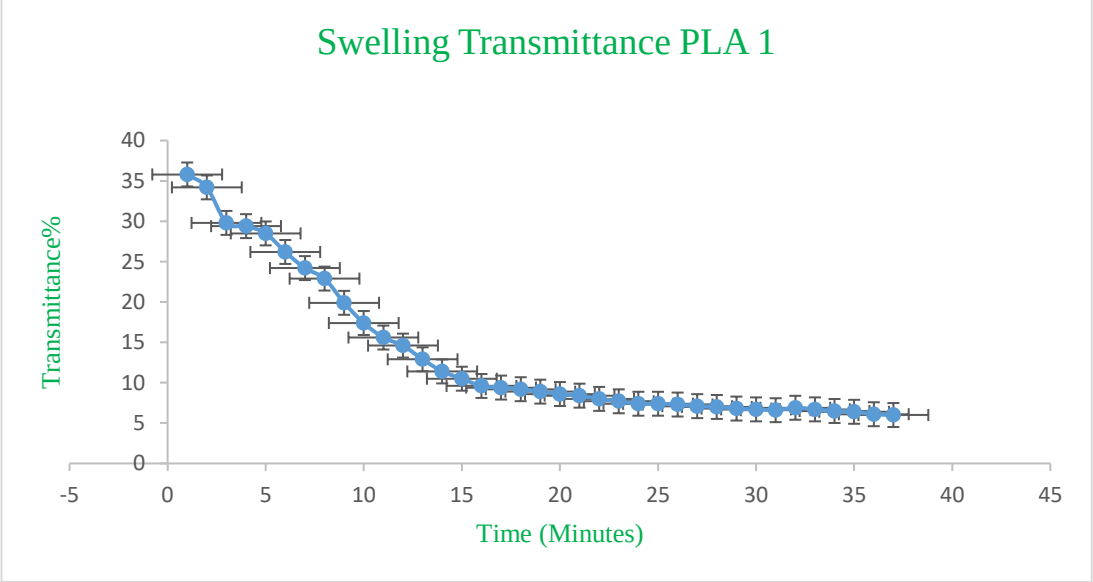

Fig.33: Swelling transmittance graph for PLA1 sample.

4.3.4. PLA4 Samples

The results show a decrease in the transmittance (from 5.6% to 5.1%) of PLA4 sample during Swelling process.

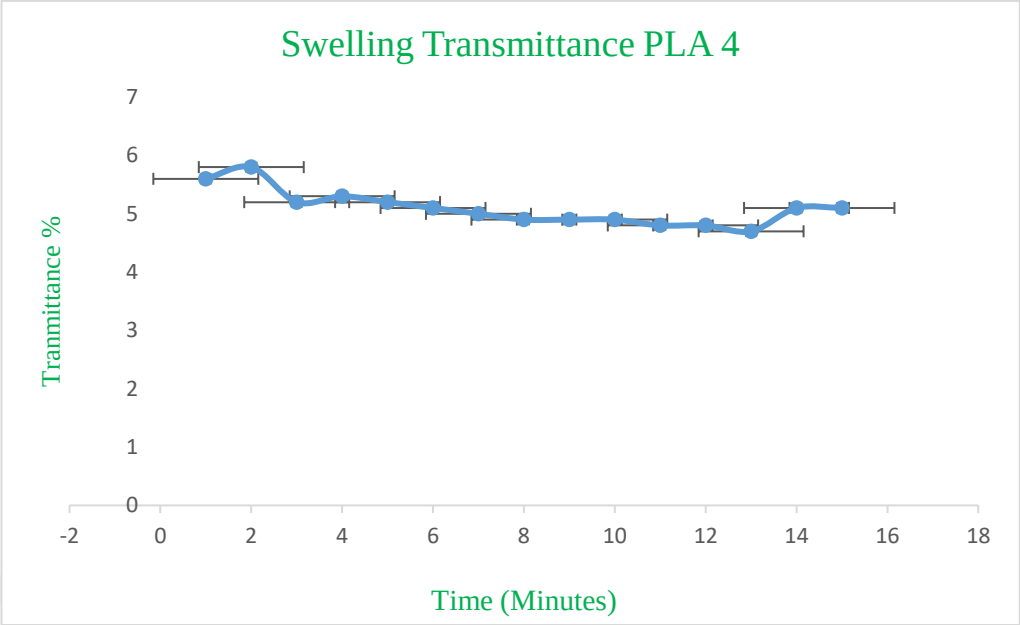

Fig.34: Swelling transmittance graph for PLA4 sample.

4.3.5. Alginic Acid 1 Sample

The results show the change in the transmittance (from 68.6% to 68.2% then a slight increase to 68.7 %) of Alginic Acid 1 sample during swelling process.

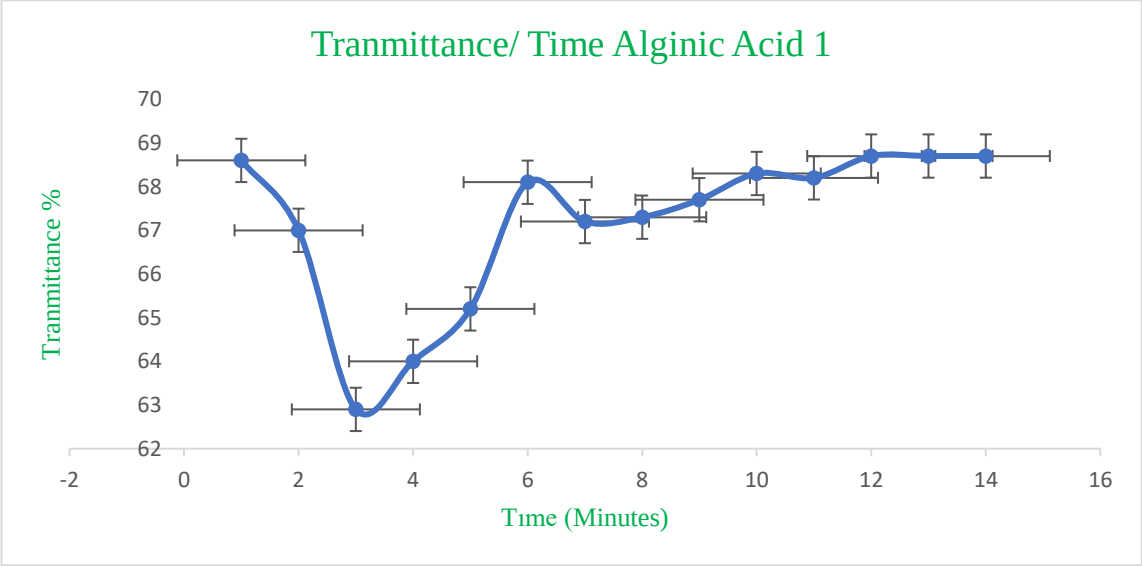

Fig. 35: Swelling transmittance graph for Alginic Acid 1 sample.

4.3.6. Alginic Acid 4 Sample

The results show the change in the transmittance (from 68% to 69% then a decrease to 68.5%) of Alginic Acid 4 sample during swelling process.

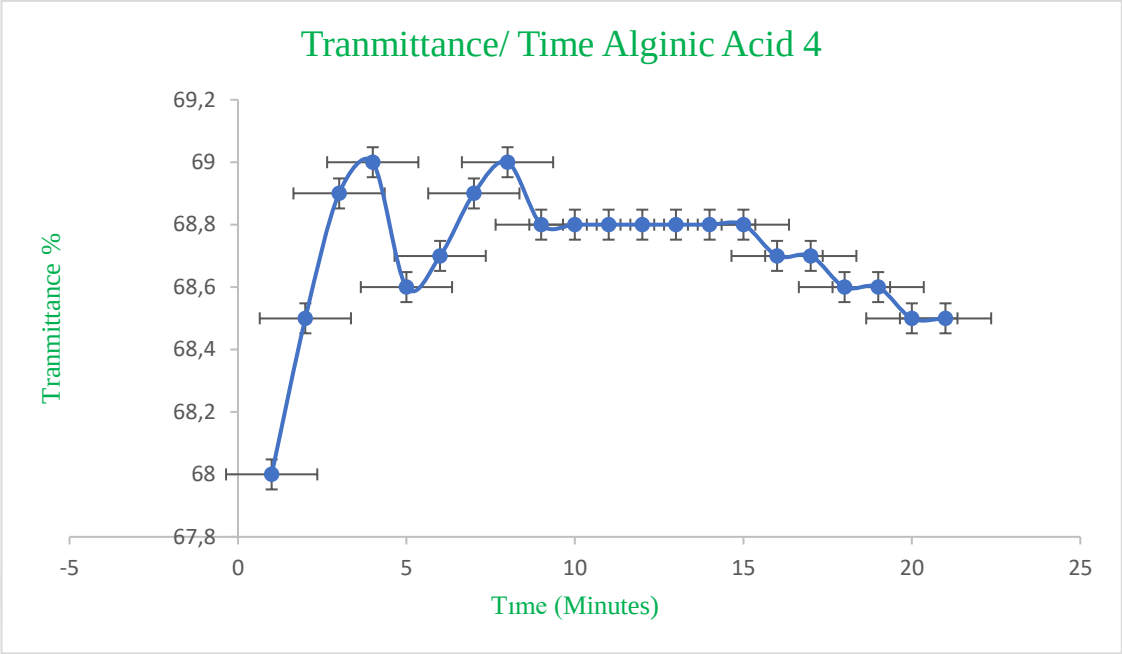

Fig.36: Swelling transmittance graph for Alginic Acid 4 sample.

4.4. The Samples:

After the synthesis of the samples, they were left to dry in a chocolate square mould. The materials take up to two days to dry. Fig. 43 shows the sample after drying.

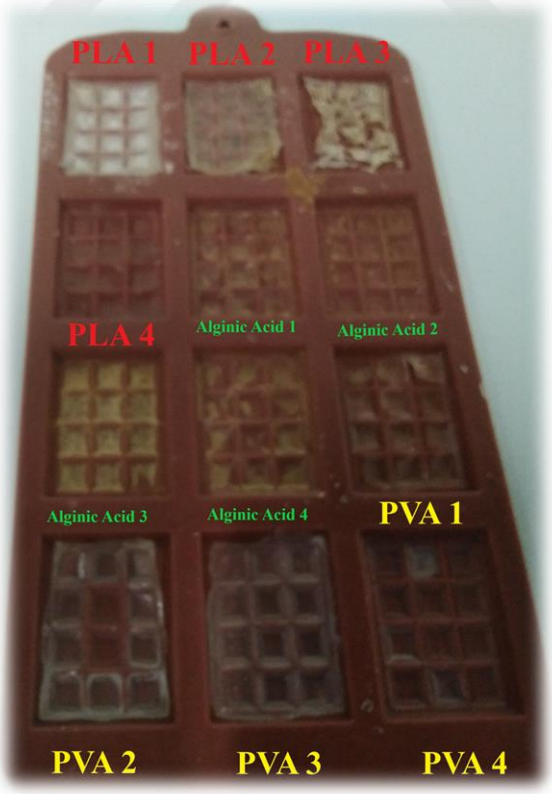

Fig. 37: Dried samples in a chocolate mould.

5. CONCLUSION

As the gelation experiment graphs demonstrate, the transmittance of the samples always decreases regardless of the composition. During the gelation process, temperature of the samples always decreases regardless of their composition.

The samples in general have a good water absorbance ability as all the samples showed a significant increase in its weight as it absorbed distilled water. The transmittance ability of the samples should increase during the absorption process as more water was getting into the samples making it more transparent. The swelling transmittance of Alginic acid/chitosan/ WS_2 composite increases the most as the graphs show.

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