Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon
College of Materials Engineering
Department of polymer and petrochemical industries

Preparation and Characterization of Gelatin Blends Membranes by Electro Spinning Method for Anti-Bacterial Appliations

A Thesis

Submitted to the Council of the College of Materials Engineering University of Babylon in Partial Fulfillment of the Requirements for the Master Degree in Materials Engineering / Polymer

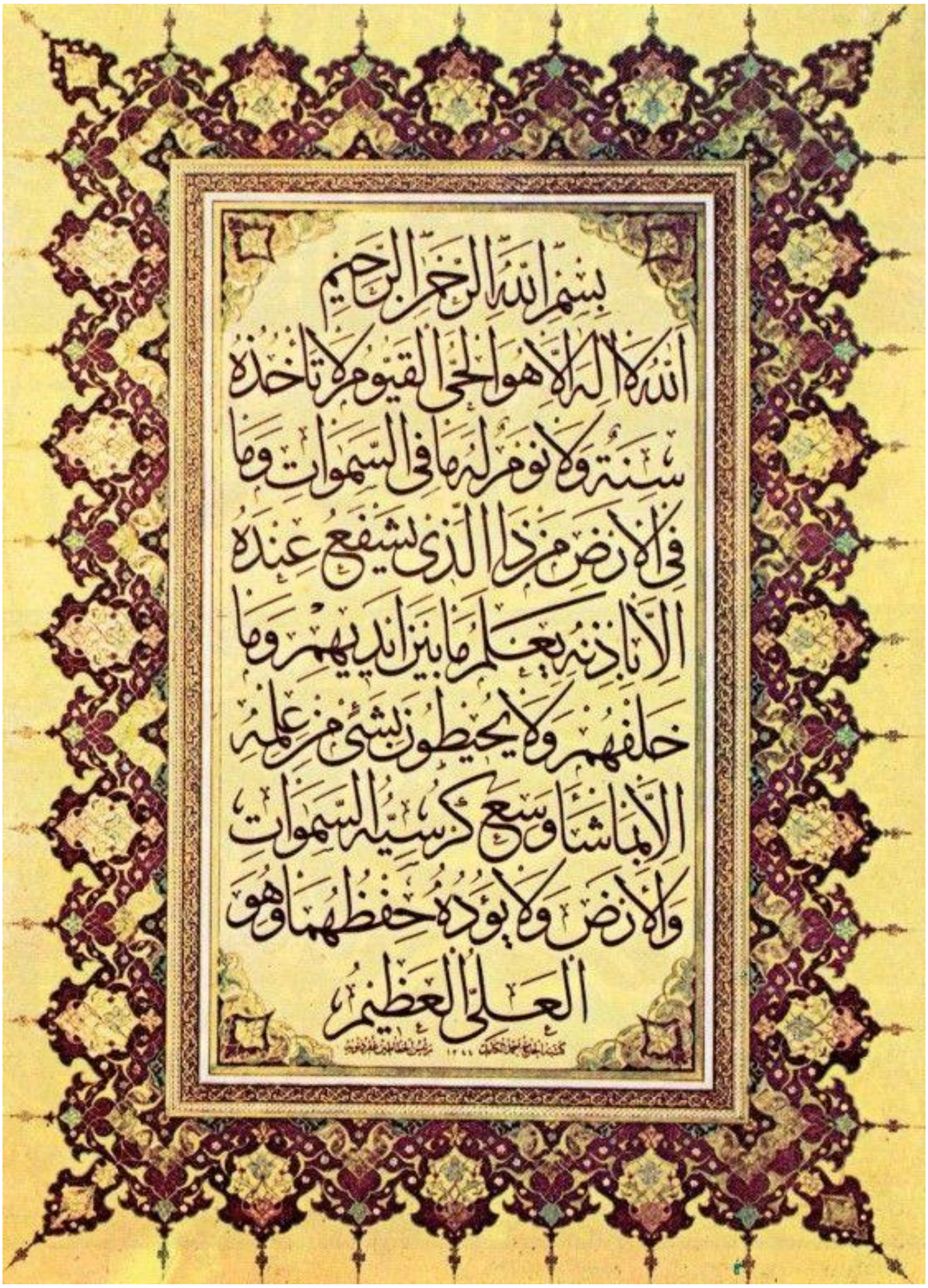
By

Mushreq Kareem Abdulkadhim Shubaduh
(B. Sc. in Materials Engineering, 2000)

Supervisor

Asst. Prof. Salih A. Habeeb (Ph.D.)

2022 A.D 1444 A.H


بسم الله الرحمن الرحيم
الله لا إله إلا هو الحي القيوم لا تأخذه
سنة ولا نوم له ما في السموات وما
في الأرض من ذا الذي يشفع عنده
إلا بإذنه يعلم ما بين أيديهم وما
خلفهم ولا يحيطون بشيء من علمه
إلا بما شاء وسع كرسيه السموات
والأرض ولا يؤده حفظهما وهو
العلي العظيم

Supervisors Certification

We certify that this thesis entitled **(Preparation and Characterization of Gelatin Blends Membrane by Electro Spinning Method for Anti-Bacterial Appliations)** was prepared by (Mushreq Kareem Abdulkadhim) under our supervision at Babylon University / College of Materials Engineering /Department of polymer and petrochemical industries, in Partial Fulfillment of the requirements for the Master Degree in Materials Engineering / Polymer.

Signature

Assist Prof. Dr. Salih A. Habeeb

(Supervisor)

Date: / / 2022

Dedication

To
The One whose throne is in the sky ... ...
The One whom we praise and thank… Allah
To
Our great prophet Mohammad and his relatives (peace and blessings of Allah be upon him and them) ….
To
The One whose the words would not be able to thank him,
Assist Prof. Dr. Salih. A. Habeas
To
The one who is my light and teacher in life, my dear father….
To
The one who is the mercy of Heaven to the secret of my existence, strength and joy to the fountain of love and compassion my beloved mother….
To
The shoulder of tenderness and miracle of all Time my brothers... ... my sisters ... ...
To
The warm heart and generous to the best and most beautiful wishes in all my life... ... ...my wife... ... ... my children with respect.

Mushreq Kareem 2022

Acknowledgments

- Firstly, all the thank and praise be to **ALLAH** in enabling me to achieve this study.
- I would like to express my deepest gratitude to my supervisor, Assist. Prof. Dr. Salih Abbas Habeeb, for his constant encouragement and guidance throughout this work. Thank you for your guidance and great effort in this study.
- Thank you to all my professors at the College of Materials Engineering / Department of Polymer Engineering and Petrochemical Industries for their appreciative support.
- I extend my sincere thanks and gratitude to the laboratory staff of the College of Materials Engineering / Department of Polymer Engineering and Petrochemical Industries for helping me in my tests to complete this research.
- I also thank the staff of the College of Science / Department of Chemistry / Physics Laboratory, University of Babylon, especially Mrs. Suhaila Mahdi Wahoudi for helping me conduct some tests to complete my research.
- Finally, I would like to express my appreciation and love to my family members and friends for their support and assistance in completing my research requirements.

Mushreq Kareem 2022

Abstract

This present study designed to prepared and characterize nanofibers and to improve the electrospinning possibility when mixing two groups of natural polymers, each group consisting of three biopolymers. The first group consists of solutions for gelatin, chitosan and sodium alginate (G:CS:SA) according to a series of mixing ratios of 80:10:10, 60:20:20, 40:30:30, and 20:40:40. Whereas the second group consists of solutions for gelatin, chitosan, and pullulan (G:CS:PUL) at series mixing ratios of 80:10:10, 60:20:20, 40:30:30,and 20:40:40. The properties effect of the solutions for the two groups was studied by evaluating the electrical conductivity, viscosity and surface tension. In addition to studying the morphological properties of the bio-nanofibers using field emission scanning electron microscopy (FE-SEM) to evaluate the diameters of the nanofibers, their distribution and the formation of beads .The results showed that increasing the ratio of CS with SA above 30% in(G:CS:SA) led to a decrease in polymer viscosity and average fiber diameters, while increase the formation of beads. The nanofibers with mixing ratios of 80:10:10 and 60:20:20 of G:CS:SA are more uniformity and contain few beads. In addition to that, X-Ray Diffraction(XRD) showed the highest crystallinity in the above ratios and good bonding between G, CS, and SA molecules in nanofibers according to the Fourier Transform Near- Infrared Spectroscopy(FT-NIR). As for the second group of natural polymers, the increase in chitosan with pullulan resulted in lower viscosity and average diameters without beads formation up to ratio 40:30:30 from (G:CS: PUL), while the crystallinity ratio were high at 80 :10:10 and 60:20:20 as a result of increased bonding between nanofiber molecules, which was shown by X-Ray Diffraction and Fourier Transform Near - Infrared Spectroscopy. The electrical conductivity of natural polymer solutions is an indicator of the increase in surface tension and contact angle with water depending on the increase in the proportions of chitosan, pullulan and sodium alginate in the polymeric solutions. In accordance with the above results,

80:10:10 and 60:20:20 represent the best blending ratios of the two groups, ratios used in the release analysis for the purpose of determining the efficiency of biopolymer fibers in food packaging and protecting from bacteria. Therefore, The mixing ratio of 60:20:20 in the second group was the best in both groups which has better resistance to bacterial growth and highest inhibition zones with diameter(24mm and 22mm) against Escherichia coli and Staphylococcus aureus respectively.

List of Contents

Chapter Four : Results and Discussion

Chapter Five : Conclusions and Recommendation

List of Abbreviation and Symbols

Abbreviation	**Meaning**
AA	Acetic Acid Glacial
AFM	Atomic Force Microscopy
ASTM	American Society for Testing Materials
°C	Celsius
CS	Chitosan
DIW	Deionized Water
E.coli	Escherichia coli
FDA	Food and Drug Administration
FE-SEM	Field- Emission Scanning Electron Microscopy
FT-NIR	Fourier Transform Near-Infrared Spectroscopy
G	Gelatin
GA	Guluronic Acid
ISO	International Standards Organization
K	Consistency Coefficient
MA	Mannuronic Acid
MW	Molecular Weight
N	Power law Index
Nm	Nanometer
PEO	polyethylene Oxide
PUL	Pullulan

R^2	Correlation Coefficients
SA	Sodium Alginate
S.aurens	Staphlococcus aurens
Sa	Surface Roughness
Sdr%	Developed Interfacial Area Ratio
Sq	Root-Mean-Square Height
Sz	Maximum Height
Tg	Glass Transition Temperature
UV	Ultraviolet-Visible Spectrophotometer
W.C.A.	Water Contact Angle
Wt.%	Weight Percent
XRD	X-Ray Diffraction
Γ	Shear Rate
δ	Shear Stress

List of Figures

List of Tables

Chapter One

Introduction

1.1 Introduction

Electrospinning is a simple and effective technique in which natural and synthetic polymers are used in the formation of nanofibers. Because of the high efficiency of nanofibers, they have been used in several different fields, such as efficient food packaging [1, 2], water filtration [3], drug delivery system, and antibacterial [4]. Because of the dangers of synthetic polymers and their effects on human health, it has drawn the attention of scientists to the increased use of biopolymers such as (polysaccharides and proteins), as many biopolymers have been tested for the production of fibers by electrospinning. There are many variations on the use of biopolymers in the electrospinning process, so that several biopolymers can be used as a group, which is implemented by applying a solution containing several compounds [5]. Nanotechnology is an effective technology that has been used in the food industry and processing, as this technology serves as a new tool for pathogen discovery, food packaging, drug delivery systems, and the delivery of biologically active treatments to affected areas. The application of this technology in the food industry will provide new ways to protect food from spoilage and maintain its nutritional value [6].

Gelatin (G) is a natural polymer used in many biomedical and industrial applications due to its biocompatibility and biodegradability. The nanofibers are formed from gelatin using organic solvents such as acetic acid, which improve the electrospinning process of solutions [7], and it has good functional properties, which are used in a wide range of industries such as pharmaceuticals, cosmetics, foodstuffs, and packaging. Pure gelatin is brittle and has a high moisture absorption. To improve some of the properties of gelatin, it is mixed with polymers, such as chitosan [8].

Pullulan(PUL) is a polymeric polysaccharide commonly extracted from the yeast-like fungus aureobasidium pullulans. It dissolves easily in water and is an FDA-approved food additive , making it ideal for food preservation. Because it lacks functional groups, it must form a copolymer with proteins that provide these bindings, such as gelatin [9].

Pullulan has good mechanical properties, tasteless, non-toxic, edible, possesses the property of an oxygen barrier, retains moisture well, and also prevents the growth of fungi. These important properties make pullulan a good material for food preservation and the food industry [10].

Chitosan (CS) is a natural biopolymer used in a wide variety of applications such as food storage, water filtration, and wound healing due to its biocompatibility, solid structure, non-toxicity, resistance to mold, biodegradability, and antibacterial effect against many strains of bacteria from By incorporating it into the electrospinning process [11]. Sodium alginate (SA) is a polysaccharide polymer that has been widely used in the food industry because of its edible nature, excellent biocompatibility, non-toxicity, and relatively low cost [12].

Biodegradable polymers from natural sources are divided into two groups: edible and non-edible. Biodegradable materials derived from food sources such as proteins, polysaccharides, and lipids are edible as they can be used to manufacture edible films or food contact packaging instead of standard plastic [13]. Polysaccharides, such as chitosan, starch, and alginate, have been used as biopolymers to create edible membranes to reduce petrochemical packaging used in food packaging that needs a place to dispose because it is non-biodegradable and non-renewable. Thus, there is a need to use alternative, renewable and biodegradable packaging materials, such as natural polymers which are materials made from natural ingredients that can be eaten by humans or animals without health risks. Natural polymers are used in the manufacture of edible coatings and membranes that surround the surface of food. Natural polymers are classified into polysaccharides, proteins, and lipids [14].

The formation or dissociated of protein - polysaccharide complexes and their solubility depends on parameters such as the type of biopolymers involved and their concentration, the pH, ionic strength, calcium ion concentration, and temperature [15]. The polysaccharides could be divided into three categories according to their electrospinning behavior. The first category was able to form fibers, the second category was able to form a jet but no fibers, and the third category was not able to form a jet at all [16].

Many natural biomaterials have high viscosities at low concentrations and simply form strong hydrogen bonds in aqueous solution, making them unsuitable for electrospinning. Therefore, reducing the surface tension and improving the mobility in the electric field is one of the most practical ways to increase and improve the spin capacity of natural biomaterials .To overcome this difficulty, it is necessary to choose a solvent that evaporates quickly, while preserving the molecular structure of the biopolymer, as well as to physically blend the bio-based polymers [17].

Highly concentrated solutions form more uniform fibers with a few beads and large diameters. The number of entanglements increases with increasing viscosity, resulting in uniform fiber movement (low whipping instability) with a small number of beads. These entanglements also prevent the plane from collapsing during the process [18,19].

The beads are formed mainly when the surface tension increases, and as the surface tension decreases, nanofibers without beads are obtained. Another important parameter, the solvent, can affect the number and size of the beads, and the morphology of the nanofibers as well [20].

The concentration and molecular weight of the polymers affect significantly and importantly on the diameter of the nanofibers compared to the effect of surface tension and electrical conductivity where are less [21]. The selection of the solvent is one of the key factors for the formation of smooth and beadless electrospun nanofiber. Usually two things need to be kept in mind before selecting the solvent. First, the preferred solvents for electrospinning process have polymers that are completely soluble. Second, the solvent should have a moderate boiling point gives an idea about the volatility of a solvent[22], in general, volatile solvents are chosen because their high evaporation rates promote easy evaporation of the solvent from the nanofibers during their journey from the needle tip to the collector. However, highly volatile solvents are mostly avoided because their low boiling points and high evaporation rates cause the jetting to dry out at the needle tip. This drying will clog the tip of the needle and thus will hinder the electrospinning process. Similarly, less volatile solvents are also avoided because their high boiling points prevent their dehydration during nano jets. Deposition of solvent-containing nanofibers on the collector will lead to the formation of granules in the nanofibers [23].

To overcome the challenges of chitosan's electrospinning ability, a blend with a polyelectrolyte polymer for its ability to be negative due to pH conditions, and thus gelatin, a natural biopolymer produced by hydrolyzed extraction of animal collagen, is proposed as an alternative to blending with chitosan [24]. Chitosan and gelatin are both biocompatible, non-toxic, biodegradable and non-immunogenic. Chitosan has antibacterial properties and aids in wound healing. A mixture of chitosan and gelatin, especially in a nanofiber form, appears to be a suitable polymer blend for biomedical applications and food packaging based on all these outstanding features [25,26], In addition, these fibers have a high surface area and low pore size [27]. Nanofiber membrane is used in food packaging to preserve some perishable foods such as fruits, meat, fish, and mushrooms after they are processed into commodities or purchased by consumers. This kind of food has strict storage requirements. The most common storage method is refrigeration or freezing. However, this method has drawbacks, such as the loss of nutrients or change in texture after thawing. Electrospinning nanofiber membrane has the characteristics of containing numerous antioxidant and antibacterial polymers active sites, which can achieve lasting and efficient protection improvement from food surrounding environment or from the surface environment of the food , so as to defend food from microbial or oxidation [28].

Nanofibers can be fabricated from different polymers, and where desirable properties cannot be achieved by using a single polymer, blending two or more polymers can result in a new material with desired properties while preserving the positive features of each individual polymer [25].

Preserving food through the packaging process is one of the important basics in the field of the food industry, as innovation has been activated in this field due to consumer need, increasing population, long-distance travel, and maintaining food quality without spoilage [29].Gelatin/chitosan nanofiber has excellent physicochemical properties such as antibacterial and antioxidant property, and can effectively preserve practical foods such as (meat, vegetables, fruits) [30]. For this purpose, different techniques of electrospinning are used to form different fibers to deliver various nutrients. Electrospun fiber is not only used for packing dehydrated foods such as wheat flour, rice, grain and dried fruits, but also widely used for preserving various fruits, vegetables, meat and other cooked food [31]. In this study, gelatin is chosen as a basic material for its high electro-spinning ability to form a uniform nanofiber from blends with two groups of natural biopolymers such as (gelatin, chitosan and sodium alginate) and second group (gelatin, chitosan and pullulan) for antibacterial applications.

1.2 The aims of this study

The main aims of this work is:-

1. Produce nanofibers from edible natural polymers used in food packaging and to protect them from microbes that cause spoilage.
2. Use of natural biopolymers that are non-toxic, available in the markets and of low cost.
3. Improve the ability of natural polymers to electrospinning without using synthetic polymers by mixing natural polymers, which classified by two groups as (G: CS: SA) and (G: CS: PUL) for antibacterial applications.

Chapter Two

Theoretical Part

&

Literature Review

2.1 Introduction

This chapter consists of two main sections. The first section focuses on the techniques used in the fabrication of nanofibers, the advantages and disadvantages of each method, the factors affecting the quality and uniformity of the fibers during the electrospinning process, especially solution parameters (such as concentration, viscosity, surface tension, conductivity, etc.), and environmental factors such as the temperature and humidity. Natural polymers used in the electrospinning process, their properties and applications in the field of food packaging, especially polysaccharides and proteins.

The second section includes previous studies of the relevant electrospinning and bio-nanofibers used in food packaging and protection.

2.2 Natural polymers

Natural polymers are compounds from nature whose fundamental components are the macromolecules formed by the repetition of small molecules called monomers. Among these large molecules are polysaccharides and proteins [32]. Similarly, polysaccharide films operate as barriers because they are odorless, tasteless, transparent, oxygen-resistant, and semi-permeable to carbon dioxide [33]. Polysaccharides have a wide range of molecular properties in weight, solubility, and structure, making them essential factors when applied in biopolymer-based fields. Delivery systems can be used not only to prevent encapsulated parts from collapsing during processing and storage, but to promote continuous and slow release [34]. Chitosan, b-glycan, modified starch, pullulan, and dextran are the most common polysaccharides used to develop nanofibers. On the other hand, the production of nanofibers from proteins by electrospinning is still a challenge because their chemical structure is very complex and their structure is three-dimensional [35], Currently, proteins are used in electrospinning by making mixtures with other polymers and several solvents [36].

2.2.1 Classification of natural polymer

Natural polymers are classified into two source: -

1. Plant source such as (Starch, Pectin, Cellulose, pullulan).
2. Animal source such as (Sodium alginate, chitosan, gelatin) [37].

2.2.2 Advantages of natural polymers

1. Biodegradable – Naturally occurring polymers produced by all living organisms. They show no adverse effects on the environment or human being.
2. Biocompatible and non-toxic – Chemically, nearly all of these plant materials are carbohydrates in nature and composed of repeating monosaccharide units. Hence they are non-toxic.
3. Economic - They are cheaper and their production cost is less than synthetic material.
4. Safe and devoid of side effects – They are from a natural source and hence, safe and without side effects.
5. Easy availability – In many countries, they are produced due to their application in many industries [37].

2.2.3 Polysaccharide

Polysaccharides are defined as macromolecular polymers composed of simple saccharides . The long-range electrostatic interactions in Polysaccharides make them a challenge for electrospinning [38].

Polysaccharides are the most important biopolymers in food, formed by monosaccharides linked together by glycoside bonds, it can be applied in the food industry given their advantages of nontoxicity, edible, and biodegradability [32], due to their hydrophilic character and the structure of chains, they exhibit good gas permeability and low moisture barrier properties, which are two important advantages for food preservation [39].

2.2.4 Proteins

Protein is a heteropolymer of more than a hundred amino acids (monomers) linked by peptide bonds; each of these amino acids contains a central carbon bonded to (a) a hydrogen, (b) a carboxyl group, (c) an amino group, and (d) R-group. Structure of proteins is categorized into four: primary (linear arrangement of amino acids by covalent bond), secondary (amino acids fold to form the shape of helix or sheet by hydrogen bond), tertiary (three dimensional structure as a result of hydrogen bonding, dipole–dipole interactions, covalent bonding or ionic bonding), and quaternary (binding multiple polypeptides to make a large macromolecule by non-covalent bonds) [40]. The basic difference between proteins and polysaccharides is that proteins are heteropolymers consisting of more than at least 20 amino acids, while polysaccharides are mostly homopolymers consisting of a monomer, for instance, glucose [41]. Proteins exist in two forms: fibrous and globular. Fibrous proteins (collagen and fish myofibrillar proteins) are generally water soluble, while globular proteins (soy protein, egg albumin and wheat glia din) are water insoluble, and should be denatured before the film formation [42].

2.3 Methods of forming nanofibers

2.3.1 Drawing

Drawing is another method used to produce fibres, similar to dry spinning. This method requires a sharp tip or a small pipette, which is the main advantage of this technique . In this method, a sharp tip is used to pull liquid fibers from a drop of polymer solution. Due to the large surface area the solvent is gradually evaporated, which causes the liquid fibers to solidify. Hollow glass pipettes can be used in place of the sharp tip with a continuous dose of polymer to avoid the problem of volume shrinkage, which limits the continuous pulling of the fibers and affects their diameter as shown in Figure (2.1) [43].

After being submerged into polymer solution droplet, the micropipette is softly removed from the droplet at a lower speed (about 10^{-4} m/s). As a result, nanofibers are formed by contact the nanofibers drawn from the needle end with the substrate, this process repeated many times on each drop of polymer solution [44]. This method may be used to make continuous nanofibers in any configuration. Furthermore, accurate control of main drawing parameters like viscosity and drawing velocity may be achieved, allowing for repeatability and control of generated fiber measurements [45].

Although this technique is simple, it is restricted to the laboratory level because nanofibers formed singly. It is a discontinuous method with low productivity, and it can also control the fiber diameter. Only viscoelastic materials can withstand the tension generated by the dragging, and depending on the orifice size, just fibers with diameters above 100 nm may be formed.

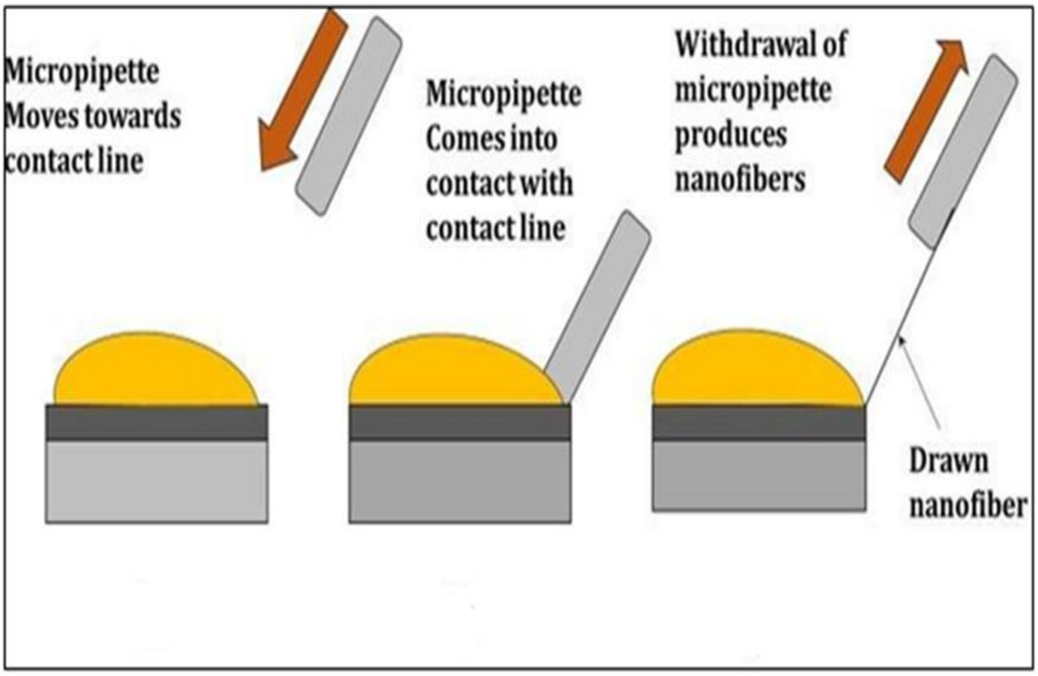


Fig. (2.1) Drawing process to produce nanofibers [43]

2.3.2 Template synthesis method

Using this method, polymeric, metallic, semiconductor or ceramic nanofibers can be produced, using chemical or electrochemical oxidative polymerization, and a porous membrane containing many cylindrical pores of varying thickness (5–50 mm) was also used. This method produces nanofibers using a mold made of aluminum oxide (AL2O3) to obtain the final structure. In this technique, nanofibers are created by passing a polymer solution through nano scale diameter pores under water pressure on one side, causing extrusion of polymer and giving the fibers when in contact with the curing solution, as shown in Figure (2.2). This method cannot produce nanofibers with a long fiber length since only a few micrometers are obtained, and the diameter of these fibers is determined by the size of the membrane pores [46, 47]. Some of the benefits of using this technology are the ability to produce nanofibers of different diameters using multiple moulds.

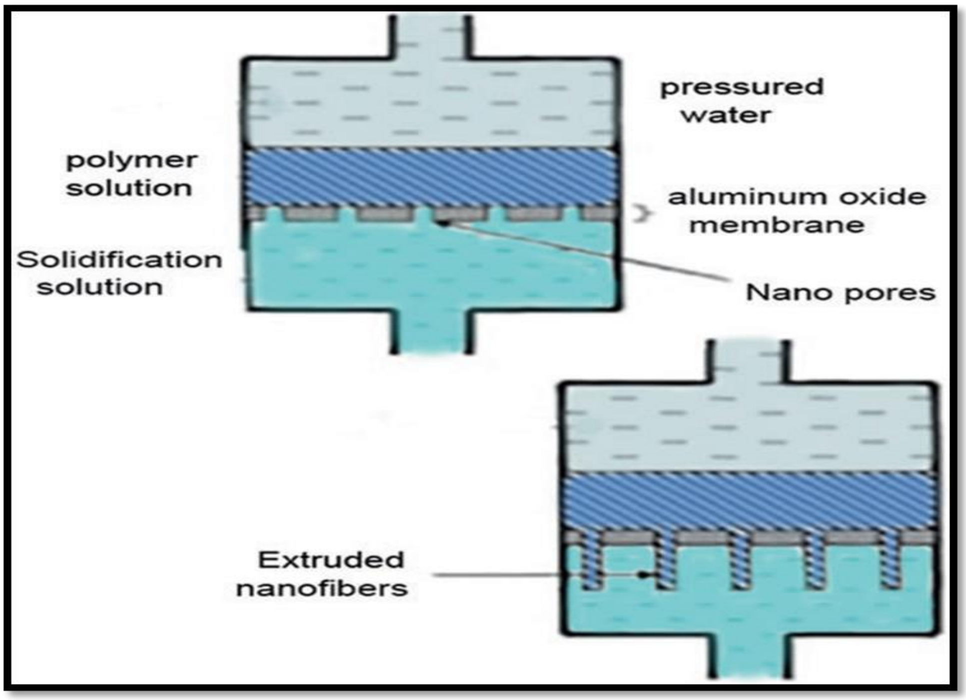


Fig. (2.2) Temple synthesis Technique [45].

2.3.3 Phase separation method

Using this method, the phases will separate due to physical incompatibility. The solvent layer is then separated from the solution while the other phase remains. There are four main stages in this method:

1. Dissolved the polymer with using convenient solvent at room temperature or above.
2. The gelation step is a difficult step because of the difficulty in regulating the porosity of the nanofibers.
3. Extraction of solvent step to separate and remove the solvent from the produced gel by using water.
4. Freezing followed by drying under vacuum step

The characteristics of nanofiber are determined by the concentration of polymer used; if the polymer percentage rises, porosity of the fiber decreases, and the mechanical properties of the fiber increase [45].

The first step in this method is dissolving the polymer at room temperature to make homogeneous solution, after that removing the gel from the solution by holding in temperature of gelation, where nanofibrous matrixes are formed due to phase separation, and eventually the solvent will be extracting followed by the matrix drying step, this eventually leads to the formation of nanofibers. As shown in Figure (2.3). This method requires minimal equipment, and may directly produce a nanofiber matrix, with mechanical properties adjusted by altering the polymer concentration [48].

This technology cannot produce long continuous fibres, and not all polymers can undergo phase separation to form nanofibers since it requires gelation capacity which limits the use of this technology [46].

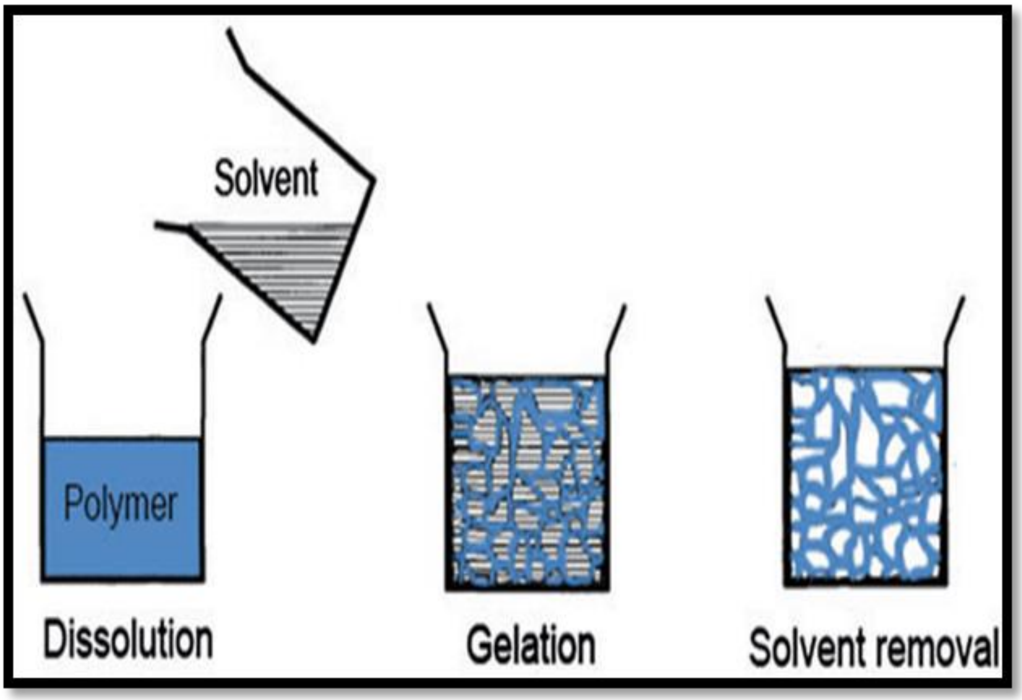


Fig. (2.3) Phase separation process [48].

2.3.4 Self-assembly method

This method involves rearrangement and reassembly of the molecules by themselves to form different shapes and configurations through non covalent forces like hydrogen bond forces and electrostatic interactions, as shown in Figure (2.4) [49].

This method is suitable for manufacturing smaller diameter nanofibers with average diameter lower than 100 nm and length in micrometer scale. Mechanism of his method is by the intermolecular forces which hold the molecules together. Orientation of the molecules determined the final shape of it. The main disadvantages of this method is the complexity, slow, low production scale and complex in maintaining fiber diameters produced [45, 50].

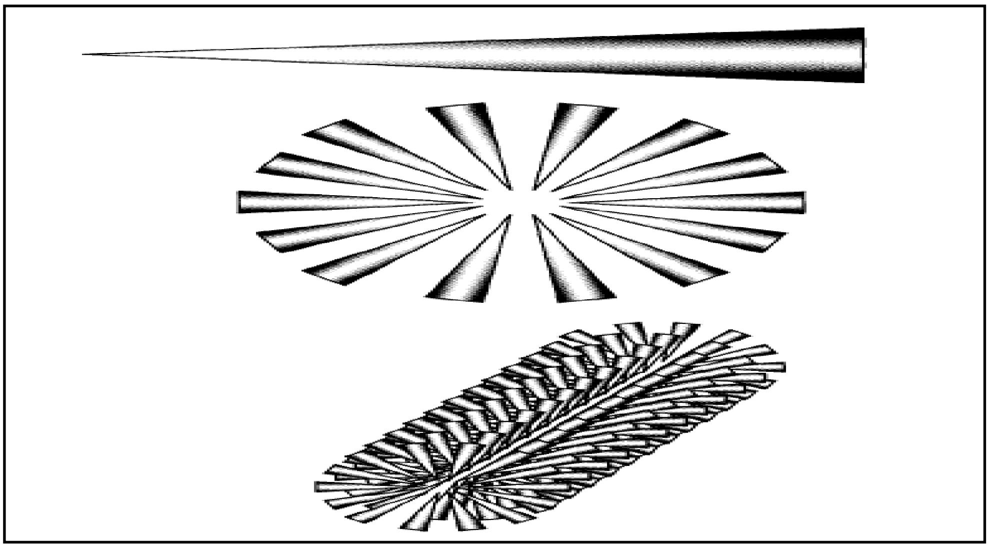


Fig. (2.4) Self-assembly process to create nanofibers [50]

2.3.5 Electrospinning

Electrospinning is a promising, high-throughput and simple method for fabricating nanofibers for polymers, composites and inorganic materials, including carbides, oxides, nitrides and hybrid compounds. In this technique, electrostatic forces are used to produce nanofibers from polymeric solutions [51]. Electrospinning is the most appropriate technology for nanofiber production. The advantages include relative ease, low cost, high speed, wide material selection, and versatility. In addition, this technique allows control of fiber diameter, microstructure, and arrangement [52]. Electrostatic fiber spinning is a technology that uses electrostatic forces to form fibers with diameters ranging from nanometers to micrometers. This technique is a simple method for creating continuous fibers with high porosity and specific surface area [53,54].

Tools used in electrospinning technique:

1. High-voltage DC power supply.
2. Syringe pump.
3. Spinneret (a small diameter needle connected to the syringe).
4. Metal collector.

Before electrospinning, the appropriate solvent is selected to completely dissolve the polymer, in which a homogeneous polymeric mixture is formed, which is placed inside the injection cylinder to conduct the electrospinning process. The positive terminal of the DC power supply is connected to the hollow needle [55], and the negative terminal is connected to the metal collector. With the increase of intensity of the electric field, the repulsive electrostatic force overcomes the surface tension, and the charged jet of the fluid is ejected from the tip of the Taylor cone. The discharged polymer jet undergoes an instability and elongation process, which allows the polymer in the jet to become very long and reduces the diameter of the extruded polymer fiber. The solvent that is used to dissolve the polymer evaporates, and the polymer in the jet is dried. The solvent evaporation depends on the distance between the tip and collector, the solution vapor pressure, and the inside chamber temperature. Stable environmental conditions are therefore important in getting good quality nanofibers. The maximum applied voltage for a needle electrospinning setup is normally less than 30 kV and is also highly humidity dependent [56]. as shown in Figure (2.5) and Table (2.1) shows the advantages and disadvantages of the above-mentioned techniques.

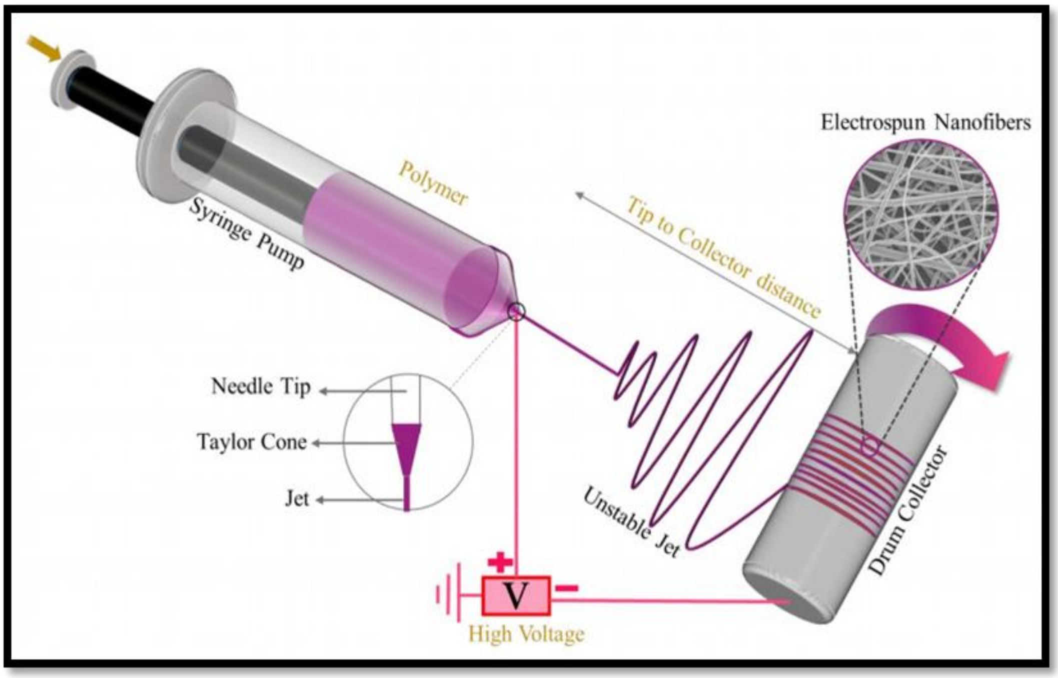


Fig. (2.5) Electrospinning Setup [57]

Table (2.1) Advantages and Disadvantages of nanofibers methods [58].

Process	**Advantages**	**Disadvantages**
Drawing	Lower equipment needs	Discontinuous process
Template Synthesis	Produces fibers with different diameters	
Phase Separation	Directly manufacture nanofiber matrix using minimum equipment. Consistency from batch to batch is easy to achieve. The matrix's mechanical characteristics may be modified by adjusting the polymer concentration.	Limited to specific polymers.
Self-Assembly	Suitable for producing a smaller nanofiber	Complicated process
Electrospinning	Can produce long and continuous nanofibers, cost- effective	Instability of jet

2.4 Important electrospinning Parameters

The electrospinning process is governed by a number of parameters that are categorized into three: process parameters, solution parameters, and ambient variables. By adjusting these parameters, which have a significant impact on the shape of the electrospinning fibers, nanofibers will be manufactured with the required shape and diameter [59].

2.4.1 Solution parameters

2.4.1.1 Concentration

The concentration of the polymeric solution plays a crucial impact in the specific properties of Nano-fibers structures produced by the electrospinning process; for example, lower solution concentrations produce a large number of beads, while higher concentrations produce fewer beads. The diameter of the fiber is also affected by the concentration of the solution; the higher concentration increases the diameter of the fiber. It has been shown that highly concentrated solutions generate more homogeneous fibers with few beads [60,61], because of the difficulty in maintaining the flow of the solution at the tip of the needle, resulting in the formation of larger fibers, there must be an optimised solution concentration for the electrospinning. At low concentrations, beads are formed instead of fibers, and at high concentrations, the formation of continuous fibers is prevented [62]. With gelatin electrospinning, researchers attempted to find a relationship between solution concentration and fiber diameter and discovered a power law relationship, in which increasing solution concentration increases fiber diameter [63,64]. The range of concentrations from which continuous fibers can be generated in electrospinning is also determined by solution surface tension and viscosity [65]. Increasing the concentration of the polymeric solution leads to a change in the shape of the beads from spherical to spindle-like. Additionally, electrospinning variables like polymer concentration have a significant impact on nano-fiber alignment [66]. The polymer concentration, electrostatic field, and gap distance are all influence the rate of solidification. As a result of the quick stretching and solidification of the polymer chains, another crucial impact occurs: the crystallization process is slowed since the stretched chains do not have enough time to form crystals. The nano-fibers are strongly orientated, despite the fact that the polymer chains are not crystalline at high concentrations. This causes a crystallization defect and lowers the glass transition temperature (Tg) [67].

2.4.1.2 Molecular weight.

This is another key for polymer that impacts the structure of the electrospun fiber. In electrospinning, high-molecular-weight polymer solutions are generally used because they give the appropriate viscosity for fiber formation. It has been shown that polymer with a low molecular weight tends to produce beads rather than fibers, whereas a solution with a high molecular weight produces fibers with greater average diameters as shown in Figure (2.6). The amount of entangled polymer chains in a solution is reflected in the molecular weight of the polymer, resulting in solution viscosity [68]. In general, it is preferable to use high molecular weight polymers because they cause strong entanglement in the chains and facilitate the production of nanofibers through electrospinning technology. As for solutions with lower molecular weights, they form granules or break into droplets during the electrospinning process [69]. The polymer's molecular weight has a big impact on rheological and electrical characteristics, including viscosity, dielectric strength, surface tension, and conductivity [70].

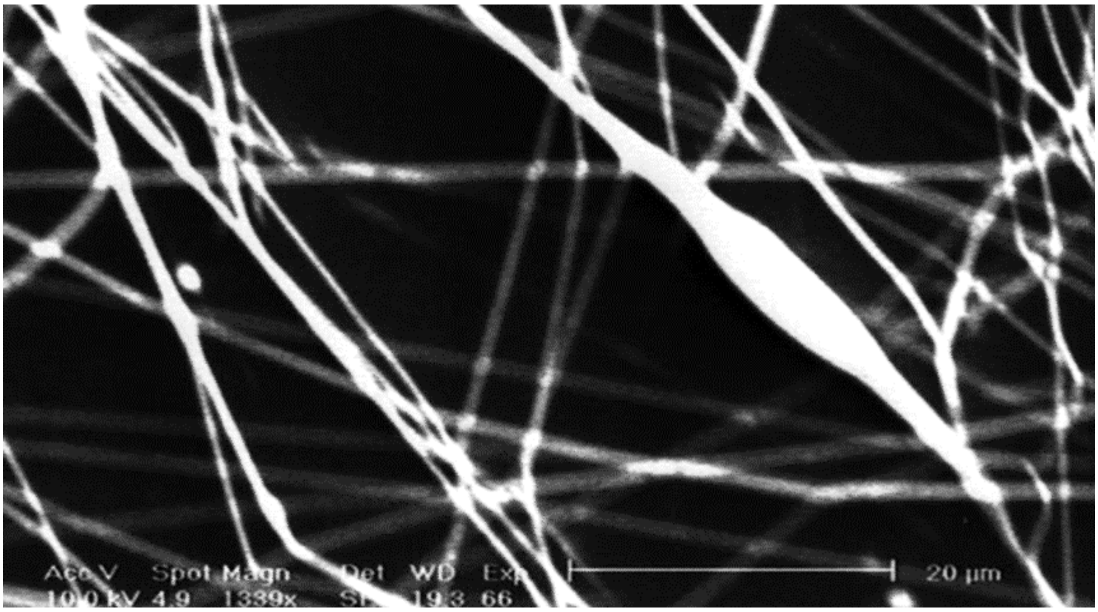


Fig. (2.6) Electron micrograph of electrospinning nanofibers forming with varying diameters [70].

2.4.1.3 Surface tension

Surface tension is very important in the electrospun process, and fibers can be created without beads by reducing the surface tension of a Nano fibrous solution as shown in Figure (2.7), which may be most dependent on the type and composition of the solvent used in the electrospinning process, while high surface tension produces droplets instead of nanofibers in the electrospinning process due to the instability of the jets [20].

The surface tension of a solution affects the production of droplets, beads, and fibers, and a reduced surface tension of the solution allows electrospinning to happen at a lower electricity field [65].

The polymeric solution should have enough charges to overcome the electrical forces induced by the ions in the solutions. When a solution's surface area is reduced by surface tension, it forms a spherical droplet. When the solution's polymer concentration is low, the greater proportion of solvent molecules has a stronger propensity to collect, and surface tension causes a spherical shape to form, leading to the creation of beads. When a fluid is very viscous, the polymer and solvent molecules interact more, and the solvent molecules spread out over the tangled polymer molecules, leaving a uniform fiber on the collector [71].

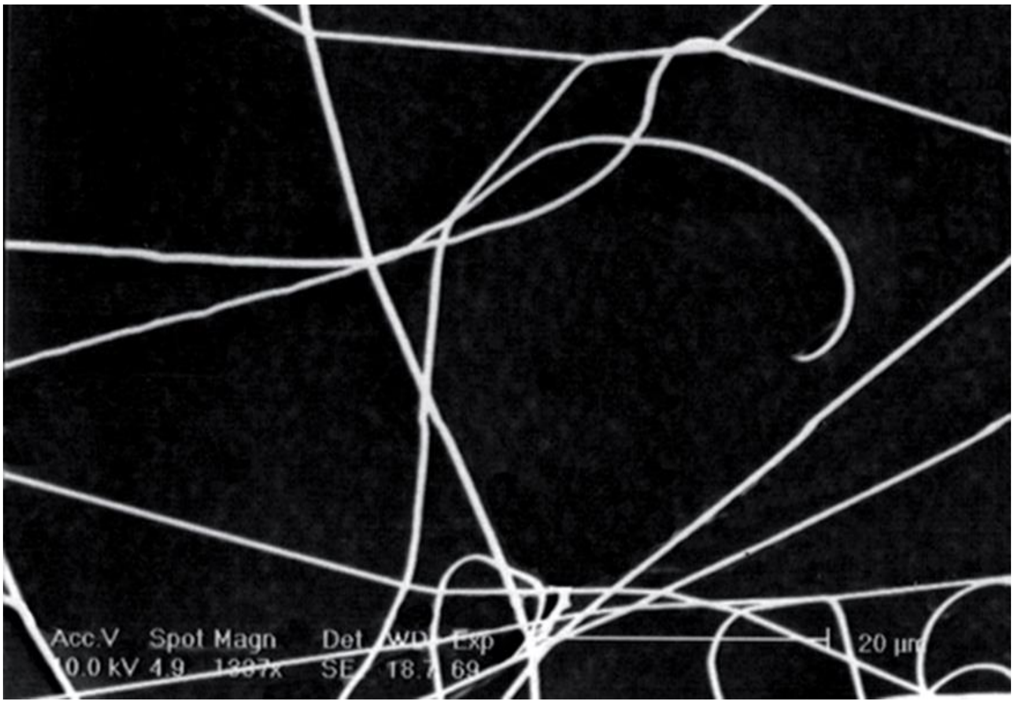


Fig. (2.7) Electrospun nanofiber electron micrograph without bead formation. [70]

2.4.1.4 Viscosity

The viscosity of the polymeric fiber solution plays a critical role in determining fiber size and form during electrospinning. Continuous fiber production is difficult with very low viscosity, and ejection of jets from the polymeric solution is impossible with very high viscosity. This means that an optimized electrospinning viscosity is needed [62]. For low-viscosity solutions, beads have a circular droplet-like form, but as the solution viscosity increases, the beads' morphologies shift from a stretched droplet or elliptical shape to a smooth fiber-like structure [72]. The solution viscosity is an important parameter that influences the fiber size and morphology when stretched from the needle tip to the collector during the electrospinning process. The entanglements of the highly viscous polymer solution prevent the jet from breaking up during the process, thus forming uniform fibers without beads. The low viscous polymer solution jet breaks up into small droplets or creates beaded fibers. If the viscosity is too high, it will be difficult to pump the solution through the capillary, and the solution may dry up or drop at the tip [71], when the viscosity is high there is a strongly interaction occurred between the molecules of solvent and the molecules of polymer as shown in the Figure (2.8).

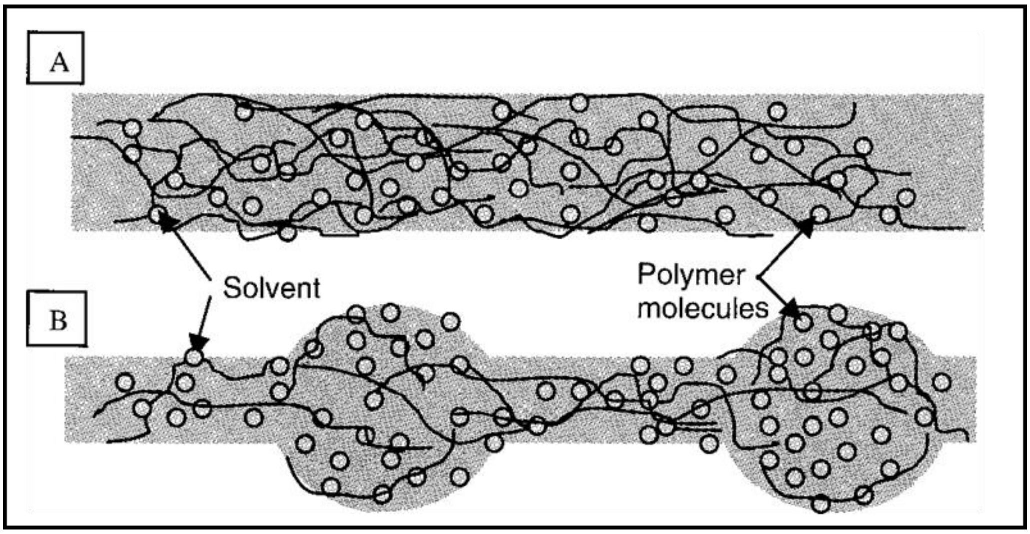


Fig. (2.8) [A] High viscosity, aligned and oriented solvent molecules between polymer molecules. [B] Solvent molecules with a lower viscosity tend to agglomerate due to the surface tension impact [46].

2.4.1.5 Conductivity/surface charge density

Solution conductivity not only affects the development of Taylor cone, but it also helps in the control of nanofiber diameter. If the solution has poor conductivity, a Taylor cone will not develop on the droplet's surface, preventing electrospinning. An increase in solution conductivity leads to a decrease in fiber diameter as well as an increase in the charge on the droplet's surface, producing a Taylor cone [73]. The solution's electrical conductivity is very important for obtaining smooth fibers with smaller diameters; hence, as the solution's electrical conductivity improves, charges in the solution and on the transfer jets rise. Adding salts or ions to a solution can enhance conductivity; when proteins and medicines breakdown in water, they form ions. Charges are important in extending the solution, and increasing the charges allows the solution to stretch more, leading to a smoother fiber diameter [74]. Another advantage of increasing charges in the solution in which bending instability improved this leads to increase the area where fibers being deposited [75].

Additionally, when ions improve the electrical conductivity of a solution, the amount of critical voltage required in the electrospinning process is reduced [76]. For example, in nature, gelatin is a polyelectrolyte polymer. When an electric field is applied, the ions raise the ability to carry a charge of the plane, making it more surface tension. As a result, gelatin is less able than synthetic polymers to produce fibers [67].

Most polymers are conductive. With the exception of dielectric materials, charged ions in the polymer solution have a major impact on jet production. The solvent used, polymer type, and amount of ionisable salts all have a role in solution conductivity. When the electrical conductivity of a solution increases, the diameter of the nanofibers decreases significantly. When the solution's conductivity is low, a jet's elongation by electrical force is unable to generate a homogenous fiber, and beads can be seen [77].

2.4.1.6 Effect of humidity and temperature

The diameter and shape of nanofibers are affected by environmental (ambient) factors such as temperature and relative humidity, as well as electrospinning and solution parameters [78,79]. When the relative humidity is low, fewer beads are made. While the high relative humidity leads to the occurrence of porous microstructures due to the effect of evaporation or phase separation [80]. Temperature affects the average diameter of the nanofibers in two ways: (1) it increases the rate of solvent evaporation and (2) it decreases the viscosity of the solution, as increases in solvent evaporation and decreases in solution viscosity which lead to a decrease in the average fiber [81].

2.4.1.7 Effect of solvent dielectric constant

Higher the dielectric constant of the solution gives less bead formation and a smaller diameter of nanofibers. As the dielectric property is increased, there is increase in the bending instability of the jet and the deposition area of the fiber is increased. As jet path length is increased fine fiber deposit on the collector [82]. For the electrospinning method to be efficient, the solutions must have a suitable dielectric constant to form nanofibers [83].

2.4.1.8 Effect of solution volatility

When increasing volatile solvents are used for polymer solution preparation, however, intermittent spinning occurs due to the solidification of a polymer jet at the needle's tip. When the proportional volatility of a polymer solution is raised, the shape and porosity of the fiber change, making a porous microstructure [80].

2.4.1.9 Effect of solvent volatility

The volatility of the solvent solution affects the electrospinning method. Therefore, the generation of smooth and bead-free nanofibers is influenced by the volatility of solvents. Suitable solvents in the electrospinning method should be fully soluble polymers with reasonable boiling points related to solvent volatilization. Low-volatility (high boiling point) solvents should not be used in creating nanofiber jets because they either prevent dehydration during nanofiber jet production or slow down the solidification process due to reduced solvent evaporation [84,85]. In the electrospinning process, common volatility solvents with fast evaporation rates are used to ensure that the solvent is easily evaporated from the tip of the needle to the collector [84]. Table (2.2) also shows the effect of the previously mentioned electrospinning parameters on the morphology of nanofibers.

Table (2.2) showing the parameters affecting the electrospinning method [69]

Solution Properties	**Symbol**	**Effect on morphology and structure**	**Effects on diameter**
Concentration	C ↑	Increasing concentration leads to increase in fiber diameter.	Nanofiber diameter ↑
Viscosity	η ↑	Increasing viscosity leads to thicker nanofibers without beads, but too high viscosity causes generation of beads.	Nanofiber diameter ↑
Solution Electrical conductivity	σ ↑	Increasing conductivity leads to thinner nanofibers.	Nanofiber diameter ↓
Surface tension	Υ	No conclusive correlation has been established between the surface tension and the nanofiber morphology.	-
Molecular weight of polymer	Mr ↑	Increasing polymer molecular weight leads to formation of a nanofiber with fewer beads.	-
Volatility of solvent	$A_{solvent}$	Higher volatility requires higher flow rate and leads to formation of a nanofiber with fewer beads.	-
Solution relative Volatility	A	Porous microstructure appears because of higher volatility.	-
Dielectric constant	ε	Sufficient dielectric constant of the solvent is needed for successful electrospinning.	-
Flow rate	Q↑	Higher flow rate results in thicker nanofibers. Too high flow rate causes the generation of beads.	Nanofiber diameter ↑
Applied voltage	V↑	Higher applied voltage leads to thinner nanofibers.	Nanofiber diameter ↓
Needle diameter	D	Minimum is required to get smoother nanofibers	Nanofiber diameter ↓
Needle tip to collector distance	D↑	Beaded morphology occurs when the distance is too short and the electric field is too strong.	Nanofiber diameter ↓
Geometry of collector	-	Metal collectors with conductive frame or rotating drum are preferred	-
Relative humidity	Ψ	Porous microstructure appears due to evaporation-cooling effects .Lower humidity enables higher flow rate and the generation of beads is reduced.	-
Temperature	T↑	Higher temperature leads to thinner nanofibers.	Nanofiber diameter ↓

2.5 Electrospinning of proteins and polysaccharides

Polysaccharides are the most abundant renewable resource in the environment. The electrospinning capacity of a polysaccharide depends on the length of the molecular chains and the bonding of the molecular chains to each other during expansion [16,86]. Therefore, molecular weight, the concentration of biopolymer solution, their conformation structure and dilution in shear thinning affect their electrospinning properties. The concentration required for the electrospinning of polysaccharides is a concentration in which the polysaccharide molecules become closer to each other so that the molecular bonding of the chain to the adjacent chain could be possible. This situation is associated with stretching and electrospinning. Polysaccharides with a random spiral structure and a low-shear thinning behavior are suitable for electrospinning [16,87].

On the other hand, the creation of nanofibers by electrospinning of proteins remains a challenge. Because of its highly complex chemical structure, it has a three-dimensional structure and multiple electrical properties. This is a common defect [35]. Currently, proteins are used in electrospinning by making mixtures with other polymers as well as a number of solvents [36].

2.6 Biodegradable polymers

According to ASTM and ISO standards degradable polymers are those which undergo a significant change in chemical structure under specific environment conditions. The chemical changes occur in loss of physical and mechanical properties, besides their biodegradability, biopolymers have other characteristics, such as air permeability, low temperature ‘sealability’, availability, and cost. Moreover, natural polymers can have a positive effect on delaying deterioration, extending shelf life, and preserving quality and food safety [88].

2.6.1 Type of Biodegradable polymers

Biodegradable polymers are classified into several different groups depending on their source, chemical composition, synthesis method and application.

1. Natural biopolymers are available in large quantities from renewable sources such as (proteins, Starch, cellulose).
2. Synthetic polymers are produced from non-renewable petroleum resources. besides of their biodegradability such as (PLA, PGA, PBS).
3. Semi-synthetic polymers are produced by blending different biopolymers (natural / synthetic) such as (starch/PLA blends, starch/PCL blends) [89]. shown in the Figure (2.9)

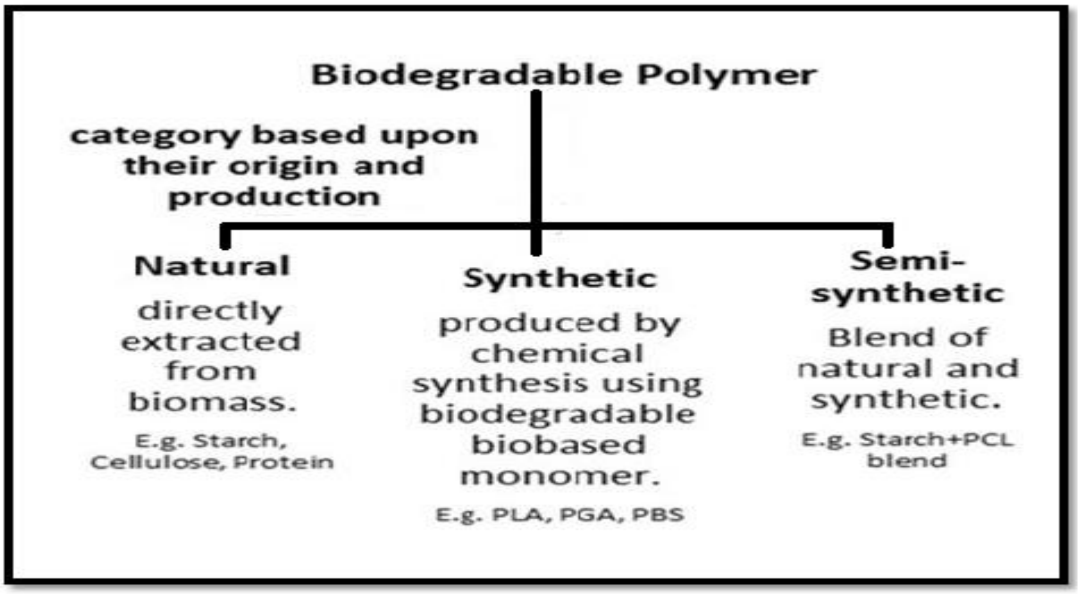


Fig. (2.9) describes the classification of biodegradable polymers [89].

2.6.2 Advantages and disadvantages of biodegradable polymers

Biodegradable polymers have a number of advantages and disadvantages, which are shown in the table (2.3).

Table (2.3) Advantages and disadvantages of biodegradable polymer [90]

Advantages	Disadvantages
Renewable	the lack of arable land (future)
good for the environment	Composability
less energy to produce	single bad properties
easier recycling	the awareness of people
it is not toxic	installations for the production
reduced dependence on oil	processing plants
Reduced emissions co2	short lived

2.6.3 The types of degradation polymers

1. Degradable polymer: The chemical structure changes with the influence of moisture and oxygen.
2. Biodegradable polymers: Polymers are degraded by the influence of naturally occurring microorganisms.
3. Hydrolytic degradable polymers: Polymers are degraded by hydrolysis.
4. Oxidation-degradable polymers: Polymers are degraded by oxidation.
5. Photodegradable polymers: Polymers degrade under the influence of natural light.
6. Thermal degradable polymers – thermally degradable [90].

2.7 Application of nanofiber

Nanofibers are lighter material with higher surface area in comparison to polymeric film. The ease of producing functional nanofiber is another advantage over many nanomaterials. Functional nanofiber in particular has attained a greater interest in recent years. The applications of functional nanofibers are increasing in various technical fields such as water filter membranes, tissue engineering, biosensors, drug delivery systems, food packaging, wound dressings, catalysis, antibacterial [4]. Figure (2.10) shows some applications of nanofibers.

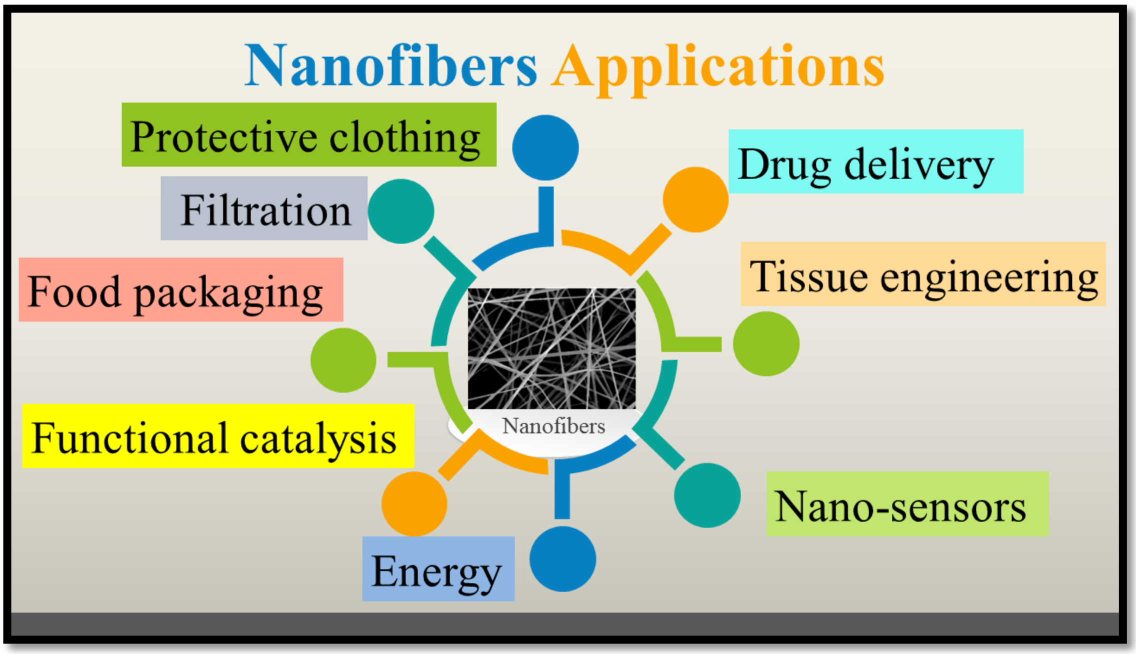


Fig. (2.10) applications of nanofibers [91].

2.7.1 Active packaging

In the active food packaging industry, edible packaging is viewed as a sustainable and biodegradable alternative that improves food quality when compared to conventional packaging. The value of edible packaging can be demonstrated in its ability to preserve food quality, prolong shelf life, reduce waste, and contribute to packing material efficiency. Due to their versatility, potential to be manufactured from a range of materials, and ability to carry various active compounds, such as antioxidants and/or antibacterial agents, edible films are one of the most promising disciplines in food science. The food packaging made of edible materials such as natural polymers, which may be consumed by humans without posing any health risks [92]. Synthetic or natural polymers can be used to make the nanofibers, with the latter possessing biodegradable properties [93]. As a result, an advantage of the electrospinning method is its ease of measurement, repeatability and uses in various fields, as well as the ability to produce continuous and long nanofibers with diameters ranging from 10 to 1000 nanometers, it also has flexibility, high strength, and surfaces area, which allows transporting nutrients and loading biologically active ingredients [94]. Electrospinning uses natural, synthetic, and mixed materials. Biopolymers, or natural polymers, contain proteins, polysaccharides, and fats that are used in food. Because it is biocompatible, low in toxicity, and biodegradable when compared to synthetic polymers, it is preferred to use in industry [95]. The use of nanofibers in food packaging, drug delivery, and wound treatment presents some problems that limit its use. One such problem is that nanofibers are highly soluble. This makes it difficult for compounds to stay for a long time and reduces their protective effect due to the high water property of most of the polymers used in their manufacture [96], in order to reduce the solubility in recent periods, some alternatives have been used, such as a mixture of natural polymers, mainly proteins and polysaccharides, and to increase the active ingredients [97].

The application of electrospun fiber membrane in actual food packaging have been widely studied in recent 10 years, and it is also worthy of further research and discussion in the future [28], which is shown in the Figure (2.11).

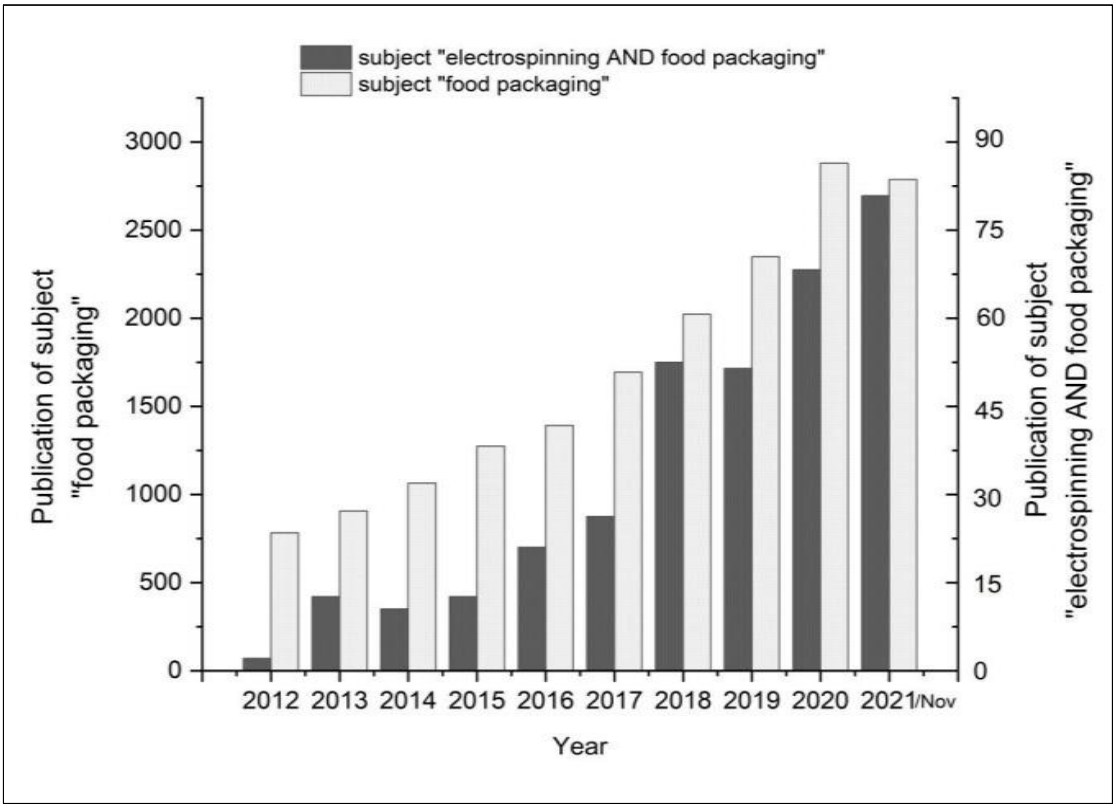


Fig. (2.11) Web of Science counts of the subject of “food packaging” and “electrospinning AND food packaging” from 2012 to November 2021[28]

2.7.2 Packaging

Food packaging is an important technology to ensure food safety in the process of storage and transportation of all kinds of food, food package plays an important role in protecting food from external environmental damage and handing food from producers to consumers [98]. The packaging process is an important area in daily life where biodegradable polymers are used to reduce waste quantities. It has other characteristics such as scalability at low temperatures, air permeability, etc. The use of biodegradable polymers in packaging requires different physical properties that depend on the storage conditions and the product to be packaged [99]. Packing polymer's physical qualities are heavily controlled by its chemical structure, molecular weight, crystallinity, and manufacturing circumstances. The physical properties of packaging need to be taken into consideration depending on what is being packaged and where it is going to be stored [100]. Industrial polymers based on petrochemicals have been used in the field of packaging and on a large range, Because of its poor biodegradability, it has become a large gathering of waste after utilize. As a result, the right approach in order to reduce the difficulties of getting rid of waste and ensure the improvement of product quality is to use natural and biodegradable polymers in making packaging materials due to the increasing demand in the packaging sectors concerned with the environment [101].

2.7.3 Advantages of natural polymer packaging

1- Biodegradable
2- Supplement the nutritional value for foods
3-Reduced packaging volume, weight, and waste
4- Incorporated antimicrobial and antioxidants
5- Low cost and abundant
6- Annually renewable resources [13].

Packaging, agriculture, medicine and other fields are all using biodegradable materials. Biodegradable polymers have seen a rise in popularity in recent years. Biodegradable polymers can be divided into two categories: synthetic and natural. It is possible to make polymers using non-renewable and renewable sources of feedstock [100]. Packaging waste has increased environmental problems. Increasingly emphasis has been placed on the development of biodegradable packaging materials [99].

2.8 Release mechanisms

The mechanism can be classified follows:

1- Degradation-controlled monolithic system: The active ingredient is distributed uniformly throughout in a matrix. The active is released on degradation of the matrix. The diffusion of the active is slow as compared with degradation of the matrix.

2- Diffusion-controlled monolithic system: The active ingredient is released by diffusion prior to or concurrent with the degradation of the polymer matrix. Rate of release also depend upon where the polymer degrades by homogeneous or heterogeneous mechanism.

3-Diffusion-controlled mononuclear (core–shell) system: The active ingredientis encapsulated by a rate-controlling membrane through which the active diffuses and the membrane erodes only after its delivery is completed.

4- Erosion: Erosion of the coating due to pH and enzymatic hydrolysis causes the release of active ingredients [102].

2.9 Materials used

2.9.1 Pullulan(PUL)

Pullulan is widely used in the food industry in the form of edible food bags that dissolve in water or in hot liquids. That is, pullulan acts as an edible coating that is applied directly to the surface of the food that can be eaten with the product. Likewise, it has been used as a drug wrapper due to its biocompatibility and low cytotoxicity. For this reason, since then, research has been carried out on the functional properties of pullulan in films, coatings, and fibers for use in food and medical packaging [103]. pullulan is a linear glucosic polysaccharide produced by the polymorphic fungus Aureobasidium pullulans, is a nontoxic, non-mutagenic Tasteless and edible compound composed primarily of maltotriose units linked in α 2:1 ratio by α -(1,4) and α -(1,6) bonds ratio, this linkage provides oxygen with adhesive properties to form fibers and films that are strong and impervious [104].

Pullulan increases the food’s shelf life as it is not an easily assimilable source of carbon for food spoilage caused by bacteria, molds, and fungi. In water retention, pullulan is also superior to starch, thus preventing food spoilage by drying out [105].

2.9.1.1 Chemical structure of the pullulan

Pullulan is a linear, homopolysaccharide consisting of maltotriose as a building block. Three glucose units of maltotriose are linked through α-(1→4) glycosidic bonds, where as maltotriose units are linked through α-(1→6) bonds. Molecular weight of the polymer is between 10 and 400 kDa, which is shown in Figure (2.12). The chemical formula of pullulan is $(C_6H_{12}O_5)_n$ [106].

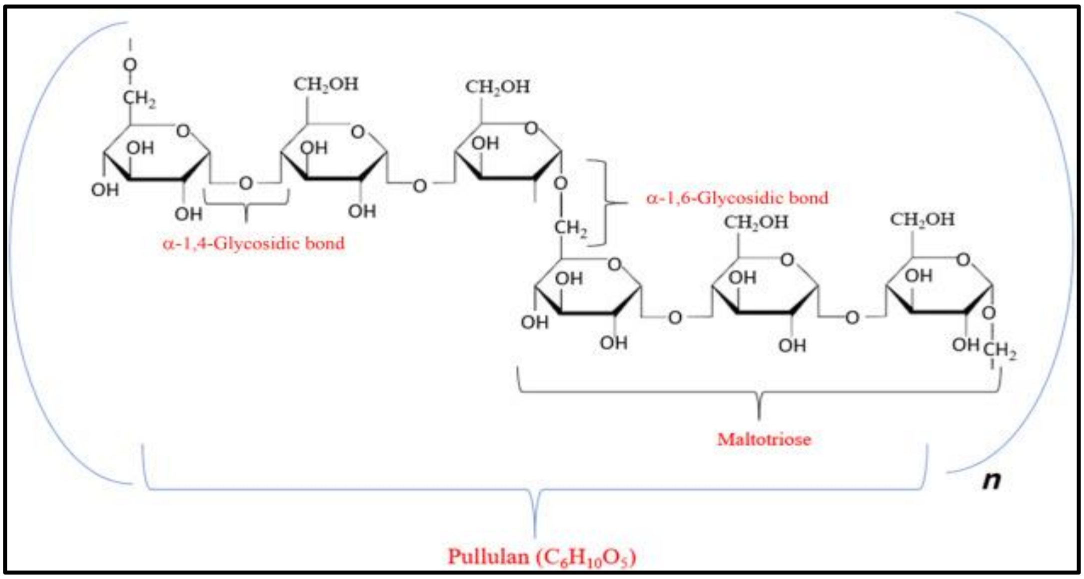


Fig. (2.12) Chemical structure of the pullulan [106].

2.9.1.2 Pullulan properties and applications

Pullulan dry powder is white in color, tasteless, odorless, nontoxic, noncarcinogenic, biodegradable, and edible. Pullulan is highly hygroscopic in nature, and decomposes at high temperatures (250–280 °C). It has high film formability, considerable mechanical strength, and possesses the ability to form thin layers, nanofibers, nanoparticles, and flexible coating. These characteristics make pullulan an ideal alternative candidate to petroleum-based polymers [107]. Degradation of pullulan results in nontoxic monomers. It is stable in thermal conditions and in solution form over a wide range of pH conditions. The aqueous solutions of pullulan are viscous but do not create gels. The viscosity of pullulan water solutions is proportional to the molecular weight of the pullulan.

This also makes it appropriate as a binder for agglomerated products. Pullulan also has excellent oxygen barriers and is designed for food packaging [108].

2.9.2 Chitosan (CS)

It is a cation alkali polysaccharides produced from chitin that has been deacetylated. Chitosan has excellent film forming, bio-degradability, and anti-bacterial properties. As a result, it is an excellent polymer for use in the food packaging applications. Not being water soluble or an alkaline solution. Chitosan, on the other hand, causes a rise in surface tension due to its polycation nature, making spinning harder. As a result, chitosan is frequently combined with other types of polymers to increase its fiber capacity. Increasing the concentration of acetic acid in a chitosan solution reduces surface tension, which is required for electrospinning [17].

Biodegradability, Biocompatibility, mucoadhesive, and antibacterial capabilities are the most well-known characteristics of chitosan. It exhibits high mechanical strength and shows high affinity towards proteins and, because of its excellent biological activities, it can be used in a wide range of applications, such as food, biomedical, cosmetic, chemical, and pharmaceutical industries [109].Chitosan possesses antibacterial capabilities given the presence of positive charges amine group in that composition, which interact with bacterial membranes and damage them, causing bacterial death [110,111]

2.9.2.1 Chemical structure of the chitosan

Chitosan is a straight chain, cationic polysaccharide that occurs naturally or can be obtained by deacetylation of chitin. It is representing cationic copolymers consisting of 2-amino-2-deoxy-β-D-glucose (60-100%) and 2-acetamino -2-deoxy-β-D-glucoside (0-50%), bound together by ß (1→4) bonds. Chitosan is the main derivative of chitin deacetylation in which the mean degree of deacetylation representing the percentage of free NH 2 groups is greater than 60% [112], as it is shown in the Figure (2.13).

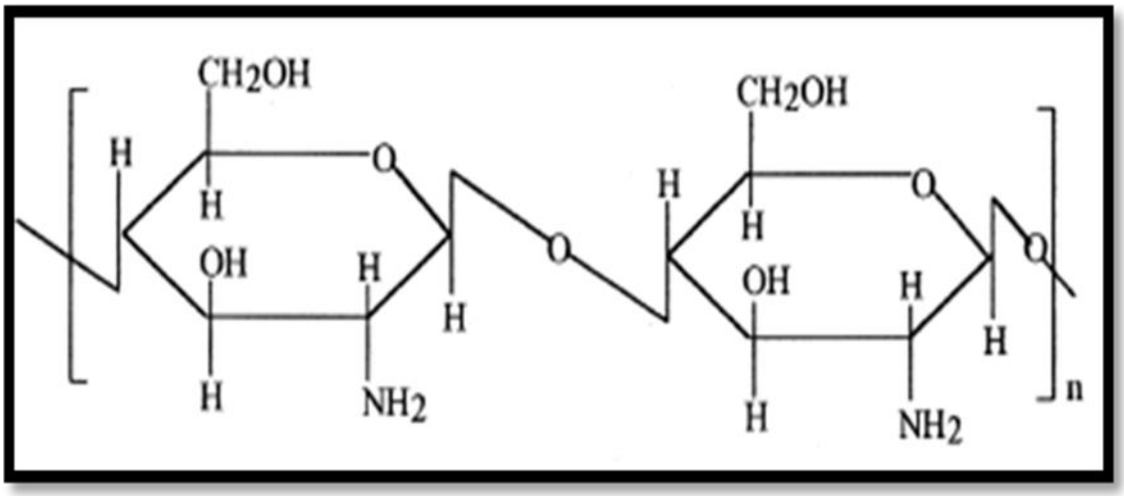


Fig. (2.13) Chemical structure of the chitosan [113]

2.9.2.2 Chitosan properties and applications

The properties and functions of chitosan are suitable for many applications in the food industry, such as binding to and removing certain elements and molecules such as dyes and fats from foods. The antibacterial and antifungal properties of chitosan can also be used during food storage and preservation. Due to chitosan's many properties such as biodegradability, natural origin, abundance, reactivity, etc., used many area of application including medical, agricultural, food processing, nutritional enhancement, cosmetics, waste and water treatment [114]. Fibrous structures are successfully formed by electrospinning chitosan solutions in 90wt% aqueous acetic acid solution [17].

2.9.3 Gelatin(G)

Gelatin has many properties, alone and in combination, promising material for tissue engineering. However, due to the gelling processes, gelatin can form a triple helix due to the abnormalities that occur in this polymer. This generates difficulties during electrospinning, so that becomes necessary to use a combination of solvents and parameters such as temperature and pH to destabilize the formation of the triple helix [97]. Because of its unique functional properties, gelatin is commonly applied in the pharmaceutical, food, cosmetic, and package industries. However, the pure gelatin is brittle and has high moisture absorption. Therefore, gelatin is usually blended with other polymers to prevail over the weakness. For example, mixing gelatin with hydrophilic molecules such as chitosan can greatly improve the properties [8]. Gelatin, made by heat or the chemical breakdown of collagen, has received a lot of attention as a natural polymer due to its environmental friendliness, biodegradability, and low cost [115].

2.9.3.1 Chemical structure of the Gelatin

Gelatin is composed of 18 types of complicated amino acids; glycine, hydroxyproline and proline make up around 57 percent of the composition, while the remaining 43 percent consists of arginine, alanine, aspartic acid and glutamic acid. There is 25.2% oxygen, 50.5% carbon, 17% nitrogen and 6.8% hydrogen in gelatin, which is made up of singly and double-unfolded hydrophilic chains. Also, gelatin's chemical structure is made up of various polypeptide chains, such as α-chains (one polymer/single chain), β-chains (two α -chains covalently linked), and γ-chains (three covalently linked α -chains) [116]. shown as in Figure (2.14).

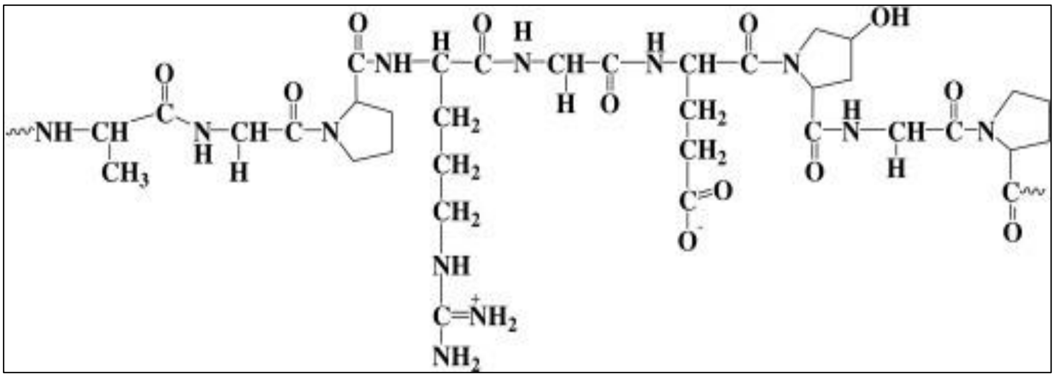


Fig. (2.14) Chemical structure of the Gelatin [117]

2.9.3.2 Gelatin properties and applications

Gelatin is one of the biopolymers with animal protein origin, which is present in the bone and skin of animals. Gelatin has many advantages such as availability, economically, polymerization، good antibacterial, antioxidant properties، biocompatibility, and biodegradability. Gelatin films have exceptional barrier properties against gas and oxygen at low or intermediate moisture. Gelatin has often been utilized as an appropriate starting material to yield biodegradable or edible films for effective food packaging [118,119].

2.9.4 Sodium alginate(SA)

Sodium alginate (SA) is a linear polysaccharide, a derivative of alginic acid comprised of β-D-mannuronic acid (MA) and α-L-guluronic acid (GA) linked by 1-4 glycosidic bonds. Sodium alginate is a cell wall component of marine brown algae and contains approximately 30 to 60% alginic acid [120]. shown as in Figure (2.15).

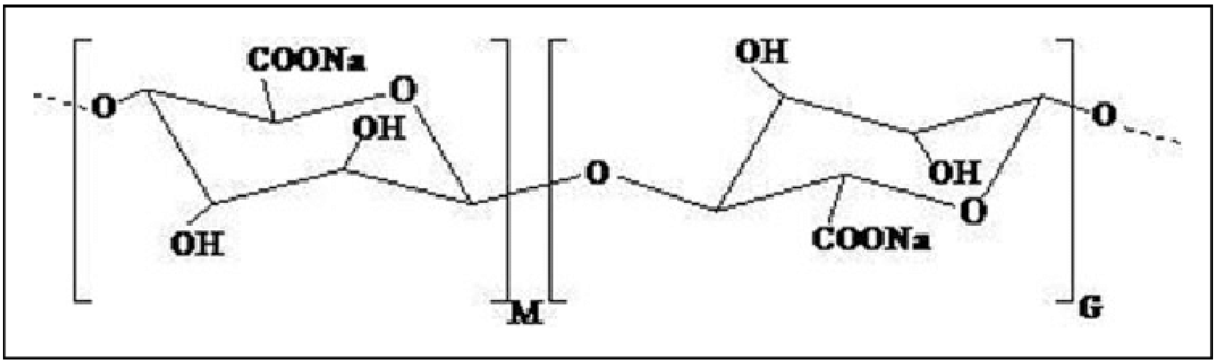


Fig. (2.15) Chemical structure of the Sodium alginate [121].

2.9.4.1 Sodium alginate properties and applications

Sodium alginate is a linear, water-soluble, non-toxic, biodegradable, hydrophilic, biocompatible, safe, perishable, non-immunogenic, poly-anionic copolymer, and polysaccharide, thus, much attention has been paid to the application of SA and its derivatives. However, films made from SA always exhibit poor strength and flexibility for the high hydrophilicity of SA [121]

SA has a potential to form edible films which exhibit poor water resistance due to hydrophilicity owing to presence of carboxylic and hydroxyl groups. To overcome this, alginate has been crosslinked with polyvalent metal cations like Ca^{2+} or polyamines like chitosan, with which it can interact to produce strong gels or insoluble polymers, this polysaccharide has many applications in all aspects of food, drug delivery, gene delivery, tissue engineering, and wound dressing and for wastewater [122].

2.9.5 Glacial Acetic acid (AA)

Acetic acid that contains very little water (less than 1%) is called anhydrous (waterless) acetic acid or glacial acetic acid. The reason it is called ice is that it solidifies and turns into solid acetic acid crystals at a temperature of 16.6 degrees Celsius(°C) .Acetic acid is an organic compound with the chemical formula CH3COOH. It is a carboxylic acid consisting of a methyl group attached to a carboxylic functional group [123], as it is shown in Figure (2.16).

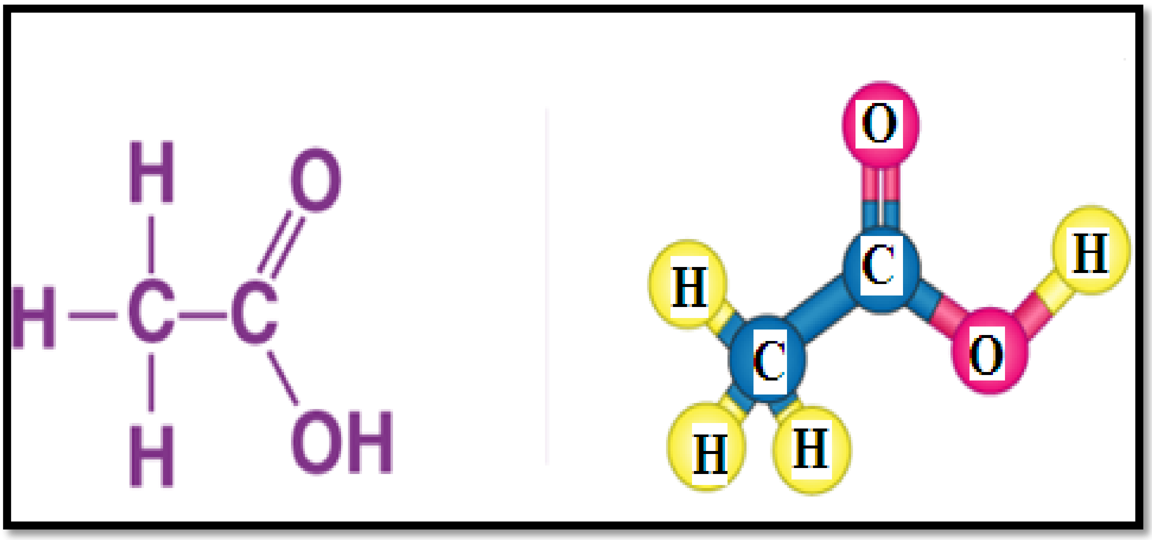


Fig. (2.16) Chemical structure of the Glacial Acetic acid(CH3COOH) [123]

2.10 Literature Review

In 2013, Saquing et al. studied the possibility of manufacturing nanofibers by mixing a sodium alginate solution with a polyethylene oxide solution with different molecular weights, as well as the basic rules for understanding the electrospinning mechanism of the carrier polymer, such as PEO. The carrier polymer must be electrolysable, has a high molecular weight, and be crosslinked. The inability of the sodium alginate solution to form nanofibers. The synthetic polymer (PEO) reduces the electrical conductivity and the surface tension of the sodium alginate solution and creates the molecular entanglement required for the electrospinning process. More than 70% (SA) must be using a high molecular weight synthetic polymer with the use of Trition x-100, a nonionic surfactant. It has also been observed that adding sodium chloride salt and a mixture of glycerine and water does not contribute to the formation of nanofibers because it does not increase the entanglement processes of the solution used to manufacture nanofibers [124].

In 2014, Islam et al. produced nanofibers by electrospinning from aqueous solutions of polyvinyl alcohol (PVA) /pullulan (PUL) with different weight ratios. The total polymeric concentrations were also changed to identify optimum electrospun condition for forming uniform and nanofibres without beads. Were studied by using (SEM), (TGA), (DSC), (XRD), (FTIR), and mechanical properties measured by tensile strength. The results showed that polymer concentrations and blend ratios have direct influence on the morphologies and properties of PVA/PULL nanofibres. 12 wt.% total polymer concentration and higher PVA content has found to be favourable for obtaining uniform and bead free PVA/PULL blend nanofibres with improved mechanical and thermal properties. These fibers are used in food packaging and medical applications [125].

In 2016, Kusumastuti et al. this study was conducted to optimize the fabrication of chitosan/alginate/PVA nanofibers by varying the conductivity of solution, solvent concentration, and polymer composition by electrospinning method. By adjusting the conductivity of blended solution at 0.5 – 2.0 Mho.cm-1, the nanofibers were created. Were characterized using Scanning Electron Microscope (SEM) to observe the morphology and Fourier Transform Infrared (FTIR) identify the constituent group. The resulted nanofibers showed a uniform nanofiber with average diameter around 200- 400 nm. Although higher acetic acid concentration (as a chitosan solvent) did not change conductivity significantly, However, it tends to create more beads on the fibers. As the concentration of sodium alginate increased, the beads were observed. The conductance of blended solution also increased along increasing of sodium alginate concentration. At lower conductivity, it resulted in the uniform nanofiber. The Nanofibers CS/SA/PVA for biopolymers are widely used in medical and industrial applications due to their unique characteristics such as the high surface area to volume ratio and biocompatibility [126].

In 2016, Antunes et al. produced nanofibers for polymeric solutions containing 20, 30, and 40% (w/v) of zein using ethanol (70% v/v) as a solvent by electrospinning method. Electrical conductivity and apparent viscosity of the zein polymeric solutions were evaluated. The morphology of the electrospun nanofibers was evaluated by scanning electron microscope(SEM) and size distribution. Results showed that increasing zein concentration increased viscosity and the average diameter of the fibers but did not affect the electrical conductivity of the solutions, the lowest concentration of zein produces nanofibers of small diameters and fewer beads, while fibers prepared from zein solution at the highest concentration had ribbon-like shapes with the highest average diameter. The fibers electrospun from 30% zein had greater uniformity and average diameter of 417 nm. These nanofibers have potential to use as encapsulants of compounds for food and active packaging development [127].

In 2016, Wen et al. prepared Nanofibres from polyvinyl alcohol (PVA) / cinnamon essential oil (CEO) / β-cyclodextrin (β-CD). An antimicrobial nanofiber film with an average diameter of 240 ± 40 nm was successfully produced under the best conditions using electrospinning technique. The addition of CEO/β-CD produced the nanofibrous film hydrophilic, according to water contact angle analysis. The PVA/CEO/β-CD nanofibrous film had excellent antimicrobial activity, and its minimum inhibitory concentration (MIC) against Staphylococcus aureus and Escherichia coli of approximately 0.9–1 mg/mL (corresponding CEO concentration 8.9–9.9 µg/mL) and minimum bactericidal concentration (MBC) was approximate 7–8 mg/mL (corresponding CEO concentration 69.3–79.2 µg /mL). Furthermore, the electrospinning process was allowed for maintaining more CEO in the film resulting in an enhanced antibacterial activity compare with casting film. The PVA/CEO/β-CD nanofibers film can effective extend the shelf-life of strawberry, that indicated it has the potential to be used in active food packaging [128].

In2017, Liu et al. produced nanofibers from poly lactic acid (PLA) and chitosan- cinnamon essential oil (CS-CEO) nanoparticles for active packaging materials by using electrospinning. The effect of the different loading amounts from CEO (1%, 1.5%, 2%, and 2.5% *v*/*v*) on the structure, morphology, and release behavior of the composite fibers was studied using FTIR, SEM, UV, AFM analyses. The results indicated that incorporating CEO could improve the antibacterial properties of the PLA/CS-CEO fibers. The optimal composition was found to be PLA/CS-CEO-1.5, which showed the highest antibacterial for a long time and a significant potential for active food packaging and other applications where antibacterial activity is required [129].

In2018, Altan, el at. produced composite nanofibers from zein and poly (lactic acid) (PLA) by incorporating carvacrol at three different concentrations (5, 10 and 20%) using electrospinning. The field emission scanning electron microscope (FE-SEM) images showed that bead free fibers were obtained from zein and PLA polymer solutions containing carvacrol at different concentrations. The Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), Antioxidant activity, and antimicrobial test. The results showed that carvacrol was encapsulated in nanofibers from zein and PLA. The antioxidant activity of carvacrol loaded zein fibers ranged from 62 to 75%, while antioxidant capacity of PLA fibers varied from 53 to 65% for 5 to 20% carvacrol content. The composite nanofiber from zein and PLA at 20% carvacrol content inhibited 99.6 and 91.3% of the growth of mold and yeast. Carvacrol- loaded nanofibers with antioxidant and antimicrobial properties can be used to extend the shelf life of fresh foods as a new approach to electrospun fibers in food applications [130].

In 2018, Yue et al. produced nanofibers from solution of carboxymethyl chitosan and polyoxyethylene (CMCS/PEO) by using electrospinning technology. The purpose of this study is to explore the potential application of electrospun (CMCS/PEO) nanofiber membranes in fruit preservation. The microstructure, antibacterial activity, hydrophilic, and air permeability of the nanofiber membrane have been tested. For compared, the fresh-keeping effects of typical conventional coatings and CMCS/PEO nanofiber membranes on strawberries' rotting rate and weight loss were studied. The CMCS/PEO nanofiber membrane exhibited antibacterial capability to both Escherichia coli and Staphylococcus aureus. The measured gas permeability was on a scale of 40–50 mm s^{-1} in the 200 Pa for nanofiber. These results showed that the CMCS/PEO nanofiber membrane might be used as a fruit packaging material. Compared with typical conventional coatings. Additionally, the composite

(CMCS/PEO) nanofiber membrane is non-toxic and edible, making it suitable for application in the food industry [26].

In 2018, Deng et al. produced composite nanofibers of chitosan(CS) / poly ethylene oxide (PEO) /lauric arginate (LAE) by electrospinning method. The physical properties of the mixture (CS/PEO) such as viscosity, surface tension, and conductivity were calculated without (LAE) and after adding to the mixture at different weight ratios of 0.1%, 0.25%, and 0.5% (w/v), the addition of LAE did not change the physical properties of chitosan/PEO in acetic aqueous solutions. Were studied using by scanning electron microscopy (SEM) showed nanofibers with an average diameter of 150 nm without beads, Fourier transform infrared (FTIR), X-ray diffraction (XRD), thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), water contact angle. The Fourier transform infrared spectra and thermal analysis indicated that the LAE molecules were homogeneously dispersed within the chitosan/PEO nanofibers. The formation of electrostatic and hydrogen bonding interactions induced by the LAE addition changed the inter and intermolecular interactions between PEO and chitosan and further affected the mobility of the polymer molecules, leading to the increased crystallinity and decreased melting point. The hydrophilicity of the nanofibrous films was significantly increased by the incorporation of LAE, as indicated by the decreasing water contact angle from 39° to 10°. the chitosan/PEO/LAE nanofibrous films showed LAE concentration dependent antimicrobial activity against E. coli and S. aureus. The fluorescent staining experiments demonstrated that the antimicrobial mechanism of the nanofibers films was cell membrane damage. The nanofiber CS / PEO / LAE films has the potential to be used in the production of antimicrobial food packaging [131].

In 2019, Alehosseini et al. produced nanofibers from protin such as (gelatin and zein) loaded curcumin by electrospinning to make an effective coating for food packaging. Green Tea Extract (GTE) has been added to formulations to evaluate its effects on the stability, protective ability and release properties of curcumin from the fibers. Due to the low solubility of curcumin in aqueous solutions, it is combined with liposomes to facilitate incorporation into gelatin fibres. The addition of GTE resulted in strong interactions with proteins, and in the case of gelatin in particular., it improved the protective effect and slowed down the curcumin release from the fibers, although it did not prevent their collapse in water. the incorporation of GTE into the zein structures did not produce any significant improvements. The higher stability of the zein fibers in water and their better release performance in the polar food simulant. Were studied using by scanning electron microscopy (SEM), Fourier transform infrared (FTIR), Small angle X-ray scattering (SAXS), thermal gravimetric analysis (TGA), and transmission electron microscopy (TEM). The results showed the developed gelatin coatings a promising release behavior in contact with fatty food, and zein-based coatings would be more adequate for packaging of high water content food products. The coatings can be optimized for packaging structures in contact with more hydrophilic or hydrophobic food products by selecting the suitable protein matrix [132].

In 2019, Somsap et al. produced antibacterial nanofibers by the electrospinning method of polymeric solutions from chitosan (CS), cellulose acetate (CA), and gelatin (G) were blended at a volume ratio of 4: 1: 5, with the incorporation of eugenol at different concentrations of 0–10% v/v) into the mixed polymers solutions. Scanning electron microscopy (SEM) showed that the average diameter after entering the eugenol ranges from (152.32 ± 41. 48 - 288.92 ± 77.69 nm). the diameter increases with the increase in the eugenol concentration, were using Differential scanning calorimetry (DSC) analyses, release of the eugenol from nanofiber and Antibacterial test. The results showed that the release of eugenol was related to eugenol diffusion through the polymer and/or erosion of the polymer، and Thermal analysis proved better thermostability when the concentration of eugenol was less than 5.0% (v/v). The CS/CA/G electrospun nanofiber mats with incorporated eugenol were better in controlling Salmonella typhimurium and Staphylococcus aureus. This approach may effectively extend the shelf- life of food with potential applications in active food packaging and others products [133].

In 2021, Wang et at. produced nanofibers from gelatin/zein (G/Z) loading with different ratios of perillaldehyde (P) by using electrospinning method. The morphology showed that the G/Z/P film had a uniform microstructure and a nano diameter at a weight ratio of 5:1:0.02 (G/Z/P). The results of FTIR and XRD indicated that these three ingredients had good compatibility and strong interaction via hydrogen bonding. water contact angle results showed that the G/Z/P films gradually change from hydrophilic to hydrophobic with the increase of perillaldehyde. Thermal analysis indicated that the G/Z/P (5:1:0.02) film has good thermal stability. Antibacterial and storage analysis indicated that G/Z/P (5:1:0.02) film is effective to inactivate Staphylococcus aureus and Salmonella enteritidis, and obviously reduces the increasing rate of total bacteria counts and volatile basic nitrogen of chicken breasts. The results tests for the stability, morphology, and antibacterial activity of the different prepared films indicated that G/Z/P (5:1:0.02) has a potential application in antibacterial food packaging [134].

2.11 Summary of literature reviews

Previous studies showed that there is a possibility to manufacture nanofibers by electrospinning technique using natural polymers and synthetic polymers or without the use of synthetic polymers by mixing natural polymers with each other using more than one solvent to improve the possibility of electrospinning of natural polymers. In addition to the possibility of using these fibers in the field of food packaging to protect them from harmful bacteria and external environmental conditions to maintain food quality, as a result of their high surface area to volume ratio and their biocompatibility. Some studies showed that the polymer concentration and mixing ratios have a direct effect on the shapes and properties of nanofibers, and other studies showed the effect of some solvents on natural polymers by evaluating the basic parameters of the solutions, where the increase in the concentration of natural polymers led to an increase in the diameter of the nanofibers, and the use of a polymer with a weight A high molecule to create the required entanglement between the chains for the success of the electrospinning process. This thesis will study what happens if natural polymers are mixed with each other and with different weight ratios on the properties of nanofibers, and investigate the possibility of using models of these fibers to protect food from bacteria that cause food spoilage.

Chapter Three

Experiment Part

3.1 Introduction

This chapter includes the properties of the polymers used in this work, gelatin, chitosan, pullulan, sodium alginate, as well as acetic acid and deionized water used as a solvent in the preparation of polymeric solutions used in the production of nanofibers by the electrospinning process. And physical tests of polymeric solutions (such as surface tension, electrical conductivity, and viscosity) and devices used in the characterization and analysis of nanofibers such as (FE-SEM, FT-NIR, AFM, XRD, UV, contact angle, and antibacterial)

3.2 Materials used

3.2.1 Chitosan (CS)

Chitosan low molecular weight (L.M.W.) was purchased from Verdean house, Daryaganj, New. Delhi-110002 (India). Table 3.1 describes properties of the chitosan. Cash number:9012-76-4

Table 3.1 Properties of the chitosan.

Property	**Data**
Molecular formula	$(C_6H_{11}NO_4)_n$
Appearance	Off- White to yellow- beige powder/chips
Molecular weight	50000- 190000 Da
Viscosity	20- 300 Cp
Loss on drying (105 °C)	10.0%
Ash	2.0%
Solubility	Aqueous solutions of acetic acid
Deacetylation	75 – 85 %

3.2.2 Sodium Alginate (SA)

Sodium alginate (SA) was purchased of HiMedia Pvt.Ltd., company, Dindori, Nashik, India. Table 3.2 describes properties of the sodium alginate. Cash number:9005-38-3

Table (3.2) Properties of the sodium alginate

Property	Data
Molecular formula	$(C_6H_7NaO_6)_n$
Appearance	White to yellow fibrous or granular powder
Solubility	Soluble in water
Density (g/cm^3)	1.601
Molecular weight(g/mol)	216.12

3.2.3 Pullulan(PUL)

Pullulan (PUL) was purchased of HiMedia Pvt.Ltd., company, Dindori, Nashik, India. Table 3.3 describes properties of the pullulan. Cash number:9027-02-7

Table 3.3 Properties of the pullulan

Property	Data
Molecular formula	$(C_6H_{12}O_5)n$
Appearance	White powder
Solubility	Soluble in water
Molecular weigth (g/mol)	90000

3.2.4 Gelatin(G)

Gelatin (G) was purchased from “HiMedia Laboratories Pvt. Ltd. A-516,” Swastik Disha Business Park, Via Vadhani” (India). Table 3.4 describes properties of the Gelatin.Cash number:9000-70-8

Table 3.4 Properties of the Gelatin

Property	Data
Molecular formula	$C_{102}H_{151}N_{31}O_{39}$
Appearance	White to slightly yellow powder
Molecular weigth (g/mol)	180000
Solubility	Soluble in hot water and acetic acid
Density(g/ cm^3)	1.2

3.2.5 Acetic acid glacial (AA)

Acetic acid glacial (AA) was purchased from HiMedia Laboratories Pvt. Ltd., company India. Table (3.5) shows properties of the Acetic acid glacial. Cash number 64-19-7

Table (3.5) Properties of the Acetic acid glacial

Property	Data
Molecular formula	CH_3COOH
Molecular weigth(g/mol)	60.05
Density(g/ ml)	1.049
Assay	99.7- 100.0%
Freezing point	16.6 °C
Boiling point	117.9°C
Solubility	Miscible with water

3.3 Preparation of Solution

3.3.1 Preparation samples for blending (G: CS: SA)

In this work, the solutions are prepared by dissolving gelatin (14g) in aqueous acetic acid 86g (77.4g AA+8.6g DIW) and the chitosan (2g) in aqueous acetic acid 98g (1.96g AA+96.04g DIW) by using a hot plate stirrer at temperature (50 °C) for two (2) hours. While sodium alginate solution (1g) was prepared in deionized water (DIW) using a stirrer at room temperature (25°C) for two (2) hours. All solutions were produced at room temperature (25°C) with a stirrer for (24) hours to obtain a homogeneous mixture with different weight percentages which was used for electrospinning. The mixing ratios of the three natural biopolymers (gelatin, chitosan, and sodium alginate) were 100:0:0, 80:10:10, 60:20:20, 40:30:30, 20:40:40, and 0:50:50, as shown in Figure 3.1A and Table 3.6.

3.3.2 Preparation samples for blending (G: CS: PUL)

The solutions are prepared by dissolving gelatin (14g) in aqueous acetic acid 86g (77.4g AA+8.6g DIW) and the chitosan (2g) in aqueous acetic acid 98g (1.96g AA+96.04g DIW) by using a hot plate stirrer at temperature (50 °C) for two (2) hours. While pullulan solution (10g) was prepared in deionized water (DIW) using a stirrer at room temperature (25°C) for two (2) hours. All solutions were produced at room temperature (25°C) with a stirrer for (24) hours to obtain a homogeneous mixture with different weight percentages which was used for electrospinning. The mixing ratios of the three natural biopolymers (gelatin, chitosan, and pullulan) were 100:0:0, 80:10:10, 60:20:20, 40:30:30, 20:40:40, and 0:50:50, as shown in Figure 3.1B and Table 3.6.

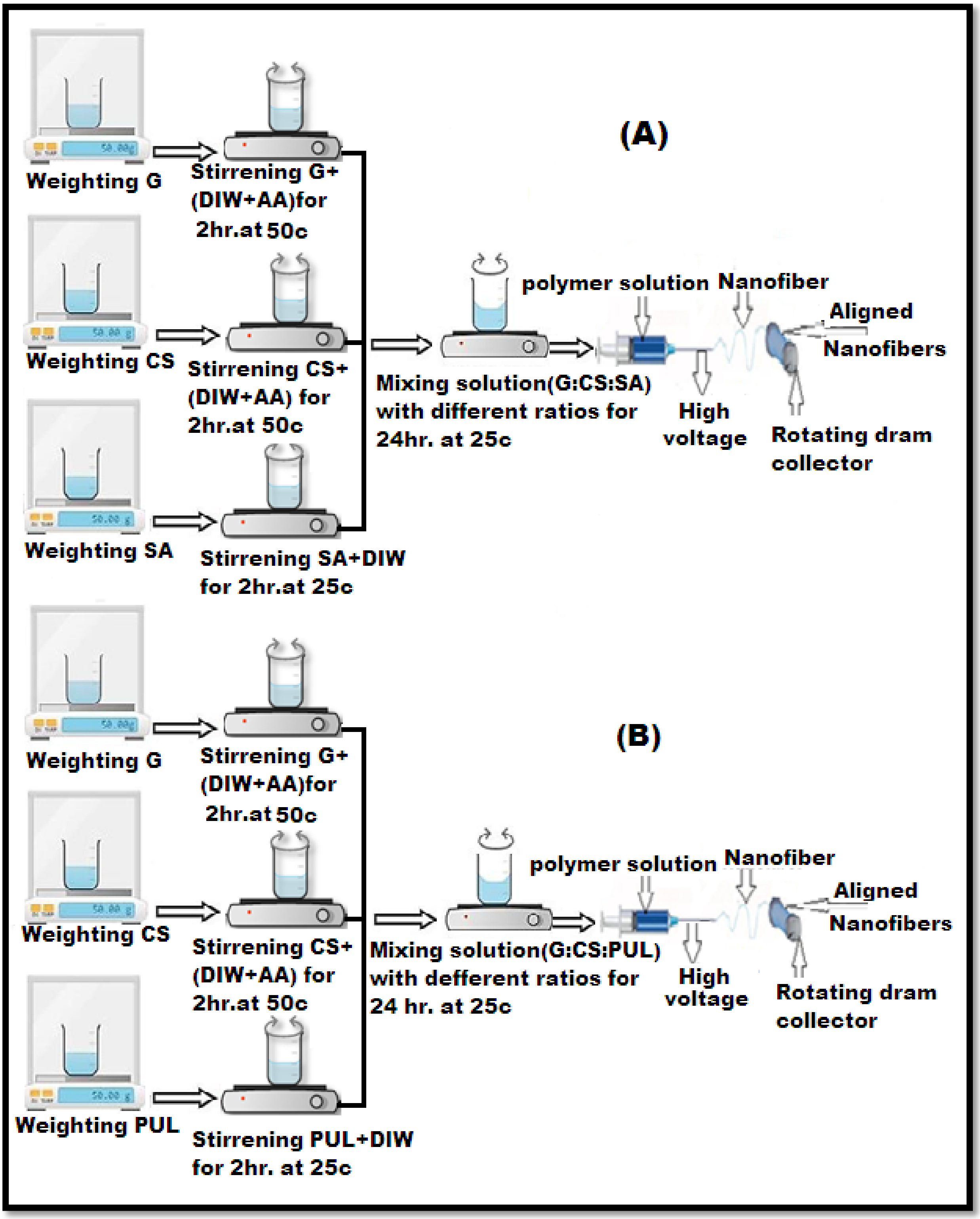


Fig. (3.1) Schematic diagram of preparation tertiary natural biopolymers solution and electrospinning process for (A) G: CS: SA (B) G: CS: PUL

Table (3.6) Materials used percentages

(G: CS: SA) blends				(G: CS: PUL) blends			
G (g)	CS (g)	SA (g)	Blend Ratio (%)	G (g)	CS (g)	PUL (g)	Blend Ratio (%)
14	2	1	100:0:0	14	2	10	100:0:0
14	2	1	80:10:10	14	2	10	80:10:10
14	2	1	60:20:20	14	2	10	60:20:20
14	2	1	40:30:30	14	2	10	40:30:30
14	2	1	20:40:40	14	2	10	20:40:40
14	2	1	0:50:50	14	2	10	0:50:50

3.4 Electrospinning process

After the solution mixing process is completed. The prepared solutions were placed into a standard 1 mL syringe attached to a stainless steel needle as a jet. A needle-jet was placed in front of the metal collector, which was arranged horizontally, during the electrospinning process, the positive electrode was connected to the tip of the needle-jet, while the negative electrode was connected to the metal collector, which used as a target for nanofiber deposition. The process conditions used were: the applied voltage was 22 KV, the tip-collector distance was 20 cm, the temperature was 25 °C, the rotation speed was 900 rpm, the flow rate was 0.5 ml/hr, and the humidity was below from 45%, as shown in Figure (3.2).

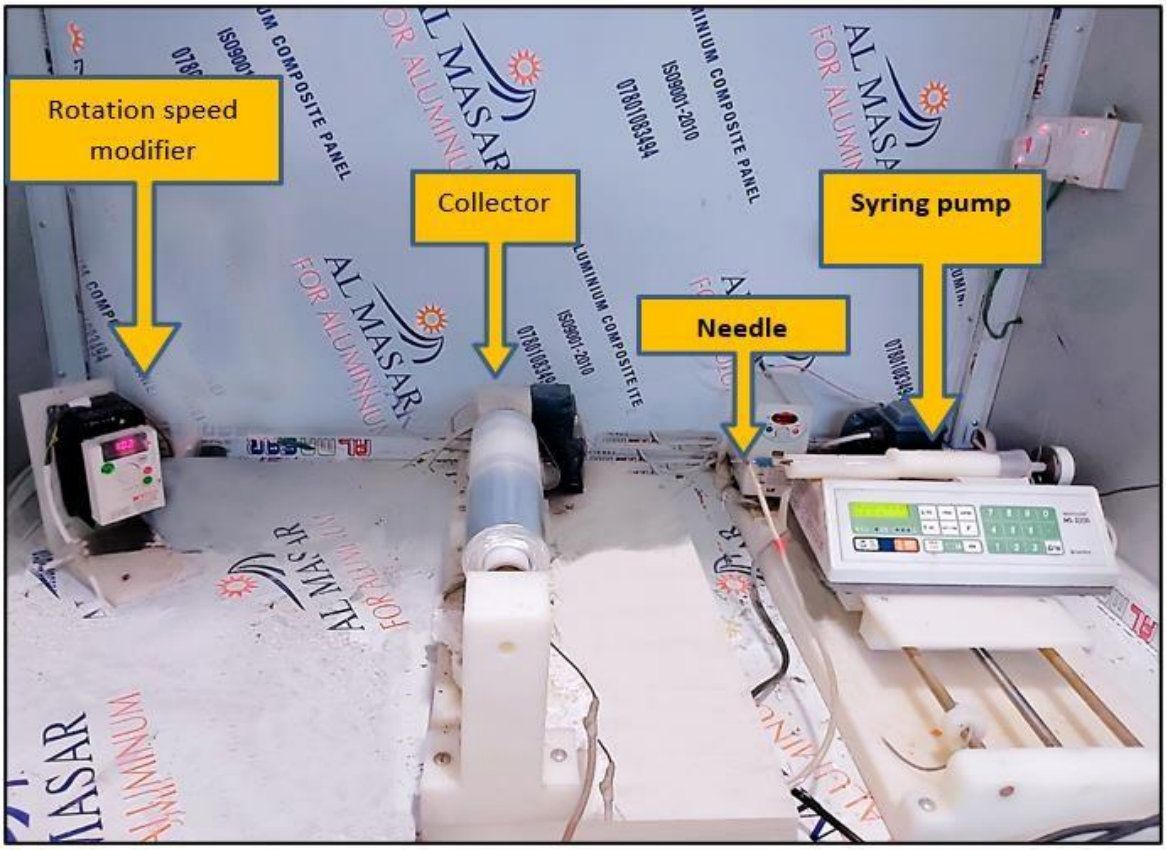


Fig. (3.2) Electrospinning device.

3. 5 Testing Procedure

The nanofibers and the solutions used in preparing them were tested using different techniques, as shown in the Figure (3.3)

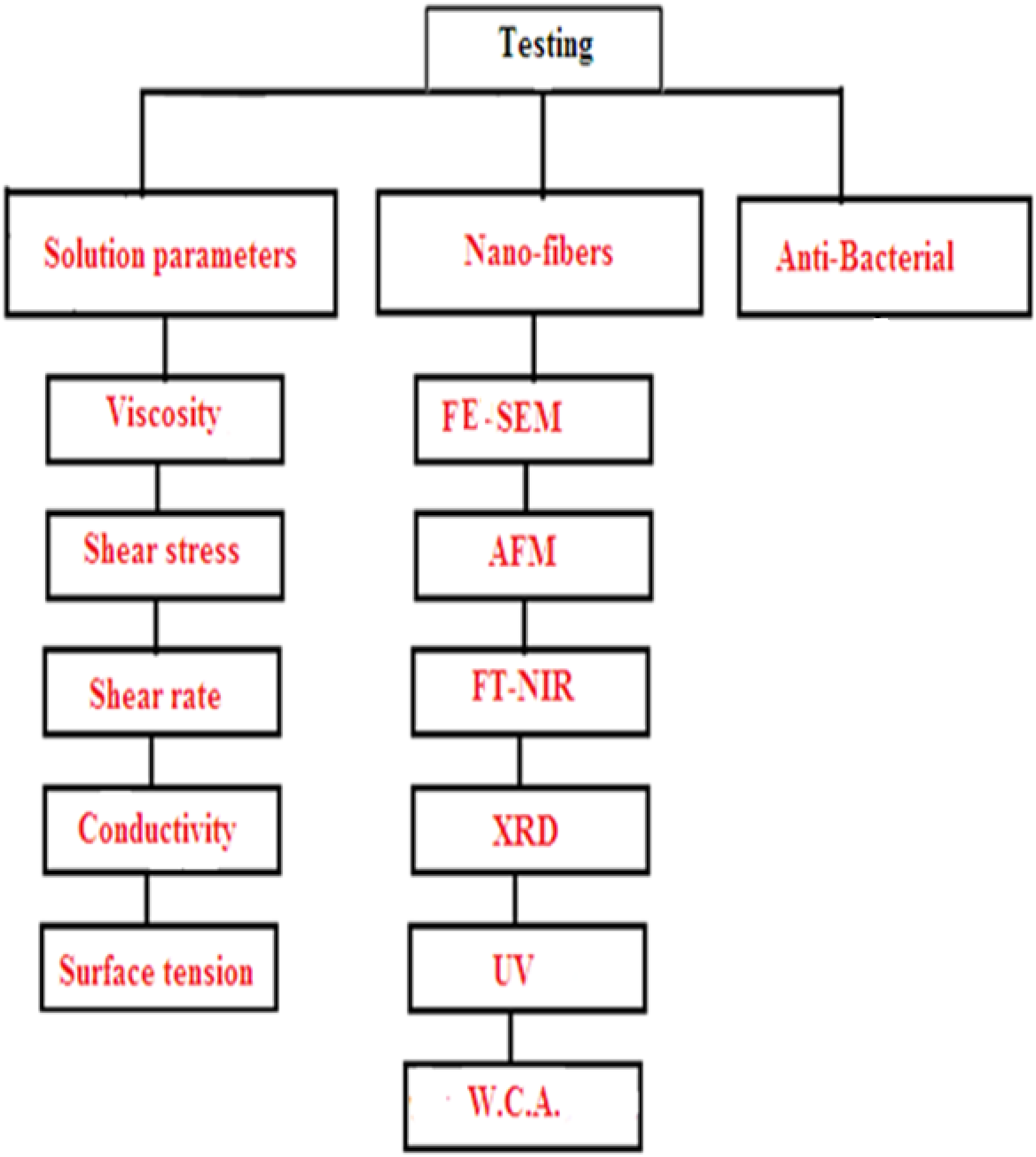


Fig. (3.3) Procedure of Testing the nanofiber

3.5.1 Viscosity

Figure (3.4) shows the Brookfield DV-III Ultra Rheometer used to measure the viscosity of solutions in units of mPa.S with shear rate (0.00-200) s-1 and the relationship between shear stress and shear rate. This test according to ASTM D7395. This test was performed in Materials Engineering College Babylon University.

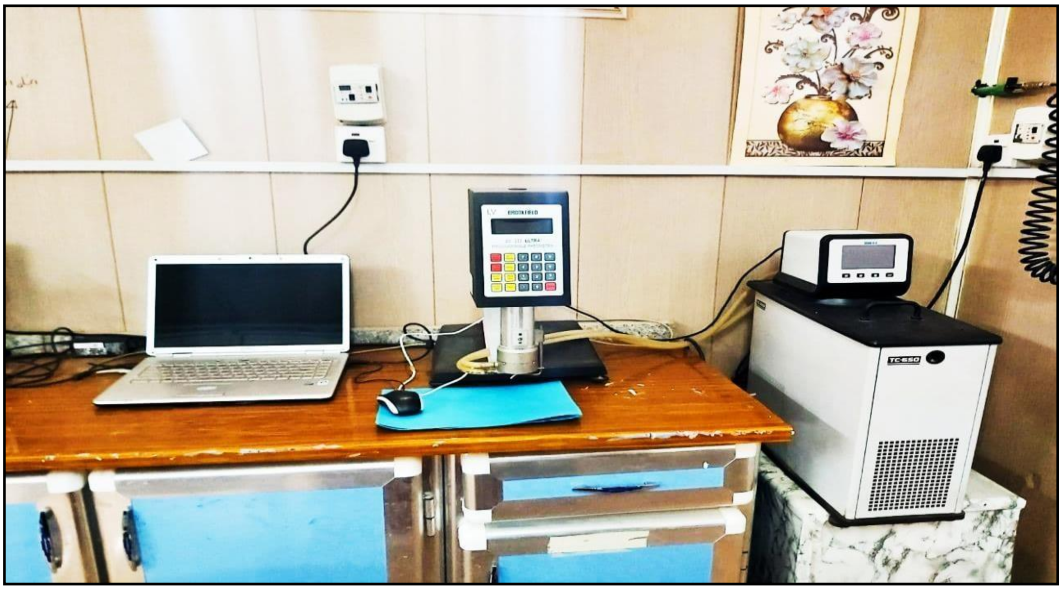


Fig. (3.4) Brookfield DV-III Ultra Rheometer

3.5.2 Surface tension

A JZYW-200B automatic interface tension meter from Beijing United Test Co., Ltd., Surface Interfacial Tensiometer, uses a platinum ring immersed in the solution placed in a cylindrical Petri dish. The ring is pulled across the surface of the solution. The solution sticks to the ring some distance above the surface. When it separates, the surface tension of the solutions is measured in mN/m، as shown in Figure (3.5). This test was performed in Materials Engineering College Babylon University.

Figure (3.5) Surface tension measuring device

3.5.3 Water Contact Angle (W.C.A)

A water contact angle (circle fitting mode) was used to test the wettability of the bio nanofibers by using" SL200B Optical Dynamic / Static Contact Angle Meter" shown in the Figure (3.6). This test was performed in Materials Engineering College Babylon University.

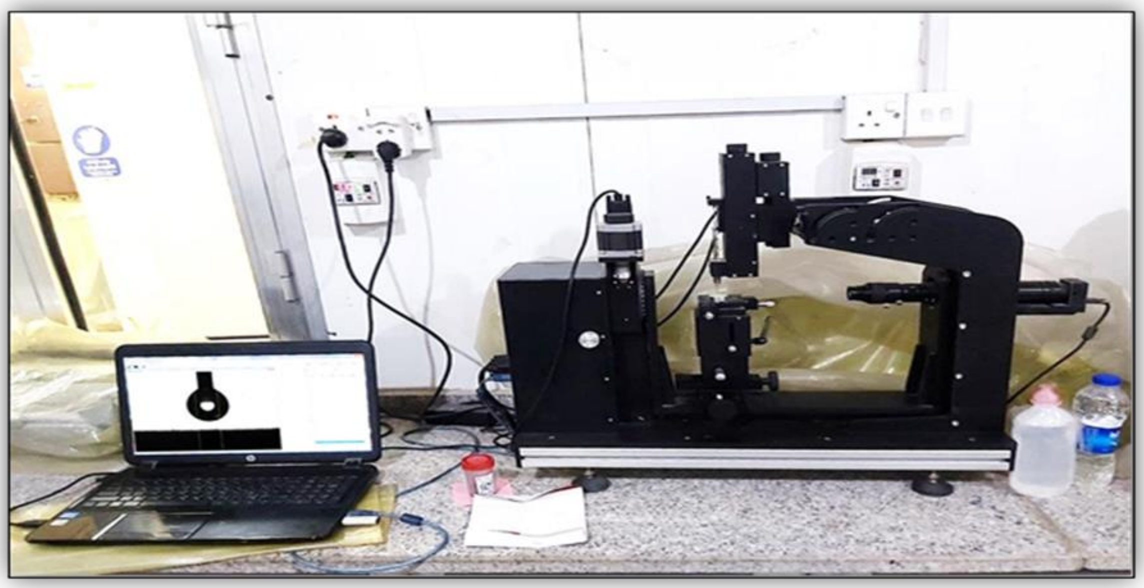


Fig. (3.6) Water contact angle measurement device

3.5.4 Electrical Conductivity

This test was performed by the electrical conductivity device "HANNA Instruments Model-Conductivity Meter 214 EC" shown in Figure (3.7). To find out the electrical conductivity values of polymeric solutions used in the production of nanofibers.

Fig. (3.7) conductivity measuring device

3.5.5 Atomic Force Microscopy (AFM)

The technique of atomic force microscopy (TT-2 AFM) using a scanning Prope microscope, as through this technique that surface properties such as roughness can be determined. Modern atomic force microscopes include not only a probe and piezoelectric scanner, but additional hardware for bringing the probe rapidly into the proximity of a surface. A video optical microscope is very helpful for operating an AFM. The video microscope helps with aligning the light lever and probe approach, and for finding features for scanning. The control computer is a standard IBM/PC-type computer with a Microsoft Windows operating system. There are two programs required to operate the TT-2 AFM: the AFM control software and the software for the color video camera. As shown in the Figure (3.8). this test was performed in Islamic republic of Iran by CAC Laboratories.

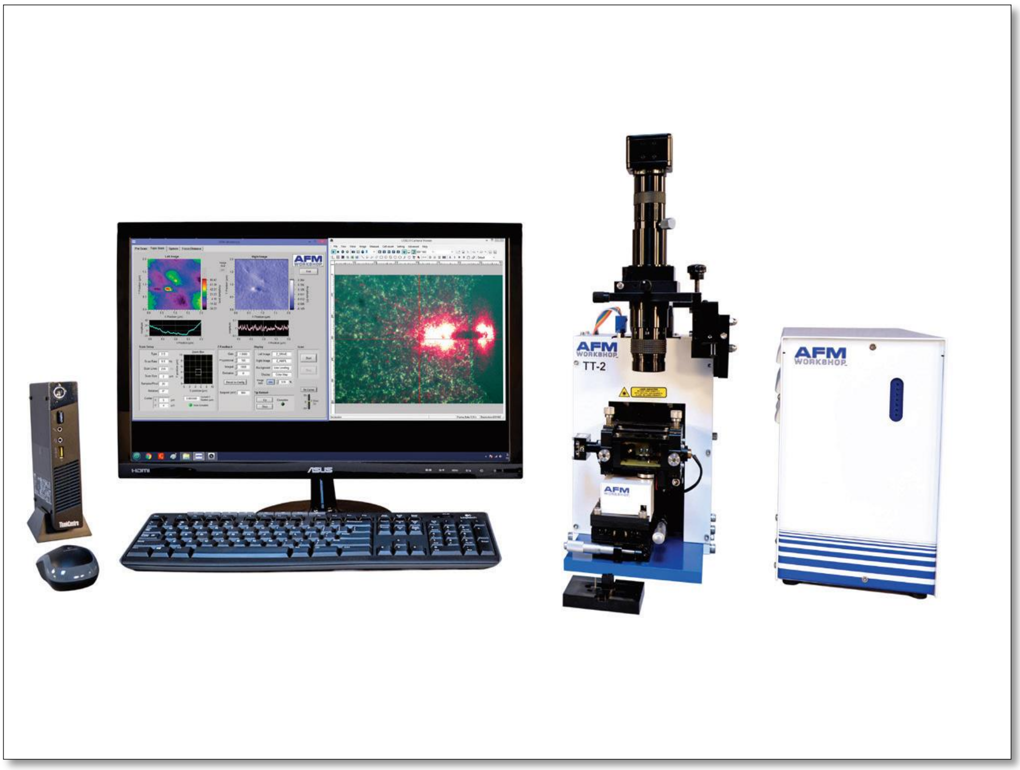


Fig. (3.8) TT-2 AFM Atomic force microscopy device

3.5.6 Field- Emission Scanning electron microscopy (FE-SEM)

The morphology (size, shape, and diameter) of the nanofibers was evaluated using a field emission scanning electron microscope “FESEM (Zeiss Sigma 300- HV) GERMANY”. The Digitizer Image Analysis Software application was used to calculate the standard deviation diameter and the nano-fiber diameters ranges, as shown in the Figure (3.9). this test was performed in Islamic republic of Iran by CAC Laboratories.

Fig. (3.9) FE-SEM (Zeiss Sigma 300- HV) device

3.5.7 X-ray diffraction analysis(XRD)

It is a rapid analytical technique, primarily used for phase identification of crystalline materials, and can also provide information on unit cell dimensions. XRD is generated in a cathode ray tube by heating a filament to produce accelerated electrons toward the target by applying a high voltage. The crystal structure of the nanofibers was investigated by X-Ray Diffraction

(2700AB, HAOYUAN Co., China) at room temperature, shown in Figure (3.10). this test was performed in Islamic republic of Iran by CAC Laboratories.

Fig.(3.10) X-Ray Diffraction (2700AB HAOYUAN co., china) device

3.5.8 Fourier transform near- infrared spectroscopy(FT-NIR)

The Fourier transform Near- Infrared Spectrometer (FT-NIR) "Spectrum Two N^{TM} FT-NIR, PerkinElmer, Inc.-USA" includes a spectrogram between transmittance or absorption and wavelength to find out the type of interaction between components and types of bonds. This test was performed according to ASTM E1252, as shown in Figure (3.11). this test was performed in Islamic republic of Iran by CAC Laboratories.

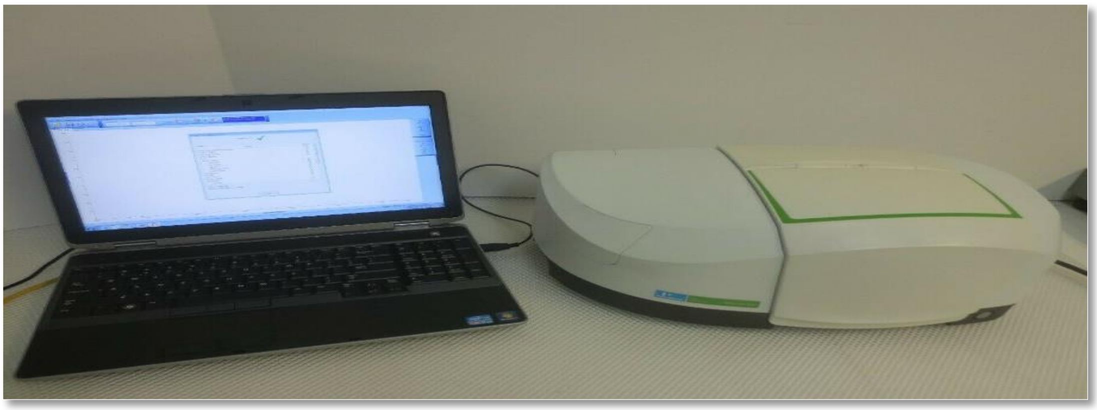


Fig. (3.11) Fourier transform near- infrared spectroscopy(FT-NIR) device

3.5.9 Ultraviolet-visible spectrophotometer(UV)

Device UV-Visible spectrophotometer type UV-1800, (Shimadzu-Japan). It is used to determine the absorption of light from the sample. The liquid sample was put in the quartz cell of device (4 cm), where a double beam spectrophotometer utilizes two beams of light: a reference beam and a sampling beam that passes through the sample. Some double beam spectrophotometer has two detectors that allow the two beams to be measured at one time. The aim of this test was to measuring optical absorbance spectra of solutions, as shown in Figure 3.12. This test was performed in Materials Engineering College Babylon University.

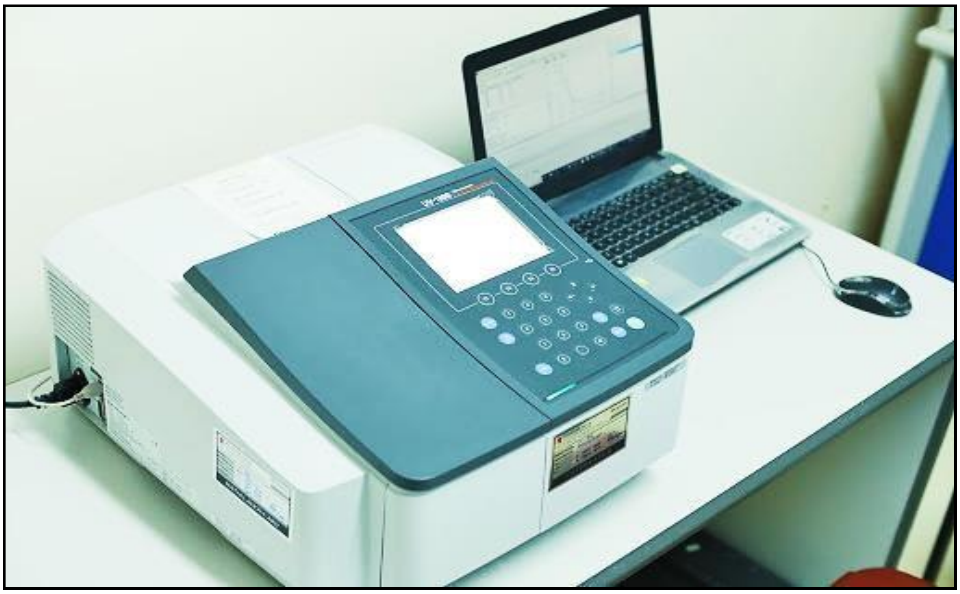


Fig. (3.12) UV- visible- spectrophotometer device

3.5.10 Anti-Bacterial Test

In the Muller-Hinton agar plate test, an antimicrobial film is placed in an agar medium containing the test microorganism (Escherichia coli, Staphylococcus aureus). The agar plates are incubated until growth is visible using an incubator device. (Binder Incubator BD115 with natural convection, model BD115-Incubators Avantgarde), as shown in Figure (3.13). The clear zone surrounding the nanofiber indicates antimicrobial diffusion from the nanofiber and growth inhibition, the diameter of the inhibitory zone was measured and recorded in millimeters to determine the antibacterial activity, as shown in Figure (3.14). The method of testing the agar plate mimics food packaging and may indicate what can happen when membranes contact contaminated surfaces and the antimicrobial agent migrates from the membranes to the food [135]. This test was performed In College of Science, Department of Life Sciences in Babylon University.

Fig. (3.13) Incubator device (model Binder Incubator BD115)

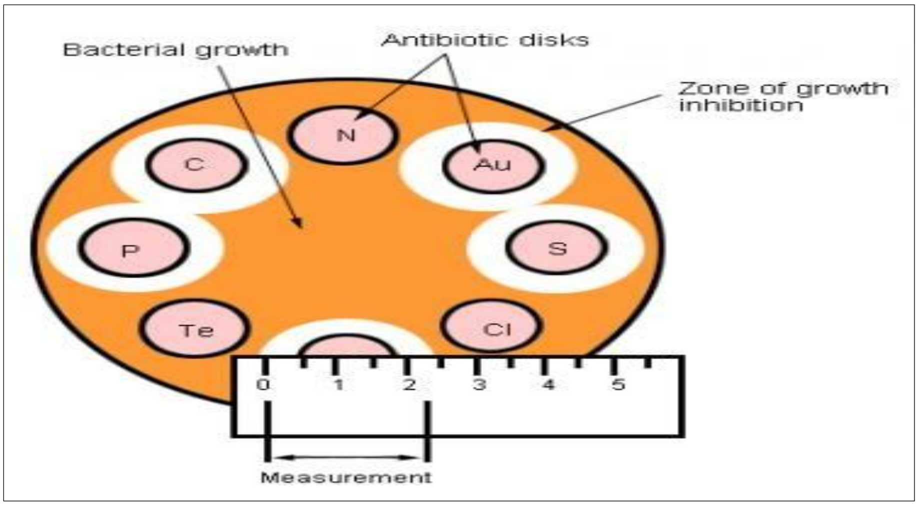


Fig. (3.14) Schematic diagram showing for measuring the diameter of the inhibitory zone [136]

Chapter Four

Results

and

Discussion

4.1 Introduction

This chapter includes a discussion of the results of the tests of nanofibers and the polymeric solutions used in their production and observing the effect of changing the proportions of polymeric solutions on the production of nanofibers and their morphological and structural properties.

These tests include (AFM, FE-SEM, FT-NIR, XRD, UV, contact angle, and anti-bacterial) for nanofibers, while tests of polymeric solutions include viscosity, surface tension and conductivity.

4.2 Rheological Properties

4.2.1 viscosity test

The viscosity of the polymer solution is one of most important factors in influencing the structural morphology of the nanofiber structure. The viscosity of pure polymer solutions increases with increasing polymer concentration [137].The ideal viscosity of a polymer solution varies based on the polymer and solvent used.The polymer concentration affects the spinnability and homogeneity of the nanofibers produced . It is important that the solution is not too dilute or overly concentrated. However, the concentration of the solution must be high enough for chain entanglement to occur[138] . The addition of gelatin to (chitosan / sodium alginate) and (chitosan / pullulan) solutions improved the electrospinning process.The chemical and physical properties of solutions, such as viscosity, have an important role in the producing of regular and continuous nanofibers . As the increasing in the proportion of gelatin in the solutions leads to an increase in the viscosity and improves chain entanglement for fiber formation as shown in Figure (4.1) and Tables(4.1 and 4.2) . The values of electrospun viscosity were calculated from the Figures(1 and 2) at shear rate($24S^{-1}$) as shown in the index (A).

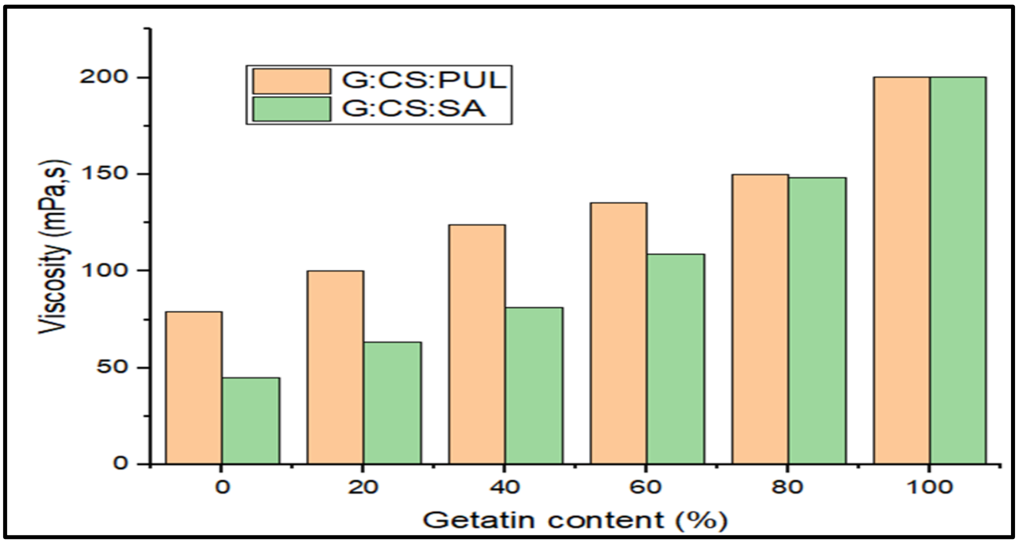


Fig (4.1) explain viscosity (mPa.S) and gelatin content for mixtuers solutions(G:CS:PUL)and(G:CS:SA)

4.2.2 surface tension test

Surface tension is an important factor at electro spinning, change solutions may give various surface tensions. Uniform nanofibers without beads can be manufactured by keep the concentration constant and lowering the surface tension [70]. Table 4.1 shows the results of the rheological properties of pure gelatin solutions and mixtures (G: CS: SA) with different proportions .we notice the increase in surface tension when the percentage of gelatin decreases from (80% to 20%) in the biopolymer mixture (G: CS: SA). As for the mixture of gelatin, chitosan, and pullulan with different weight ratios, we also noticed an increase in surface tension when reducing the percentage of gelatin from (80% to 20%) in the biopolymer mixture (G: CS: PUL), as shown in Table 4.2. The reason for the increase in surface tension is the large number of internal faces formed in solutions [139].

4.2.3 Electrical Conductivity

High conductivity polymer solutions has a higher charge carrying capacity than low conductivity polymer solutions. The Create of a Taylercone and the controlling of nanofiber diameter are both affected by solution conductivity. The weak conductivity solution cannot produce nanofibers with the electrospinning technique because the surface of the droplet will be free of charge, preventing the Taylor cone from forming. On the other hand, the increase in the conductivity of the solution will induce the Taylor cone to develop due to the increased charge on the surface of the droplet . We note from the results presented in Table 4.1 that the electrical conductivity of the solution decreased from 660 to 580.3 µs/cm, when the percentage of gelatin increased from (20% to 80%) in the mixture of biopolymers solutions (G:CS:SA) [140]. In addition, from the results presented in Table 4.2 solutions of gelatin, chitosan and pullulan with different weight ratios, we noticed a decrease in the electrical conductivity of the solution from 490.45 to 400.23 µs/cm, when the percentage of gelatin increased from (20% to 80%) in the mixture of biopolymers solutions (G: CS: PUL). These results indicated that increasing in electrical conductivity is due to the increase in free ions in solutions and agree with previou study [141].

4.3 Contact Angle

The contact angle plays an important role in converting nanofibers from hydrophobic polymeric materials to hydrophilic materials, which leads to increasing the nanofiber release efficiency and improving its antibacterial efficiency. From Figure 4.2, we observed that the values of contact angles increased from (27.516°) to (54.988°) with decreasing gelatin content (80%-20%) in the blends nanofibers (G:CS:SA). The contact angle also increased from (25.5°) to (56.545°) when the percentage of gelatin was reduced from 80% to 20% in the blends nanofibers (G:CS:PUL). This result because of increased the ratio of chitosan for all nanofibers and the good interconnection between the components of the nanofibers [142].

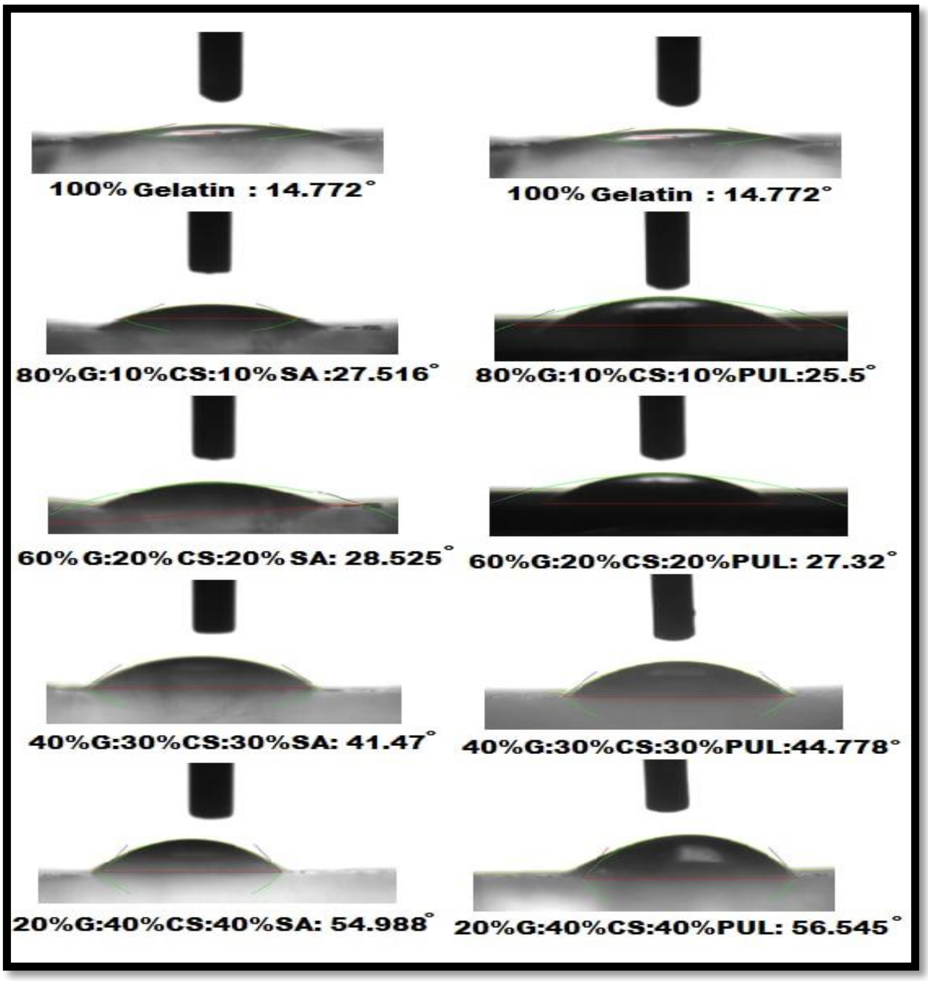


Fig. (4.2) shows the contact angles of pure gelatin fibers and bio-nanofibers (G:CS:SA) and (G:CS: PUL) with different weight ratios

Table 4.1 represents the results of pure gelatin and polymer blends solutions as Conductivity (µS/cm), Surface tension (mN/m), Viscosity (mPa.S) at shear rate ($24S^{-1}$)

Gelatin ratio (%)	Conductivity	Surface tension	Viscosity
0.0 (50% CS: 50% SA)	693.3	38.006± 0.104	45.2
20 (20 % G : 40 % CS:40% SA)	660	32.56±0.235	63.3
40 (40 % G : 30 % CS: 30% SA)	635.2	27.616±0.205	81.4
60 (60 % G : 20 % CS: 20% SA)	600.7	24.194±0.322	108.8
80 (80 % G : 10 % CS: 10% SA)	580.3	23.703±0.210	148.3
100 (Pure Gelatin)	570.6	23.061±0.671	200.4

Table 4.2 represents the results of pure gelatin and polymer blends solutions as Conductivity (µS/cm), Surface tension (mN/m), Viscosity (mPa.S)) at shear rate ($24S^{-1}$)

Gelatin ratio (%)	Conductivity	Surface tension	Viscosity
0.0 (50% CS: 50% PUL)	567.8	39.423±0.412	79.12
20 (20 % G : 40 % CS:40% PUL)	490.45	39.17±0.160	99.97
40 (40 % G : 30 % CS: 30% PUL)	425.32	34.716±0.210	123.72
60 (60 % G : 20 % CS: 20% PUL)	415.51	32.96±0.395	135.22
80 (80 % G : 10 % CS: 10% PUL)	400.23	32.27±0.0472	149.76
100 (Pure Gelatin)	570.6	23.061±0.671	200.4

4.4 AFM Analysis of nanofiber

Figure (4.3) and Table (4.3) show the roughness parameters of nanofibers for pure gelatin and nanofibers for solutions of gelatin, chitosan and sodium alginate with different weight ratios. We noticed a decrease in the (Sdr%) from (281.7 %) to (36.37 %) when the percentage of gelatin in the mixtures decreased from (100%) to (60%), and then increased from (45.35%) to (96.97 %) with decreased ratios gelatin in the mixtures from 40% to 20%, because of formation beads. These results indicated that the high viscosity or high nanofibers diameter lead to high surface roughness and interaction between the blends polymers lead to low surface roughness [143]. As well formation of beads in the nanofibers leads to an increase in the surface roughness when the percentage of gelatin decreases from (40%) to (20%).

On other hand, the roughness parameters of blends biopolymers nanofibers as (Sdr%) of (G:CS: PUL) decreased from (281.7) to (27.21) when decreased the ratio of gelatin in blends from (100 % to 40 %). Then increased to (42.95) at 20 % gelatin blend, because of formation only beads. These results indicated that the high viscosity or high diameter of the nanofibers leads to a high surface roughness, and that the interaction between the polymer mixture leads to a decrease in the surface roughness [70]. As well formation of beads in the nanofibers leads to an increase in the surface roughness when the percentage of gelatin decreases to (20%), shown in Table (4.4) and Figure (4.4).

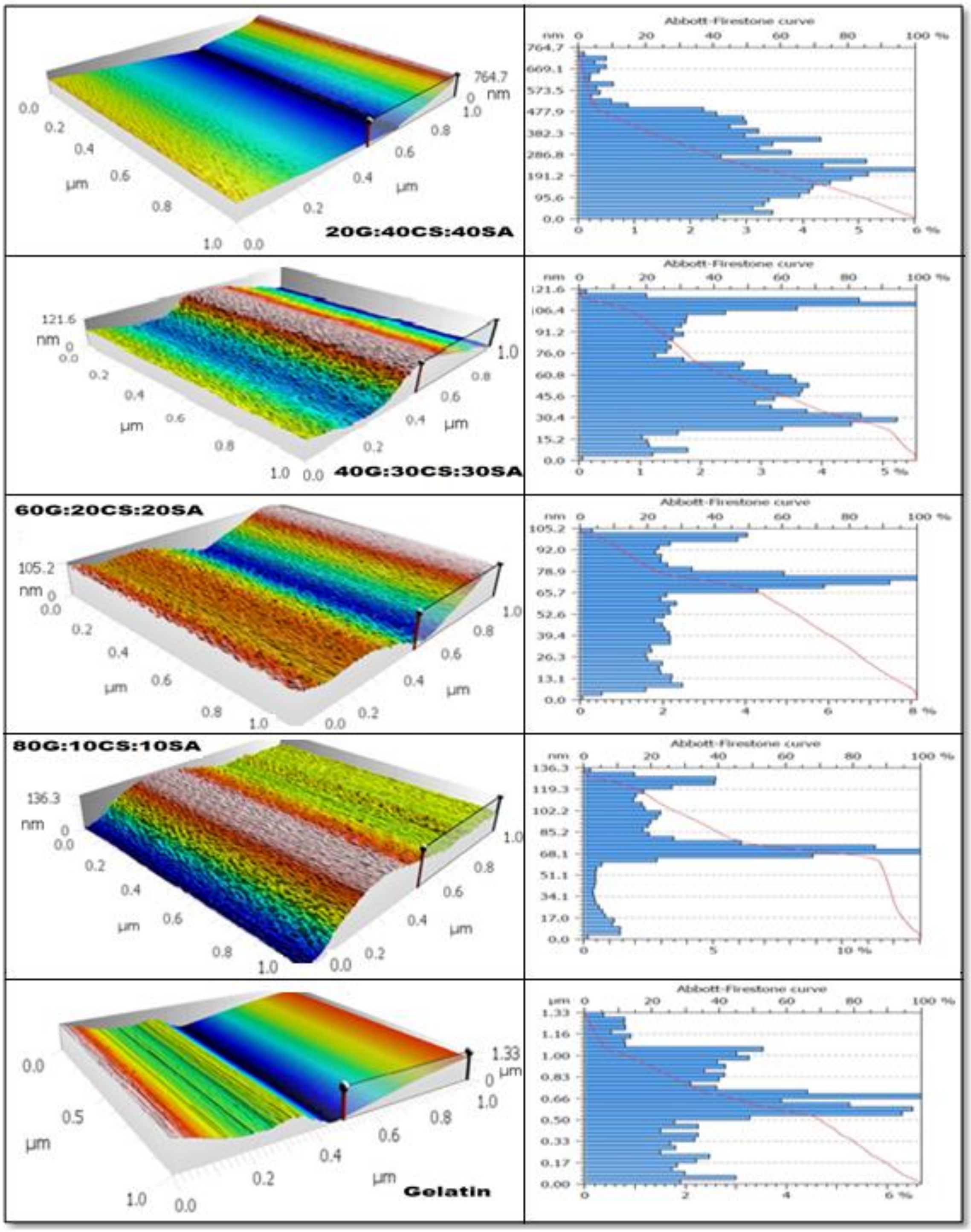


Fig. (4.3) AFM images by using MountainsSPIP® Expert 8.2.9621 according to ISO 25178 - Primary surface for pure gelatin and bio nanofibers blends (G: CS: SA) with different weight ratios

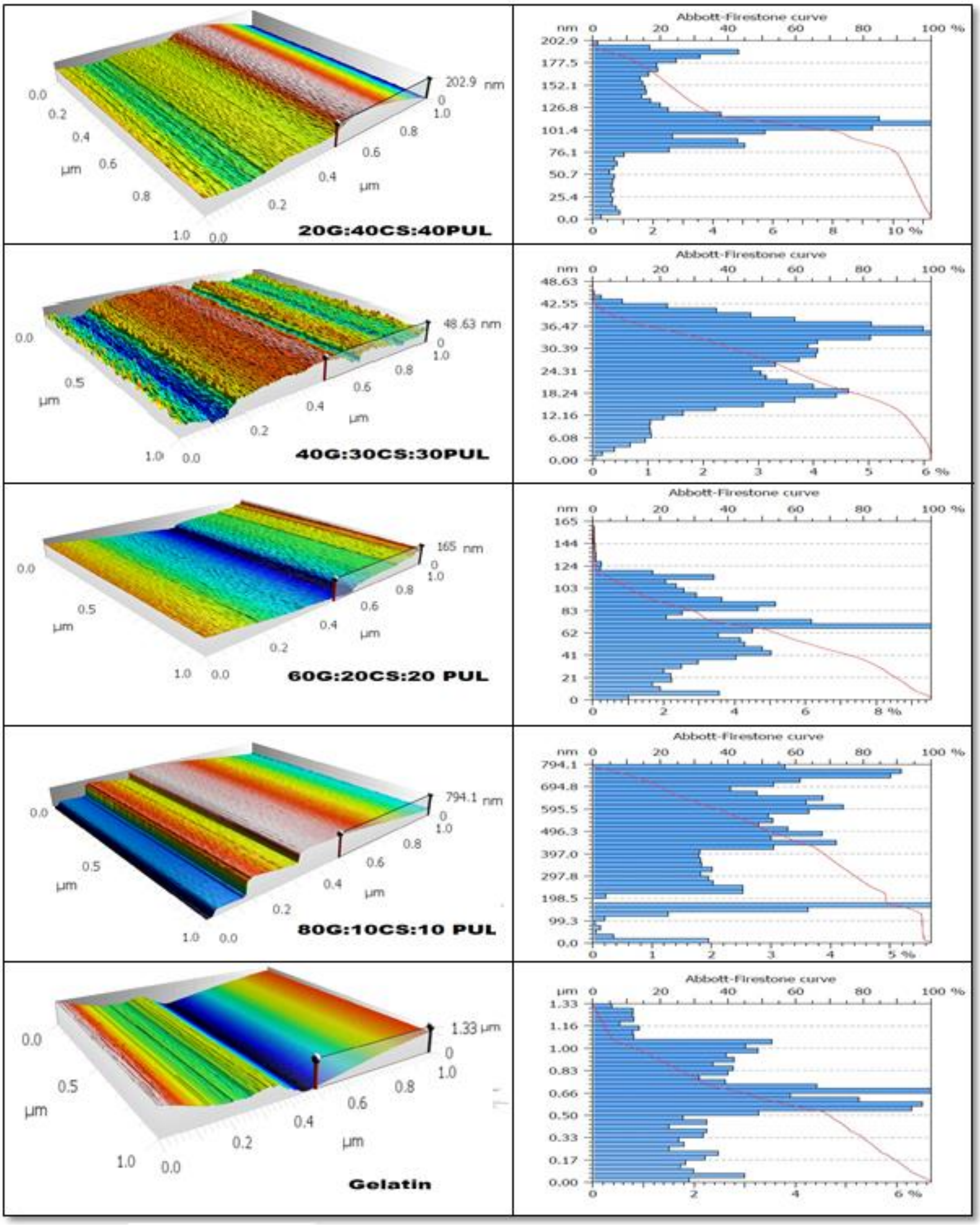


Fig. (4.4) AFM images by using MountainsSPIP® Expert 8.2.9621 according to ISO 25178 - Primary surface for pure gelatin and bio nanofibers blends (G: CS: PUL) with different weight ratio

Table (4.3) Sq, Sz, Sa, Sdr (%) Roughness parameters of pure gelatin fibers and nanofibers for (G: CS:SA) with different weight ratios

Gelatin ratio (%)	**Sq(nm)**	**Sz(nm)**	**Sa(nm)**	**Sdr(%)**
20(20%G:40%CS:40%SA)	152.1	764.7	124.6	96.97
40(40%G:30%CS:30%SA)	32.20	121.6	27.50	45.35
60(60%G:20%CS:20%SA)	26.99	105.2	22.87	36.37
80(80%G:10%CS:10%SA)	30.01	136.3	23.33	53.11
100%G	308.7	1330	246.6	281.7

Table (4.4) Sq, Sz, Sa, Sdr (%) Roughness parameters of pure gelatin fibers and nanofibers for (G:CS: PUL) with different weight ratios

Gelatin ratio (%)	**Sq(nm)**	**Sz(nm)**	**Sa(nm)**	**Sdr(%)**
20(20%G:40%CS:40%PUL)	42.32	202.9	32.25	42.32
40(40%G:30%CS:30% PUL)	19.546	48.63	18.163	27.21
60(60%G:20%CS:20% PUL)	30.13	165.0	24.68	32.94
80(80%G:10%CS:10% PUL)	206.9	794.1	174.3	219.9
100%G	308.7	1330	246.6	281.7

4.5 FE- SEM Analysis of nanofiber

The results of the morphological properties of the pure gelatin nanofiber and blends nanofiber from gelatin: chitosan: sodium alginate with different composition ratios were shown in Table (4.5) and Figure (4.5). There is no possibility of electrospinning the solutions at mixing ratios of 50% CS: 50% SA, as a result of high surface tension, as shown in the Table (4.1), while there was electrospinning ability for solutions at a mixing ratio of 20% G: 40% CS: 40% SA, as shown in Figure 4.7A; without nanofibers, only a large amount of drops formation, due to the low percentage of gelatin, the viscosity of the mixture decreased. The ability of the electrospun nanofibers improves with increasing gelatin in the mixing ratio 40% G: 30% CS: 30% SA, 60% G: 20% CS: 20% SA, and 80% G: 10% CS: 10% SA. The nanofibers are denser and uniform with 100% G, 80% G: 10% CS: 10% SA, 60% G: 20% CS: 20% SA with a decrease in the diameters and range of the nanofibers and absence of beads than nanofibers with decrease gelatin proportion. The fibers were uniform and the average diameter (AD) begins to decrease from (256.86±56.56 nm) to (142.35±59.05 nm) when decreasing the gelatin ratio from 100% to 60 % and range of nanofiber diameter distribution gradually decreasing from (152.94 - 400 nm) to (47.06-352.94 nm), while the decrease in the percentage of gelatin to 40% leads to the formation of nano-fibers with beads as a result of decreasing the viscosity of the mixture solution (gelatin, chitosan, and sodium alginate).The ratios (80:10:10) and (60:20:20) of gelatin: chitosan: sodium alginate are the best blending ratio had a uniform nanofiber with very low beads formation [140] On the other hand, for a pure gelatin solution and a mixture of gelatin, chitosan, and pullulan solutions with different weight ratios. There is no possibility of electrospinning the solutions at mixing ratios of 50% CS: 50% PUL, the result of the high surface tension shown in the Table (4.2). In addition, there is the possibility of electrospinning the solutions and drops formation only with a ratio of 20% G: 40% CS: 40%, as shown in Figure 4.7B, as a result of the decrease in the percentage of gelatin, which led to a decrease in the viscosity of the solutions. The ability of electrospun nanofibers improves with the increase of gelatin in the blending ratio 40 % G: 30 % CS: 30% PUL, 60 % G: 20 % CS: 20% PUL, 80 % G: 10 % CS: 10% PUL. the nanofibers are denser and uniform at the ratio at 100 - 40% gelatin with decrease in the diameters and range of the nanofibers. The average diameter (AD) begins decrease from (256.86±56.56 nm) to (82.32±33.91 nm) when decreasing the gelatin ratio from 100% to 40 %

and range of nanofiber diameter distribution gradually decreasing from (152.94-400 nm) to (30.76-209.13nm). The ratios (80:10:10) and (60:20:20) of gelatin: chitosan: pullulan were the best blending ratio which had a uniform nanofiber with very low beads formation [144]. This result is shown in Table (4.6) and Figure (4.6).

Table (4.5) Average fibers diameters, ranges of Nanofibers (nm) , and ability of electro spun for blends gelatin :chitosan: sodium alginate with different weight ratios.

Gelatin ratio (%)	**Ranges of Nanofibers (nm)**	**Ability of Electro spun**
0.0 (50% CS: 50% SA)	None	None
20 (20 % G : 40 % CS:40% SA)	None	Yes, with drops formation
40 (40 % G: 30 % CS :30% SA)	23.53 – 105.88	Yes, with (Fiber+ Beads) formation
60 (60 % G: 20 % CS: 20% SA)	47.06 - 352.94	Yes, with Fiber formation
80 (80 % G : 10 % CS: 10% SA)	105.88-294.12	Yes, with Fiber formation
100 (Pure Gelatin)	152.94 - 400.00	Yes, with Fiber formation

Table (4.6) Average fibers diameters, ranges of Nanofibers (nm) , and ability of electro spun for blends gelatin :chitosan: Pullulan with different weight ratios.

Gelatin ratio (%)	Ranges of Nanofibers (nm)	Ability of Electro spun
0.0 (50% CS: 50% PUL)	None	None
20 (20 % G : 40 % CS:40% PUL)	None	Yes, with drops formation
40 (40 % G : 30 % CS :30% PUL)	30.76-209.13	Yes, with Fiber formation
60 (60 % G : 20 % CS: 20% PUL)	39.13-145	Yes, with Fiber formation
80 (80 % G : 10 % CS: 10% PUL)	39.43-198.37	Yes, with Fiber formation
100 (Pure Gelatin)	152.94-400.00	Yes, with Fiber formation

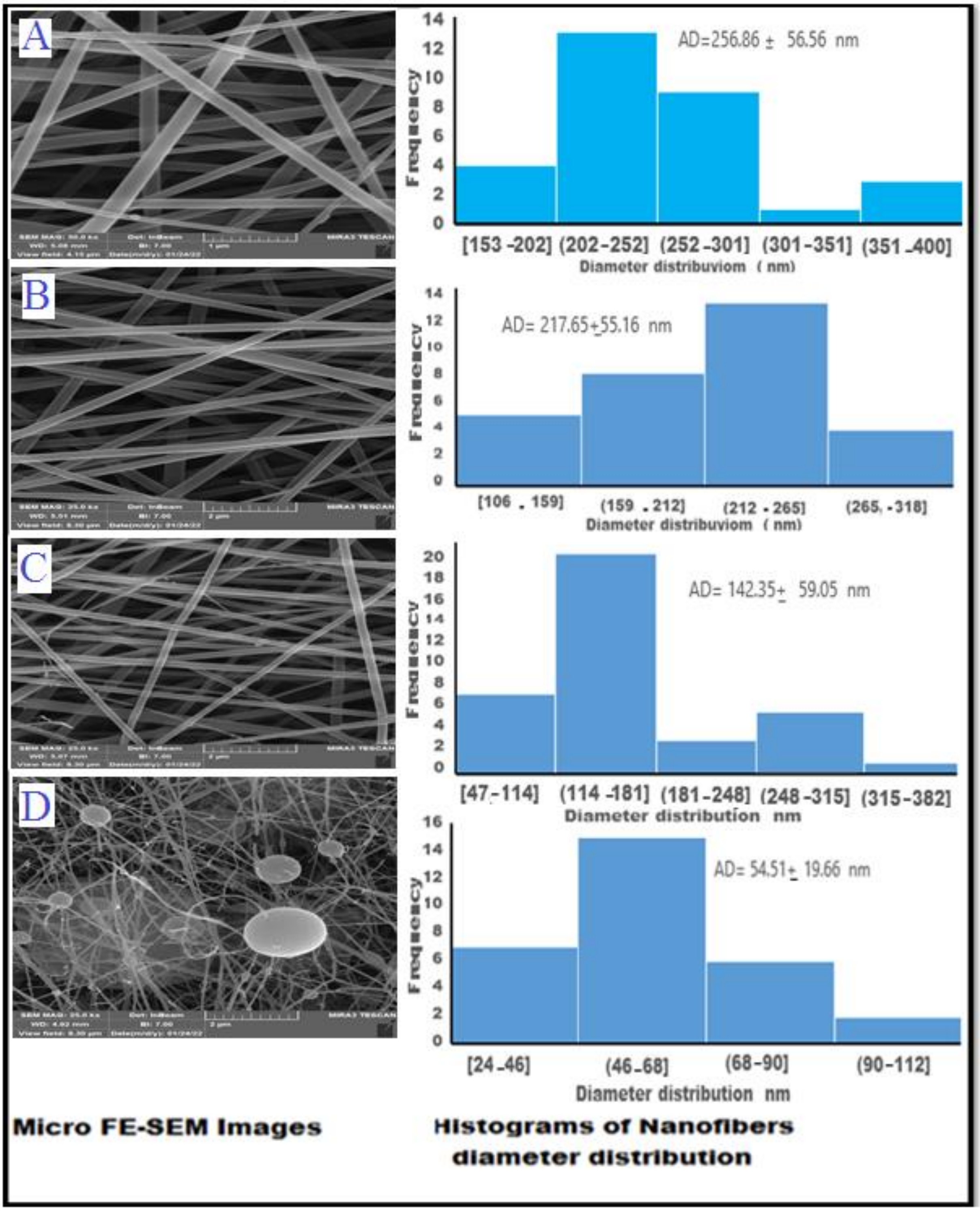


Fig. (4.5) FE-SEM-histograms of bio nanofibers diameter distribution for three blends biopolymers nanofibers (A) Gelatin(100%) ,(B) 80% G: 10 %CS: 10% SA , (C) 60% G:20 %CS: 20% SA ,and (D) 40% G:30 %CS: 30% SA

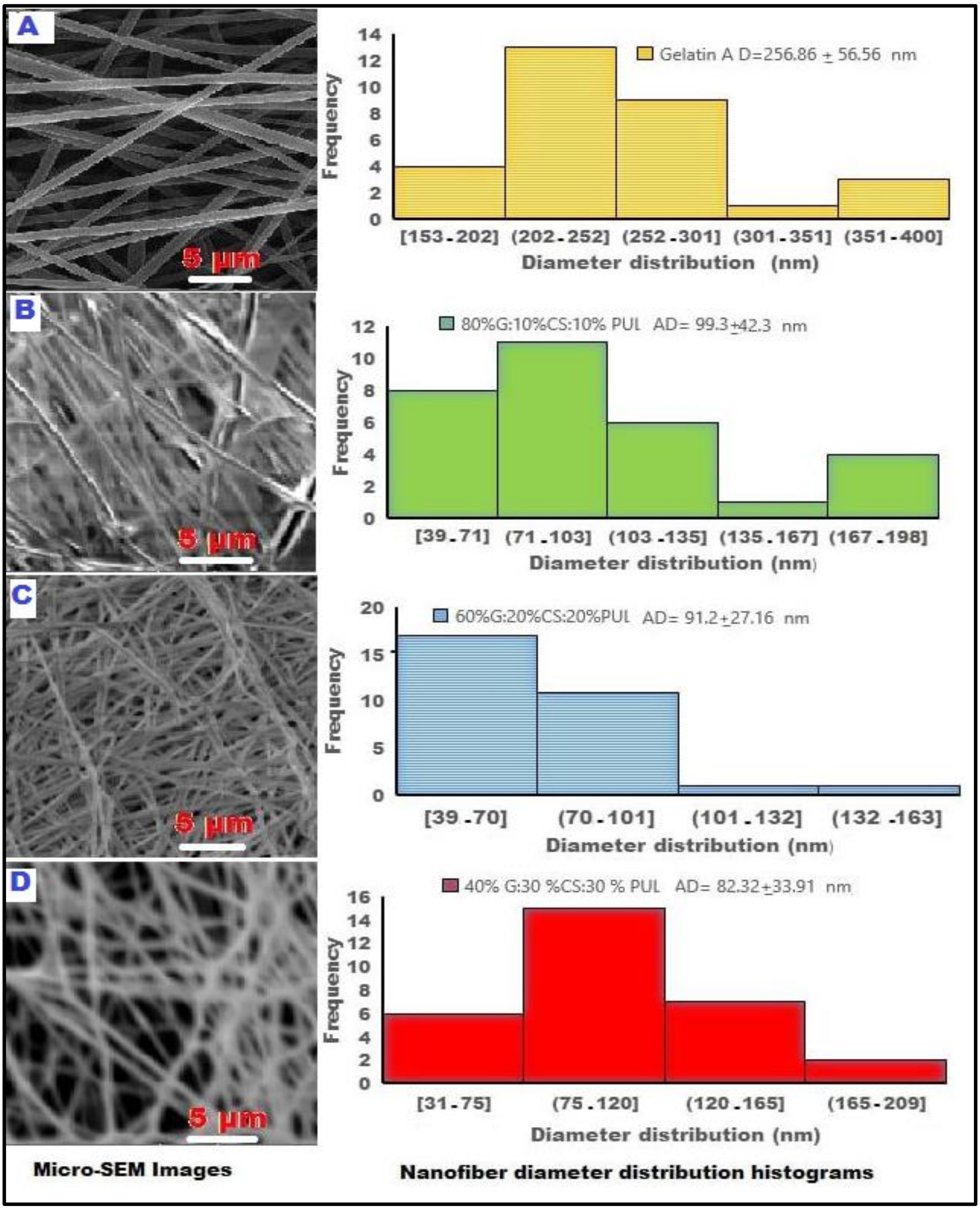


Fig. (4.6) FE-SEM histograms of bio nanofibers diameter distribution for three blends biopolymers nanofibers (A) Gelatin(100%) ,(B) 80% G: 10 %CS: 10% PUL , (C) 60% G: 20 %CS: 20% PUL ,and (D) 40% G:30%CS:30%PUL

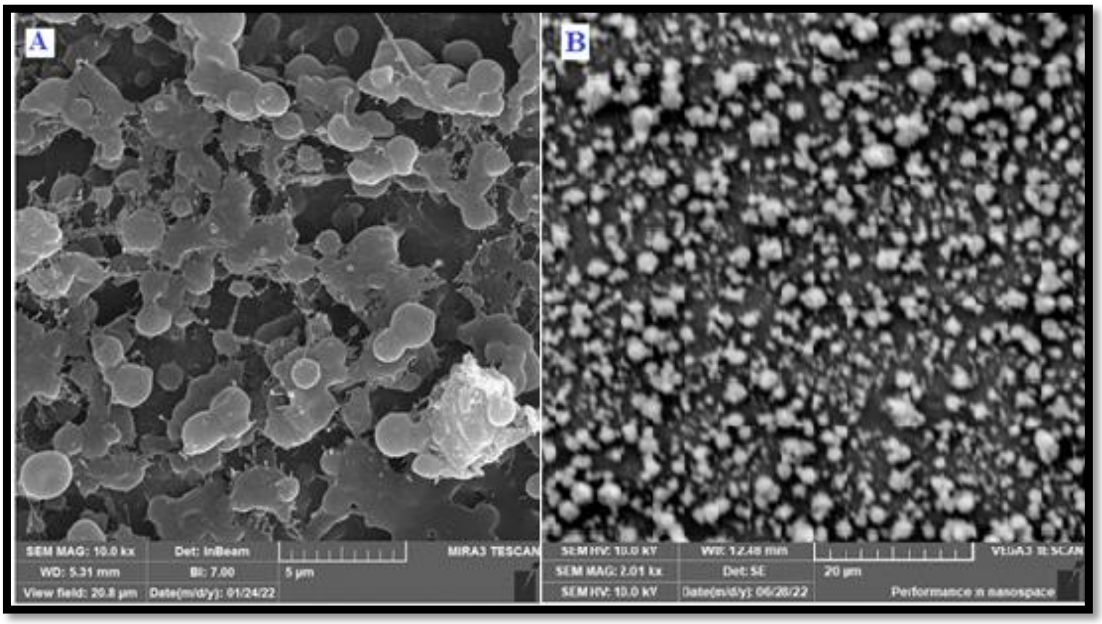


Figure (4.7) shows SEM images of droplets with different weight ratios (A) 20% G: 40% CS: 40% SA (B) 20% G: 40% CS: 40% PUL

4.6 (FT-NIR) Analysis of nanofiber

The chemical bonds that occur between the nanofibers components during their formation can be determined using FT-NIR. Whereas, the FT-NIR (500-4000) cm^{-1} analysis range was used. Figure 4.11(a) to (d) shows the FT-NIR spectra of gelatin (G), chitosan(CS), sodium alginate(SA), and blending nanofibers(G:CS:SA), respectively. The FT-NIR spectrum of gelatin (as seen in Figure 4.8 (a) the absorption peaks at (3388, 2922, 1651, 1557, 1240, and 1094 cm^{-1}, these peaks represent secondary amide NH stretching, CH stretching, C=O stretching (amide I), NH bending (amide II), and C–O–C stretching, respectively. Figure 4.8 (b) shows the absorption peaks of chitosan (CS) at 3314, 2922, 2851, 1651, 1557, 1423, 1380, and 1157cm^{-1}. this peak 3314 cm^{-1} represents NH and OH stretching vibrations [145]. The CH stretching vibration caused the bands at 2922 and 2851 cm^{-1} [146]. The bands are at 1651 and 1557 cm^{-1} amino groups (amide I (C=O str.) and amide II (N-H bend and C-N str.) [147]. The CH2 and CH3 vibrations are assigned bands about 1423 and 1380 cm^{-1}, respectively. The characteristic bands of C–O–C linkage were found in the

1157 cm-1. The typical absorption peaks of sodium alginate(SA) at 3430, 2900, 1610, 1417, 1100, 970, and 828cm^{-1}, this peaks represents OH stretching of hydroxyl groups, CH stretching groups, asymmetric COO- stretching, symmetric COO- stretching, C-O stretching, C-O stretching (uronic acid), C-H stretching (mannuronic acid), respectively, shown in Figure.4.8(c). In the spectrum for (G: CS:SA) showing a high electrostatic attraction between sodium alginate's negatively charged carboxylic acid salts (COO-) and chitosan's positively charged amino groups (-NH3+) at 1545 cm-1[148], and (C=O) group for (G) with N–H groups of (CS) result in strong hydrogen bonds. shown in Figure.4.8(d) [149].

The interaction of the carboxyl group of alginate with the amino group of gelatin leads to the formation of the polyelectrolyte region of 1500-1650 cm^{-1} [150].

Figure 4.9 (a) and (d) show the FT-NIR spectra of gelatin (G), chitosan (CS), pullulan (PUL), and blending nanofibers (G: CS: PUL), respectively. The FT-NIR spectra of pullulan(PUL) were seen at 3310 cm^{-1} (O-H stretching vibrations), 2930 cm^{-1} (C-H vibrations),1636 cm^{-1}(O-C-O stretching), 1354 cm^{-1}(C-O-H stretching), 1081 cm^{-1}(C6-OH stretching), and 929 cm^{-}1 (α-(1, 6)-D-glycosidic) shown in Figure 4.9 (c) [151]. In the spectrum for (G: CS: PUL) the hydrogen bonds of pullulan and gelatin were formed between groups (O–H) of pullulan and (N–H, O–H) of gelatin. Ionic bonds form between chitosan, which has a positive charge, and the amino acids in gelatin, which have a negative charge. The amino acids in gelatin also contain -COOH, -NH2 and -OH groups that can form hydrogen bonds with -OH and -NH2 groups on the chitosan structure, as shown in Figure 4.9 (d) [144]. The FT-NIR spectra of the G:CS:SA and G:CS: PUL nanofiber films had the characteristic peaks of G, CS, SA, and PUL and did not have additional peaks. These results indicated that all the interactions among these three ingredients during the formation of nanofiber films were physical.

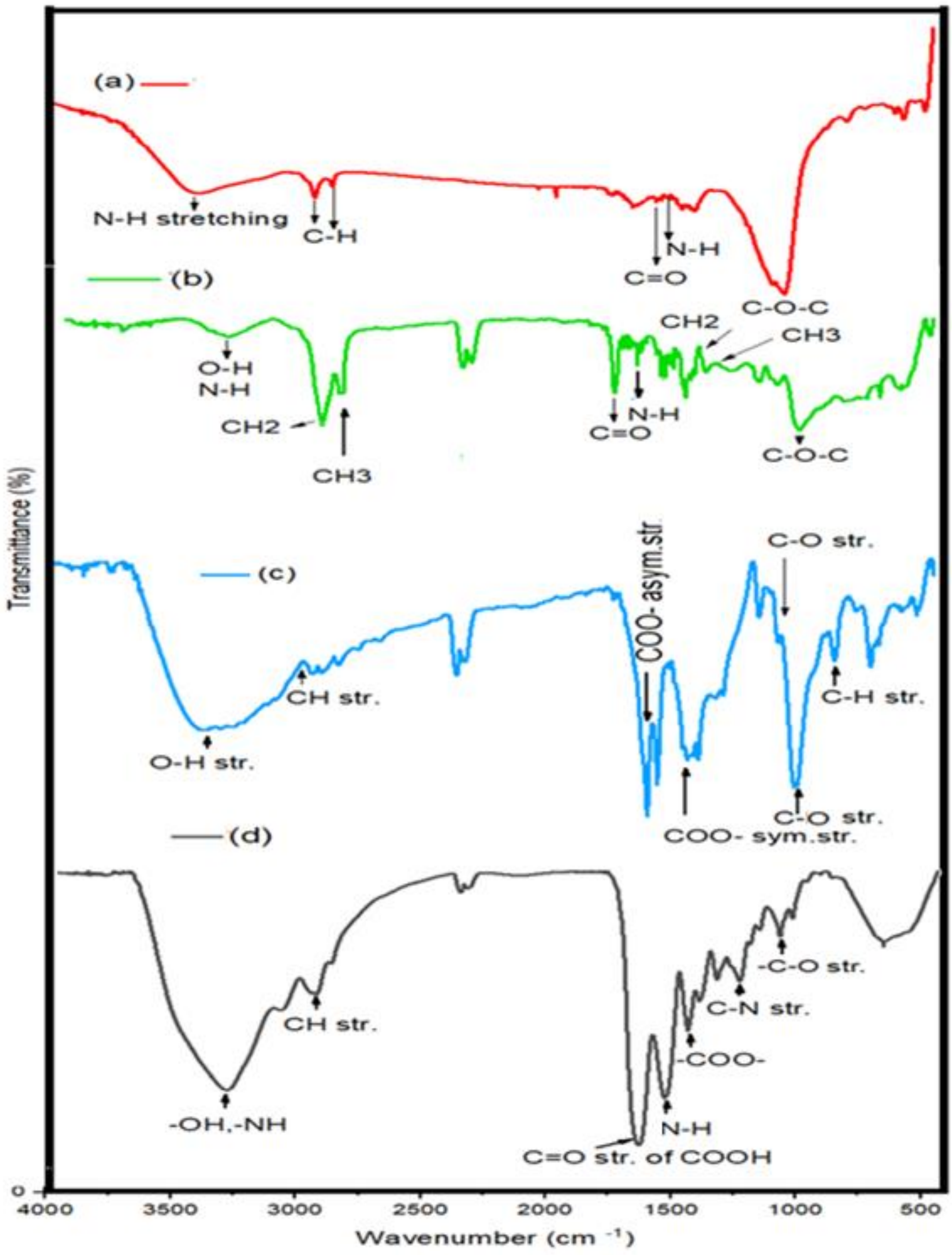


Fig. (4.8) shows the FT-NIR spectra for(a) G, (b) CS, (c)SA, (d) G:CS: SA bio-nanofiber

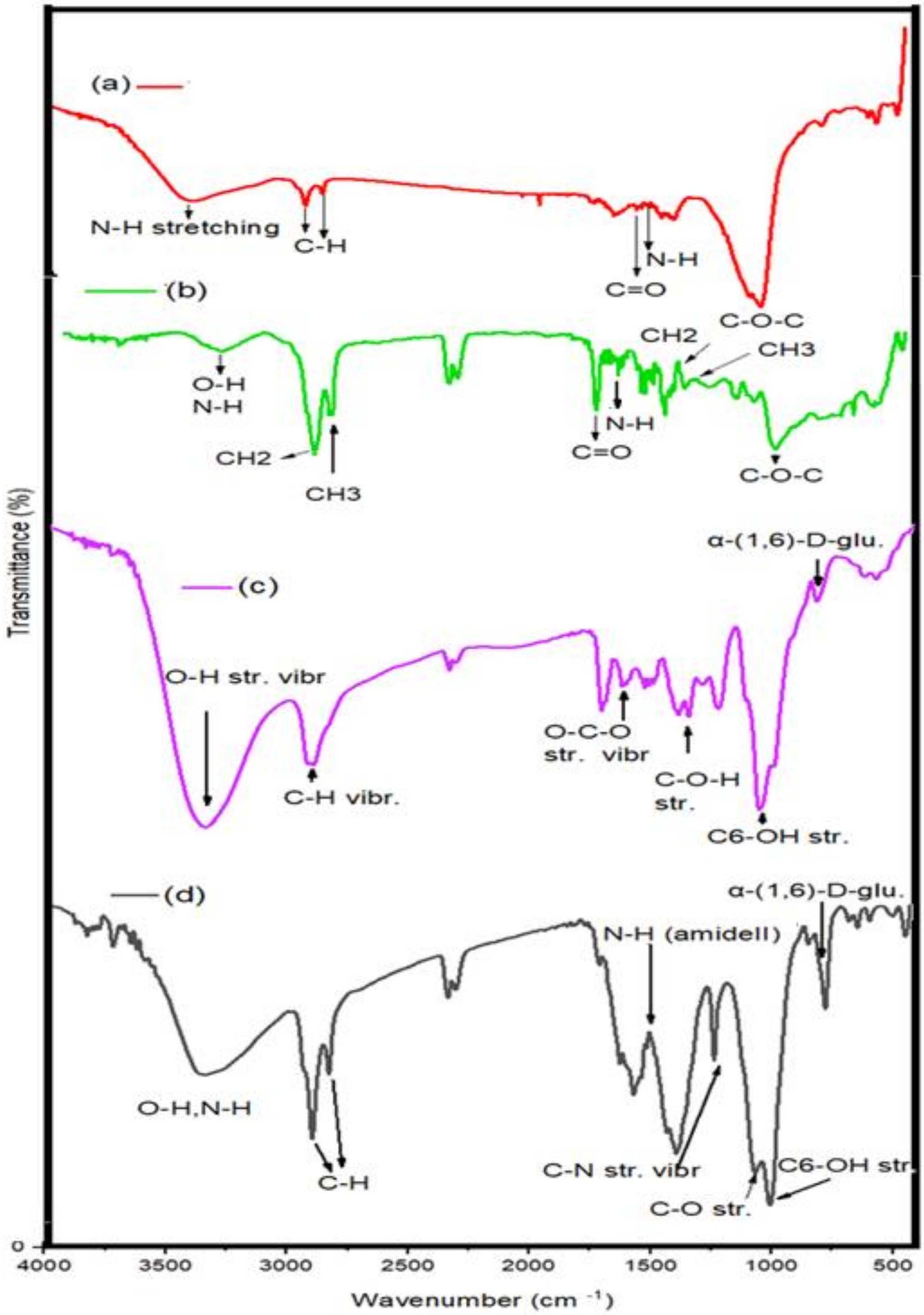


Fig. (4.9) shows the FT-NIR spectra for(a) G, (b) CS, (c)PUL, (d) G:CS: PUL bio-nanofibers.

4.7 (XRD) Analysis of nanofiber

The crystalline structure of the bio- nanofibers were determined, as the crystallinity % was the most important physical properties of the pure nanofiber and blends nanofiber. Therefore, the X-ray diffraction (XRD) pattern was used to characterize the crystallization changes of pure (G) nanofibers, nanofibers from mixture of G:CS:SA, and nanofibers from mixture of G:CS: PUL at different weight ratios.

The crystal structures of nanofibers prepared by electrospinning methods were obtained by XRD analysis. According to Figure (4.10) and Table (4.7), the pure (G), (80G: 10CS: 10SA), (60G: 20CS: 20SA), and (40G: 30CS: 30SA) nanofibers showed broad diffraction peaks at 2θ = 19.39°, 22.1°, 21.4°, and 20.89°, respectively, as well as a small peak at 2θ = 51.24°, 53.65°, 54.03°, and 52.82° for the nanofibers for (G), (80G: 10CS: 10SA), (60G: 20CS: 20SA), and (40G: 30CS: 30SA), respectively. After calculation, the crystallinity of the (G), (80G:10CS:10SA), and (60G:20CS:20SA) nanofiber were 6.03%, 7.19%, and 8.1%, respectively. Compared with the pure (G) the crystallinity% increased, these results lead to improve crystalline properties of gelatin by bends with the CS and SA, while the crystallinity % of the(40G:30CS:30SA) is decreased, due to the formation of beads and low fiber density, as shown in images (FE-SEM) Figure (4.5) D. The best crystallinity % at 80G:10CS:10SA and 60G:20CS:20SA blend ratio, which indicates more interaction between the intermolecular of the blends [148].

Figure (4.11) and Table (4.8),the (G), (80G:10CS:10PUL), (60G:20CS:20PUL), and (40G:30CS:30PUL) nanofibers showed broad diffraction peaks at **2**θ=19.39°, 20.4°, 19.9°, and 19.20° respectively, and the small peaks at 2θ=51.24°,51.65°, 48.53°, and 52.22° for the nanofiber for (G), (80G:10CS:10PUL), (60G:20CS:20PUL), and (40G:30CS:30PUL) respectively**.** After calculation, the crystallinity of the (G), (80G:10CS:10PUL), and

(60G:20CS:20PUL) nanofiber were 6.03%,7.59%, and 9.21%, respectively, Compared with the pure (G) the crystallinity% of nanofiber were increased, These results lead to improve crystalline properties of gelatin by bends with the CS and PUL, while the crystallinity % is decreased, Due to the low fiber density, As shown in images (FE-SEM) Figure (4.6) D. the best crystallinity % at 80G:10CS:10PUL and 60G:20CS:20PUL blend ratio, which indicates more interaction between the intermolecular of the blends .

Table (4.7) results of Orientation of pure gelatin nanofiber and nanofibers of (G: CS: SA) with different weight ratios

Gelatin ratio (%)	**Orientation**
20(20%G:40%CS:40%SA)	Beads
40(40%G:30%CS:30% SA)	3.9%
60(60%G:20%CS:20% SA)	8.1%
80(80%G:10%CS:10% SA)	7.19%
100%G	6.03%

Table (4.8) results of Orientation of pure gelatin nanofiber and nanofibers of (G:CS: PUL) with different weight ratios

Gelatin ratio (%)	**Orientation**
20(20%G:40%CS:40%PUL)	Beads
40(40%G:30%CS:30% PUL)	4.33%
60(60%G:20%CS:20% PUL)	9.21%
80(80%G:10%CS:10% PUL)	7.59%
100%G	6.03%

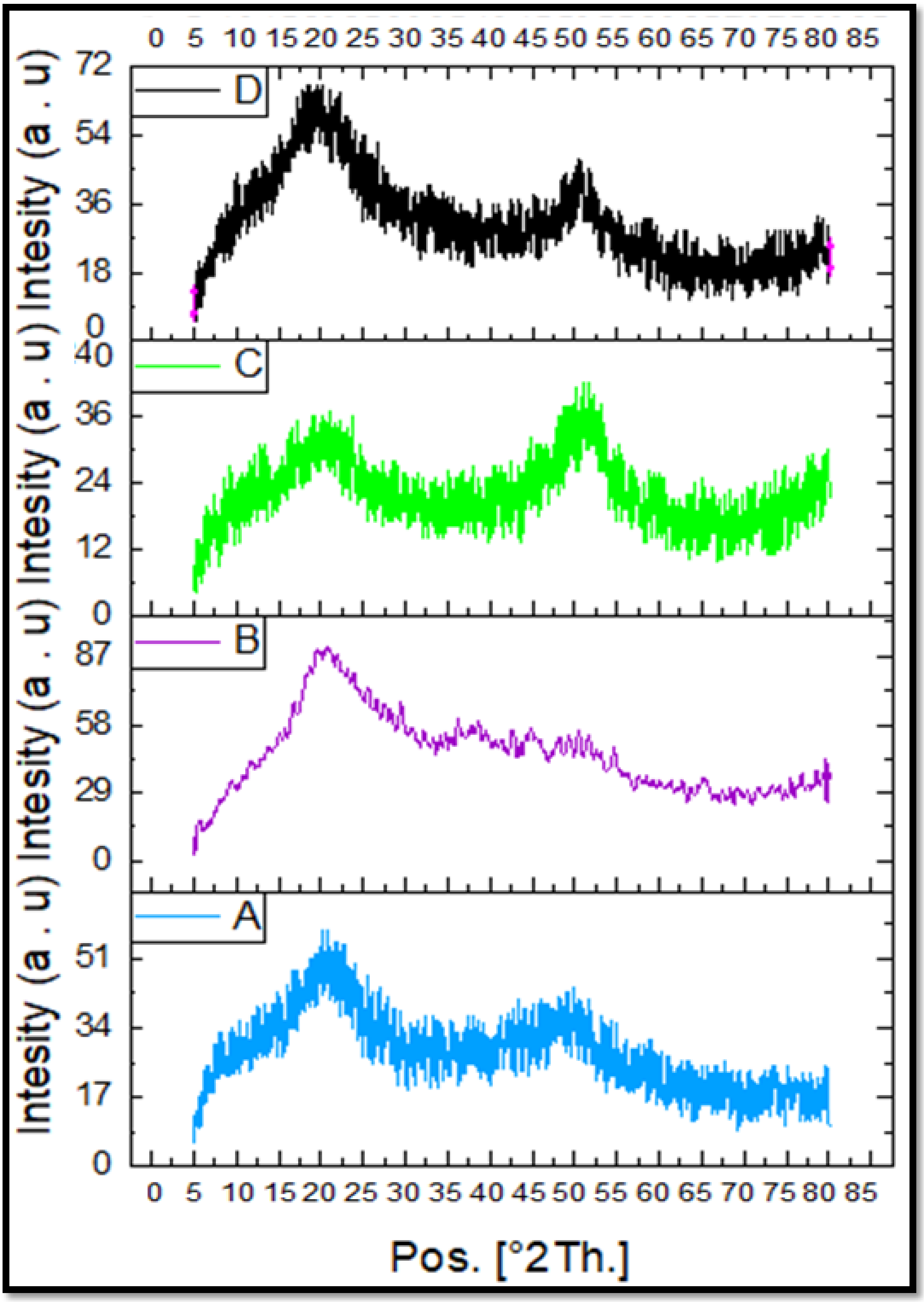


Fig. (4 .10) shows the X-ray diffractometric analysis of (A) 40G: 30CS:30SA, (B) 60 G:20CS:20SA, (C) 80 G:10CS:10SA, and (D) pure gelatin nanofibers.

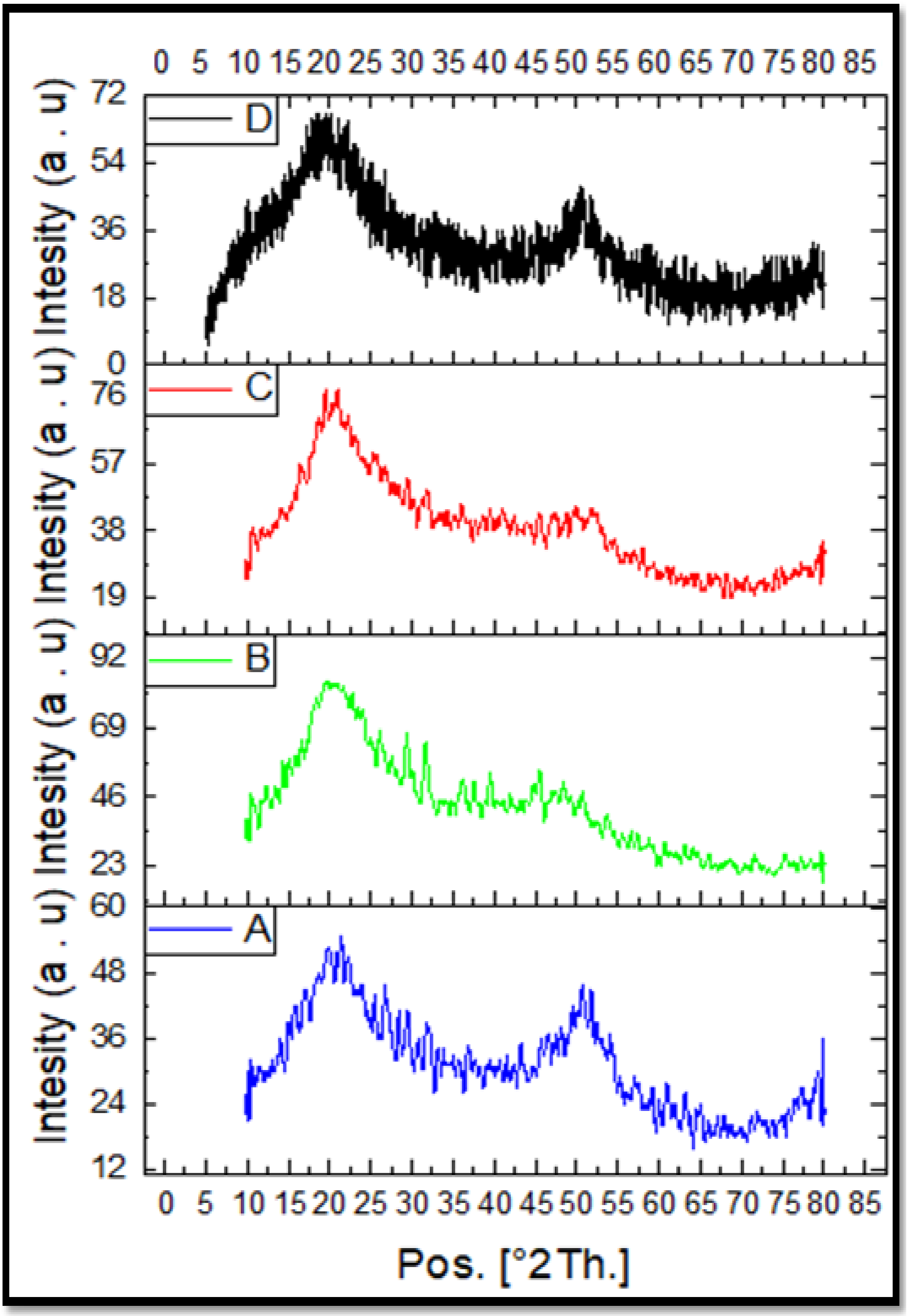


Fig. (4.11) shows the X-ray diffractometric analysis of (A) 40G: 30CS:30PUL, (B) 60G: 20CS:20PUL, (C) 80G:10CS:10PUL, and (D) pure gelatin nanofibers

4.8 Bio Nanofibers Release

For the purpose of determining the release of Anti-Bacterial polymeric substances that prevent food spoilage، 4 mg of pure gelatin nanofibers and nanofibers of polymeric mixtures represented by (G:CS:SA) and (G:CS: PUL) were dissolved in 10 mL of distilled water. The release of polymeric materials in distilled water is done by determining the wavelengths because of changing the absorption peak, using UV spectra according to the length of the wavelength from190 nm to 300 nm (as seen in the Figure 4.12). The maximum absorption peak of pure gelatin is 240 nm, Because of the presence of amino groups in gelatin, which are glycine, proline and argine, are responsible for the presence of this absorption peak [152]. while the maximum absorption peak of (60 G: 20CS:20 SA) is 227 nm and maximum absorption peak of (60 G: 20CS:20 PUL) is 224 nm. From the above results, it was found that the best release ratio for nanofibers is (224nm) for (60G: 20CS:20PUL). These results are consistent with the result of nanofiber release of (chitosan: gelatin) and agree with previous studies [152,153].

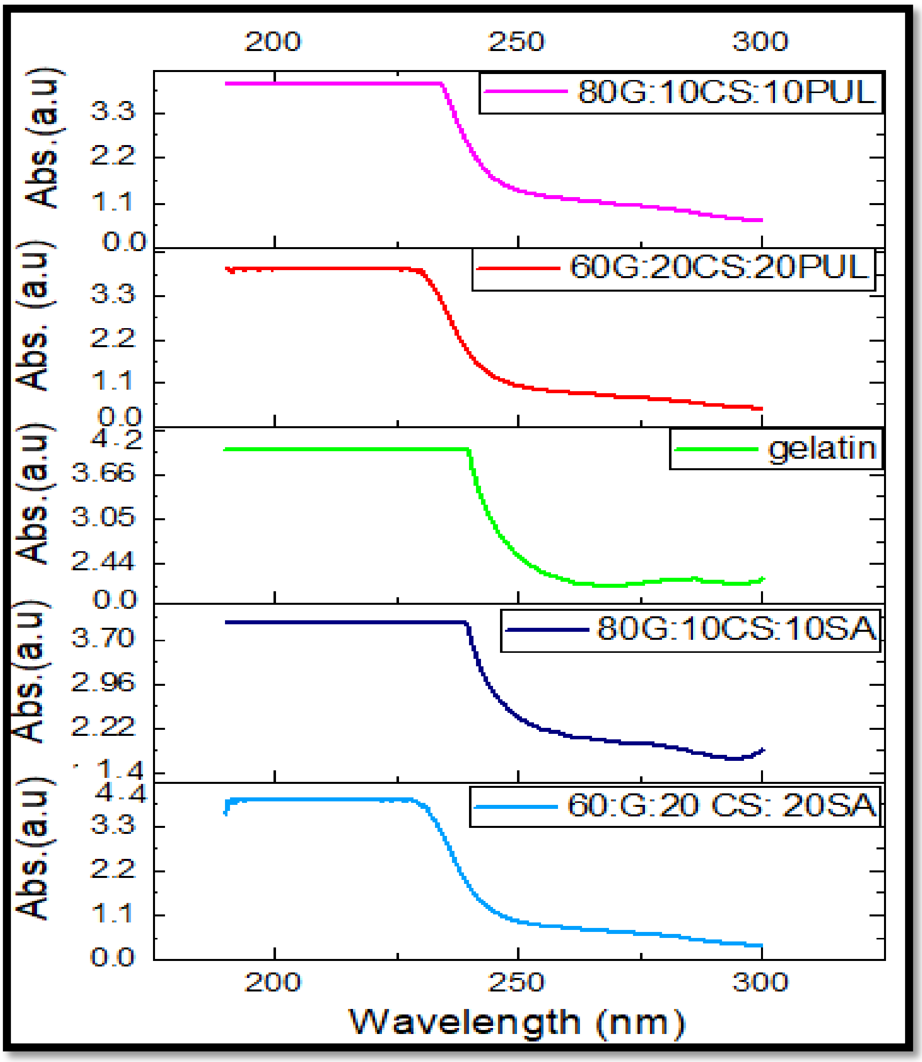
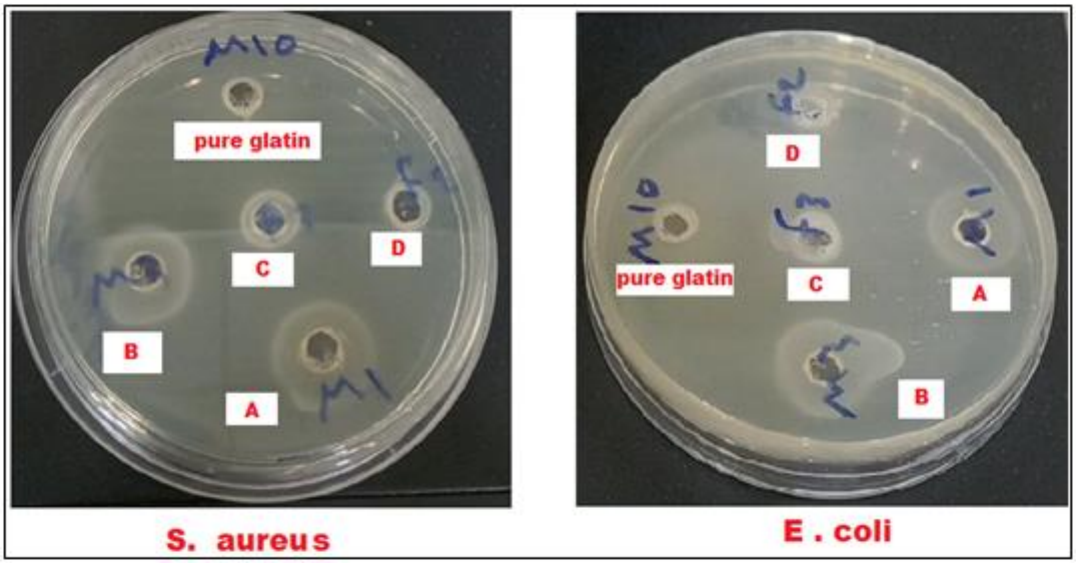


Fig. (4.12) UV- Visible spectra for pure gelatin nanofiber and nanofibers of (G: CS: SA) and (G:CS: PUL) with different weight ratios

4.9 Anti-Bacterial

The Agar Well Diffusion method achieved antibacterial activity as Mueller Hinton agar plates were prepared and inoculated with the test organisms, including gram-positive S. aureus and gram-negative E. coli by spreading the inoculum on to the surface of the medium with the help of sterile swabs. Wells (9 mm in diameter) nanofibers were drilled in agar using a sterile cork drill. The plates were incubated at 37°C for 48°, and the antibacterial efficacy against E. coli and S. aureus was evaluated by measuring the diameter of the inhibition zone and recording it in millimeters. Figure 4.13 shows the good antimicrobial activity of the blending bio-nanofibers against E. coli and S. aureus, where the inhibition zones of the nanofibers increased by decreasing the percentage of gelatin from (80%-60%) for (G:CS:SA) and (G:CS: PUL), the increased proportion of CS and PUL from 10% to 20% in the nanofiber mixture of G:CS:PUL was shown to had better resistance to bacterial growth and higher inhibition zone diameters of 24 and 22 mm against E. coli and S. aureus, respectively. as shown in Table 4.9. Because the pullulan is an important sources of antimicrobials, its incorporation into natural polymers helps to resist bacterial growth [97], and chitosan is natural polymer with high effectiveness against microbes and depends on the type of microbes, especially chitosan of low molecular weight because it works not only outside the cell but inside it due to the presence of the positively charged amino group interacting with the membranes of microbial cells negatively charged, which leads to the leakage of proteins and other components inside cells of microorganisms [154,155].



`Fig. 4.13 Zones of inhibition against S. aureus and E. coli bacteria for pure gelatin nanofiber and (A) 80 %G: 10%CS: 10% PUL, (B) 60%G: 20%CS: 20%PUL, (C) 60%G: 20%CS: 20%SA, and (D) 80%G: 10%CS: 10% SA of blends bio-nanofibers.

Table (4.9) results of inhibition zones (mm) against bacteria E. coli and S. aureus for fiber pure gelatin and nanofiber from (G:CS:SA), (G:CS: PUL) at different weight ratios

Sample	inhibition zones (mm)	
	E. coli(-)	S. aureus(+)
Pure gelatin	ND	ND
(D) 80%G: 10%CS:10%SA	15	14
(C) 60%G: 20%CS:20%SA	18	16
(B)80%G:10%CS:10%PUL	22	20
(A)60%G:20%CS:20%PUL	24	22

ND, not detected.

Chapter Five

Conclusions
&
Recommendations

5.1 Conclusions

Through this work, the following conclusions can be summarized:

1- Increase concentration, viscosity, molecular weight, decreasing surface tension and contact angle leads to an increase in fiber diameter.

2- For each blend critical values of viscosity and molecular weight to obtain optimum fiber dimensions with minimum beads depending on the shear rate range and electrospinning conditions.

3- All natural polymer in this work indicates good degradability and release.

4- Using of acetic acid solutions in the preparation of polymeric solutions reduced the surface tension of natural polymers and the success of the electrospinning process.

5- All fibers produced are hydrophilic with different mixing ratios.

6- The interaction between the components (G, CS, PUL, and SA) is a physical.

7- Increasing the percentage of gelatin improves the electrospinning process.

8- Blend nanofibers from the biopolymers (G:CS:PUL) had a higher antibacterial activity than blend biopolymers (G:CS:SA).

5.2 Recommendations

Some recommendations that may be useful for future researchs in this field include:

1. Using of nanofibers in medical applications such as tissue engineering and wound dressings.
2. Using another type of polymer, such as starch, collagen or cellulose, instead of gelatin.
3. Using the nanofibers (G:CS: PUL) in absorbing of heavy metal ions from waste water.
4. Procedure antifungal test for nanofibers.

References

References

[1] Liu, H., Gough, C. R., Deng, Q., Gu, Z., Wang, F., & Hu, X. (2020). Recent advances in electrospun sustainable composites for biomedical, environmental, energy, and packaging applications. *International Journal of Molecular Sciences*, *21*(11), 4019.

[2] Habeeb, S., Rajabi, L., & Dabirian, F. (2019). Comparing Two Electrospinning Methods in Producing Polyacrylonitrile Nanofibrous Tubular Structures with Enhanced Properties. *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, *38*(3), 23-42.

[3] Nadhim, B.A. and Habeeb, S.A., 2021. Studying the Physical Properties of Non-Woven Polyacrylonitrile Nanofibers after Adding γ-Fe2O3 Nanoparticles. Egypt. J. Chem., 64(12), pp.7521-7530.

[4] Khatri, Z., Kim, I. S., & Kim, S. H. (2016). Functional nanofibers: production and applications. *Journal of Nanomaterials*, 2016.3

[5] Aman Mohammadi, M., Rostami, M. R., Raeisi, M., & Tabibi Azar, M. (2018). Production of electrospun nanofibers from food proteins and polysaccharides and their applications in food and drug sciences. Jorjani Biomed J, 6(4), 77.

[6] Rashidi, L., & Khosravi-Darani, K. (2011). The applications of nanotechnology in food industry. Crit Rev Food Sci Nutr, 51(8), 723-730.

[7] Zhuang, X., Cheng, B., Kang, W., & Xu, X. (2010). Electrospun chitosan/gelatin nanofibers containing silver nanoparticles. arbohydrate polymers, 82(2), 524-527.

[8] Lin L, Lv S, Li B (2012) Angiotensi on-I-converting enzyme (ACE)-inhibitory and antihypertensive properties of squid skin gelatin hydrolysates. Food Chem. 131:225–230.

[9] Assani, K. (2018). M1 to M2 Macrophage Induction Using Retinoic Acid and Mesenchymal Stem Cells Loaded on an Electrospun Pullulan/Gelatin Scaffold to Promote Healing of Chronic Wounds.

[10] Rekha, M. R., & Sharma, C. P. (2007). Pullulan as a promising biomaterial for biomedical applications: a perspective. Trends Biomater Artif Organs, 20(2), 116-121

[11] Antaby, E., Klinkhammer, K., & Sabantina, L. (2021). Electrospinning of Chitosan for Antibacterial Applications—Current Trends. Applied Sciences, 11(24), 11937.

[12] Guarino, V., Caputo, T., Altobelli, R., & Ambrosio, L. (2015). Degradation properties and metabolic activity of alginate and chitosan polyelectrolytes for drug delivery and tissue engineering applications. *AIMS Materials Science*, *2*(4), 497-502.

[13] Sam, S. T., Nuradibah, M. A., Chin, K. M., & Hani, N. (2016). Current application and challenges on packaging industry based on natural polymer blending. In Natural Polymers (pp. 163-184). Springer, Cham.

[14] Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings: A review. Materials *Science and Engineering: C*, *33*(4), 1819-1841.

[15] Asma, C., Meriem, E., Mahmoud, B., & Djaafer, B. (2014). Physicochemical characterization of gelatin-cmc composite edibles films from polyion-complex hydrogels. J. Chil. Chem. Soc. 59(1), 2279-2283.

[16] Stijnman, A. C., Bodnar, I., & Tromp, R. H. (2011). Electrospinning of food-grade polysaccharides. Food Hydrocoll. 25(5), 1393-1398.

[17] Wang, F., Hu, S., Jia, Q., & Zhang, L. (2020). Advances in electrospinning of natural biomaterials for wound dressing. *Journal of Nanomaterials*, *2020*.

[18] Habeeb, S.A., 2021. Impact of Polymeric Solutions Parameters on Morphological Properties of Composite Nanofibers. Journal of University of Babylon for Engineering Sciences, 29(2), 115-120.

[19] Habeeb, S. A., Rajabi, L., & Dabirian, F. (2020). Production of polyacrylonitrile/boehmite nanofibrous composite tubular structures by opposite charge electrospinning with enhanced properties from a low-concentration polymer solution. Polymer. Compos. 41(4), 1649-1661.

[20] Hohman, M. M., Shin, M., Rutledge, G., & Brenner, M. P. (2001). Electrospinning and electrically forced jets. II. Applications. *Physics of fluids*, *13*(8), 2221-2236.

[21] Cengiz, F., Dao, T. A., & Jirsak, O. (2010). Influence of solution properties on the roller electrospinning of poly (vinyl alcohol). Polym. Eng. Sci., 50(5), 936-943.

[22] Lannutti, J., Reneker, D., Ma, T., Tomasko, D., & Farson, D. (2007). Electrospinning for tissue engineering scaffolds. *Materials Science and Engineering: C*, *27*(3), 504-509.

[23] Sill, T. J., & Von Recum, H. A. (2008). Electrospinning: applications in drug delivery and tissue engineering. *Biomaterials*, *29*(13), 1989-2006.

[24] Samimi Gharaie, S., Habibi, S., & Nazockdast, H. (2018). Fabrication and characterization of chitosan/gelatin/thermoplastic polyurethane blend nanofibers. Journal of Textiles and Fibrous Materials, 1, 2515221118769324.

[25] Amiri, N., Rozbeh, Z., Afrough, T., Sajadi Tabassi, S. A., Moradi, A., & Movaffagh, J. (2018). Optimization of chitosan-gelatin nanofibers production: Investigating the effect of solution properties and working parameters on fibers diameter. BioNanoScience, 8(3), 778-789.

[26] Yue, T. T., Li, X., Wang, X. X., Yan, X., Yu, M., Ma, J. W., ... & Long, Y. Z. (2018). Electrospinning of carboxymethyl chitosan/polyoxyethylene oxide nanofibers for fruit fresh-keeping. Nanoscale research letters, 13(1), 1-8.

[27] Woo, C. H., Choi, Y. C., Choi, J. S., Lee, H. Y., & Cho, Y. W. (2015). A bilayer composite composed of TiO2-incorporated electrospun chitosan membrane and human extracellular matrix sheet as a wound dressing. Journal of Biomaterials Science, Polymer Edition, 26(13), 841-854.

[28] Wang, Y., Xu, H., Wu, M., & Yu, D. G. (2021). Nanofibers-based food packaging. *ES Food & Agroforestry*, *7*, 1-24.

[29] Marsh, K., & Bugusu, B. (2007). Food packaging—roles, materials, and environmental issues. *Journal of food science*, *72*(3), R39-R55.

[30] Wang, H., Ding, F., Ma, L., & Zhang, Y. (2021). Recent advances in gelatine and chitosan complex material for practical food preservation application. *International Journal of Food Science & Technology*, *56*(12), 6279-6300.

[31] Dutta, P. K., Tripathi, S., Mehrotra, G. K., & Dutta, J. (2009). Perspectives for chitosan based antimicrobial films in food applications. *Food chemistry*, *114*(4), 1173-1182.

[32] Dickinson, E. (2017). Biopolymer-based particles as stabilizing agents for emulsions and foams. Food Hydrocolloids, 68, 219-231.

[33] Cruz-Morfin, R., Martínez-Tenorio, Y., & López-Malo, A. (2013). Biopolímeros y su integración con polímeros convencionales como alternativa de empaque de alimentos. Temas de Selección de Ingeniería de Alimentos, 7(2), 42-52.

[34] Fu, Y., Sarkar, P., Bhunia, A. K., & Yao, Y. (2016). Delivery systems of antimicrobial compounds to food. *Trends in food science & technology*, *57*, 165-177.

[35] Kriegel, C., Arrechi, A., Kit, K., McClements, D. J., & Weiss, J. (2008). Fabrication, functionalization, and application of electrospun biopolymer nanofibers. *Critical reviews in food science and nutrition*, *48*(8), 775-797.

[36] Khan, M. A., Yue, C., Fang, Z., Hu, S., Cheng, H., Bakry, A. M., & Liang, L. (2019). Alginate/chitosan-coated zein nanoparticles for the delivery of resveratrol. *Journal of Food Engineering*, *258*, 45-53.

[37] Kulkarni Vishakha, S., Butte Kishor, D., & Rathod Sudha, S. (2012). Natural polymers–A comprehensive review. *International journal of research in pharmaceutical and biomedical sciences*, *3*(4), 1597-1613.

[38] Coma, V. (2013). Polysaccharide-based biomaterials with antimicrobial and antioxidant properties. *Polímeros, 23(3), 287-297.*

[39] Schiffman, J. D., & Schauer, C. L. (2008). A review: electrospinning of biopolymer nanofibers and their applications. *Polymer reviews*, *48*(2), 317-352.

[40] Hanani, Z. N., Roos, Y. H., & Kerry, J. P. (2014). Use and application of gelatin as potential biodegradable packaging materials for food products. *International journal of biological macromolecules*, *71*, 94-102.

[41] Jeevahan, J., Chandrasekaran, M., Durairaj, R., Mageshwaran, G., & Joseph, G. B. (2017). A brief review on edible food packing materials. *Journal of Global Engineering Problems and Solutions*, *1*(1), 9-19.

[42] Iwata, K., Ishizaki, S., Handa, A., & Tanaka, M. (2000). Preparation and characterization of edible films from fish water-soluble proteins. *Fisheries science*, *66*(2), 372-378.

[43] Beachley, V., & Wen, X. (2010). Polymer nanofibrous structures: Fabrication, biofunctionalization, and cell interactions. Progress in polymer science, 35(7), 868-892.

[44] Kumar, P. (2012). Effect of colletor on electrospinning to fabricate aligned nano fiber (Doctoral dissertation).

[45] Alghoraibi, I., & Alomari, S. (2018). Different methods for nanofiber design and fabrication. *Handbook of nanofibers*, 1-46.

[46] Ramakrishna, S. (2005). *An introduction to electrospinning and nanofibers*. World scientific

[47] Zhang X, Lua Y (2014) Centrifugal spinning: an alternative approach to fabricate nanofibers at high speed and low cost. Polym Rev 54:677–701.

[48] Vasita, R., & Katti, D. S. (2006). Nanofibers and their applications in tissue engineering. *International Journal of nanomedicine*, *1*(1), 15.

[49] Li W-J, Shanti RM, Tuan RS (2006) Electrospinning technology for nanofibrous scaffolds in tissue engineering. Nanotechnologies Life Sci 9:135–186

[50] Malkar, N. B., Lauer-Fields, J. L., Juska, D., & Fields, G. B. (2003). Characterization of peptide− Amphiphiles possessing cellular activation sequences. *Biomacromolecules*, *4*(3), 518-528.

[51] Sridhar, R., Lakshminarayanan, R., Madhaiyan, K., Barathi, V. A., Lim, K. H. C., & Ramakrishna, S. (2015). Electrosprayed nanoparticles and electrospun nanofibers based on natural materials: applications in tissue regeneration, drug delivery and pharmaceuticals. *Chemical Society Reviews*, *44*(3), 790-814.

[52] Hanaa, J. (2014), Nano Fiber composite by electrospinning technique, department of applied sciences, University of Technology.

[53] Li, D., Wang, Y., and Xia, Y. (2003). Electrospinning of polymeric and ceramic Nano-fibers as uniaxially aligned arrays. Nano letters, 3 (8), 1167-1171.

[54] Dabirian, F., Hosseini Ravandi, S. A., and Pishevar, A. R. (2010). Investigation of parameters affecting PAN Nano-fiber production using electrical and centrifugal forces as a novel method. Current Nanoscience, 6 (5), 545-552.

[55] Li, D., & Xia, Y. (2004). Electrospinning of nanofibers: reinventing the wheel? *Advanced materials*, *16*(14), 1151-1170.

[56] Medeiros, E. S., Mattoso, L. H., Offeman, R. D., Wood, D. F., & Orts, W. J. (2008). Effect of relative humidity on the morphology of electrospun polymer fibers. *Canadian Journal of Chemistry*, *86*(6), 590-599.

[57] Salehi, M., Sharafoddinzadeh, D., Mokhtari, F., Esfandarani, M. S., & Karami, S. (2021). Electrospun nanofibers for efficient adsorption of heavy metals from water and wastewater.

[58] Teo, W. E., & Ramakrishna, S. (2006). A review on electrospinning design and nanofibre assemblies. *Nanotechnology*, *17*(14), R89.

[59] Chong, E. J., Phan, T. T., Lim, I. J., Zhang, Y. Z., Bay, B. H., Ramakrishna, S., & Lim, C. T. (2007). Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. *Acta biomaterialia*, *3*(3), 321-330.

[60] Garg, K., and Bowlin, G. L. (2011). Electrospinning jets and nanofibrous structures. Biomicrofluidics, 5 (1), 013-403.

[61] Huang, Z. M., Zhang, Y. Z., Kotaki, M., and Ramakrishna, S. (2003). A review on polymer Nano-fibers by electrospinning and their applications in nanocomposites. Composites science and technology, 63 (15), 2223-2253.

[62] Sukigara, S., Gandhi, M., Ayutsede, J., Micklus, M., & Ko, F. (2003). Regeneration of Bombyx mori silk by electrospinning—part 1: processing parameters and geometric properties. *Polymer*, *44*(19), 5721-5727.

[63] Ki, C. S., Baek, D. H., Gang, K. D., Lee, K. H., Um, I. C., & Park, Y. H. (2005). Characterization of gelatin nanofiber prepared from gelatin–formic acid solution. *Polymer*, *46*(14), 5094-5102.

[64] Zeng, J., Haoqing, H., Schaper, A., Wendorff, J. H., & Greiner, A. (2003). Poly-L-lactide nanofibers by electrospinning–Influence of solution viscosity and electrical conductivity on fiber diameter and fiber morphology. *e-Polymers*, *3*(1).

[65] Deitzel, J. M., Kleinmeyer, J., Harris, D. E. A., & Tan, N. B. (2001). The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, *42*(1), 261-272.

[66] Chen, M., Patra, P. K., Warner, S. B., and Bhowmich, S. (2006). Optimization of electrospinning process parameters for tissue engineering scaffolds. Biophysical Reviews and Letters, 1 (02), 153-178.

[67] Bhardwaj, N., and Kundu, S. C. (2010). Electrospinning: a fascinating fiber fabrication technique. Biotechnology advances, 28 (3), 325-347.

[68] Tan, S. H., Inai, R., Kotaki, M., & Ramakrishna, S. (2005). Systematic parameter study for ultra-fine fiber fabrication via electrospinning process. *Polymer*, *46*(16), 6128-6134.

[69] Vlachou, M., Siamidi, A., & Kyriakou, S. (2019). Electrospinning and drug delivery. Electrospinning *and Electrospraying-Techniques and Applications*

[70] Haghi, A. K., & Akbari, M. (2007). Trends in electrospinning of natural nanofibers. *physica* status *solidi (a), 204*(6), 1830-1834.

[71] Demir, M. M., Yilgor, I., Yilgor, E. E. A., and Erman, B. (2002). Electrospinning of polyurethane fibers. Polymer, 43 (11), 3303-3309.

[72]Shamim, Z., Saeed, B., Amir, T., Abo Saied, R., Rogheih, Damerchely, 2012. The effect of flow rate on morphology and deposition area of electrospun nylon 6 nanofiber. J. Eng. Fabrics Fibers 7 (4), 42.

[73] Sun, B., Long, Y. Z., Zhang, H. D., Li, M. M., Duvail, J. L., Jiang, X. Y., & Yin, H. L. (2014). Advances in three-dimensional nanofibrous macrostructures via electrospinning. *Progress in Polymer Science*, *39*(5), 862-890.

[74] Zong, X., Kim, K., Fang, D., Ran, S., Hsiao, B. S., & Chu, B. (2002). Structure and process relationship of electrospun bioabsorbable nanofiber membranes. *polymer*, *43*(16), 4403-4412.

[75] Choi, J. S., Lee, S. W., Jeong, L., Bae, S. H., Min, B. C., Youk, J. H., & Park, W. H. (2004). Effect of organosoluble salts on the nanofibrous structure of electrospun poly (3-hydroxybutyrate-co-3-hydroxyvalerate). *International Journal of Biological Macromolecules*, *34*(4), 249-256.

[76] Son, W. K., Youk, J. H., Lee, T. S., & Park, W. H. (2004). Electrospinning of ultrafine cellulose acetate fibers: studies of a new solvent system and deacetylation of ultrafine cellulose acetate fibers. *Journal of Polymer Science Part B: Polymer Physics*, *42*(1), 5-11.

[77] Hayati, I., Bailey, A. I., & Tadros, T. F. (1987). Investigations into the mechanisms of electrohydrodynamic spraying of liquids: I. Effect of electric field and the environment on pendant drops and factors affecting the formation of stable jets and atomization. *Journal of Colloid and Interface Science*, *117*(1), 205-221.

[78] Huan, S., Liu, G., Han, G., Cheng, W., Fu, Z., Wu, Q., & Wang, Q. (2015). Effect of experimental parameters on morphological, mechanical and hydrophobic properties of electrospun polystyrene fibers. *Materials*, *8*(5), 2718-2734.

[79] Pelipenko, J., Kristl, J., Janković, B., Baumgartner, S., & Kocbek, P. (2013). The impact of relative humidity during electrospinning on the morphology and mechanical properties of nanofibers. *International journal of pharmaceutics*, *456*(1), 125-134.

[80] Weng, L., & Xie, J. (2015). Smart electrospun nanofibers for controlled drug release: recent advances and new perspectives. *Current pharmaceutical design*, *21*(15), 1944-1959.

[81] De Vrieze, S., Van Camp, T., Nelvig, A., Hagstro¨ m, B., Westbroek, P., De Clerck, K., 2009. The effect of temperature and humidity on electrospinning. J. Mater. Sci. 44 (5), 1357–1362.

[82] Subbiah, T., Bhat, G. S., Tock, R. W., Parameswaran, S., & Ramkumar, S. S. (2005). Electrospinning of nanofibers. *Journal of applied polymer science*, *96*(2), 557-569.

[83] Sunil CU, Shridhar NB, Jagadeesh SS, Ravikumar C. Nanofibers in drug delivery: An overview. World Journal of Pharmaceutical Research. 2015;**4**(8):2576-2594. ISSN: 2277-7105

[84] Hader A, Haider S, Kang IK. A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. Arabian Journal of Chemistry. 2018; **11:1165**-1188. DOI: 10.1016/j.arabjc.2015.11.01.

[85] Akhtar A, Sahib Z, Sanati A. A review on electrospun nanofibers for oral drug delivery. Nanomedicine Journal. 2017;**4**(4):197-207.

[86] Santos, C., Silva, C. J., Büttel, Z., Guimarães, R., Pereira, S. B., Tamagnini, P., & Zille, A. (2014). Preparation and characterization of polysaccharides/PVA blend nanofibrous membranes by electrospinning method. *Carbohydrate polymers*, *99*, 584-592.

[87]. Kong L, Ziegler GR. Fabrication of pure starch fibers by electrospinning. Food Hydrocolloids. 2014; 36:20-5.

[88] Kolybaba, M., Tabil, L. G., Panigrahi, S., Crerar, W. J., Powell, T., & Wang, B. (2006). Biodegradable polymers: past, present, and future. In *ASABE/CSBE North Central Intersectional Meeting* (p. 1). American Society of Agricultural and Biological Engineers.

[89] Meena, P. L., Goel, A., Rai, V., Rao, E., Singh Barwa, M., Manjeet, C., ... & Rao, S. (2017). Packaging material and need of biodegradable polymers: A review. *International Journal of Applied Research*, *3*(7), 886-896.

[90] Ivonkovic, A., Zeljko, K., Talic, S., & Lasic, M. (2017). Biodegradable packaging in the food industry. *J. Food Saf. Food Qual*, *68*, 26-38.

[91] El Fawal, G. F. (2020). Polymer nanofibers electrospinning: A review. *Egyptian Journal of Chemisstry*, *63*(4), 1279-1303.

[92] Suvarna, V., Nair, A., Mallya, R., Khan, T., & Omri, A. (2022). Antimicrobial Nanomaterials for Food Packaging. *Antibiotics*, *11*(6), 729.

[93] Moreira, J. B.; Morais, M. D.; Morais, E. D.; Vaz, S.; Alberto, J.; Costa, V. Electrospun Polymeric Nanofibers in Food Packaging. In Impact of Nanoscience in the Food Industry; Grumezescu, A. M., Holban, A. M., Eds.; Elsevier Inc.: Amsterdam, 2018; pp 387–417.

[94] Schmatz, D. A., da Silva Uebel, L., Kuntzler, S. G., Dora, C. L., Vieira Costa, J. A., & de Morais, M. G. (2016). Scaffolds containing Spirulina sp. LEB 18 biomass: development, characterization and evaluation of in vitro biodegradation. *Journal of Nanoscience and Nanotechnology*, *16*(1), 1050-1059.

[95] Mendes, A. C., Stephansen, K., & Chronakis, I. S. (2017). Electrospinning of food proteins and polysaccharides. *Food Hydrocolloids*, *68*, 53-68.

[96] Xiao, Q., & Lim, L. T. (2018). Pullulan-alginate fibers produced using free surface electrospinning. *International journal of biological macromolecules*, *112*, 809-817.

[97] Rodríguez-Sánchez, I. J., Fuenmayor, C. A., Clavijo-Grimaldo, D., & Zuluaga-Domínguez, C. M. (2021). Electrospinning of ultra-thin membranes with incorporation of antimicrobial agents for applications in active packaging: a review. *International Journal of Polymeric Materials and Polymeric Biomaterials*, *70*(15), 1053-1076.

[98] Zhao, L., Duan, G., Zhang, G., Yang, H., He, S., & Jiang, S. (2020). Electrospun functional materials toward food packaging applications: A review. *Nanomaterials*, *10*(1), 150.

[99] Petersen, K., Nielsen, P. V., Bertelsen, G., Lawther, M., Olsen, M. B., Nilsson, N. H., & Mortensen, G. (1999). Potential of biobased materials for food packaging. *Trends in food science & technology*, *10*(2), 52-68.

[100] Chandra, R. U. S. T. G. I., & Rustgi, R. (1998). Biodegradable polymers. *Progress in polymer science*, *23*(7), 1273-1335.

[101] Sam ST, Nuradibah MA, Ismail H, Noriman NZ, Ragunathan S (2014) Recent advances in polyolefins/natural polymer blends used for packaging application. Polym Plast Technol Eng 53:631–644.

[102] Joshi, P., & Gnanarajan, G. (2016). A Review on Microencapsulation-A Novel Drug Delivery System. *World Journal of Pharmaceutical Research*, 711-728.

[103] Trinetta, V., & Cutter, C. N. (2016). Pullulan: A suitable biopolymer for antimicrobial food packaging applications. In *Antimicrobial food packaging* (pp. 385-397). Academic Press.

[104] Aguilar-Vázquez, G., Loarca-Piña, G., Figueroa-Cárdenas, J. D., & Mendoza, S. (2018). Electrospun fibers from blends of pea (Pisum sativum) protein and pullulan. *Food Hydrocolloids*, *83*, 173-181.

[105] Cheng, K.-C., Demirci, 5., and Catchmark, J. M. (2011). pullulan: biosynthesis, production, and applications. Appl. Microbiol. Biotechnol., 92(1), 29.

[106] Jindal, N., & Khattar, J. S. (2018). Microbial polysaccharides in food industry. In *Biopolymers for food design* (pp. 95-123). Academic Press

[107] Trinetta, V. (2017). The application of edible and active pullulan coatings on foods

[108] Cingh, R. C. and Caini, G. K. (2012). biosynthesis of pullulan and its applications in food and pharmaceutical industry. In: *Microorganisms in Sustainable Agriculture and Biotechnology*, Cpringer, pp. 509+557.

[109] Kakoria, A., & Sinha-Ray, S. (2018). A review on biopolymer-based fibers via electrospinning and solution blowing and their applications. *Fibers*, *6*(3), 45.

[110] Sarhan WA, Azzazy HM. High concentration honey chitosan electrospun nanofibers: biocompatibility and antibacterial effects. Carbohydrate polymers. 2015; 122:135-43.

[111] Nguyen TTT, Chung OH, Park JS. Coaxial electrospun poly (lactic acid)/chitosan (core/shell) composite nanofibers and their antibacterial activity. Carbohydrate Polymers. 2011;86(4):1799-806.

[112] Kmiec, M., Pighinelli, L., Tedesco, M. F., Silva, M. M., & Reis, V. (2017). Chitosan-properties and applications in dentistry. *Adv Tissue Eng Regen Med Open Access*, *2*(4), 00035.

[113] Pal, K., Bharti, D., Sarkar, P., Anis, A., Kim, D., Chałas, R., ... & Jarzębski, M. (2021). Selected applications of chitosan composites. *International Journal of Molecular Sciences, 22(20), 10968.*

[114] Ibrahim, H. M., & El-Zairy, E. M. R. (2015). Chitosan as a biomaterial—structure, properties, and electrospun nanofibers. *Concepts, compounds and the alternatives of antibacterial*, *1*(1), 81-101.

[115] Zhan J, Lan P. The review on electrospun gelatin fiber scaffold. Journal of Research Updates in Polymer Science. 2013;1(2):59-71

[116] Alipal, J., Pu'ad, N. M., Lee, T. C., Nayan, N. H. M., Sahari, N., Basri, H., ... & Abdullah, H. Z. (2021). A review of gelatin: Properties, sources, process, applications, and commercialisation. *Materials Today: Proceedings*, *42*, 240-250.

[117] Ali, A., & Ahmed, S. (2018). Recent advances in edible polymer based hydrogels as a sustainable alternative to conventional polymers. *Journal of agricultural and food chemistry*, *66*(27), 6940-6967.

[118] Nasrollahzadeh, M., Nezafat, Z., Shafiei, N., & Bidgoli, N. S. S. (2021). Food packaging applications of biopolymer-based (nano) materials. *Biopolymer-Based Metal Nanoparticle Chemistry for Sustainable Applications*, 137-186.

[119] Lu, Y., Luo, Q., Chu, Y., Tao, N., Deng, S., Wang, L., & Li, L. (2022). Application of gelatin in food packaging: A review. *Polymers*, *14*(3), 436

[120] Ahmad, A., Mubarak, N. M., Jannat, F. T., Ashfaq, T., Santulli, C., Rizwan, M., ... & Ali, S. (2021). A critical review on the synthesis of natural sodium alginate based composite materials: An innovative biological polymer for biomedical delivery applications. *Processes*, *9*(1), 137.

[121] Zhu, L., Zhang, L., Tang, Y., Ma, D., & Yang, J. (2015). Synthesis of kaolin/sodium alginate-grafted poly (acrylic acid-co-2-acrylamido-2-methyl-1-propane sulfonic acid) hydrogel composite and its sorption of lead, cadmium, and zinc ions. *Journal of Elastomers & Plastics*, *47*(6), 488-501.

[122] Sharma, S., Sanpui, P., Chattopadhyay, A., & Ghosh, S. S. (2012). Fabrication of antibacterial silver nanoparticle—sodium alginate–chitosan composite films. *Rsc Advances*, *2*(13), 5837-5843

[123] Bennett, C. J., & Kaiser, R. I. (2007). On the formation of glycolaldehyde (HCOCH2OH) and methyl formate (HCOOCH3) in interstellar ice analogs. *The Astrophysical Journal*, *661*(2), 899.

[124] Saquing, C. D., Tang, C., Monian, B., Bonino, C. A., Manasco, J. L., Alsberg, E., & Khan, S. A. (2013). Alginate–polyethylene oxide blend nanofibers and the role of the carrier polymer in electrospinning. Industrial & Engineering Chemistry Research, 52(26), 8692-8704.

[125] Islam, M. S., Rabbani, M. M., Yang, S. B., Choi, W. S., Choi, J. H., Oh, W., ... & Yeum, J. H. (2014). Poly (vinyl alcohol)/pullulan blend nanofibres prepared from aqueous solutions using electrospinning method. Polymers and Polymer Composites, 22(9), 779-786.

[126] Kusumastuti, Y., Putri, N. R. E., & Dary, A. R. (2016, July). Electrospinning optimization and characterization of chitosan/ alginate/ polyvinyl alcohol nanofibers. In AIP Conference Proceedings (Vol. 1755, No. 1, p. 150007). AIP Publishing LLC.

[127] Antunes, M.D., Lim, L., Pinto, V.Z., Halal, S.L., Zavareze, E.D., & Dias, A.R. (2016). ELECTROSPUN ZEIN NANOFIBERS FOR FOOD AND PACKAGING APPLICATIONS.

[128] Wen, P., Zhu, D. H., Wu, H., Zong, M. H., Jing, Y. R., & Han, S. Y. (2016). Encapsulation of cinnamon essential oil in electrospun nanofibrous film for active food packaging. Food Control, 59, 366-376.

[129] Liu, Y., Wang, S., Zhang, R., Lan, W., & Qin, W. (2017). Development of poly (lactic acid)/chitosan fibers loaded with essential oil for antimicrobial applications. Nanomaterials, 7(7),1

[130] Altan, A., Aytac, Z., & Uyar, T. (2018). Carvacrol loaded electrospun fibrous films from zein and poly (lactic acid) for active food packaging. *Food Hydrocolloids*, *81*, 48-59

[131] Deng, L., Taxipalati, M., Zhang, A., Que, F., Wei, H., Feng, F., & Zhang, H. (2018). Electrospun chitosan/poly (ethylene oxide)/lauric arginate nanofibrous film with enhanced antimicrobial activity. Journal of agricultural and food chemistry, 66(24), 6219-6226.

[132] Alehosseini, A., Gómez-Mascaraque, L. G., Martínez-Sanz, M., & López-Rubio, A. (2019). Electrospun curcumin-loaded protein nanofiber mats as active/bioactive coatings for food packaging applications. Food Hydrocolloids, 87, 758-771.

[133] Somsap, J., Kanjanapongkul, K., Chancharoonpong, C., Supapvanich, S., & Tepsorn, R. (2019). Antimicrobial activity of edible electrospun chitosan/cellulose acetate/gelatin hybrid nanofiber mats incorporating eugenol. CURRENT APPLIED SCIENCE AND TECHNOLOGY, 19(3), 235-247.

[134] Wang, D., Liu, Y., Sun, J., Sun, Z., Liu, F., Du, L., & Wang, D. (2021). Fabrication and Characterization of Gelatin/Zein Nanofiber Films Loading Perillaldehyde for the Preservation of Chilled Chicken. Foods, 10(6), 1277.

[135] Singh, G., Joyce, E. M., Beddow, J., & Mason, T. J. (2012). Evaluation of antibacterial activity of ZnO nanoparticles coated sonochemically onto textile fabrics. Journal of microbiology, biotechnology and food sciences, 2(1), 106-120.

[136] Frieri, M., Kumar, K., & Boutin, A. (2017). Antibiotic resistance. Journal of infection and public health, *10*(4), 369-378

[137] Faris, D., Hadi, N.J. and Habeeb, S.A., 2021. Effect of rheological properties of (Poly vinyl alcohol/Dextrin/Naproxen) emulsion on the performance of drug encapsulated nanofibers. Mater. Today: Proc., 42, pp.2725-2732

[138] Stepanyan R, Subbotin AV, Cuperus L, Boonen P, Dorschu M, Oosterlinck F, Bulters MJH, Nanofiber diameter in electrospinning of polymer solutions: Model and experiment, Polymer (2016), doi: 10.1016/j.polymer.2016.05.045

[139] Gomes, S., Rodrigues, G., Martins, G., Henriques, C., & Silva, J. C. (2017). Evaluation of nanofibrous scaffolds obtained from blends of chitosan, gelatin and polycaprolactone for skin tissue engineering. International journal of biological macromolecules, 102, 1174-1185.

[140] Muthu Kumar, T. Senthil; Kumar, K. Senthil; Rajini, N.; Siengchin, Suchart; Ayrilmis, Nadir; Rajulu, A. Varada (2019). A comprehensive review of electrospun nanofibers: Food and packaging perspective. Compos. B. Eng., 107074–. doi: 10.1016/j.compositesb.2019.107074

[141] Liu, F., Liu, Y., Sun, Z., Wang, D., Wu, H., Du, L., & Wang, D. (2020). Preparation and antibacterial properties of ε-polylysine-containing gelatin/chitosan nanofiber films. International journal of biological macromolecules, 164, 3376-3387.

[142] Amjadi, S., Nazari, M., Alizadeh, S. A., & Hamishehkar, H. (2020). Multifunctional betanin nanoliposomes-incorporated gelatin/chitosan

nanofiber/ZnO nanoparticles nanocomposite film for fresh beef preservation. Meat science, 167, 108161.

[143] Zhong, S., Teo, W. E., Zhu, X., Beuerman, R. W., Ramakrishna, S., & Yung, L. Y. L. (2006). An aligned nanofibrous collagen scaffold by electrospinning and its effects on in vitro fibroblast culture. J Biomed Mater Res A.: An Official Journal of the Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 79(3), 456-463

[144] Wang, Y., Guo, Z., Qian, Y., Zhang, Z., Lyu, L., Wang, Y., & Ye, F. (2019). Study on the electrospinning of gelatin/pullulan composite 2nanofibers. Polymers, 11(9), 1424.

[145] Li, C., & Li, J. B. (2017). Preparation of chitosan-ferulic acid conjugate: Structure characterization and in the application of pharmaceuticals. International journal of biological macromolecules, 105, 1539-1543.

[146] Sun, L., Sun, J., Chen, L., Niu, P., Yang, X., & Guo, Y. (2017). Preparation and characterization of chitosan film incorporated with thinned young apple polyphenols as an active packaging material. Carbohydrate Polymers, 163, 81-91.

[147] Mathew, S., & Abraham, T. E. (2008). Characterisation of ferulic acid incorporated starch–chitosan blend films. Food hydrocolloids, 22(5), 826-835.

[148] Liu, J., Liu, S., Wu, Q., Gu, Y., Kan, J., & Jin, C. (2017). Effect of protocatechuic acid incorporation on the physical, mechanical, structural and antioxidant properties of chitosan film. Food Hydrocolloids, 73, 90-100.

[149] Jafari, J., Emami, S. H., Samadikuchaksaraei, A., Bahar, M. A., & Gorjipour, F. (2011). Electrospun chitosan–gelatin nanofiberous scaffold: Fabrication and in vitro evaluation. Bio-medical materials and engineering, 21(2), 99-112

[150] N. Devi, D. Hazarika, C. Deka & D. K. Kakati (2012): Study of Complex Coacervation of Gelatin A and Sodium Alginate for Microencapsulation of Olive Oil, Journal of Macromolecular Science, Part A: Pure and Applied Chemistry, 49:11, 936-945

[151] Saber-Samandari, S., Gulcan, H. O., Saber-Samandari, S., & Gazi, M. (2014). Efficient removal of anionic and cationic dyes from an aqueous solution using pullulan-graft-polyacrylamide porous hydrogel. Water, Air, & Soil Pollution, 225(11), 1-14

[152] Mathew, S. A., & Arumainathan, S. (2022). Crosslinked Chitosan–Gelatin Biocompatible Nanocomposite as a Neuro Drug Carrier. ACS Omega.

[153] Qin, Z. Y., Jia, X. W., Liu, Q., Kong, B. H., & Wang, H. (2019). Fast dissolving oral films for drug delivery prepared from chitosan/pullulan electrospinning nanofibers. International journal of biological macromolecules, 137, 224-231.

[154] Ke, C. L., Deng, F. S., Chuang, C. Y., & Lin, C. H. (2021). Antimicrobial actions and applications of chitosan. Polymers, 13(6), 904.

[155] Jridi, M., Hajji, S., Ayed, H. B., Lassoued, I., Mbarek, A., Kammoun, M., ... & Nasri, M. (2014). Physical, structural, antioxidant and antimicrobial properties of gelatin–chitosan composite edible films. International journal of biological macromolecules, 67, 373-379.

Index (A)

The relationship between viscosity and shear rates for various biopolymer ratios(G:CS:SA),(G:CS:PUL) are shown in Figure (1 and 2) , the non-Newtonian flow behavior for all polymer blends , a significant reduction in shear viscosity with increase the shear rate for the initial shear rates until 100 S^{-1} , then slightly decreasing from 120 to 200 S^{-1} for all . Shear-thinning events resulted from the biopolymer chains in the blends aligning with the increased velocity gradient[137] .

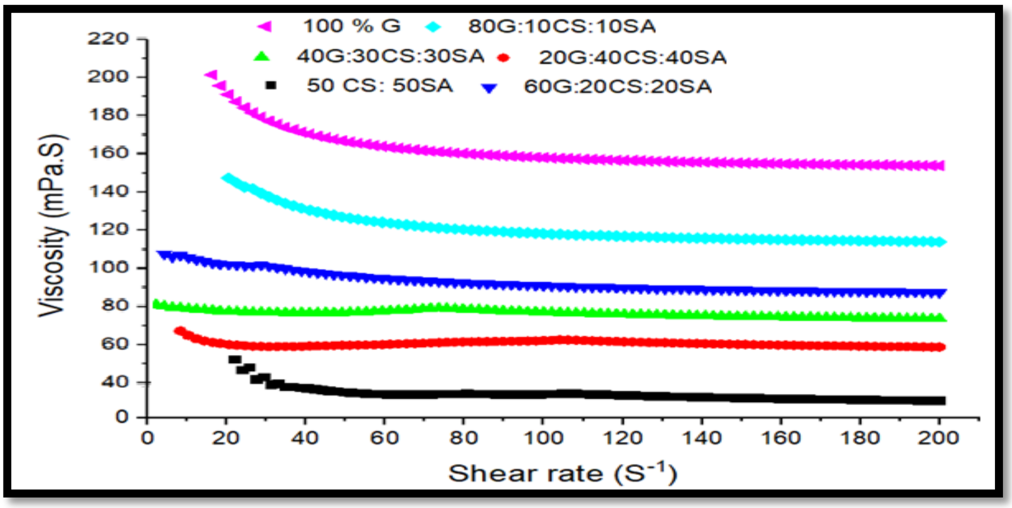


Fig (1) explain the relationship between the viscosity (mPa.S) and shear rate (S^{-1}) for pure gelatin and mixtures solutions of gelatin, chitosan and sodium alginate in different proportions.

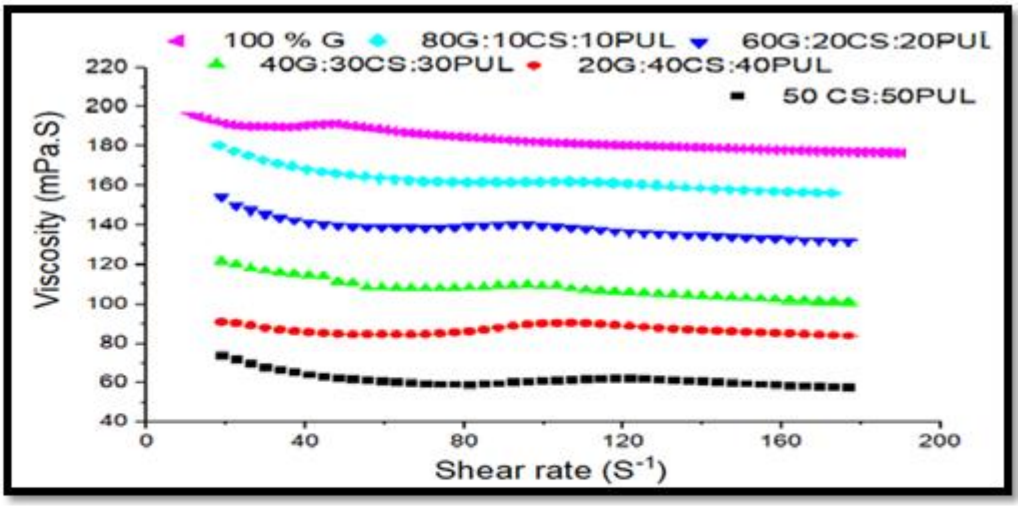


Fig (2) explain the relationship between the viscosity(mPa.S) and shear rate(S^{-1}) for pure gelatin and mixtures solutions of gelatin, chitosan and Pullulan in different proportion.

The relationship of shear stress with the shear rates for various biopolymer ratios (G:CS:SA)and(G:CS:PUL) are shown in Figures (3 and 4) and Table (1 and 2) where the shear stress increases with the increase in shear rates, indicating that the behavior of blends was non-Newtonian, and the behavior of the shear stress-shear rate curves follows the power law system. The power law index (n) and the consistency coefficient (K) can be determined using the equations below [138]:

$\delta = K\gamma^n$ eq. (1)

$\text{Log } \delta = \text{Log } K + n \text{ Log}\gamma$ eq. (2)

Where (δ) shear stress, shear rate (γ) and flow behavior index (n) represented the slope and (log K) represented the logarithmic consistency coefficient (interception). All values of the flow index (n) were lower than unity, which indicates pseudo plastic behavior of blends, while the increase in the values of (R^2) correlation coefficients indicates an improvement in the homogeneity of gelatin blends . The best homogeneous blends ratio containing cross-links were 80:10:10 and 60:20:20 from (G:CS:SA) and (G:CS:PUL).

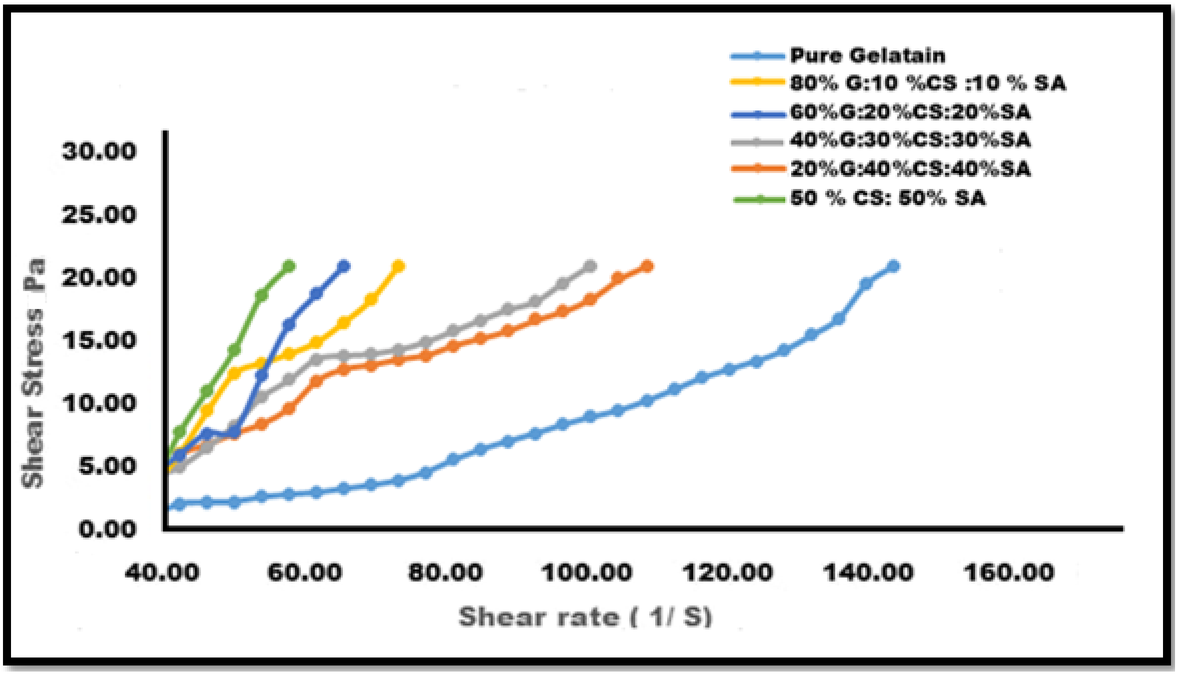


Fig. (3) shows the shear stress- shear rates curves for pure gelatin and three biopolymers solution with different ratio percentage (G:CS:SA)

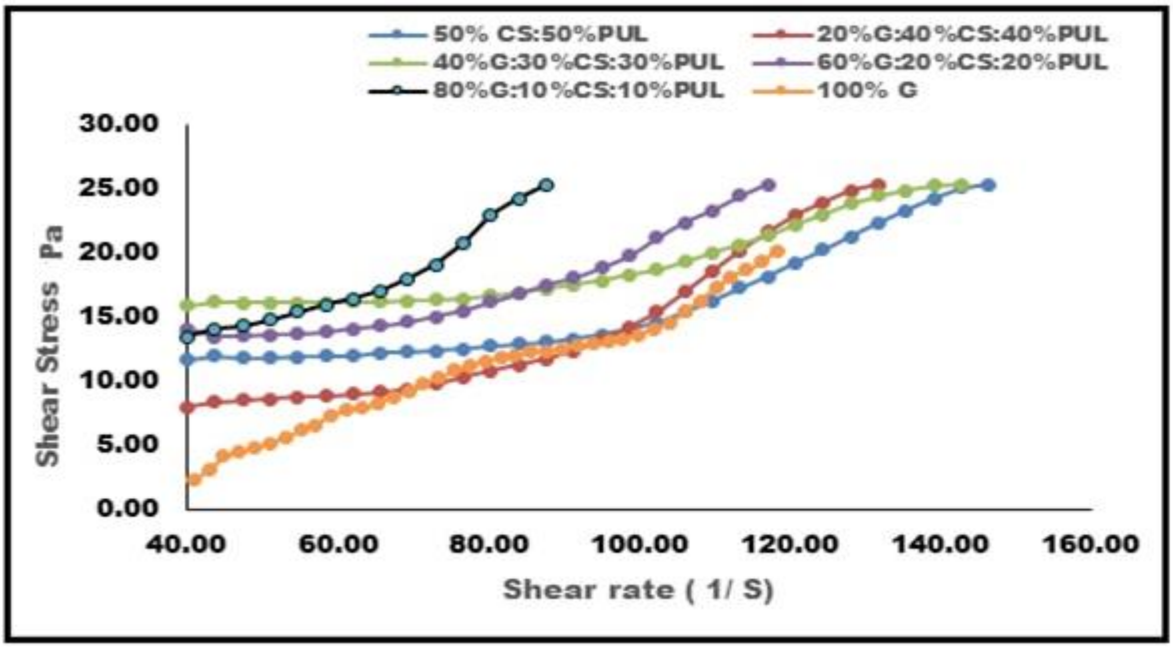


Fig. (4) shows the shear stress- shear rates curves for pure gelatin and three biopolymers solution with different ratio percentage (G:CS:PUL)

Table (1) represents the results of logarithmic shear stress – shear rate fitting equations of three blends (G:CS:SA).

Gelatin ratios (%)	**Correction coffient (R^2)**	**Fitting equations**
0.0 (50% CS: 50% SA)	0.9635	$\delta = 0.0381\ \gamma - 1.505$
20 (20 % G : 40 % CS:40% SA)	0.9642	$\delta = 0.0311\ \gamma + 1.449$
40 (40 % G : 30 % CS :30% SA)	0.9663	$\delta = 0.0443\ \gamma - 1.3598$
60 (60 % G : 20 % CS: 20% SA)	0.976	$\delta = 0.101\ \gamma + 1.334$
80 (80 % G : 10 % CS: 10% SA)	0.9801	$\delta = 0.1075\ \gamma - 1.267$
100 (Pure Gelatin)	0.9848	$\delta = 0.1754\ \gamma + 1.235$

Table(2) represents the results of logarithmic shear stress – shear rate fitting equations of three blends (G:CS:PUL).

Gelatin ratios (%)	**Correction coffient (R^2)**	**Fitting equations**
0.0 (50% CS: 50% PUL)	0.9624	$\delta = 0.6187\ \gamma - 1.4142$
20 (20 % G : 40 % CS:40% PUL)	0.9655	$\delta = 0.930\ \gamma + 1.\ 5562$
40 (40 % G : 30 % CS :30% PUL)	0.9722	$\delta = 0.2344\ \gamma + 1.0799$
60 (60 % G : 20 % CS: 20% PUL)	0.9743	$\delta = 0.5931\ \gamma + 1.1154$
80 (80 % G : 10 % CS: 10% PUL)	0.9804	$\delta = 0.632\ \gamma - 1.3885$
100 (Pure Gelatin)	0.9848	$\delta = 0.1754\ \gamma + 1.235$

الخلاصة:

صممت هذه الدراسة لتحضير وتوصيف الألياف النانوية ولتحسين إمكانية الغزل الكهربائي عند خلط مجموعتين من البوليمرات الطبيعية ، تتكون كل مجموعة من ثلاثة بوليمرات حيوية. تتكون المجموعة الأولى من محاليل الجيلاتين و الشيتوزان و ألجينات الصوديوم (G:CS:SA) وفق متسلسلة بنسب مزج 80:10:10 ، 60:20:20 ، 40:30:30، و 20:40:40 . بينما المجموعة الثانية فتتكون من محاليل الجيلاتين والشيتوزان والبولولان (G: CS: PUL) بنسب خلط متسلسلة 80:10:10 ، 60:20:20 ، 40:30:30 ، 20:40:40. تمت دراسة تأثير خواص المحاليل للمجموعتان من خلال تقييم التوصيل الكهربائي واللزوجة والتوتر السطحي بالإضافة إلى دراسة الخصائص المورفولوجية للألياف النانوية الحيوية باستخدام المجهر الإلكتروني لمسح الانبعاث الميداني (FE-SEM) لتقييم اقطار الالياف النانوية وتوزيعها وتشكيل الخرز. أظهرت النتائج أن زيادة نسبة CS مع SA أعلى من 30٪ في G: CS: SA أدى إلى انخفاض في لزوجة البوليمر ، ومتوسط أقطار الألياف ، وزيادة تكوين الخرز. تعتبرالألياف النانوية ذات نسب الخلط 80:10:10 و60:20:20 من (G:CS:SA) أكثر انتظامًا وتحتوي على عدد قليل من الخرز ،بالإضافة إلى ان حيود الأشعة السينية (XRD) أظهر اعلى نسبة تبلور في النسب المذكورة أعلاه والترابط الجيد بين جزيئات G و CS و SA في الألياف النانوية وفقًا لتحويل فورييه الطيفي القريب من الاشعة تحت الحمراء(FT-NIR) . بالنسبة للمجموعة الثانية من البوليمرات الطبيعية ، أدت الزيادة في CS مع PUL إلى انخفاض اللزوجة ومتوسط الأقطار دون تكوين خرز حتى نسبة 40:30:30 من G: CS: PUL، بينما كانت نسبة التبلور عالية عند 10:10: 80 و 60:20:20 نتيجة لزيادة الترابط بين جزيئات الألياف النانوية ، والتي أظهرها حيود الأشعة السينية وتحويل فورييه الطيفي القريب من الأشعة تحت الحمراء. تعتبر الموصلية الكهربائية لمحاليل البوليمر الطبيعية مؤشرًا على زيادة التوتر السطحي وزاوية التلامس مع الماء اعتمادًا على الزيادة في نسب الشيتوزان والبولولان وألجينات الصوديوم في المحاليل البوليمرية. وفقًا للنتائج أعلاه ،

تمثل 80:10:10 و 60:20:20 أفضل نسب مزج للمجموعتين ، وهي النسب المستخدمة في تحليل الإطلاق لغرض تحديد كفاءة ألياف البوليمر الحيوي في تغليف المواد الغذائية والحماية من البكتيريا. لذلك ، كانت نسبة الخلط 60:20:20 في المجموعة الثانية هي الأفضل في كلا المجموعتين التي تتمتع بمقاومة أفضل للنمو البكتيري وأعلى مناطق تثبيط بقطر (24 ملم و 22 ملم) ضد الإشريكية القولونية والمكورات العنقودية الذهبية على التوالي.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية هندسة المواد
قسم هندسة البوليمرات والصناعات البتروكيمياوية

تحضير وتوصيف أغشية خلائط الجيلاتين بطريقة الغزل الكهربائي للتطبيقات المضاده للبكتريا

رسالة
مقدمة الى قسم هندسة البوليمر والصناعات البتروكيمياوية
كلية هندسة المواد/ جامعة بابل
وهي جزء من متطلبات نيل درجة الماجستير في هندسة المواد/البوليمر

من قبل الباحث
مشرق كريم عبد الكاظم شباده
(بكالوريوس هندسة المواد ٢٠٠٠)

بإشراف
أ.م.د.صالح عباس حبيب

٢٠٢٢ م **١٤٤٤هـ**