NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF BIOMEDICAL ENGINEERING

DESIGN OF INTRAVITREAL INJECTABLE HYDROGELS FOR SUSTAINED DRUG DELIVERY IN GLAUCOMA TREATMENT AND THERAPY

PhD THESIS

Kassahun Alula AKULO

Nicosia

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Approval

We certify that we have read the thesis submitted by Kassahun Alula AKULO titled "**Design of Intravitreal Injectable Hydrogels for Sustained Drug Delivery in Glaucoma Treatment and Therapy**" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Biomedical Engineering.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Kassahun Alula Akulo

25/06/2022

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Kassahun Alula Akulo

Abstract

Design of Intravitreal Injectable Hydrogels for Sustained Drug Delivery in Glaucoma Treatment and Therapy

Akulo Kassahun Alula
Prof. Terin Adeli
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Injectable hydrogels load drugs and achieve the controlled release of loaded drugs at the site of actions within the desired time and significantly maintains a massive local concentration for a prolonged time. This study deals with the preparation of injectable hydrogels by combining natural polymers with different proportion which are administered intravitreally by loading a drug for the treatment of glaucoma and reduce the swift clearance of the loaded drug before reaching the target place. Swelling tests, biodegradability, SEM, FTIR, X-RD and drug release were performed to characterize the hydrogel. *In-vitro* coagulation tests were performed using activated partial thromboplastin time (APTT), prothrombin time (PT), total serum albumin and cholesterol level all the above test analyses confirm the hemocompatibility of the hydrogel for *in-vitro* applications.

Keywords: glaucoma, silk fibroin, gelatin, curcumin, injectable hydrogel

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List of Abbreviations

ABS	Acid phosphate Buffer Saline
AMD	Age related Macular Degeneration
APTT	Activated Partial Thromboplastin Time
CUR	Curcumin
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
ECM	Extracellular Matrix
EDTA	Ethylene diamine tetra acetic acid
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
GE	Gelatin
GG	Gellan Gum
HA	Hyaluronic Acid
INR	International Normalized Ratio
iPSCs	Induced Pluripotent Stem cells
IOP	Intraocular Pressure
MBA	N, N methylene bisacrylamide
NIH	National Institute of Health
NMR	Nuclear Magnetic Resonance
NPs	Nanoparticles
NTG	Normal Tension Glaucoma
PACG	Primary Acute Angle Closure Glaucoma
PBS	Phosphate Buffer Saline
PEG	Polyethylene Glycol
pH	Power of Hydrogen
POAG	Primary Open Angle Glaucoma
PT	Prothrombin Time
RPE	Retinal Pigment Epithelium
RNA	Ribonucleic Acid
SEM	Scanning Electron Microscopy
SF	Silk fibroin
STF	Simulated Tear Fluid

UV	Ultraviolent
Wd	Initial weight of the sample
WHO	World Health Organization
Ws	Weight of the swollen sample
XRD	X-ray Diffraction

CHAPTER I
Introduction

Disease-related visual impairment or loss of vision has become the foremost problem universally, Studies by the World Health Organization (WHO) have shown that an estimated 2.2 billion globally have a visual impairment or blind out of which at least 1 billion have a visual impairment that has not yet been treated.(Bourne et al., 2017).

These 1 billion visually impaired people include those with moderate or severe distance vision impairment or blindness due to untreated refractive errors, glaucoma, cataract, diabetic retinopathy, corneal opacities, and conjunctivitis, as well as near vision impairment due to untreated (Fricke et al., 2018).

The health difficulties related to retinal sicknesses become the reason for a large proportion of those blind, with ocular diseases, mostly constraints like glaucoma, Age-related Macular Degeneration (AMD) as well as macular edge abnormalities of the macular hole according to the studies conducted by (Rossi et al., 2017).

The loss of vision is one of the severe health impairment and very common difficult health situations that may affect every individual across the world (Bourne et al., 2017) The chief cause of visual impairment is called AMD affecting 30-40 million people all over the world who are aged over 60 (Rozing et al., 2020).

Parts of the eye called macula were responsible for vision degenerates in parallel with age brings unclear vision and blind spots in the central portion. The formation of an extracellular ocular deposit called drusen is a sign of age-related macular degeneration (Rozing et al., 2020). The prevalence of such disease is forecast to double in 2050 based on demographic age (Wang & Han, 2017).

Next to cataract glaucoma is the next leading cause of irreversible blindness in the world. According to recent reports from the WHO, approximately 80 million people worldwide suffer from glaucoma, and about 50% of those affected are unaware that they have it. It is estimated that this number will increase to 112 million people by 2040(Bro et al., 2021), (Jonas et al., 2014).

There are different types of glaucoma based on their origin (Nuzzi & Tridico, 2017). Primary Open Angle glaucoma (POAG) and Primary Acute Angle Closure Glaucoma (PACG) are common types. Normal Tension Glaucoma (NTG), pigmentary

glaucoma and Uveitis glaucoma categorized as secondary glaucoma (Pakravan et al., 2013).

Open-Angle Glaucoma

POAG is the most prevalent type of glaucoma in the world. Researchers suggest that it is caused by a gradual blockage of the eyes drainage channel leading to an increase in IOP which in turn leads to optic nerve damage that develops slowly, as the exact causes are not yet known (X. Sun et al., 2017). Early detection and treatment are very crucial to protect from the total damage of retinal nerve fibers optic discs in the particular and visual field in general (Bertaud et al., 2019).

Elevated IOP of the eye is the major factor for POAG (Voss et al., 2015). IOP of the normal eye is in between 10 to 21 mmHg (Yadav et al., 2019). The range of intraocular pressure increases up to 40 mmHg even though an elevated intraocular pressure above 23 mmHg is not the only indicator for glaucoma but it is the primary risk factor as shown in the studies (Lee et al., 2020), (G. Z. Chen et al., 2013) as it is shown in the figure 1 elevated intraocular pressure is the defect happened in the loss of controlled balance of aqueous humor secretions that regulate the pressure inside the eye to be at a normal level which is in the range of 12 mmHg (Al-shohani, 2016).

Acute Narrow - or Closed Angle Glaucoma

Structural and anatomic deformities of the anterior chamber of the eye are the main features of primary acute angle closure glaucoma (PACG) (Fu et al., 2020). This type of glaucoma is primarily caused by an increase in aqueous humor outflow at the angle of closure due to mechanical obstruction of iris and blockage of Schlemm's canal (C. L. Zhang et al., 2020). This blockage causes obstruction and degeneration of the optic nerve. PACG is reversible disease if the angle-closure process is treated in the early stage of eye discomfort (Artero-Castro et al., 2020; X. Sun et al., 2017).

Uveitis Glaucoma

Uveitis is a complication characterized by intraocular inflammation of the iris, uveal tract, choroid layer and ciliary body that is possibly expanded to inflammation of nearby intraocular structures such as retina vitreous and optic nerve (Kesav et al., 2020) additionally the friction created between the ciliary body and artificial intraocular lens become the reason.

Normal-Tension Glaucoma

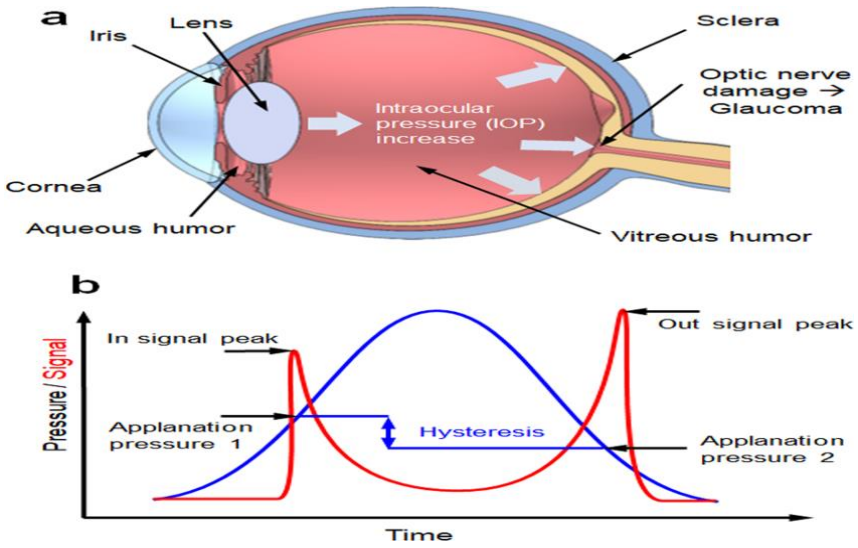
Normal-tension glaucoma is an exclusive and progressive disease caused by an abnormally high translaminar pressure although IOP is considered as the most significant risk factor (Killer & Pircher, 2018; Lee et al., 2020).

Pigmentary Glaucoma

Pigmentary glaucoma is characterized by exorbitant pigmentation of the anterior segment of the eye, thick trabecular meshwork, midperipheral iris, and pigment deposits on the posterior surface of the central cornea (Tong et al., 2017). Accumulation of ocular pigmentation in the trabecular meshwork plummets the outflow of aqueous humor, resulting in elevated IOP which inevitably leads to the loss of optic nerve functionality linked with visual field as it is indicated in (Fig. 1). Pigments associated with pigmentary glaucoma are released when anterior lens zonules rub against the iris pigment epithelium (Tandon et al., 2019)

Figure 1.

Anatomy of Human Eye and Ocular Response Tonometry



a. Cross-section of eye b. Corneal hysteresis tonometry. Corneal hysteresis measurement is demonstrated on a curve, which equates the corneal application signal and the air pressure history over time (Y. Kim et al., 2019).

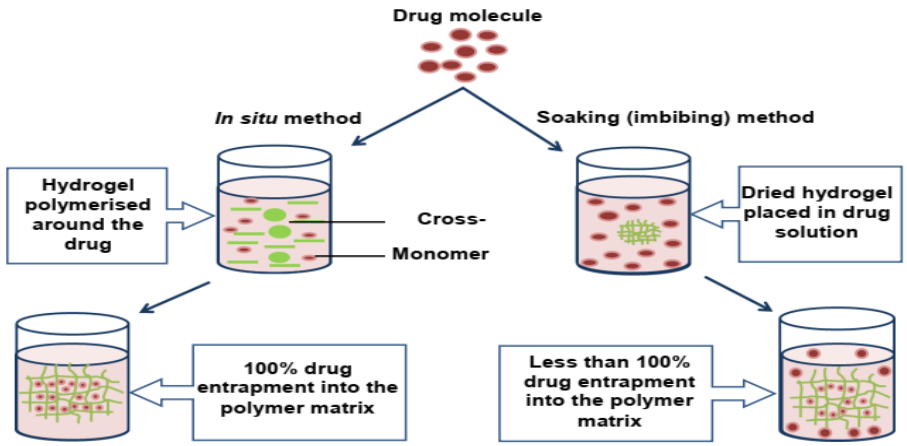
For the past years, different researches were made to improve the adequacy of treatments of ocular diseases. Various advanced drug delivery techniques have been developed including nanoparticles, Nano-emulsions, and hydrogels aimed to improve the residence time of loaded drugs and bioavailability. Among the strategies drawn *in situ* forming, stimuli-responsive injectable hydrogels took the attention of researchers (Song et al., 2018).

Injectable hydrogels, which are in situ injectable gelling systems ideal for drug delivery, are nowadays preferred in biomedicine due to their suitable properties and ease of administration. (Sun et al., 2020). Injectable hydrogels load drugs and achieve the controlled release of loaded drugs at the site of actions within the desired time and significantly maintains a massive local concentration of loads for a prolonged time (N. Chen et al., 2019), (Song et al., 2018).

The presence of massive water in the hydrogel creates an opportunity for the loading of hydrophilic drugs. Drugs can be loaded in either of the following ways *in situ* method or soaking method as shown in (Fig. 2). *In situ* method uses the monomer or/and chemical cross-linkers polymerized in the existence of the intended drugs (K. Wang & Han, 2017).

Figure 2

Drug loading techniques of hydrogels

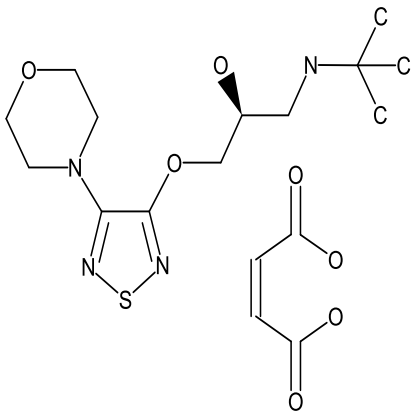


Timolol maleate ($C_{17}H_{28}N_4O_7S$) is a beta-adrenoceptor blocker (Fig. 3) used to treat glaucoma which is caused by high pressure inside the eye (Erk, 2002). Timolol maleate is the drug that is currently applied effectively to treat glaucoma so that lowers

high pressure inside the eye helps to prevent blindness by decreasing the amount of fluids within the eye than other anti-glaucoma agents (Pakzad et al., 2020).

Figure 3

Chemical Structure of Timolol maleate



This study focuses on applications of biomaterials in the preparation of hydrogels by the combination of different proportions used for intravitreal delivery of drug-loaded substances. The hydrogels aim to reduce the quick removal of the loaded drug from the nasolacrimal system of the orbit, effectively prolonging residence time of the drug, which minimizes the frequent administration of eye drops to sustain the concentration of effective therapeutic drug. (Meng et al., 2020).

Biomaterials for Drug Delivery

Biomaterials may enhance the release of instilled drugs moreover facilitates the integration of *in-vivo* applications. Furthermore, naturally obtained biomaterials can act as a reserve growth factor as the regular extracellular matrixes do (Jain et al., 2017). Proposed biomaterials made from different biopolymers like chitosan, polyethylene glycol(PEG), Silk Fibroin (SF) and other naturally obtained polymers nowadays widely applied in the field of biomedical science (Mej & Delgado, 2019).

Such biomaterials should have the desirable characteristics that match the original cells and should exhibit good quality on biocompatibility and biodegradability that maintain the sustainability of transplanted cells and nearby cells in undertaking regular cell functioning like a cell to cell adhesion, cellular proliferation and extracellular matrix production as well as used materials should exhibit an appropriate

degradation with timely matched allowing the synthesis of new cellular matrices by cells (Hunt., et al., 2018).

Mechanical properties, manufacturing technology and architectural visibility of manufactured hydrogels are the crucial properties that should be taken into solid consideration while working with biomaterials in that, naturally made biomaterials preferably consistent with the physiology of extracellular matrix where to be applied which prevents toxicity to the nearby tissues (Y. Chen et al., 2019).

Comparatively biomaterials are easily affordable, locally available, accessible and easily characterizable where the process is compatible furthermore, the architectural structures match normal tissue with the pore size and interconnectivity which allows appropriate diffusion of nutrients (Datta et al., 2020).

Importance of Biomaterials for the Treatment of Glaucoma

Biomaterials have gained so many attention in medicine throughout the last century, these naturally occurring polymers found in the forms of Polysaccharide such as chitosan, silk fibroin, dextran, alginate starch, galactans, cellulose, hyaluronic acid (HA), xanthan; DNA, RNA, proteins derived from plants and animals like polypeptides and polyesters (Abbasian et al., 2019).

Biomaterials and their derivatives widely applied in pharmaceuticals, biomedicine, tissue engineering mainly as scaffolds for tissue and cell regeneration, for drug delivery in the form of injectable hydrogel and imaging applications (Xu et al., 2020).

Naturally derived biomaterials increasingly applied in an area of regenerative medicine particularly tissue engineering, due to their exceptional biodegradability and biocompatibility. In particular, naturally derived hydrogel polymers have high potential for drug delivery in ocular diseases due to their higher biologic values compared to synthetic polymers (Samadian et al., 2020).

Biopolymers

Due to the fact that biomaterials are biodegradable and induce less inflamatory reactions they are widly applied in a number of medical fields (Xu et al., 2020). They possess similar features with extracellular matrix (ECM) components which help prevent immunological response and chemical toxicity exerted by synthetic

biopolymers. Other important properties of these biomaterials are the biodegradability properties which give them distinct characteristics for tissue engineering and drug delivery over other classes of biomaterials (Helanto et al., 2019).

Biopolymers especially natural ones get attention over synthetic is that due to the incredible ability in transporting bioactive materials to distinct places of tissues, cells and even organelles of the cell. The mechanical properties and biodegradability of the biopolymers were also improved by the addition of cross linkers (Jacob et al., 2018).

Silk fibroin as Biopolymer

It is a natural polymer found from insect groups of the class Arthropoda and Lepidoptera, especially silkworms and some spider groups that produce silk fibers in large quantities. Their mechano-physical as well as biological remarkable properties of silk fibers become the interest of researchers for biomedical and pharmaceutical applications (W. Zhang et al., 2017).

Silkworm produces bombyx mori silk with extraordinary luster and great skin affinity has been utilized in materials for a large number of years in the history of mankind. As of late, silk-based composites have been widely examined as one of the promising applicants in different application fields, for example, biosensors, tissue engineering, for drug delivery systems, textile raw materials for biomedical applications, auxiliary applications, biomedical applications (Xie et al., 2019).

Fibrous protein and sericin (globular protein) are the protein components in silk fibroin, with the protein fibroin surrounded by sericin protein to form a glue-like formation. Silk fibroin (SF) is suitably used in the preparation of various products such as hydrogels, film particles, sponges and scaffolds for biomedical applications. These forms can also be used for drug delivery due to their remarkable properties such as biodegradability, biocompatibility and low toxicity (Li et al., 2013).

It is proved that silk fibroin can be potential raw material due to its friendly properties with natural physiology that makes it to be preferable in the study of tissue reconstruction and age-related visual impairments (Suzuki et al., 2019). The aim of the present study is also to optimize silk fibroin in the formation of hydrogel to load drugs in the treatment of ocular disease, particularly glaucoma without causing an adverse effect in the nearby cells.

Molecular Structure of Silk fibroin. Silkworm a protein fiber that is a naturally obtained biopolymer primarily composed of two proteins particularly fibroin (70-80%) and sericin (20-30%) synthesized by silkworms explicitly by the silk gland. The morphological structure and properties of silk fibroin are better understood by undertaking characterization setups such as nuclear magnetic resonance (NMR), X-ray diffraction and other various tests that give information for the compatibility of *in vivo* applications (Arthe et al., 2019).

Silk fibers basically considered as an excellent biopolymers due to its flexibility, low thrombogenicity, biocompatibility, biodegradability, having high tensile strength, best elasticity and good toughness, which maintenances the attachment, support and proliferation of bioreceptors (Thu-Hien Luong1 et al., 2015).

Research analysis confirmed that silk fibroin is the total product of 80 amino acids serine, alanine and glycine are amino acids that account for 80% of total amino-acids present. Gly-Ala-Gly-Ala-Gly-Ser (GAGAGS) is the repetitive sequence pattern that amino acids follow in the construction of silk fibers which self-assembly into an anti-parallel β-sheet structure (Siavashani et al., 2020).

Chitosan as Biopolymers

Chitosan with linear structure composed of β-(1→4)-linked D-glucosamine (deacetylate unit) and N-acetyl-D-glucosamine (acetylated unit), which essentially produced from chitin shells of shrimp and other crustaceans with a basic substance of sodium hydroxide (Bakshia et al., 212019).

Due to the formation of polyoxy salts, chitosan reveals basic properties that differ from other polysaccharides. Like other biomaterials chitosan become an ingredient in the formation of hydrogels, particles and films that can be used for biomedical applications in the form of drug delivery agent, tissue engineering, and as a platform for cancer diagnosis. Its low toxicity, high biocompatibility and ease of degradation are aligned with many natural extracellular matrices (Highley et al., 2015).

While working with polymers the value of pH is crucial it should be given special attention, for instance, a buffer solution whose pH reads below 6.2 degrades chitosan due to the functional groups present on it furthermore, gelation can immediately happen by modifying the pH because of the equalization among different bonds like hydrogen bonding, the inter-chain electrostatic interactions and the

hydrophobic interactions (Kumorek et al., 2020). According to the survey done by different scholars, major restriction while working with injectable hydrogels is monitoring gelation period. Rapid hydrogel formation as well as slow gelation brings loss of accuracy on the drugs made. On the other hand, the combination of self-healing and injectable hydrogel properties of a chitosan-based was developed (H. Wang et al., 2014).

Properties of Chitosan. Chitosan is traced from partial alkaline deacetylation of chitin, which contained two single sugar units called, N-acetyl glucosamine (GlcNAc) and glucosamine (GlcN). Each monosaccharides units of chitosan consist of reactive functional groups. The position of attachment for the functional groups is two of the hydroxyl groups at C-3 and C-6 position whereas the amino acid in the C-2 position in their structure.

Different ratios of two monosaccharides present in chitosan equip different primary physiochemical properties like molecular weight, viscosity and degree of deacetylation. These properties meaningfully regulate anti-biofilm and antimicrobial activities of chitosan, and deacetylations further determine chitosan viscosity and solubility (Zheng et al., 2019).

For use as biomaterials, chitosan exhibit necessary properties (biocompatible, biodegradable) that simulate matrix of extracellular cells, tissues, and organs. Chitosan can be used either as a gel or dried form, depending on the temperature used and the amount of water present in the structure, which provides the properties of flexibility, which is a tissue structure, and the possibility of injection into target cells without difficulty (Skwarczynska et al., 2019).

The study conducted by the team Franca (Franca et al., 2019) noted that chitosan widely used in the treatment of glaucoma acting as a basis for controlled delivery of drugs into the eye due to polycationic nature which allows interaction with the polyanionic surface through hydrogen bonding of the ocular mucosa.

Alginic Acid

Alginic acid $(C_6H_8O_6)_n$ consists of a-L-guluronic acid and D-mannuronic acid structures linked by alpha-1,4 bonds. Due to the carboxyl group attached as a chain to the C5 carbon, exhibits an acidic character which has properties such as high hydrophilicity, the ability to gel and pH-dependent viscoelasticity. In addition,

biocompatibility and biodegradability are some of the physiological properties used in the development of films and gels for medical and food applications (Matsumoto et al., 2017).

Pullulan

A biomaterial named Pullulan is a polysaccharide that originated from the black yeast (Aureobasidium Pullulan) fermentation which is non-ionic properties non-toxic, non-mutagenic, less immunogenic reaction, non-carcinogenic (cancer-free) furthermore it is odorless, tasteless, and can be also used as a source of food. Due to its nature and important properties, pullulan is widely used in all biomedical fields, especially in targeted drug delivery, tissue therapy and wound healing. Pullulan responds to external stimuli, so it can be used to develop hydrogels that are used for targeted delivery of drugs, nutrients and other molecules to a specific area of the host (Saeaeh et al., 2019).

Gellan Gum

Gellan gum (GG) is an exopolysaccharide and water-soluble derived through *Sphingomonas elodea* and grouped in the sphingans family composed of repeating units of tetrasaccharide (1,3-β-D-glucose,1,4-β-D-glucuronic acid,1,4- β-D-glucose,1,4-α-L-rhamnose) having a functional group chain called carboxyl responsible for the negative charge of the structure (Salvatore et al., 2019).

Gellan Gum is known for its gelling agent broadly used for thickener of samples, an agent of viscosities and stabilizer. The properties that gellan gum has also allowed to be used for other sophisticated bio-based applications such as food and drug formulations, production of self-supporting gels at exceptionally low concentrations, acts as modulators *in vitro* load release protein-based hydrogels (Babaei et al., 2019). In the treatment of ophthalmologic impairments upon topical administration eye blinking, drainage of nasolacrimal fluids highly reduces the effective absorption of the drug to alleviate this constraint different studies are conducted through times out of them the study done by the group of researchers (Destruel et al., 2020) comes with the formation of in situ gelling drug delivery systems by using gellan gum polymer becomes the promising ophthalmologic instillation dosage form.

Polyethylene Glycol (PEG)

Polyethylene glycol (PEG) well known in its linear structure of synthetic polyether can be molded in different sizes depending on the intended functional group. Currently, Polyethylene glycol holds the attention of researchers by its properties of solubility in water and other organic solvents this may increase its biocompatibility, its low toxicity and non-immunogenicity (Ivanova et al., 2014).

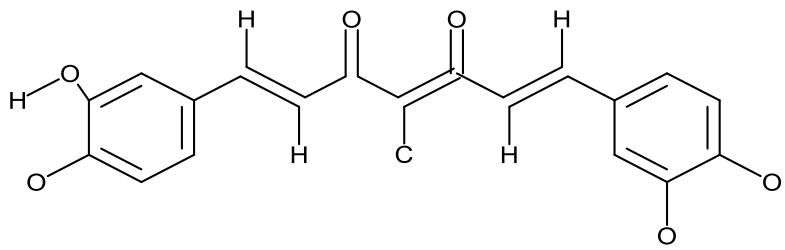
Due to hydroxyl as a side chain and electron donors on the structure undergo reaction with hydrophobic molecules via hydrogen bonding as well provides immediate drug release. Polyethylene glycol has also a low melting point due to a weak bond in between the chains, so that exhibits the rapid formation of solid above all it is non-toxic and cost-effective as well (Fellows & Dalton, 2017).

Curcumin (CUR)

Curcumin (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) is a part of turmeric root, a bright yellow chemical produced naturally as Curcuma longa (Fig. 4), and consists of various chemicals that play a key role as antimicrobial activities, antioxidants, anti-inflammatory agents, wound healing, and potential chemotherapeutic properties (Thapa et al., 2020).

Figure 4

Chemical structure of curcumin



Biomedical Applications of Hydrogels

Hydrogel is three-dimensional structure that has the potential to absorb and bind water within itself (Ilochonwu et al., 2020). Hydrogels are suitable for the formation of polymer networks through the cross-linking of chemicals or through the influence of physical actions or enzymatic actions that allow the hydrogel to freely allow the movement of particles or materials loaded on it (Bahram et al., 2016)

Nowadays, hydrogels are very attractive biomaterials in the field of biomedical applications, ranging from tissue engineering to drug delivery, due to their inherent properties. They are produced either by intermolecular attraction, covalent bonding, or physical crosslinking involving external factors such as heat and pH. (An et al., 2020).

The swollen equilibrium state, the morphological structure of the meshes and the defined chemical structure of the hydrogel provide the basis for the movement of the materials, which can be freely moved to the place where they are needed, and for the mechanical strength, which supports the adaptation to the new environment. Moreover, the large volume of water they can absorb and their delicate consistency is the absolute explanation for part of the beneficial properties of hydrogels, such as biocompatibility, biodegradability, and mimicking the extracellular matrix of living host cells (Mellati et al., 2021).

Hydrogels, as biopolymers, form a network in which the crosslinking chains are either covalently bonded or undergo a non-covalent interaction. The formed crosslinking of hydrogels allows for a permeable network that enables the movement of molecules such as drugs, essential nutrients, and oxygen through the entangled structure (Leijten et al., 2017).

To alleviate the problem faced while using solid implants for different treatment injectable hydrogels are used widely, because hydrogels can hold water and easily dissolution nature encapsulate drugs to easily release in the local area. Injectable hydrogels still have hydrophilic nature so that aids in preserving the activity of the protein not to go the formation of semisolid. It is mandatory to study and know more about the properties and behaviors of polymers more while working hydrogel as a drug delivery system (Al-shohani, 2016).

Hydrogels for Drug Delivery Units

Biomaterials, in general, are a unit being developed as helpful therapeutic modalities for a large variety of applications, as well as for the delivery of cells and molecules, cell scaffolds regenerative drugs, for cell culturing in tissue engineering and for mechanical support and tissue bulking (Mealy et al., 2018).

Nowadays, due to their biocompatibility and associated suboptimal inflammatory response, hydrogels are a potential solution to current treatment complications, especially preventable neural retinal diseases such as age-related

macular degeneration and glaucoma, which are common in the world. There are several treatment options available, but many of them have a number of problems and limitations. For all these problems, the use of hydrogels as drug delivery material offers a solution (Al-shohani, 2016).

Significance of the Study

This study was designed to prepare injectable hydrogels for sustained intraocular drug delivery systems by using natural polymers of SF/gelatin (GE)/ and curcumin with chemical crosslinking of N, N' methylene bisacrylamide under an *in-vitro* condition so that the developed hydrogels become so far useful for derivation, transport and inoculate drugs into the local place of ophthalmologic disease.

This study indicates a direction and strengthens the idea of using natural polymer for the regeneration of injured cells of neural retinal and directs treatment of neural problems and purpose of drug delivery as well and so forth represents a significant step towards the use of drug delivery therapies for the treatment of ophthalmologic constraints of glaucoma.

Aims and Objectives of the Study

Aim

This study aimed to design injectable hydrogels for sustained intraocular drug delivery systems using natural polymers pure silk fibroin, gelatin, curcumin and MBA as cross linker *in vitro*.

Specific Objectives

- To be able to prepare and characterize hydrogels by using natural polymers.
- To design highly biocompatible injectable hydrogels with a pure solution of silk fibroin, gelatin, curcumin and MBA as cross linker for sustained intraocular drug delivery systems.
- To evaluate the maximum swelling nature of hydrogels in acidic and basic buffer solutions
- To determine the biodegradability of hydrogels in simulated tear fluids

- To perform drug release study and compare retention time with the conventional administration of drugs

CHAPTER II
Literature Review

Drug Delivery System

From its very definition drug delivery is a technique used to transport and instilled therapeutic drugs in the host where to safely achieve the desired therapeutic effect. The system can be categorized depending on the administration methods and the intended site of action where the drugs or any loaded materials are instilled in the host's body as local and systematic. (Ji & Kohane, 2019)

Drug delivery systems are a way of continuous progress since the 1950s by improving its way of design, the materials used for developing the carrier, its drug release time, increasing the solubility in aqueous and improving chemical stability, reduce its immunogenicity and improve its accuracy in pharmacological activities. Furthermore, the final aim of this system according to the studies is to provide and secure therapeutic activities in the concentration of drugs at the intended site (C. Li et al., 2019).

Drug carriers in a topical dose of drugs using injectable hydrogels transfer the drug to the required site in a better way compared to systemic administration and also allow sustained release of drugs in better bioavailability. Hydrogels are known for their capability of loading hydrophilic drugs and also compress water-insoluble drugs all this becomes possible because of *in situ* formations of the 3D network (Y. Sun et al., 2020).

Drug release can be stimulated or driven either by some environmental mechanisms for instance temperature, light energy the given pH changes and the formulation of the hydrogels crosslinking types either physical or chemical crosslinking all these have an impact on the bioavailability and targeting the delivery of the drugs (Qureshi et al., 2019)

Role of Particles Nano/micro on Efficient and Organ-based Drug Delivery

Loading of drugs especially on hydrogels containing particles (Nano/ micro) size becomes the area of attention and the promising work now on for effective and efficient drug delivery techniques. The physicochemical properties of the hydrogel as a carrier having these particles in a specific condition of the disease or constraints of

each will improves the therapeutic efficiency of the drugs at a high level the characteristics of particles will have a magnified impact on composition, surface chemistry, size and elasticity on successful drug delivery system (Nejati et al., 2019).

Particles in drug delivery techniques play a significant role as the study indicates that can extend the half-life of loaded drugs for therapeutic activities and increase the chance of uptake by the target host cells. In the area of biomedical science micro-particles and Nano-particles are confirmed to be beneficial for vaccine preparation, bio-imaging and early detection and therapy of various health constraints. (Nejati et al., 2019)

Currently there are tremendous studies released on drug delivery systems out of all nanoparticle-based studies took the attention of researchers and believed to be the promising research area in biomedical applications and for therapeutic activities in the area of pharmacology because of its high degree of potential in effectively deliver drugs loaded on in the targeted positions. In addition to drug delivery systems, the science of nanoparticles is also highly used for biomedical imaging due to its potential in targeting molecules explicitly which permits a combination of distinct agents and the ability to tune pharmacokinetic profile (Pettinelli et al., 2020).

All researches were done in the development of nanoparticles drug delivery system targeting the diseased tissue without bringing any adverse effect to the healthy tissue (Raza et al., 2019).

Ophthalmologic Diseases and Possible Solution

The center of the study conducted by Lakowski and his friends is about ocular impairments related to photoreceptor cells as a result of degeneration of retinal cells besides they also address the current achievement in the area of cell replacement therapy as the promising future treatment options. For out grading treatment there is a need of ensuring the efficacy of this approach, developing identified protocols for isolation of cells and purification perfectly be done.

Their investigation additionally talked about the biomarkers used in the process of separation of photoreceptor cells from embryonic stem cell cultures and from the mouse of developing retina. This approach was also applied to the human Induced Pluripotent Stem cells (iPSCs) system by identifying biomarkers (46) with substantial

expression levels in the human retina and human pluripotent stem cell differentiation cultures. (Lakowski et al., 2018).

Ophthalmologic constraints that are believed to be the major cause of blindness all over the world for instance are glaucoma, macular degeneration related to age currently become the leading problems. To alleviate these problems different therapeutic techniques are currently in use like topical eye drop, systemic drug delivery intra- and peri-ocular injection but still have limitations in effectively deliver the drugs reaching their site of the local area where they undertake actions. (Jung et al., 2019)

This study focuses on assessing the way of therapy that aid to overcome retinal degeneration via different strategies and their drawbacks. The study also goes through cellular transplantation approaches for repairing visual function in patients with retinal pigment that have been impaired.

According to the assessment done in this paper, there are a lot of barriers obstructing further study of new therapy techniques, cellular therapy applications and improvement to medicate retinal sicknesses is restricted to the source of retinal pigment epithelium and photoreceptors. Moreover, different studies also confirmed that undifferentiated cells specifically human pluripotent stem cells, offer a perfect source of retinal pigment epithelium and photoreceptors for cellular treatments treating retinal impairments, as they can be essentially extended *in vivo* and many can separate into these cell types. (Rowland et al., 2012)

According to the study done by the team members of this paper, the major reason for the untreatable retinal degeneration was inherited and age-related which causes blindness worldwide, numerically over about 30million individuals around the world are harmed by different forms of ocular degenerations which are revealed from early childhood which is inherited from the early beginnings and whereas an effect happened because of age-related degeneration of the retina. Now a day's stem cell therapies come with promising alternatives increasing community consciousness, interest, and hope over patients with several various devastating diseases. The survey was done by this group also confirms stem cell-based treatments are imagined as potential medicines, or perhaps even remedies for a few at present untreatable types of retinal degeneration (Singh et al., 2019).

The alternative therapy that alleviates the challenge of treating by injecting stem cell suspensions directly into the open surface of the retina that causes massive cell harm, so the best way is using three-dimensional (3D) hydrogel for controlled

injectable drug delivery to repair or replace the damaged cells. This study also assessed the importance of 3D hydrogels for cells to grow and become proficient mature genetic expression sequences and external morphology using biodegradable hydrogels also have additional benefits in that it is easily absorbed by the host upon injection (Yang et al., 2009).

The scope of this review done by Tsou and his colleague covers most broadly the uses of hydrogels its applications, regulations and boldly roles to play in the applications of stem cell treatments in regenerative therapies as well as tissue culturing and other therapeutic applications. This study also addresses the chemical nature of hydrogels by defining its properties (Tsou et al., 2016).

Hydrogels in the structure have 3D arrangements with a polymeric crosslinking structure with properties of hydrophilic nature characterized by having high water content capability such condition grants for hydrogels to be easily changeable chemical properties, have high biocompatibility, elasticity and the potential to be a growth medium and the capability to mimic the surrounding extracellular matrix so that chosen for broad uses in biomedical research in different areas such as drug delivery, in the field of regenerative medicine, tissue engineering as well as their ability to encapsulate cells makes them hold attention in the field (Narayanaswamy & Torchilin., 2019). The study also tried to categorize hydrogels as both natural and synthetic with their advantages and limitations (Tsou et al., 2016).

General Histological Information of the Human Eye

The eye is one of the complex organs of human physiology, specialized in detecting light stimuli and converting them into meaningful information. As it is indicated in (Fig. 5) the cornea is the opening allows the movement of light rays reflected from the object to the lens to be refracted and focus into the retina where there are sensory cells that can detect and transmit image to the brain and the exact image of the object is formed. (Karimi et al., 2016)

The iris is responsible for automatically regulate the amount of light passing to the inner part of the eye and adjusts the eye to the difference of light rays on the visual fields. The visual system of the eye is in many ways far more complicated and complex than an artificial camera. For example, the eye can track moving objects with

coordinated eye movements. The eye can also secure, maintain, self-fix, and clean its transparent optical framework (Rose, Michael, 2011).

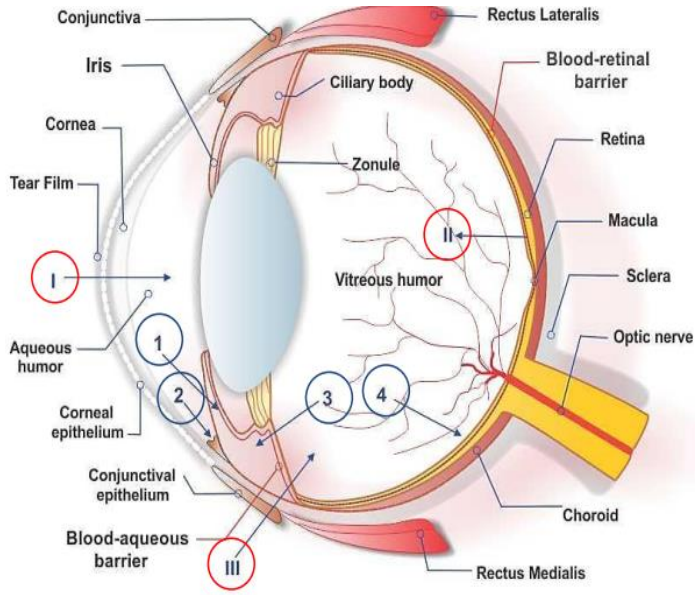
To easily alleviate the health defects related to visual impairments by using intraocular drug delivery systems is mandatory to have a better understanding of major parts of the eye and their functions. The posterior and anterior segments are important parts that embrace all physiology of the eye. The eye gets firsthand information from the reflected light rays back from the environment via receptor cells inside the eye. (Krishnaswami et al., 2018)

Cornea is a very important part of the eye acting as a transparent window that covers the front of the eye allows light to refract from the stimuli directly to the inner portion of the eye. Soon after the refracted light can be controlled by the iris which allows the passage of controlled rays to the next part by refracting and transmitting to the lens and then to the retina where the impulse was transmitted to an electrical signal and converted to an image. Aqueous humor takes the responsibility to nourish and supply nutrients and oxygen to the organelles like cornea, iris and lens which lack vessels carrying important ingredients in addition to these aqueous humor is also used to remove by-products from the area. (Nguyen et al., 2015)

It is the cornea that creates a barricade for simply absorption of drugs into the eye because of the membranes on the surface of it (Janagam., et al 2017). Because human eye has natural protection to remove foreign things entering in by its tear turnover, by undergoing reflex blinking and lacrimal drainage using liquid droplets ma wash away easily before moving to the action where they are injected so to alleviate all these limitations studies showed that using injectable hydrogels in a controlled retention time will treat appropriately the periocular disease. (Song et al., 2018)

Figure 5

Main parts of the human eye



I. Tear film and layer of cornea II. Vessels of blood and retinal walls

1. Venous blood 2. Aqueous humor outflow pathway 3. Diffusion into the anterior 4.diffusion through the blood-retinal barrier (Platania et al., 2018).

Layers of the Neural Retina

The retina, which is the inner layer, includes an outer shading epithelium, the inner neural retina, and the epithelium of the ciliary body and iris. The neural retina is uninterruptedly connected to the focal sensory system via the optic nerve. The retinal ganglion cells are confined to the vitreous layer, and long axons originating from the retinal ganglion cells extend to various brain areas where message translation occurs. The retinal ganglion cells form the bridge through which the signals of external stimuli are transmitted from the retina to the brain via the optic nerve and converted into real images (Maekawa et al., 2016).

Anatomical Structure of Neural Retina

The retina is a thin tissue made out of a collected neuronal system within the eyeball (Fig. 6). It is a fragment in the central nervous system and is in charge of changing outside world natural scenes into important data to the mind. It is here where light rays are converted into electrical signals to be sent to the brain via nerve fibers to

be interpreted into meaningful information to construct our perception of the visual world (Rossi et al., 2017).

The thickness of the retina in mankind is about 0.4 mm with a bowl shape it is an all-rounded requested structure with three fundamental layers of neurons named the retinal membranes these fundamental layers are isolated by plexiform layers (two membranes having synapses made by axons and dendrites). The three retinal membranes are located at the back of the retina, far from the entrance of the light, in their position the inner nuclear cell layer is in the center whereas the ganglion cell layer closest to the focal point of the eye. (D.L. et al., 2017)

Photoreceptor layer is the place where exceptionally very specialized cells are amassed in that light is identified and changed into electrical signals cells of photoreceptors, cones and rods namely of two vitally specialized cells cones are well known in their sensitivity to bright light responsible in controlling fine detail and recognize the color vision.

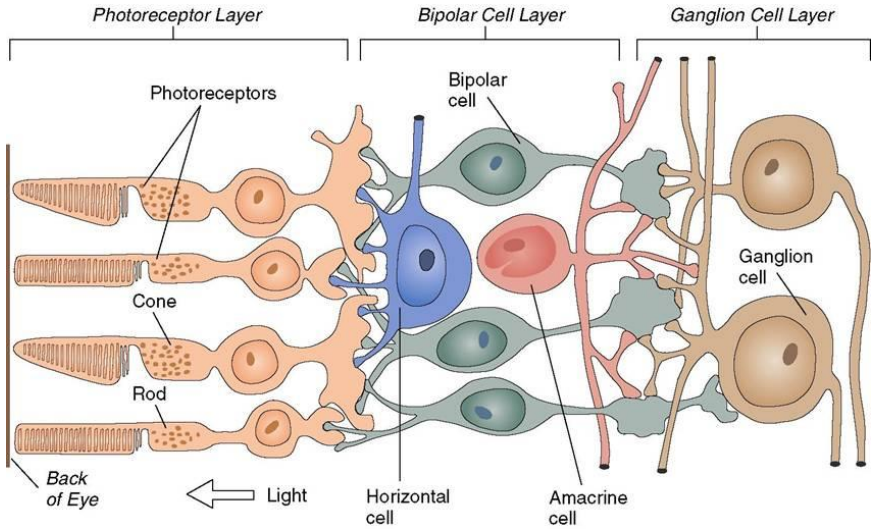
The other pigment called rods are responsible for dim light vision (Gao et al., 2020) they are distributed across the retina with very diverse sketches, in the retina, there is a part which is called fovea where cones are highly concentrated (up to 160,000 cones/mm^2) so that our fine vision is utmost comprehensive, but the density of cones drops rapidly as we move far from the fovea so then cones become inactive in the dark light, but their number increases promptly to reach a topmost somewhere in the range between 5mm and 7mm, beyond which they progressively decreasing in number (Clements & Wright, 2018).

In human photoreceptor cells, there are three different types of cones and one type of rod particularly exhibited in the human photoreceptor. Individual cones around the fovea become responsible for distinguishing vision in the respective color they have, then they are highly sensitive to different subdivision spectrums of light (J. Wang et al., 2016).

These three cones namely red, green, and blue cones are dispersed throughout the retina in such a way that only 10% are blue cones, and they are absent from the fovea. Though red cones and green cones are randomly mingled about ~2 times more blue cones than green cones in the area of distribution (Dagnelie, 2011).

Figure 6

Major Cell types of the retina



Biomaterials

Depending on its source biomaterials could be either natural (those obtained naturally either from animal or plant products) or synthetic which is artificially prepared, generally subdivided into ceramics, metals, polymers and composites. At present, for the diagnosis and treatment of different human diseases, biomaterials are primarily applied in practice. Currently, different studies are in progress to develop the most efficient biomaterials regarding improving their viability in terms of preparation method, physicochemical characterization, and biological properties. (Kargozar., 2019)

The science of biomaterials has meaningfully contributed to the rapidly growing field of medicine of therapeutic, surgical and medical technologies. Important properties that the biomaterials have is a very crucial issue within the rapidly growing fields, many studies have been devoted to the understanding of biocompatibility phenomena. Biocompatibility from its very definitions the capacity of a material to carry out functions with suitable host response in a specific application without causing adverse effect (Ghasemi-Mobarakeh et al., 2019).

According to the study done by Nikolova & Chavali of the biomaterials, they tried to bold the importance and the role of it in the growing disciplines of medical treatments for renewal and restoration of various tissues and organs. Now a day's implantable 3D scaffolds are widely used for the treatment of imperfections while

promoting the renewal of vessels, muscles, bones and nerves as well as for new cell attachment, proliferation. In this study, the importance of 3D scaffold described as due to its porous structure and potential to retain massive water in it plays a pivotal role which facilitates the movement of body fluids, gases (oxygen), nutrients across the cells, support cell to cell interaction, sustainability and extracellular matrix deposition with least adverse effect and toxicity wherein the process of biodegradation in a manageable and controlled rate. (Mealy et al., 2018)

Biomaterials have specific, mechanical, chemical as well as physical properties this property is the one that grants the capacity for processing and control of 3D structural shape and geometry however scaffolds or hydrogels produced depends on the type of materials used. Currently, many studies are ongoing aimed to identify a variety of new natural biomaterials having high quality ensuring increased cell viability, cell differentiation and proliferation by applying additional impurities of hormones growth factors ECM proteins which gives a high chance to mimic the given tissue (Nikolova & Chavali, 2019).

Recently biomaterials, have taken the attention of scholars to be used as bioactive compounds, herbal analysis and drug-target interaction evaluation. Components derived from nature that exhibit high biodegradable nature, wide selectivity, which possesses high biological activity and having a potential to mimic the extracellular matrix of the cells are believed to be a biomaterial.

According to the studies done by H. Zhang and his colleagues, there is a potential to immobilize the surface of the biomaterials to make more convenient and selective in their work by using various chemicals and applying carrier materials, nanomaterials and natural polymers including porous materials are some biomaterials to be immobilized (H. Zhang et al., 2019).

Ceramics as Biomaterials

The natural properties of biomaterials help to interact with human cells and tissues by mimicking the extracellular membranes and also body fluids which support treating, improving and/or substituting anatomical structures of the human body. Ceramics non-metallic inorganic materials which are commonly manufactured by combining particles with water and organic binder so then shaped in the form they are needed using appropriate heat the binder burned out and the water evaporate to dry,

the thermal treatment is the crucial steps in determining the final microstructure of the ceramics(Affatato et al., 2015).

Ceramics used as biomaterials called bioactive, bio-resorbable and bio-inert based on the feedback obtained from the host tissue it is long ago that biomaterials used to solve imperfections of orthopedics and dentistry because of its excellent properties exhibited like the resistance of corrosion, excellent biocompatibility, and nonrusting (H. Xie et al., 2019).

Metals as Biomaterials

To alleviate drug delivery barriers in the field of bio medication Nano systems play an unreplaceable role as is indicated in the guideline of National Institute of Health (NIH). The institute elaborates the importance of nanoparticles in the formulation of a drug in their size of less than 1 micron (Zhou et al, 2016).

The latest data showed that there are several nanomaterials like Nano shells, nanoparticles and nanotubes are used as part of nano-medicines. Furthermore, metals as nanoparticles hold an implausible potential in diverse biomedical applications including in drug delivery systems. Some of the commonly used nanoparticles and their role in the field of biomedical therapy are, silver made nanoparticles AgNPs used to treat leukemia, AgNPs acting as antibacterial activity (against various bacteria), polyethylene glycol stabilized chitosan-g-polyacrylamide modified AgNPs applied in the delivery of gene, Gold nanoparticles (AuNPs) like AuNPs used for Cancer detection, drug delivery, imaging tissue engineering and Platinum nanoparticles (PtNPs), PtNPs are biomedically applicable for cancer detection, prevention of bone loss, treatment of Parkinson's disease, dental adhesives and many more important in biomedical applications (Rai et al., 2015).

Polymer as Biomaterials

Nowadays different types of polymers are in use for biomedical applications. Those naturally obtained polymers have some advantages that keep them promising in the area of tissue engineering (Q. Zhang et al., 2019).

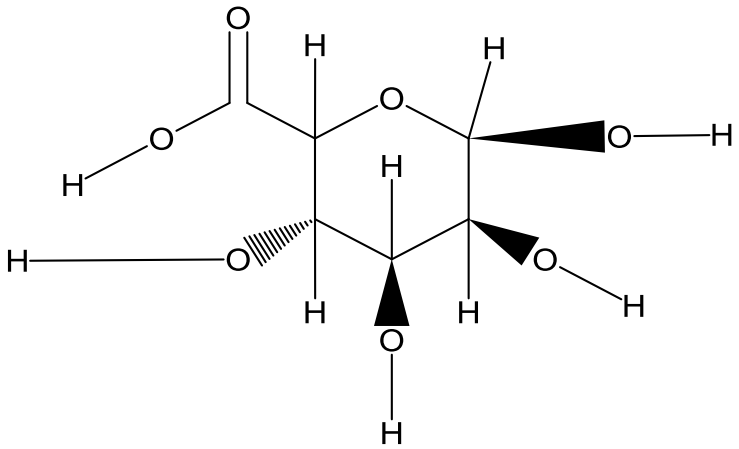
Alginic acid as Natural Polymers

According to the study by (Dekamin et al., 2018), Alginic acid is one of the naturally obtained polymers that are most importantly biodegradable and highly biocompatible with other unique properties that the sectors need to have and brings a change on the applicability of natural polymers in different disciplines such as medicine, production of cosmetics, hydrogel formation, for the application of drug delivery unit, in the food packaging process acting as a polymeric catalyst and as adhesives.

Alginic acids from its very nature biocompatible, nontoxic biodegradable and also affordable for users with low-cost polymer (Połomska et al., 2011). The name Alginate is given in general for the group of polysaccharides made in replicating two hexuronic acids of d-mannuronic acid (M) and -1-guluronic acid (G) residues, bonded by a glycosidic bond in the position of C-1 and C-4 in a linear fashion (Fig. 7). This polysaccharide which is obtained in nature found in the cell wall of brown seaweed and Azotobacter and pseudomonas species of gram-negative bacteria (Dekamin et al., 2018).

Figure 7

Structure of Alginic Acid



Therefore, due to functional groups present on the structure of alginic acid gets the properties of hydrophilic, high viscoelasticity, pH dependence and ability to form gelation by multivalent metal cations. Moreover, Alginate develops gels, films, and/or

alginate particles for the purpose and application of medicine and drug delivery systems. (Caner et al., 2007)

Alginic acid consists of the active site which allows the binding of metals, inorganic ions so that makes it to have important properties that make to be a primary choice to be used for biomedical applications. Besides using monovalent cations consents alginic acid to form water-soluble complexes through using bi or trivalent cations with alginic acid exhibit opposite properties forming insoluble complexes (Sharma et al., 2017).

Alginic acid is a straight-chain colloidal polymer obtained in nature capable of dissolving in water and composed of D-mannuronic acid of uronic derivatives and L-guluronic acid which differs in C-5 configuration typically poly uronic acid. Due to its unique properties, it's also chosen to be used for water treatment to remove some heavy metals and also used for manufacturing dressing materials for medical applications as well (Maureira & Rivas, 2009).

Silk fibroin as Natural Polymers

Bombyx mori silk cocoons are the source for the production of a natural protein called silk, whose structural arrangement, the hydrophobic B-sheet, helps to have strong physical, mechanical toughness and strength. Silks are the best biomaterials due to their molecular arrangements that allow them to mimic the extracellular membrane of cellular tissues and organs without causing diverse reactions, so they are very commonly used for tissue engineering to produce scaffolds, hydrogels, and other types both in mixture with other biopolymers (Shan et al., 2018).

It is an essential protein polymer-forming material used for biomedical applications because of its best natural properties of biocompatibility interaction with the extracellular matrix of cells, hemocompatibility and the capacity to facilitate tissue formation. Silk fibroin also easily designed in the level of molecular solution structure so then this all properties lead to various applications (Adalı & Uncu, 2016).

Properties of Silk Fibroin. The silk is considered an excellent biomaterial because of its great biocompatibility, flexibility, low thrombogenic, biodegradability, high tensile strength, elasticity and a good degree of toughness, which supports specific cell interaction to substrates of silk fibers bio-receptors binding, tissue support and proliferation. In addition, the surface of fibroin films has unique properties that

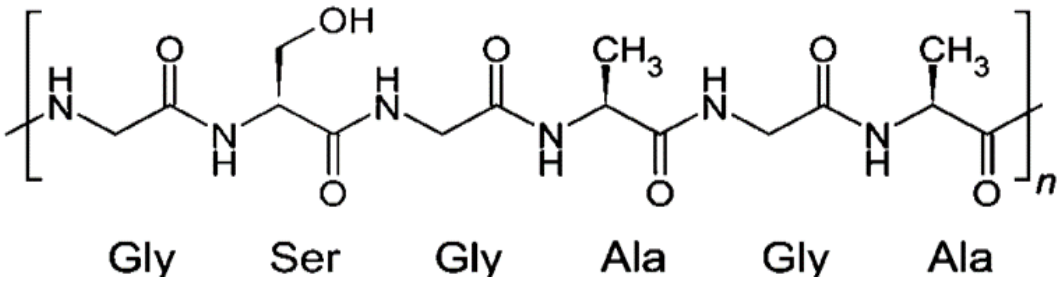
enable the expression of genes encoding high levels of extracellular matrix proteins that are associated with mobility (Terada et al., 2016).

Further investigation indicates eighteen different types of amino acids in combination forms the protein (fibrous protein) and sericin (globular protein) of silk fibroin out of all 80% of them are a repetition of glycine (Gly), serine (Ser), and alanine (Ala), the structure holds repetitive Gly-Ala-Ser-Gla-Ala-Gla-Ser as shown in the (Fig. 8) in which assembled into in anti-parallel β-sheet fashion.

Silk fibroin satisfies the preconditions required to be used for tissue engineering as it holds strong mechanical properties in compared with other natural biomaterials in the area which gives environmental stability and because of β-sheet on the structure exhibit good intensity and toughness, as a result, takes the attention of scientists to work with it because of its mechanical properties (Z. H. Li et al., 2013)

Figure 8

Structure of Silk fibroin proteins



The content of silk fibroin is suitable for immobilization of bioreceptors and thermodynamically stable, mechanically highly resilient proteins (N.Nikhom, et al., 2012). It is approved by the U.S. Food and Drug Administration (FDA) for use in medicine and these biomaterials have a variety of applications, for example, it is used for the design of biosensors, the manufacture of diagnostic devices for medical purpose and it is also used for the manufacture of scaffolds used for tissue engineering and widely used as drug delivery units (W. Zhang et al., 2017).

Gelatin

Gelatin is obtained and produced by the process of partial hydrolysis of natural collagen (Huang et al., 2017). It has exceptional gelling behavior and common functional properties with proteins, which is why it is widely used in many areas of the processing industry, such as food processing, cosmetics, and medicine (Leone et al., 2020).

Gelatin is a widely used food items due to its toxin-free nature and is also used in pharmaceuticals and biomedical device manufacturing. The most important property of gelatin is its transparency, which is more or less turbid (Maki & Annaka, 2020).

Polymers for Hydrogels

A 3D structure hydrogel is one of the polymers made, semi-solid that have the potential to hold water and don't dissolve rather swell up when immersed potentially used for the function in response to external stimuli. However, hydrogels made from different polymers are highly sensitive to the environment like temperature, change of pH which swells up or shrinks the hydrogel and so a good opportunity to manage the drug retention and release depends on the physical state of the hydrogel (Affatato et al., 2015).

Hydrogels in their chemical structure fundamentally constitute the following functional groups - $CONH_2$, -COOH, -OH, - NH_2, - SO_3H - and - CONH that grants the material to be hydrophilic that reacts with water thoroughly and due to water holding capacity hydrogels mimics natural tissue via the degree of flexibility. As like chemical stimuli, the same conformational change exhibited because of physical stimuli such as pressure applied, increase or decrease of temperature, field coverage of electric and magnetic, the composition of solvents, light intensity however the magnitude of response determined by the degree of the applied stimulus (Bahram et al., 2016).

The Swelling Properties of Hydrogels

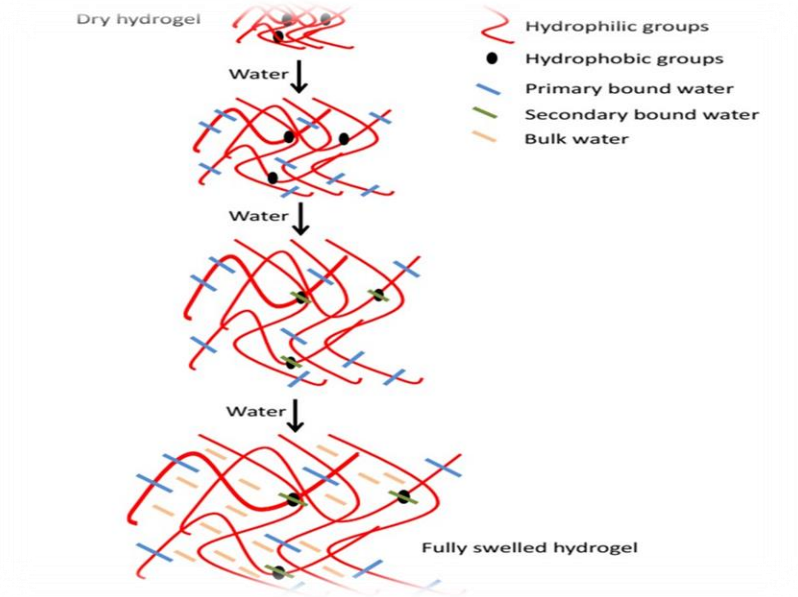
One of the characteristics that make hydrogel a noble biomaterial is its ability to retain a massive amount of water bound inside of it (Fig. 9). The water inside the hydrogel is believed to perform transporting and managing the loaded ingredients for

instance drugs and nutrients additionally grants flexibility and creates an environment that mimics the tissue of the hosts.

The massive amount of water at the surface of hydrogel plays a pivotal role again in the biocompatibility adhesion of platelets and protein binding and ease delivery of drugs to the place of actions. (Cooper & Yang, 2019). The water absorbed inside the hydrogel is associated non-covalently and forms as bulk (free) and bound (either tightly or slightly) within the hydrogel. The water binds to the hydrogel tightly with hydrogen bonding in the polar side of the hydrophilic (Nguyen et al., 2015).

Figure 9.

Swelling nature of the hydrogels



Primary bond with hydrophilic tail, secondary bound of massive water with hydrophobic groups and formation of bulk water filling the voids in the hydrogel

CHAPTER III
Materials and Methods

Materials

Bombyx mori cocoons and Curcuma longa (Turmeric) rhizome were obtained from a local market of North Cyprus. Gelatin, Tween 80, Calcium chloride ($CaCl_2$), Sodium triphosphate Pentabasic ($Na_5O_{10}P_3$), Ethanol (C_2H_5OH, 98%), MBA were all purchased from Sigma Aldrich. SnakeSkin® Dialysis Tubing of 3,500 molecular weight cut out membranes was purchased from Thermo Scientific USA. Anhydrous sodium carbonate $Na_2CO_3.6H_2O$ and $CaCl_2.6H_2O$ obtained from EMSURE® Merck chemicals in Darmstadt, Germany, Timolol maleate ($C_{17}H_{28}N_4O_7S$) purchased from local pharmacy of North Cyprus.

Extraction and Purification of Silk Fibroin

Cocoons are naturally obtained protein-rich made by silkworms which are nontoxic collected and purified to finally ended up pure silk fibroin solution passing through serious steps as discussed below. The cocoons are locally available and affordable can be purchased from the local markets of TRNC. After obtained from the market surface cleaning should be done by removing unnecessary parts like pupa, dust, impurities, and other foreign particles so that it may increase efficiency of silk fiber.

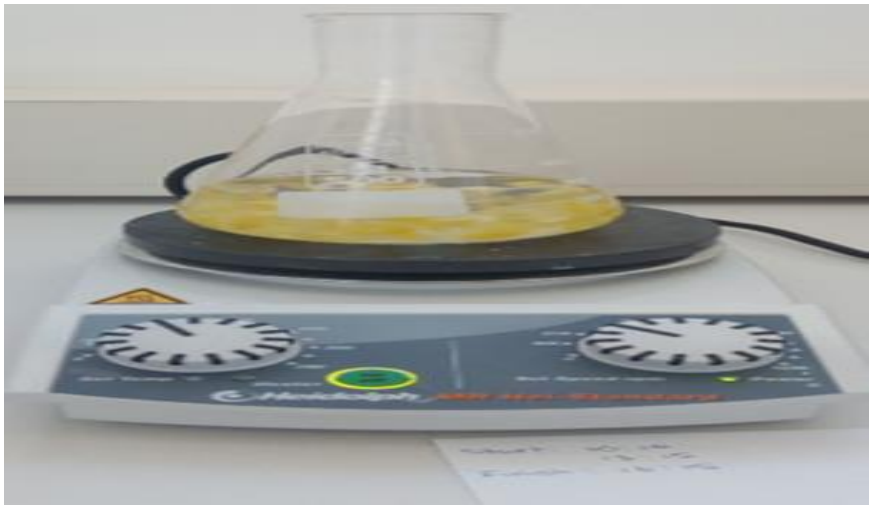
Degumming Process

The process of removing the glue protein and sericin (Fig. 10). The overall process is done using the protocol formulated by Adali and Uncu via thermochemical treatment over the cocoons (Adalı & Uncu, 2016).

In this procedure, a sodium carbonate solution was prepared by weighing 12g of sodium carbonate and mixed with 200ml of deionized water. Then 2g of cut silk cocoons were weighed and added to the flask, which was mixed with the sodium carbonate solution by rotating the magnetic stirrer for three hours, This step is repeated three times, changing only the sodium carbonates, and the silk cocoons are washed thoroughly with deionized water several times until the yellow color disappears, and finally the fibers are released to dry so that they dissolve easily (Adalı & Uncu, 2016).

Figure 10.

A process of silk cocoons degumming with 0.1M $Na2CO_3$ Solution



Dissolution Process

Silk cocoon mainly consists of hydrophilic proteins called sericin consists of water-insoluble fiber and fibroin protein component. By the process of degumming 70% of glue and sticky substance removed from silk cocoons. Degummed fibers were dissolved in the strong electrolyte (27.79gm of $CaCl_2$ + 29.13ml of C_2H_5OH +36ml of deionized water) at 70℃ with continuous stirring until the total dissolution and silk fibroin solutions obtained as shown in (Fig. 11).

Process of Dialysis

A solution of silk fibroin poured into the dialysis tube then immersed into a beaker filled with deionized water, this process repeated three times by changing the deionized water every three ours by doing so strong electrolyte molecules removed from the solution. Finally, the purifies SF extracted from the dialysate with a syringe and filled into a bottle following the procedure established by (Adalı & Uncu, 2016). The concentration of the dialyzed pure regenerated silk fibroin solution was adjusted as shown here below.

Figure 11.

Purification Method of Pure SF Protein

Extraction and Isolation of Curcuminoids

Method of percolation-extraction were applied in this process where 144g of *Curcuma longa* powder mixed with acetone. After curcuminoid extraction completed, the extracts were concentrated in a rotary evaporator and the concentrated curcumine extract was purified by column chromatography, using silica gel (SiO_2) and dichloromethane as adsorbents then by heating, dichloromethane was removed, and final products were collected in the form of powder and directed to thin layer chromatographic analysis. For isolating pure curcumin column chromatography set up was carried out as its shown in the (Fig. 12, 13 and 14) sequentially.

Figure 12.

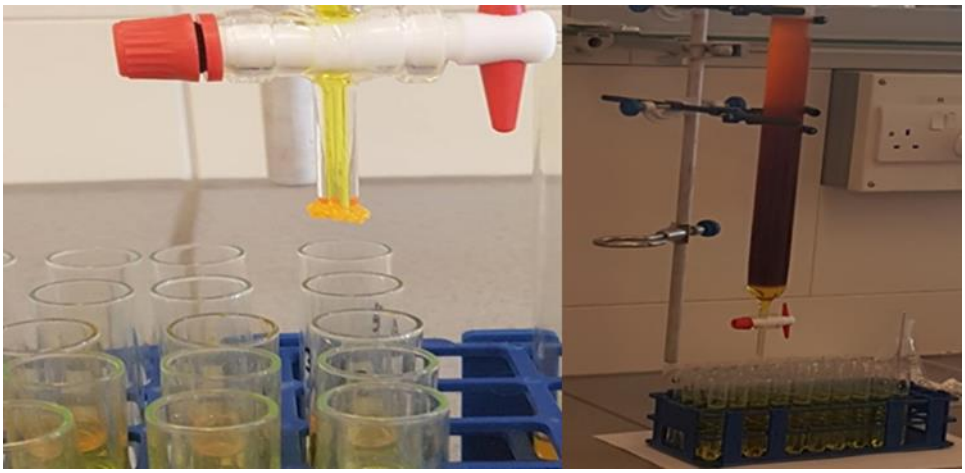
The Percolation Extraction Method of Curcuma longa

Figure 13.

The Process of Rotary Evaporation and Remains After Solvent Had Evaporated

Figure 14.

Column Chromatography Method for Separating Curcumin



Hydrogel Preparation

For preparing injectable hydrogel, gelatin, purified SF solution, curcumin, and MBA were used. Different proportion of dialyzed SF mixed with gelatin, curcumin and MBA in each trail and putted in the refrigerator under 4℃ and also checked under room temperature. Out of the many trials done the following three compositions successfully form the hydrogel and are used for further experiments.

Table 1.

Proportion applied in the preparation of hydrogel

Sample code	**GE (ml)**	**SF (ml)**	**CUR**	**MBA(μl)**	**Status**
Ia	5	1.5	0	25	Hard gel
Ib	5	2.0	0	25	Soft gel
Ic	3	2.0	0.5	25	Softer gel

Test for Swelling Properties

Swelling tests were carried out for checking changes in weight of samples after immersed in a certain volume of PBS (pH 7.4) and ABS at 37°C at various time points. The pH ranges were chosen to mimic physiological conditions of the human body. Swelling ratio calculated by

$$\text{Swelling ratio\%} = \frac{\text{Weight of the swollen sample} - \text{Weight of sample}}{Initail\ weight\ of\ the\ sample} \times 100\%$$

Test for Coagulation and Fibrinogen Activity

In-vitro anti-coagulation test analysis were done by STA compact coagulation auto analyzer. Fresh blood samples were collected according to Clinical and Laboratory Standards Institute (CLSI) guidelines from healthy donors into specimen collection tubes having anticoagulant trisodium citrate 0.109M (3.2%) and centrifuged for 10minutes at 850 RCF. Separated plasma were collected and mixed with sample of hydrogel then incubated under static conditions at 37°C for 15 minutes. PT, APTT, and fibrinogen analyses were performed. results of APTT and PT were expressed in seconds and analysis result for INR and fibrinogen were mg/dL.

Protocol of Activated Partial Thromboplastin Time (APTT) Test.

Human blood samples were collected in tubes containing citrate which stops blood clotting binding with calcium in the blood. The intrinsic pathway was activated by adding calcium to reverse the anticoagulant effect of the oxalate, and an activator was mixed into the plasma sample, and the time to clot formation was measured.

Test procedure for Prothrombin Time (PT)

Blood plasma were used for measuring PT. Sample of blood taken from human placed in sample collection tube containing citrate an anticoagulant mixed and centrifuged which separates plasma part of the blood (Adalı & Uncu, 2016). Tissue factor (factor II) added into the plasma and the time required for the sample to clot was measured.

International Normalized Ratio (INR)

INR is a calculation used to normalize PT. The INR is based on the ratio between the patient's PT and the normal mean PT. The test called PT were done to determine how fast the blood clots in patients treated with oral anticoagulants (Adalı & Uncu, 2016). INR uses the ISI to equate all thromboplastins to the reference thromboplastin as follows.

$$\text{INR} = \left(\frac{\text{patient PT ISI}}{\text{meannormalPT}}\right)^{\text{ISI}}$$

Where ISI is International Sensitivity Index.

Fibrinogen Activity Test

Fibrinogen is a soluble protein in the blood that plays a role in blood clotting and wound healing and creates a favorable environment for new tissue development. 150-400 mg/dl is the normal range of fibrinogen concentration in human blood (Factor, 2019), although the range may vary in different laboratories and depending on the method they use.

Test for Total Serum Albumin and Cholesterol Level

Nearly half of healthy blood plasma contains serum albumin ranging in between 3.5g/dl -5g/dl. Albumin plays a critical role, regulating the transport and availability of numerous chemical compounds and molecules in the vascular systems of the blood. They also interact with various drugs to enhance the delivery of various ligands such as drugs. (Moman & Varacallo, 2018).

Cholesterol is a natural substance about 75% of which is produced by the liver and the rest obtained from the diet we eat and it is required by the body for the normal physiological activates. A total cholesterol level of 200 mg/dl or less is considered optimal. (Sung et al., 2017).

Erythrocyte Morphology and Blood Count Analysis

For complete blood count analysis hematology analyzer was used. Fresh blood was collected in lavender tubes containing K_2EDTA as an anticoagulant, and the samples (hydrogel with and without drug) were then immersed in the whole blood in test tubes. Samples were mixed for 20 minutes with a shaker (300 rpm) that enables the interaction between the sample surface and blood cells.

***In-vitro* Analysis of Platelet Adhesion**

Human blood were collected freshly from a healthy donor by sample collection tubes and then centrifuged for 15minutes at 100 RCF. Platelet-rich plasma was collected after centrifuged completed mixed with sample of hydrogel by immersing for about 15minutes at 37°C. Peripheral smears with May-Grunwald and Giemsa staining were applied to determine adhesion morphology and platelet microparticle formation on the surface of the hydrogel with a light microscope at low (100×) and high (400×) magnification.

Loading of Drug and Release

Timolol maleate ($C_{17}H_{28}N_4O_7S$) is the drug that has been chosen as the model drug in this study. The process of loading the drug was carried out by immersing the piece of hydrogels weighing 0.0249gm in 5ml solutions of timolol maleate for 50hrs. Then using the UV visible spectrometer at 295nm, the absorbance of the drug has been taken and the number of loaded drugs can be calculated from the curve of calibration (Morsi et al., 2016).

The efficiency of the loaded drug and drug loading capacity has been calculated by the formula mentioned below.

$$\text{Drug loading efficiency} = \frac{\text{weight of loaded drug}}{\text{weight of the drug in feed}} \times 100\%$$

$$\text{Drug-loaded capacity} = \frac{\text{weight of loaded drugs}}{\text{weight of the hydrogel}} \times 100\%$$

Drug release experiment was done by immersing Timolol maleate loaded hydrogel in 5ml solution of simulated tear fluid (STF) of pH 7.4. for a predetermined interval of *in vitro* release experiment, 1ml medium solution samples were withdrawn at periodic interval and replaced by equal volume of fresh solution and analyzed spectrophotometerically at 295nm in contrast to standard reference of simulated tear

fluid. Amount of cumulative release was determined from interpolation of standard calibration curve at λmax = 275nm using the formula:

$$\text{Cumulative percent release} = \frac{\text{amount of } Timolol\ maleate \text{ released}}{\text{total amount of } Timolol\ maleate \text{ loaded on the hydrogel}} \text{ X } 100\%$$

Analysis of Scanning Electron Microscope (SEM)

Analysis of SEM for the samples was performed at TUBITAK-MAN using a JEOL/JSM-6510LVF scanning microscope.

Analysis of Fourier Transform Infrared (FTIR)

FTIR spectra of the studied samples were obtained in the Department of Pharmacy of the State Laboratories using a Perkin Elmer Spectrum 65 FTIR

X-ray Diffraction Analysis (XRD)

TUBITAK-MAM Gebze Turkey is the place where the XRD analysis of samples was carried out, using a Shimadzu XRD-6000 model diffractometer with Cu X-ray tube (λ = 1.5405 A˚ (10^{-10}m) the crystallinity index is calculated by the technique based on the method proposed by Kim and his collegues (U. J. Kim et al., 2005). It consisted of measuring the maximum intensity, I_{110}, at 2θ = 16˚. The crystallinity index was calculated by the equation stated (Teimouri et al., 2015)

$$\text{Crlpeak} = \frac{I110 I\text{am}}{I110}$$

Statistical Analysis

All data obtained were presented as mean ± standard deviation. Significance difference were performed by a student's t test at a probability level of 0.05 and one-way (ANOVA) used for determining the difference among the groups using Graphpad prism version 8.0.2 software.

CHAPTER IV
Finding and Discussions

Swelling Properties

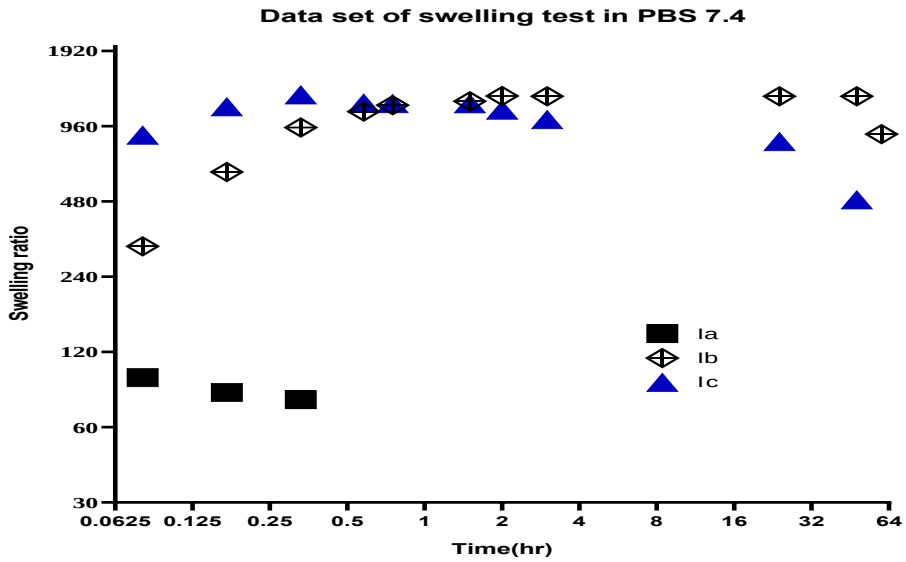
Swelling nature of freshly prepared hydrogels were examined in acidic solution with pH 1.2 and pH 4.7 and basic solution pH 7.4 solutions. It is observed in the experiment that samples coded by Ib and Ic swells uniformly and accordingly reaching its maximum equilibrium ratio. Samples encoded with Ib swell within 180 minutes and Ic within 45 minutes in a solution at pH 7.4. While sample Ib saturates its swelling equilibrium within 90 minutes and Ic within 45minute in a solution at pH 4.7 and in acidic buffer solution at pH 1.2 sample Ic reaches its maximum swelling capacity within 35 minutes and sample Ib within 20 minutes, one of the sample encoded with Ia swells rapidly within 10 minutes in both ABS and PBS and then decays completely.

percentage swelling results in a solution at pH 7.4 for Ia, Ib and Ic shown in Fig. 15 are 98%, 1469.2% and 1393%, respectively. Fig. 16 and 17 shows percentage swelling in a solution at pH 4.7 for the same samples Ia, Ib and Ic respectively are 145%, 700.4% and 1242.6%, and in a solution at pH 1.2 for the same samples Ia, Ib and Ic 125%, 1440.9% and 1092.1%, respectively.

The result shows that hydrogels are able to absorb large amounts of liquids due to their structure. Sample Ia swells immediately within 10 minutes both in acidic and basic buffer solutions because of its amorphous structure and contains a small amount of silk fibroin which allows the nature of swelling.

Figure 15

Swelling % Hydrogels of Sample Ia, Ib, Ic in PBS pH 7.4



The maximum percent swelling result in PBS pH 7.4 for each sample coded Ia, Ib and Ic is 98%, 1469.2% and 1393%, respectively. It can be concluded that swelling is very rapid in the first 30 minutes and gradually increases thereafter.

Figure 16

Swelling % of Hydrogels of Sample Ia, Ib, Ic in ABS Ph 4.7

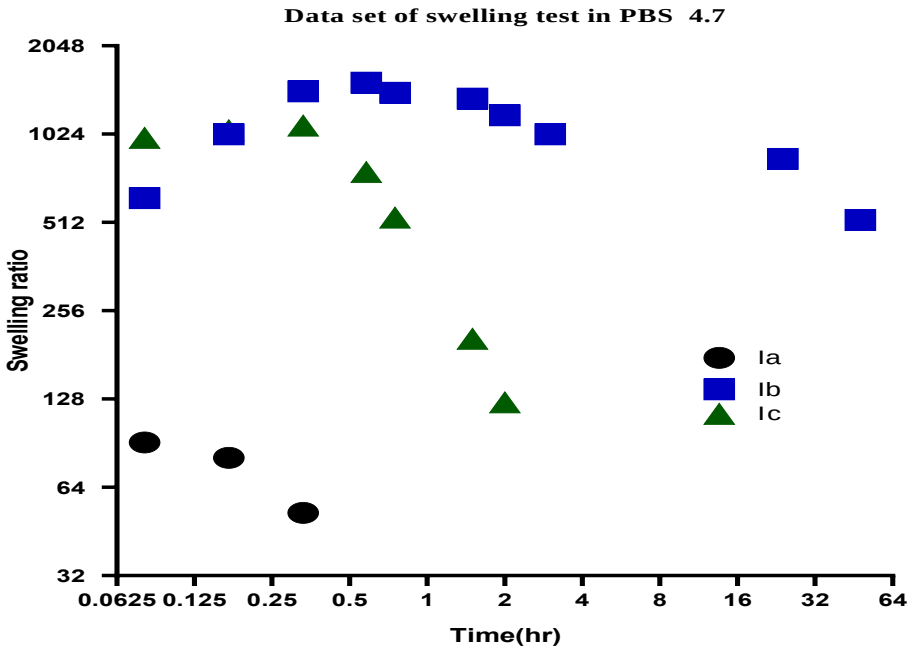
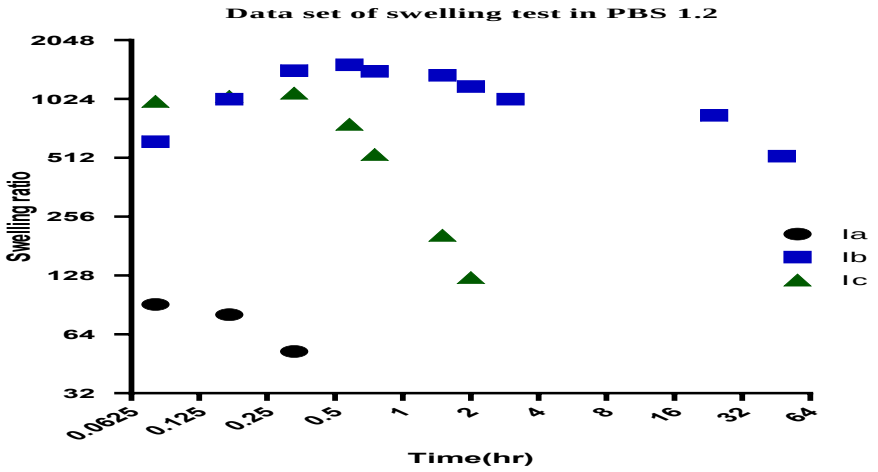


Figure 17

Swelling % of Hydrogels of Sample Ia, Ib, Ic In ABS Ph 1.2



***In-vitro* Coagulation Analysis for Hydrogels (Combinations Ia, Ib Ic)**

The anticoagulant effect of the hydrogel was evaluated by APTT and PT measurements, which were used to assess secondary hemostasis. The values of PT and APTT activation were measured for control plasma containing citrate and after contact with samples (Ia, Ib & Ic) at a temperature of 37±1℃.

The result of the test for APTT, PT and INR is in the normal range, confirming that the prepared injectable hydrogels are biocompatible in terms of clotting activity. The total cholesterol level and serum albumin were also within the desirable limits according to the National Cholesterol Education Program (NCEP) standard.

Table 2

Coagulation Analysis for Hydrogels (Combinations Ia, Ib Ic and Hk (control), Total Cholesterol and Serum Albumin

Code	PT (%)	INR	PT (time)	APTT (time)	Total level of Cholesterol(mg/dL)	Albumin (g/dL)
Standard	70-120%	0.8-1.2	11.5-15s	23.6-35.2	< 200	< 5.18
Ia	92%	1.06	13.5sec	35.4sec	160mg/dl	4.6g/dl
Ib	86%	1.11	14.1sec	37.9sec		
Ic	94%	1.04	13.3sec	34.6sec		
Hk	101%	0.99	12.7sec	31.7sec	160mg/dl	4.6g/dl

In-vitro Coagulation Analysis and Fibrinogen Activity Test of Hydrogels with Drug (Combinations Ia/D, Ib/D and Ic/D)

The anticoagulant activities of a hydrogel loaded with a drug (Timolol maleate) were evaluated by APTT, PT and INR values were displayed in the unit selected (seconds, INR, % ratio). The following result was recorded for samples of (Ia/D, Ib/D & Ic/D) at a temperature of 37 ± 1℃. The results obtained are within the ranges stated on the assay value of standards which ensures that the prepared hydrogels are in the best position of biocompatibility.

A fibrinogen activity test is also done for the samples both with drugs and without drugs and it is found to be compatible according to the standard value.

Table 3

Anti-Coagulation and Fibrinogen Activity Test of Hydrogels with Drug

Code	Percent PT	INR	PT Time	APTT/sec	Fibrinogen
Standard	70-120%	0.8-1.2	11.5-15s	23.6-35.2S	200-400 mg/dl
Ia	92	1.06	13.5	35.4	237 mg/dl
Ia/D	93	1.04	13.5	35.2	242 mg/dl
Ib	86	1.30	17.1	32.6	235 mg/dl
Ib/D	85	1.47	19.1	37.0	253mg/dl
Ic	84	1.12	14.8	31.3	253mg/dl
Ic/D	89	1.08	14.3	31.5	266mg/dl

Complete Blood Count Analysis

Hydrogels were immersed in fresh blood with K_2 EDTA as an anticoagulant. No significant difference was found between all samples and the control, as shown in Table 4. This experiment confirms the fact that the polymers used for the preparation of the hydrogels are biocompatible and have no negative effect on the total of erythrocytes, leukocytes and platelets in normal blood.

Table 4

Total blood Count Analysis of Samples with Drug and Without Drug

Cells	**Control**	Ib	Ib/D	Ic	Ic/D
Wbc	**5.29 10e3/uL**	**5.12 10e3/uL**	**5.10 10e3/uL**	**5.03 10e3/uL**	**5.27 10e3/uL**
Neu	1.35	1.78	1.30	1.37	1.25
Lym	3.18	2.13	2.99	2.51	3.44*
mono	.473	.422*	.529	.445	.299*
Eus	.228	.265	.219	.637	.275
Baso	.060	.012	.068	.077	.014
Rbc	**5.37 10e6/uL**	**5.11 10e6/uL**	**5.43 10e6/uL**	**5.36 10e6/uL**	**5.76 10e6/uL**
Hgb	16.0 g/dL	15.4 g/dL	16.2 g/dL	16.0 g/dL	16.7 g/dL
Hct	48.2 %	46.0 %	49.1 %	52.5 %	52.6 %
Mcv	89.8fL	90.0 fL	90.3 fL	97.8 fL	91.2 fL
Mch	29.7 pg	30.2 pg	29.8 pg	29.8 pg	29.0 pg
mchc	33.1g/dL	33.6 g/dL	33.0 g/dL	30.5 g/dL	31.8 g/dL
Rdw	11.5 %	11.5 %	11.7 %	13.2 %	11.8 %
Plt	**248 10e3/uL**	**194 10e3/uL**	**252 10e3/uL**	**296 10e3/uL**	**265. 10e3/uL**

Peripheral Smear Test for *In-vitro* analysis of Platelet Adhesion and Erythrocyte Morphology.

A peripheral in vitro smear test was performed to evaluate the hemostatic state of the hydrogel, as shown in Fig. 18 and 19, which show microscopic images of both hydrogels with drug and hydrogels without drug. No platelets form on the surface of the hydrogel, which makes the hydrogel promising for biomedical applications. In the morphological analysis of erythrocytes, as shown in Fig. 20 and 21, clustering of erythrocytes occurs, which is due to the dissolution of the hydrogel and the hydrophilic nature of the silk fibroin, which allows the absorption of water from the hydrogel.

Figure 18

Microscope Micrograph of Hydrogel with Drug 400x

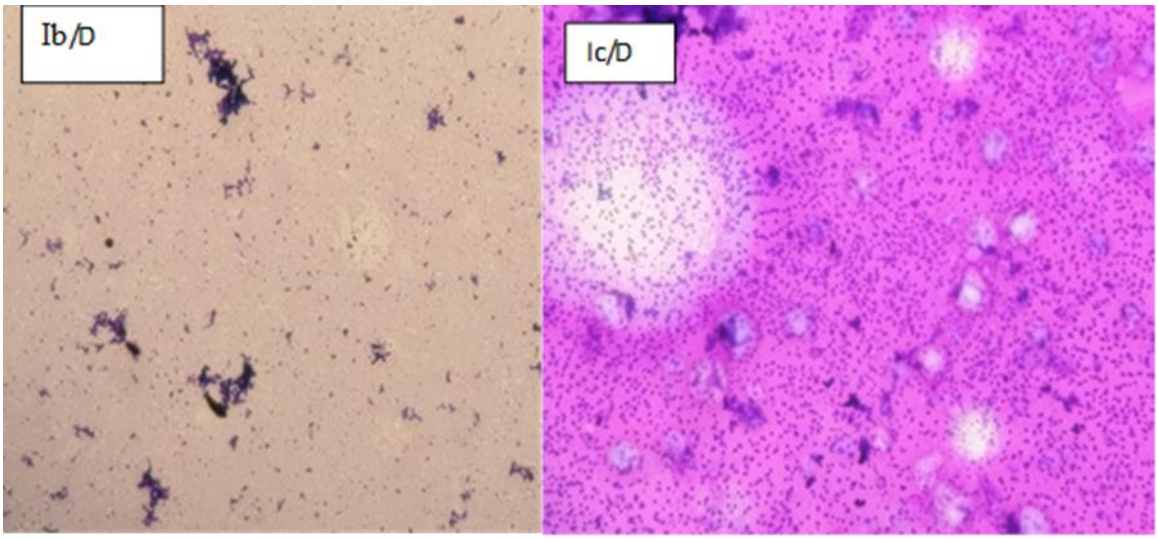


Figure 19

Microscope Micrograph of Hydrogel Without Drug 400x

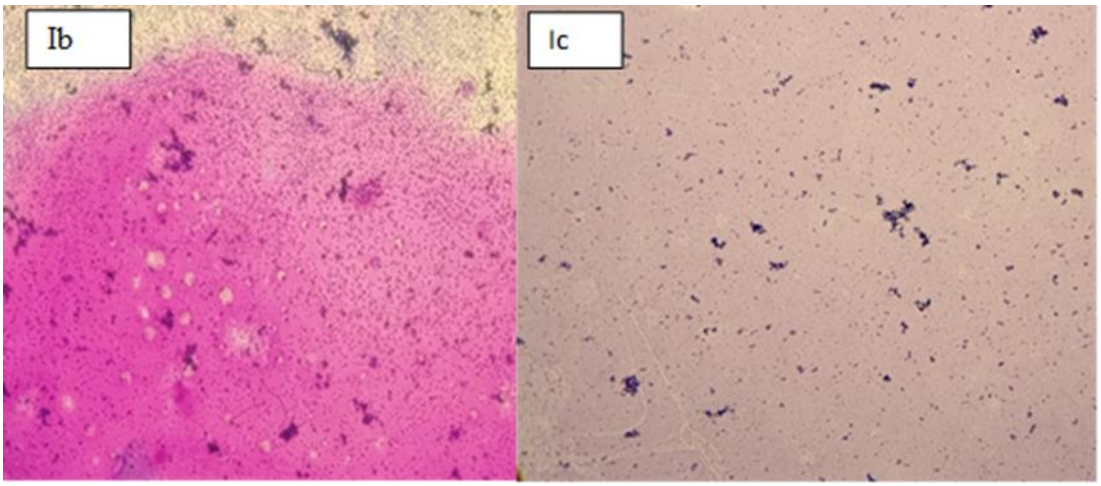


Figure 20

Erythrocyte Morphology After Mixing Samples Ib and Ic

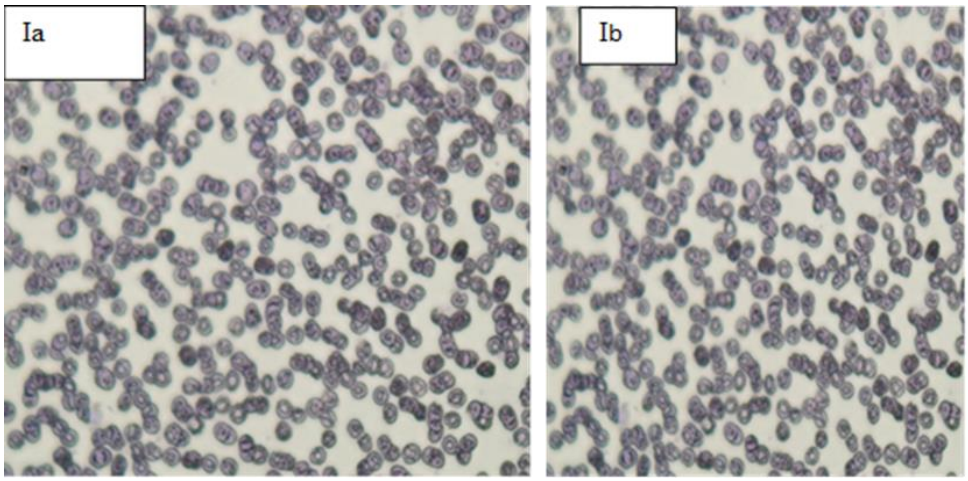
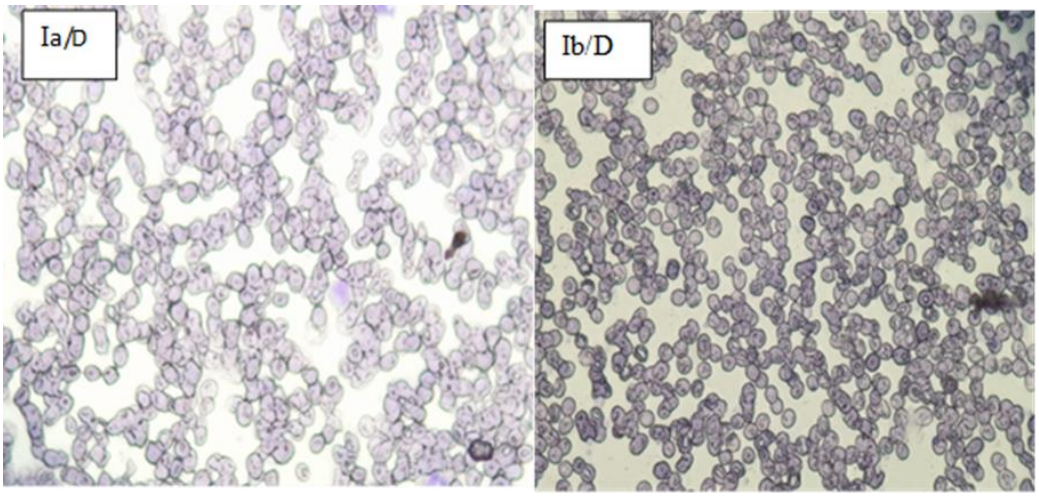


Figure 21

Erythrocyte Morphology After Mixing Samples with Drug Ib/D and Ic/D

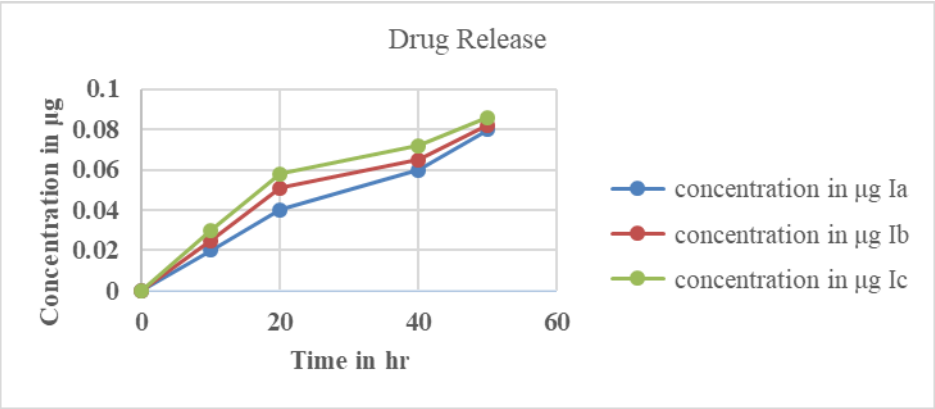


Drug release

Cumulative drug release was measured over 50hr by immersing the hydrogel loaded with a drug timolol maleate in 5ml of simulated tear fluid (STF) at pH 7.4 the hydrogels showed an initial release of the drug and subsequently stabilized over time. The characteristic release with subsequent normalization of concentration from the hydrogels is consistent with the findings of Morsi and colleagues (Morsi et al., 2016), confirming that the hydrogel is a candidate for controlled release of drug in the treatment of glaucoma (Pakzad et al., 2020).

Figure 22

Drug Release of Timolol Maleate from Hydrogels Ia, Ib and Ic



Scanning Electron Microscopy Analysis

Microscopic figure SEM shows the morphology of Ia, Ib, and Ic encoded hydrogels prepared from various combinations of SF, gelatin and MBA, and curcumin. In Ia, the number of pores was low and their morphology was rough, which could be crucial for drug loading. Ib and Ic contain visible microspheres with open pores that facilitate drug transport and aid in the controlled release of loaded drugs.

The proportion used for Ic increased amount of SF with decreasing gelatin and the presence of curcumin be responsible for the morphological features to have better pore structure and good interconnectivity, favoring drug loading capacity.

Figure 23

SEM Micrograph of Sample Ia, Ib, Ic 100µm

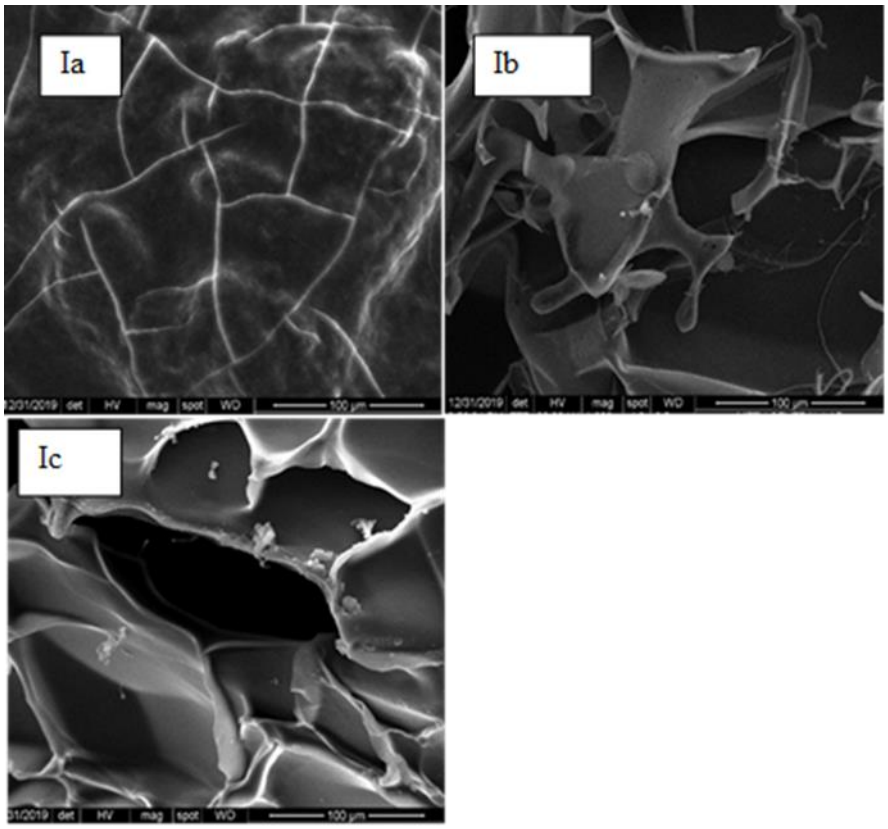
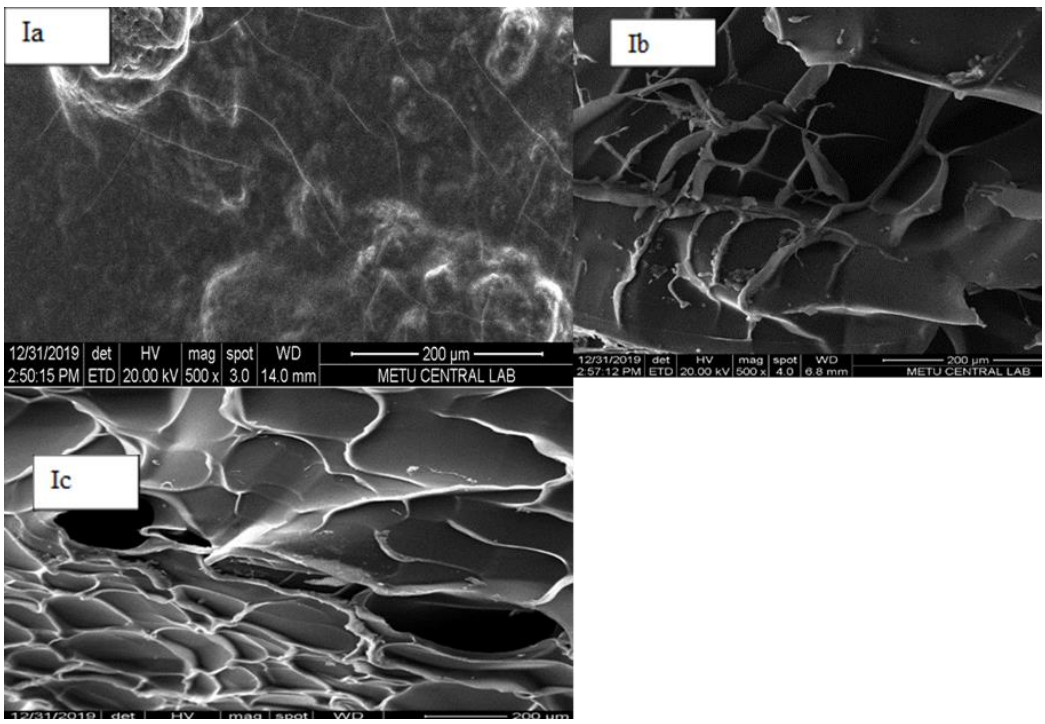


Figure 24

SEM Micrograph of Sample Ia, Ib, Ic 200µm



FTIR Analysis

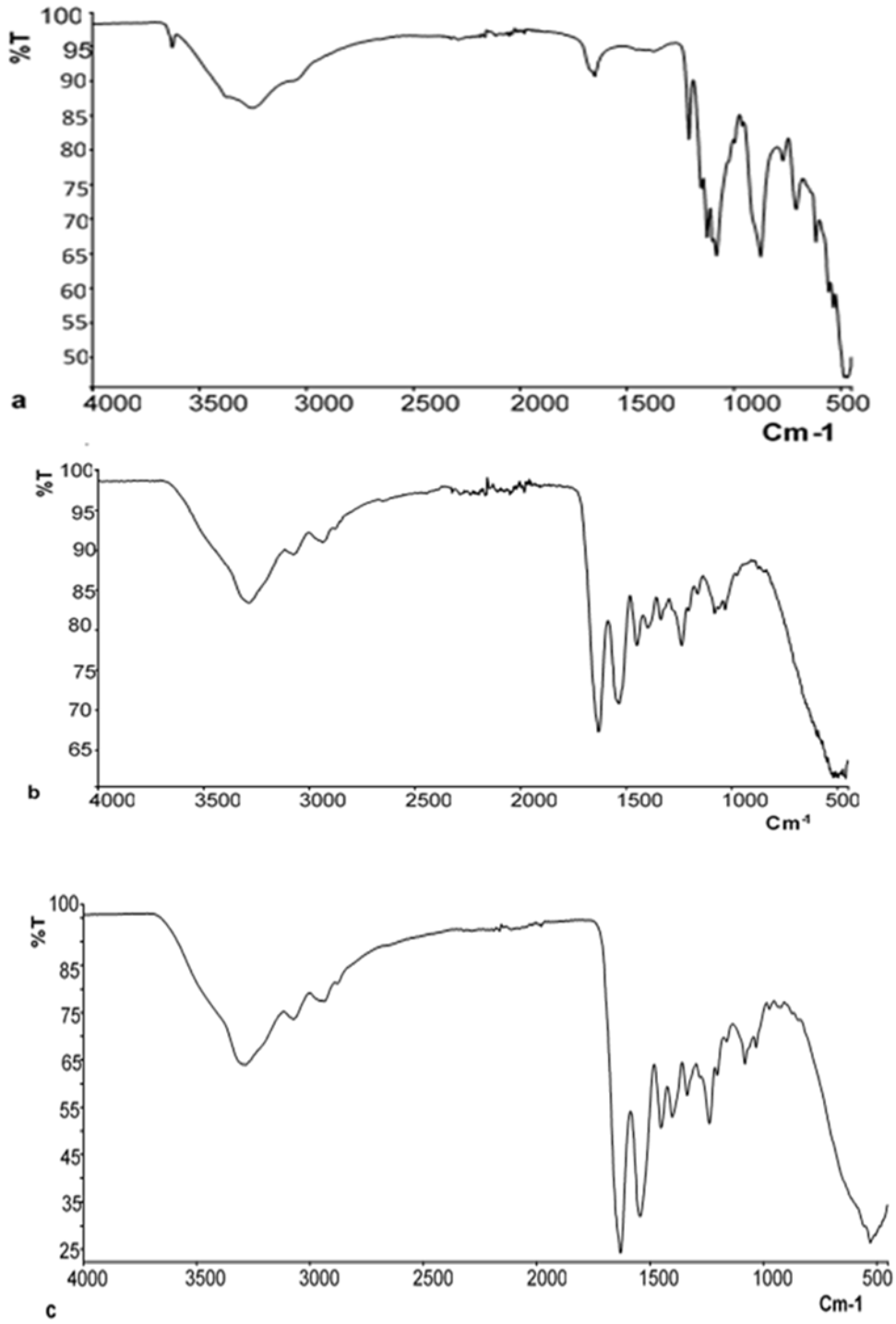
In the given hydrogel particularly, SF/GE/CUR/MBA characterized by the absorption band in FTIR. Adali and her colleagues have been well-labeled. In figure 25 the absorption band region within 1650 cm^{-1} - 1350 cm^{-1} is assigned to the amide I and 1544.09 cm^{-1} and 1532.81 cm^{-1} to the amide II absorption mainly from the NH bend in-plane bending vibrations and CN stretching in protein backbone of silk fibroin.

The bands assure in the β-sheet confirmation of the samples (Adali et al., 2019). The sample coded by figure 25c which is loaded with curcumin reads an absorption band at 1646.4cm^{-1} for amide I and 1446.5 cm^{-1} for amide II as is in agreement with previous studies (Leone et al., 2020). Since curcumin was completely mixed into the mixture the stretching vibration could be very limited and the bands disappeared in the complexes of SF (Guo et al., 2021).

Due to the presence of CO and CN vibration stretching of amide I and amide II in the gelatin The absorption band was shown to be 1638 and 1558 which is in agreement with the study of (Khade et al., 2014). The hydrogel showed an absorption band 3287.63 cm^{-1}, 3289.33 cm^{-1} and 3247.10 cm^{-1} which were due to OH and NH stretching vibration.

Figure 25

FTIR Spectra of Samples (a) Ia, (b) Ib and (c) Ic Hydrogels

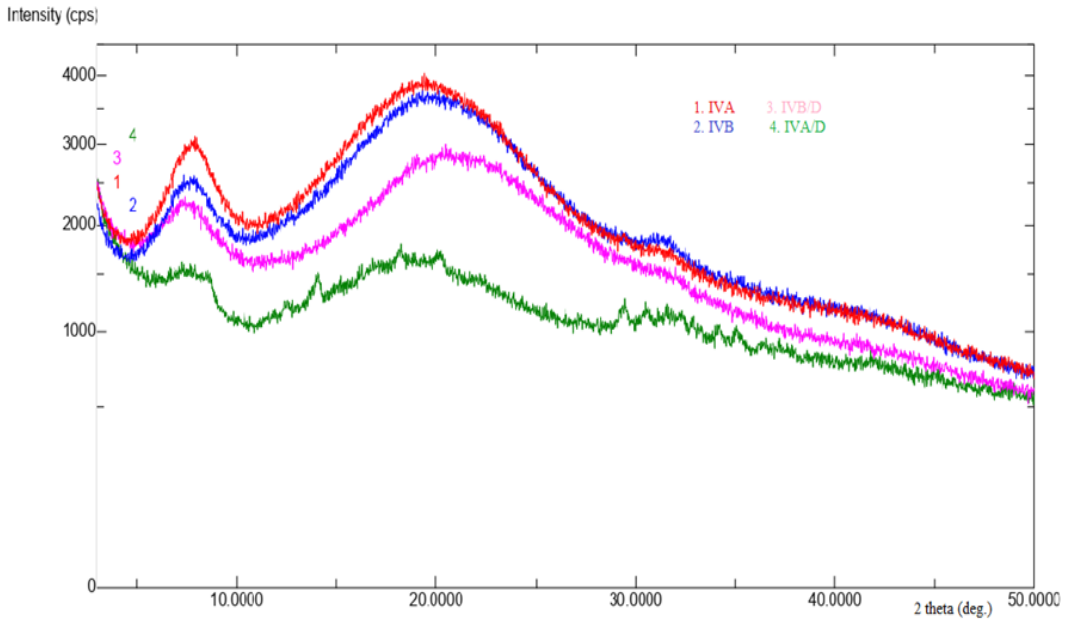


X-ray Diffraction Analyses

X-ray diffraction pattern of SF/Gel/Curcumin hydrogel gives characteristic crystallinity peaks at 2θ = 11.62◦, 20.56◦ and 10.46◦ of high intensity due to the fact that there is more periodicity because of the preferred crystal orientation, however in the drug loaded sample new weaker crystalline peaks appear at 12.58◦, 20.56◦ and 9.12◦ crystals are arranged in a random order than the heat of the peak so both crystalline structure and degree of crystallinity**.**

Figure 26.

XRD pattern of Hydrogel with drug and without drug coded by Ia, Ib, Ia/D and Ib/D containing SF/Gela/ Curcumin in different composition



CHAPTER V

Conclusion and Recommendation

We have gotten good results with our hydrogel preparations and hydrogels were characterized by some of the parameters *in-vitro* coagulation tests (PT, APTT, INR, cholesterol level and serum albumin level) and swelling test. The overall characterizations of different samples show that the samples Ia, Ib, Ic all the samples are confirmed to be candidates for blood compatible biomedical applications.

The swelling test result shows that the sample coded by Ia with a combination of increased gelatin and decreased silk fibroin swells immediately and dissolved as the exposure time raised up both in the acidic buffer and basic buffer solution. However, the result of the swelling indicates clearly that the acidic buffer solution activates the swelling ratio than the basic buffer solution in all combinations. The addition of curcumin has no significant influence on the swelling nature.

The morphological features from scanning electron microscopy analysis showed that samples coded by Ia have low and rough pores which are vital for loading drugs and samples of Ib and Ic contains interconnected structure with open pores which facilitate the transport of drugs increased the potential of controlled release of loaded drugs. The presence of curcumin in sample Ic makes better pore structure and good interconnectivity that favors the best drug load capacity and release of drug loads as well. In the X-ray diffraction crystallinity peaks with high intensity due to the fact that there is more periodicity because of the preferred crystal orientation.

In summary, hydrogel-based ophthalmic drug delivery systems have a promising future for the treatment of ocular diseases. More attention should be paid to application of hydrogels to deliver complex bio-macromolecules into the back of eye for treatment of retinal diseases particularly glaucoma, and promote their clinical translation

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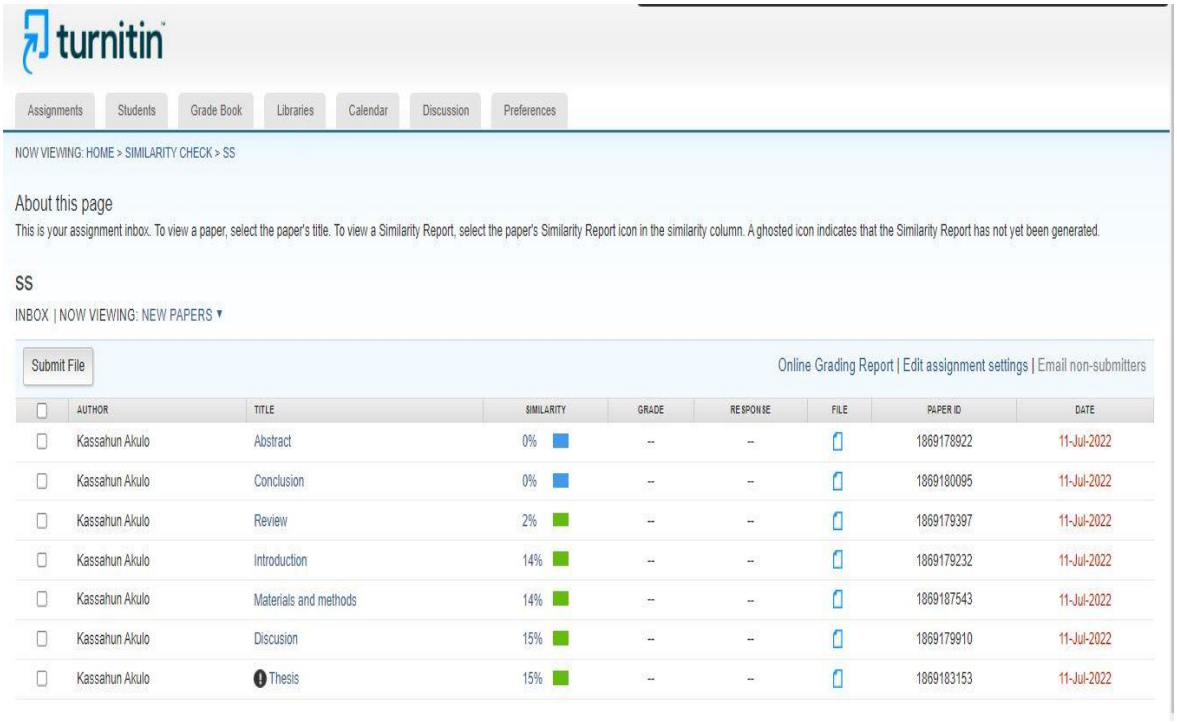
Appendices

Appendix I

Similarity Report



Appendix II

CURICULUM VITATE

1. PERSONAL INFORMATION

NAME, SURNAME: KASSAHUN ALULA AKULO BIRTH DATE: 21 Feb. 1984 Woliata Ethiopia Marital Status Married	Contact address Kasalula2012@gmail.com +251913841213/+905338550429
AFFELIATION: Senior Lecturer and Reasearcher at Mizan Tepi university ADRESS: Mizan Tepi University Ethiopia	

2. ACADEMIC EXPERIENCE

PERIOD	TITLE	DEPARTMENT	UNIVERSITY
25 Oct. 2010	Bsc	Biology	Woliata sodo university Ethiopia
11 June 2015	MSc	Biotechnology	Haromaya University Ethiopia
20 June 2022	PhD	Biomedical Engineering	Near East University Nicosia TRNC

3. WORK EXPERİANCE

Year	Place	Enrollment
2016 - Present	Mizan Tepi University	Senior Lecturer
2010 -2016	Woliata Liqa School	Lecture I

4. LANGUAGE

	Speaking	Listening	Writing
English	Fluent	Fluent	Fluent
Amharic	Fluent	Fluent	Fluent
Oromifa	Fluent	Fluent	Fluent
Woliatigna	Fluent	Fluent	Fluent
Turkish	Good	Good	Good

5. RESEARCH INTEREST

Applicaion of Biomaterilas for Tissue Engineering	Biocompatabiliy, drug delivey and tissue culture

4. PUBLICATIONS IN INTERNATIONAL REFEREED JOURNALS

Akulo, K.A.; Adali, T.;Moyo, M.T.G.; Bodamyali, T.

1. Intravitreal Injectable Hydrogels for Sustained Drug Delivery in Glaucoma Treatment and Therapy. Polymers 2022, 14, 2359. https://doi.org/10.3390/polym14122359
2. Alula, K., Zeleke, H., & Manikandan, M. (2018). In Vitro Propagation of sweet potato (Ipomoea batatas (L.) Lam) through apical meristem culture. Journal of Pharmacognosy and Phytochemistry, 7, 2386-2392.

PAPERS UNDER REVIEW FOR POSSIBLE PUBLICATIONS

Akulo KA, Adali T, Ebedal OH. Preparation Characterization and Blood Compatibility Studies of Silk Fibroin / Gelatin / Curcumin based Injectable Hydrogels. Research Square; 2022. DOI: 10.21203/rs.3.rs-1447945/v1.

5. CERTAFICATES AWARDED

1. Certificate of attendance second international Biomedical Engineering conference
2. certificate of participation awarded "Methods in Research and SPSS software Training

6. HOBBİES

Reading books and listening spritual songs

Appendix III

EK: 1037-2020

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 23.01.2020
Toplantı No : 2020/76
Proje No :955

Yakın Doğu Üniversitesi Mühendislik Fakültesi öğretim üyelerinden Doç. Dr. Terin Adalı'nın sorumlu araştırmacısı olduğu, YDU/2020/76-955 proje numaralı ve **"Hidrojel Ve Polielektrolit Yapılarda Kan Uyumluluğu Çalışmaları"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

1. Prof. Dr. Rüştü Onur (BAŞKAN)
2. Prof. Dr. Nerin Bahçeciler Önder (ÜYE) KATILMADI
3. Prof. Dr. Tamer Yılmaz (ÜYE) KATILMADI
4. Prof. Dr. Şahan Saygı (ÜYE)
5. Prof. Dr. Şanda Çalı (ÜYE)
6. Prof. Dr. Nedim Çakır (ÜYE)
7. Prof. Dr. Nurhan Bayraktar (ÜYE)
8. Doç. Dr. Nilüfer Galip Çelik (ÜYE) KATILMADI
9. Doç. Dr. Emil Mammadov (ÜYE)
10. Doç. Dr. Mehtap Tınazlı (ÜYE) KATILMADI

Appendix IV

ETHICAL APPROVAL DOCUMENT

ETHICAL APPROVAL DOCUMENT

Date: 28.06.2022

To the Institute of Graduate Studies,

For the thesis project entitled "DESIGN OF INTRAVITREAL INJECTABLE HYDROGELS FOR SUSTAINED DRUG DELIVERY IN GLAUCOMA TREATMENT AND THERAPY", the researchers declare that they have approval from the Near East University, Scientific Research Ethical Board with Decision at 23.01.2020, Meeting No: 2020/76 for project No: TDU/2020/76-955.

Title: Prof. Dr

Name Surname: TERIN ADALI

Signature:

Role in the Research Project: Supervisor