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Development of an Ocular Film Containing Ofloxacin in a Chitosan Matrix

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Abstract

Background*: Chitosan is a natural polymer that is widely used in pharmaceutical applications owing to its biodegradability and biocompatibility. High molecular weight chitosan, which is commonly found in the market (Sigma Aldrich), is acid soluble and thus limits its application for ocular purposes.* ***Objective****: This study aimed to improve the characteristics of high-MW chitosan ocular films by utilizing a water-soluble, low-MW chitosan modifier for the delivery of ofloxacin post-surgery.* ***Methods****: Various film formulas were prepared using high-MW chitosan as the main polymer matrix, glycerin and polyethylene glycol 400 as plasticizers, and low-MW chitosan as film modifiers. Glycerine was the best plasticizer that produced a good film appearance when added at an appropriate ratio, 8.33 times the weight of the high MW chitosan (TGc) and 6.25 times the weight of low- and high-MW chitosan blends at (1:1) ratio (MGb). The films were further developed as TGcs and MGbs were cross-linked using sodium tripolyphosphate to control the release of ofloxacin and improve its mechanical characteristics. Water absorption capability, mechanical characteristics, in vitro drug release, and antimicrobial activity were evaluated to determine the film formula.* ***Results****: The MGb formula showed the highest water absorption (approximately 230 %), while the lowest was shown by the TGcs formula (approximately 145 %). In contrast, the TGcs formula had the highest film elasticity (141.33±8.81%), and the MGb formula had the lowest (42.55±6.11%). Surprisingly, the best controlled release of ofloxacin for up to 24 h was produced by the MGbs film, which also showed the highest antimicrobial activity. MGbs also showed moderate film characteristics, which are suitable for ocular applications.* ***Conclusion****: The research concluded that The addition of water-soluble low-MW chitosan and a cross-linker agent can improve the controlled release and characteristics of chitosan-based ocular films.*

Keywords*: antioxidant, extraction, refrigerated storage, Smallanthus sonchifolius, stability*

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INTRODUCTION

Antibiotics have become commonplace in the prevention of postoperative infections. Fluoroquinolones, in particular, are widely used because of their effectiveness against various pathogenic bacteria responsible for intraocular postoperative infections, such as cataracts, glaucoma, and corneal surgeries (Jenna et al., 2005). Additionally, in certain surgeries, implant films are commonly used to assist in the regeneration of ocular tissues. For instance, in glaucoma and extraocular muscle surgeries, gelatin films are implanted in the eye (Pfizer, 2014). The choice of polymers for such purposes generally revolves around those that exhibit hydrophilic and water-swelling properties, as they typically have low irritation potential. Commonly used polymers in the market include gelatine-A or CMC-Na, chosen for their transparent appearance, which facilitates postoperative monitoring processes (Roreger, 1995). Moreover, ocular films must possess characteristics, such as high elasticity, sufficient mechanical strength, and easy biodegradation. Although chitosan is widely used in drug delivery owing to its excellent biocompatibility and biodegradability, its characteristics can pose challenges in drug delivery applications. Commercially available chitosan is often of high molecular weight and soluble in acid, presenting difficulties when used for visual drug delivery. It is also soluble in acid and presents difficulties when used for drug delivery through the eye, leading to irritation and discomfort. Low-molecular-weight chitosan exhibits distinct characteristics compared to its high-molecular-weight counterpart, with higher water solubility, thereby overcoming potential irritations and other weaknesses. This study focused on the development of a film formula containing low-molecular-weight chitosan, which is more soluble under neutral conditions. Preliminary studies are necessary to determine whether low molecular weight chitosan can exhibit the desired characteristics for ocular film development, which should possess adequate elasticity without being overly resistant to destruction or degradation. Therefore, the development of a film formula containing low molecular weight chitosan is crucial, followed by monitoring the characterization of the resulting films. However, it is essential to anticipate how the drug release characteristics are influenced. Hence, the use of low-molecular-weight chitosan must be complemented by a cross-linking agent to restrain and regulate drug release.

MATERIALS AND METHODS

Materials

The materials used were long-chain chitosan/high-molecular-weight chitosan (Surindo), short-chain chitosan prepared in the laboratory, ofloxacin (Sanbe Farma), technical glycerin (Brataco), and sodium tripolyphosphate (Sigma Aldrich).

Tools

Ubbelohde viscometer, mechanical tester (Textechno Favigraph), IR spectrophotometer, and UV-Vis spectrophotometer (Beckman) were employed.

Method

Preparation of low molecular weight chitosan

High-molecular-weight chitosan was processed chemically to obtain low-molecular-weight chitosan using H_2O_2.

Characterization of chitosan

Determination of molecular weight

A series of chitosan concentrations (5 concentrations) were prepared using a solvent system of 0.1 M CH_3COOH - 0.2 M NaCl (1:1). Approximately 10 mL of this solution was placed in a viscometer. The falling time of the solvent and each concentration of the solution were determined, with measurements taken three times. The approximate molecular weight was calculated using the Mark-Houwink equation.

Infrared spectrum

A pellet of KBr was prepared using chitosan powder, followed by homogenization. After homogenization, it was placed in the holes of a disc plate as a mold, closed, and compressed using a mini-hydraulic press. It was ensured that the KBr-chitosan film layer was transparent before being placed on the disc holder. The position was optimized to properly obtain the infrared light. A computer was used to perform the experiments.

Preparation of chitosan stock solution

Two grams of high-molecular-weight chitosan was dissolved in 100 mL of 1% acetic acid. Low-molecular-weight chitosan (2 g) was dissolved in 100 mL filtered distilled water. Homogenization was performed using a magnetic stirrer.

Plasticizer selection

A film formula was prepared using different plasticizers, namely glycerin and polyethylene glycol 400. Chitosan and plasticizers were mixed at various ratios ranging from 1:5 to 1:50 (chitosan-to-plasticizer ratio in weight) (chitosan to plasticizer ratio). The mixture was homogenized using a stirrer at 450 rpm for 24 h and then poured into film-forming molds. The

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mixture was dried in an oven at 50°C for 18 h, removed, and stored in tightly closed containers with desiccants. The physical appearance of the samples was observed.

Optimization of high molecular weight chitosan film formula with plasticizer

The selected plasticizer was used to prepare a film formula containing only high-molecular-weight chitosan. From previous experiments, it was determined that the best formula lies between ratios of 1:5 to 1:10. Therefore, a series of formulae with compositions within these ratios was prepared. Ratios of 1:5, 1:6.25, 1:8.33, and 1:10 (w/w) were used. The mixtures were homogenized, poured into film-forming molds, dried in an oven at 50°C for 18 h, removed, and stored in tightly closed containers with desiccants. The samples were observed for their physical appearance.

Optimization of the ratio of high molecular weight chitosan to low molecular weight chitosan film formula with plasticizer

A series of comparisons between high- and low-molecular-weight chitosan with a plasticizer were made and evaluated based on the film with the best physical appearance. The ratios for high molecular weight, low molecular weight, and plasticizer were 1:1:10 to 1:1:100, respectively. The mixtures were homogenized, poured into film-forming moulds, dried in an oven at 50°C for 18 hours, removed, and stored in tightly closed containers along with desiccants. The samples were observed for their physical appearance.

Incorporation of ofloxacin into the film

Ofloxacin was incorporated into the homogenized film mixture. Homogenization between the active substance and film mixture was performed using a stirrer for 2 h at room temperature, then poured into film-forming molds, dried, and stored in tightly closed containers along with desiccants.

Characterization of water absorption strength

The initial film weight measurements were conducted, followed by immersion in PBS pH 7.4 at 37°C. The film was retrieved at 1-, 2-, 3-, 4-, 5, and 10-minute intervals, re-weighed after immersion, and the percentage of hydration was determined to indicate the water absorption strength. The initial and final weights were measured, the weight differences were compared with the initial values, and the percentage was calculated.

Characterization of film mechanical properties

The formed films were cut into 3 cm × 2 mm pieces and clamped at a distance of 1 cm using a Textechno Favigraph device. The films were subjected to a tensile load, Eand, their elongation was monitored, and other physical parameters related to the mechanical properties were determined.

Morphological characterization

Characterization was performed using Scanning Electron Microscopy to observe the surface morphology of the formed films. The analysis was conducted at the Marine Geological Research Center, Bandung.

In vitro release testing

The drug release profile from the film reservoir was determined by immersing it in 100 mL tear fluid-like aqueous medium (equivalent to PBS pH 7.4) and stirring at 100 rpm at 37°C. A 3 mL sample was taken at time intervals of 0, 1, 2, 3, 6, 9, 12, 18, and 24 h, and each sample was added to 3 mL of solvent. Subsequently, the concentration of released ofloxacin was determined using UV-Vis spectrophotometry at a wavelength of 290 nm.

Antimicrobial activity testing

Antimicrobial activity was tested using the agar diffusion method. The medium used was Mueller-Hinton agar with gram-negative Escherichia coli and gram-positive Staphylococcus aureus bacterial suspensions as models. The films were cut into 1 cm sizes and sterilized using UV light for 10 min at a height of 25 cm. Sterile films were placed on agar media containing microbes and incubated for 24 h, after which the resulting inhibition zones were observed.

RESULTS AND DISCUSSION

The critical point for identifying and characterizing these raw materials is the determination of molecular weight, with the aim of observing the influence of molecular weight on the resulting properties. Characterization of molecular weight begins with the preparation of chitosan solutions at five concentration series using a solvent system of 0.1 M CH_3COOH - 0.2 M NaCl (1:1). The absolute molecular weight of chitosan can be determined using high-performance liquid chromatography instruments, obtaining constants that are then used in the Mark–Houwink equation. The molecular weight value is an approximation of the intrinsic viscosity obtained using the Mark–Houwink equation, as follows:

$$[\eta] = K\,(Mv)\alpha$$

where [η] represents the intrinsic viscosity and K and α are constants given for the solvent system and

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temperature, respectively. This equation correlates the intrinsic viscosity with the molecular weight of a specific solvent system and temperature. The solvent system used in this process is 0.1 M CH_3COOH with 0.2 M NaCl (1:1). According to the literature, for this solvent system, the constant values of K and (α) at 25°C are 1.81 and 0.93 (Kasaai, 2007). This determination is essentially based on the assumption that there is a proportional relationship between the viscosity and molecular weight of a component or substance. Measurements were performed using an Ubbelohde capillary viscometer, which compares the falling time of a solution with the solvent used. The falling time of the solvent was lower than that of the polymer solution tested, resulting in an actual increase in viscosity and molecular weight with increasing falling time. The critical point for determining the molecular weight based on viscosity is a constant temperature and the same solvent system. Because temperature parameters greatly influence viscosity values, the constants in the Mark–Houwink equation also differ for different solvent systems. The characterization results of the molecular weight of chitosan are presented in Table 1.

Table 1 . Chitosan Molecular Weight Values Based on Intrinsic Viscosity

No.	Chitosan	Mw (kDa)
1	High MW	1176,18±1,88
2	Low MW	17,80±0,91

It can be seen that the two main components used in this study are different and in line with the research design. Although this method is only an approximation, the results sufficiently describe the molecular weights of the materials used. More accurate determination of molecular weight can be performed using gel permeation chromatography and the two methods can be compared to evaluate the parameters K and α used in this equation. Chitosan degradation is chemically performed using H_2O_2 via an oxidative reaction that cleaves glycosidic bonds, causing a decrease in molecular weight (C.Q. Qin et al., 2002). Thus, low molecular weight chitosan was successfully prepared.

Infrared identification was used to identify the functional groups present in the material. Here, is the infrared spectrum produced during the characterization process.

No significant difference was observed in the functional groups between high-molecular-weight (HMW) and low-molecular-weight (LMW) chitosan. The spectra formed between these two materials were almost identical; the most noticeable difference between the two spectra was the transmittance of the given spectrum. The depolymerization process of chitosan did not alter the structure of the monomer units forming the chitosan polymer, and Figure 1, peaks appeared at the same wavenumber for both HMW and LMW chitosan. Characterization using this infrared instrument causes molecular bond vibrations. This response is characteristic of the bonds present in the molecular groups at a wavenumber of 3750 cm^{-1} and overlapping with vibrations from N-H and C-H in the CH_2 groups at 2920 cm^{-1}. Absorption in the wavenumber range of 1680-1480 cm^{-1} indicates the presence of carbonyl groups, particularly in the amide CO-NHR bonds; protonated amino groups, which are also visible at the peak wavenumber of 1160 cm^{-1}; and CO group vibrations, which also appear in the wavenumber range from 1160 cm^{-1} to 1000 cm^{-1}. These peaks are quite specific to chitosan and have relatively high intensities.

Film formulations require additional materials in the form of plasticizers to provide elastic effects on the mechanical properties of the film. In this study, two types of plasticizers, PEG 400 and glycerin, were used for screening purposes. Safety (low toxicity) was the basis for choosing these two plasticizers, in addition to their affordability and availability in the market being factors considered. Generally, both materials are water miscible, which can enhance the flexibility of the film. In this study, we found that the use of PEG 400 was not superior to glycerin. The most prominent aspect of films formulated with PEG 400 is poor clarity, whereas for eye preparations, one of the requirements is a preparation that does not interfere with vision, measured by the clarity parameter of the film. Meanwhile, the films formulated using glycerin showed good or clear clarity. Tables 2 and 3 show the formulation design for plasticizer screening, with HG indicating high molecular weight chitosan with glycerin, HP indicating high molecular weight chitosan with polyethylene glycol 400, MG indicating mixed high and low molecular weight chitosan with glycerin, MP indicating mixed high and low molecular weight chitosan with polyethylene glycol 400. The numbers 1-3 indicate the varying plasticizer compositions.

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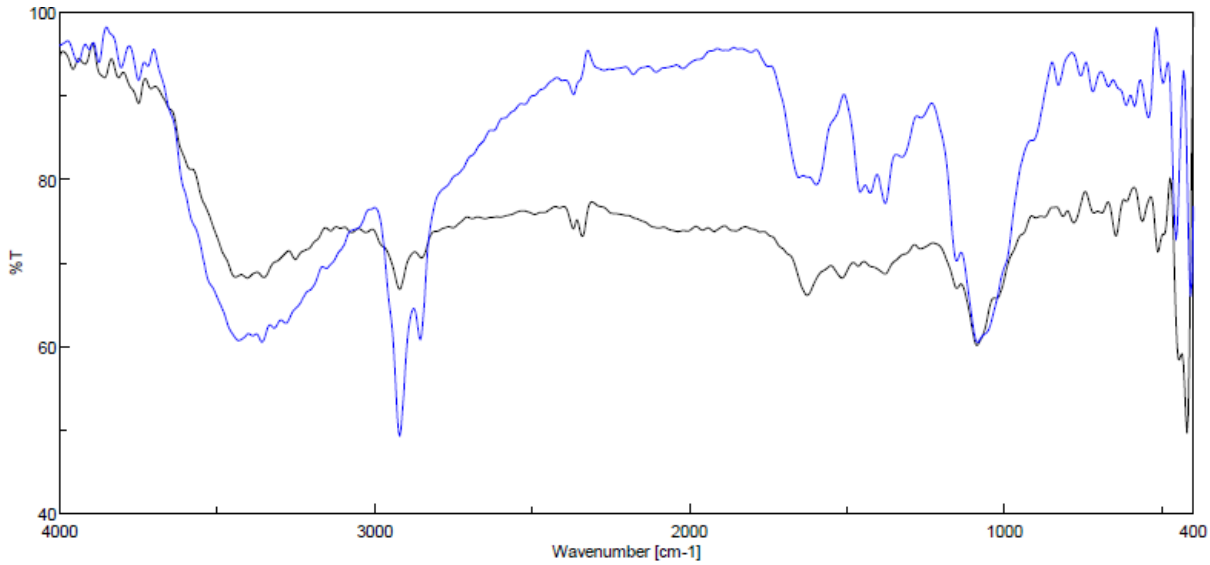

Figure 1. Infrared spectra of high molecular weight chitosan and low molecular weight chitosan

Table 1. Ratio screening for plasticizer vs high molecular weight chitosan

Plasticizer	Formula	Chitosan	Plasticizer	Clarity	Flexibility	Stickiness
Gliserin	HG1	1	5	√ √	√	-
	HG2	1	10	√ √	√	-
	HG3	1	50	√	√√	√√√
Polietilen glikol 400	HP1	1	5	-	-	√
	HP2	1	10	-	√	√
	HP3	1	50	-	√	√ √

- Poor
√ Fair
√√ Good
√√√Very good

Table 2. Ratio screening for plasticizer vs chitosan combination

Plasticizer	Formula	Mixed Chitosan	Plasticizer	Clarity	Flexibility	Stickiness
Gliserin	MG1	1	5	√√	√	-
	MG2	1	10	√√	√	√
	MG3	1	50	√√√	√	√√
Polietilen glikol 400	MP1	1	5	√	-	√
	MP2	1	10	-	-	√
	MP3	1	50	-	-	√

- Poor
√ Fair
√√ Good
√√√Very good

The flexibility and mechanical properties were evaluated qualitatively based on the data shown in Tables 2 and 3. When screening the selection of plasticizers, variations in formulas between chitosan and plasticizers based on dry weight in the range of 1:5 to 1:50 and physical observations showed good appearance only in the range of 1:5 to 1:10. Films formulated using PEG 400 showed extreme characteristics at each ratio: at a ratio of 1:50, the film was too elastic, whereas at a ratio of 1:5, the film had already hardened and hardened at a ratio of 1:10. For the results obtained with the glycerine plasticizer, the film showed sticky physical properties at a ratio of 1:50 and fairly good flexibility at ratios of 1:5 and 1:10, but still needed optimization. Thus, from this screening, glycerin was chosen as the plasticizer for the next formulation. The differences in the results obtained using these two plasticizers are highly influenced by the differences in the characteristics of the two materials used. Glycerine is a viscous liquid from the polyol group; its molecular weight is not too large, only about 92.09 g/mol, and it is widely used in the

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pharmaceutical field as a plasticizer by utilizing its structure. In general, the main mechanism used by plasticizers to increase polymer flexibility is lowering the glass transition temperature (Tg) (Rosen et al., 2003). When the plasticizer is incorporated into the polymer, an increase in flexibility occurs because of the intermolecular separation of polymer molecules. In this case, chitosan is interspersed with small glycerin molecules. The OH groups in the glycerin structure contribute significantly to the flexibility because they can easily attract water to form hydrogen bonds, and the chitosan structure interspersed with glycerin becomes less rigid in terms of bonding (bond rigidity decreases). Glycerine is known to improve the hydrophilicity of films and plasticize their mechanical properties. Plasticizer screening was performed using 400 polyethylene glycol compounds. This molecule was chosen as a candidate because of its good biodegradability, non-irritating properties, and the availability of easy-to-obtain materials. The group that plays a role in increasing plasticity is hydroxyl (-OH), which is almost the same as glycerine. However, the addition of ethylene (poly) groups to its structure provides quite different characteristics between the two plasticizers used, especially their hydrophilicity. The hydrophilicity of a plasticizer is a determining factor that affects its affinity for water. The amount of water in films plasticized using PEG 400 did not increase with increasing atmospheric humidity (Bourtoom, 2008).

Chitosan and glycerin as plasticizers were prepared with certain ratios (calculated on a dry weight basis) between 1:50 and 1:5. Good film characteristics were obtained between the ratios of 1:5 to 1:10, so the ratio was narrowed down again to between 1:5 and 1:10, and organoleptic evaluation was conducted. The best appearance was observed at a ratio of 1:8.33 (w/w) between chitosan in dry weight and glycerin. The quality parameters assessed included flexibility, film clarity, dryness or wetness, and film stickiness. The same procedure was performed for films containing high- and low-molecular-weight chitosan with glycerin at the same ratio.

The optimal ratio of chitosan to glycerine at 1:8.33 is suspected to be the most appropriate because it provides the best flexibility, which facilitates further handling and use. The flexibility data qualitatively exhibited the best elasticity characteristics. Meanwhile, in films containing high molecular weight chitosan, low molecular weight chitosan, and glycerin, the best ratio is found in formula MGb, which is 1:6.25. It is evident that the use of glycerin is reduced, which is due to the contribution of low-molecular-weight chitosan, which shares similar properties with glycerin in terms of assisting in improving the physical characteristics of the film related to elasticity.

The drying time and temperature for film formation were optimized. Initially, drying was performed at 25, 50, and 80°C for 24 h. Films dried at room temperature or approximately 25°C appeared wet and were not feasible for application, thus requiring more than 24 h for drying. Subsequently, drying was performed at 50°C, which resulted in a better film appearance in terms of dryness and integrity. However, drying at 80°C resulted in excessively dry yellowish films. Therefore, the optimal drying temperature for further processing was determined to be 50 °C. Optimization of the drying time was also conducted for 6, 12, 18, and 24 h at a temperature of 50°C. The best results were obtained with films dried for 18 h. The consistency of the formed films was sufficiently good, not too wet or too dry, and they maintained a visual appearance (clear color). The films dried for 6 and 12 h exhibited characteristics that were too wet and sticky, making them impractical for application. In contrast, films dried for 24 h showed characteristics that were too dry and difficult to apply to the eye mucosa.

Table 4. Optimization of high molecular weight chitosan with glycerine ratios

Formula	Chitosan	Glycerine	Flexibility	Clarity
Hga	1	5	√	√
HGb	1	6.25	√	√
HGc	1	8.33	√√√	√
HGd	1	10	√	√

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Table 5. Optimization of Combined Chitosan with Glycerine Ratios

Formula	Combined Chitosan	Glycerine	Flexibility	Clarity
MGa	1	5	√	√√
MGb	1	6.25	√√√	√√
MGc	1	8.33	√	√√
MGd	1	10	√	√√

One of the characteristics that need to be determined for the film is its water absorption strength. This parameter characterizes the film in biomedical applications and is closely related to its ability to absorb water (in bodily fluid applications), which is greatly influenced by the degree of chitosan deacetylation and the crystallinity of the material. The water absorption strength for ocular delivery purposes was determined by immersing the film in a PBS buffer solution at pH 7.4, and then calculating the water absorption strength. Chitosan's characteristic hydrophilicity and the penetration or diffusion of water into the film occur more rapidly, causing the film to swell before degradation. Therefore, although bond cleavage and degradation processes occur during the hydration process by the medium, the swelling process of the film by the medium is more dominant (Ren, Yi, Wang, & Ma, 2005). In this study, the water absorption strength was determined and different characteristics were obtained for each formula. Sampling was conducted every minute for 5 min. Initially, all the formulas increased, but subsequently, the water absorption strength decreased. This is suspected to be due to the residue from the buffer, which can no longer be used to expand the film but instead dissolves chitosan (Eduaro et al., 2006), such as the acetic acid component in the film, facilitating the solvation of the film itself. For formulas that only use high-molecular-weight chitosan and glycerin, the water absorption strength is quite significant, at approximately 215.16%. The highest water absorption strength was obtained using Formula 3, which was approximately 235.01%. This difference is likely due to the natural polymer properties, polymer chain flexibility, molecular weight, crystal structure, and chemical composition of the chitosan itself (Taghizadeh & Davari, 2006). The differences in solubility between chitosan affect ionization, particularly the protonation of amino groups in chitosan molecules, which affects the dissolution mechanism and results in the opening of chitosan chains and changes in their conformation. In this medium with a pH of approximately 7.4, which is less supportive of the protonation process of amino groups in films that only use high molecular weight chitosan, chitosan can still be protonated because of the presence of acetic acid in the film, which dissolves in the medium, facilitating the protonation of chitosan and water penetration into it. For films formed from combined chitosan, the protonation capacity of the buffer will be higher because the chitosan monomers are fewer, resulting in sufficient protonation by the H + source derived solely from the buffer. Cumulatively, the highest water absorption strength was shown by formula MGb, which contains high molecular weight chitosan, low molecular weight chitosan, and glycerine. This is in line with the hypothesis that the water absorption strength will increase with the addition of water-soluble components in the film, which is the purpose of improving ocular film characteristics. Notably, the use of cross-linking agents intended for release control decreases the water absorption strength of the film because the strong ionic interaction between chitosan and sodium tripolyphosphate prevents water from penetrating further, thus reducing its absorption capacity compared to films without sodium tripolyphosphate.

The formula with the highest water absorption capacity compared to formula TGc shows a difference that is not significant in terms of the water absorption strength. The glycerine ratio in these two formulas is indeed different and smaller in formula MGb due to the influence of low molecular weight chitosan, resulting in only a slight difference in the water absorption strength. The best formula based on the parameter of water absorption strength is MGb, which contains a combination of chitosan and glycerine, because the highest water absorption strength parameter will be useful for the film's biodegradation process during application.

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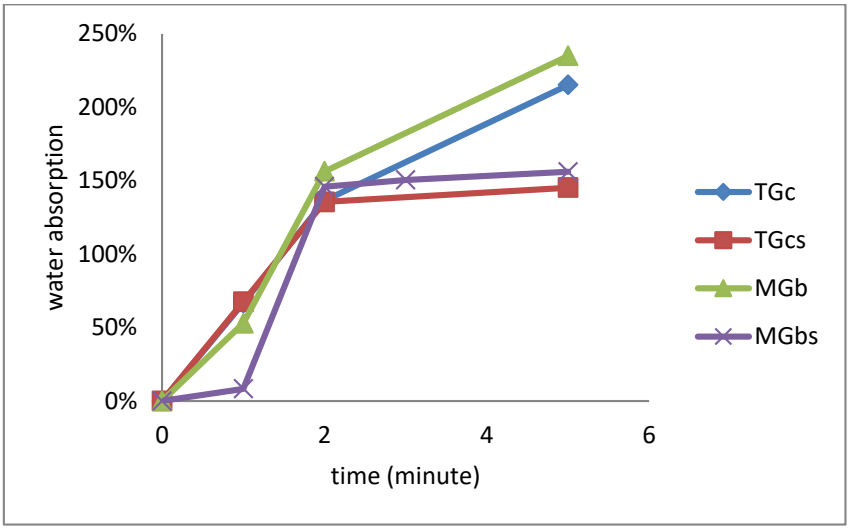

Figure 2. Graph of water absorption strength for various ocular film

Table 6. Values of mechanical parameter for various films

Formula	Break Elongation (%)	Maximum elongation (%)	Maximum Mechanical Strength
HGc	86.69±10.96	85.85±10.96	31.78±8.19
HGcs	146.13±7.39	141.36±8.81	18.07±7.39
MGb	43.16±6.06	42.55±6.11	12.28±0.76
MGbs	58.91±2.56	57.25±1.94	20.05±3.28

The mechanical characteristics of the formed film must be determined for various purposes, particularly for distribution and usage. Mechanical strength testing revealed the strength and elasticity of the film, which were reflected in the tensile strength and elongation. Mechanical strength typically determines the polymer characteristics; for example, a polymer with a low modulus, low tensile strength, and low elongation at break indicates weaker and softer characteristics. Films used for ocular delivery must be flexible and not too strong (to facilitate degradation). The mechanical characteristics of the films are presented in Table 6.

From Table 6, it can be seen that the addition of sodium tripolyphosphate increased the value of break elongation in each formula. The break elongation parameter indicates the ability of the material to withstand shape changes without cracking in the system. The addition of sodium tripolyphosphate as a cross-linking agent is intended to increase the mechanical integrity of the film (by bonding with chitosan ionically), thus requiring a greater force to form cracks when subjected to force due to increased resistance or resilience. The same effect also occurs in films containing not only high-molecular-weight chitosan but also a mixture with low-molecular-weight chitosan. Thus, the influence of the addition of sodium tripolyphosphate was observed in both materials, whether high- or low-molecular-weight chitosan. In formulas MGb and MGbs, when compared to the TGc and TGcs formulas, lower elongation values (both maximum and at break) were observed. If we trace the characteristics of these two materials, they are significantly different. Materials such as low-molecular-weight chitosan have properties that make it easier to absorb water, thus enhancing plasticity owing to the easier lubrication process of the film. This leads to a decrease in mechanical strength, as evidenced by the table showing an inverse relationship between elongation and mechanical strength. The desired film characteristics can be adjusted by considering various factors, such as the solvent pH, ionic strength, and type of acidic solvent used. The fragility of the film can be assessed based on the mechanical strength. For visual delivery purposes, the mechanical strength must be low because the film can be easily broken. This requirement is met by the formula MGb, which has the lowest mechanical strength and the highest absorption strength. The addition of low molecular weight chitosan improved the characteristics of the film.

SEM characterization showed the morphology of the films formed by the formulas. In this study, monitoring was conducted for each formula. Generally, the morphology of each formula shows the same homogeneity, with differences observed only in the

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formula containing sodium tripolyphosphate, indicating a higher bond density. The morphology of the films using the mixture was also evaluated to observe the effect of adding low-molecular-weight chitosan. Formula MGb, which is a combination of high molecular weight chitosan with low molecular weight chitosan, shows a denser film morphology compared to films containing only low molecular weight chitosan as the film former. The presence of low-molecular-weight chitosan enhances bonding with glycerine due to hydrogen bond interactions. Glycerine has -OH groups on its three carbon atoms, which can interact to form hydrogen bonds with chitosan. In low molecular weight chitosan, there are more OH groups, allowing more glycerine to bind and affect bone density.

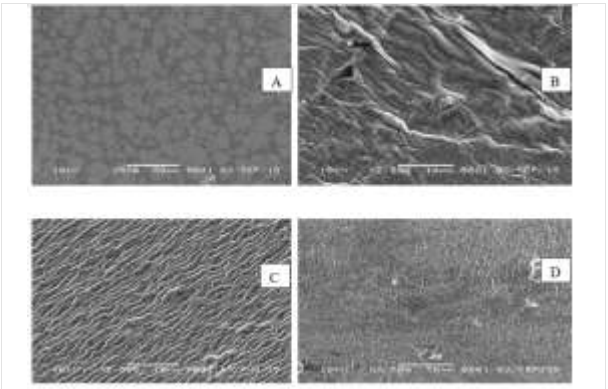

Figure 3. (A) Morphology of HGc at 500x; (B) HGcs at 2500x; (C) MGc at 2500x; (D) MGcs at 2500x

The effect of adding low-molecular-weight chitosan can be observed by comparing films using only high-molecular-weight chitosan as the film former and films using an additional low-molecular-weight chitosan. Upon observation, the structural density provided by the formula MGc is higher than that of the formula without low-molecular-weight chitosan. This can occur because low-molecular-weight chitosan, with its more hydrophilic characteristics, is more prone to interact with glycerine as a plasticizer, which is also hydrophilic. This contributed to the density of the formed structures. The effect of sodium tripolyphosphate was also observed in the morphology formed; the purpose of using sodium tripolyphosphate was used as a cross-linking agent to strengthen the integrity of the film. The diverse morphologies also indicate diverse characteristics.

Release testing was conducted to assess the ability of the system to release the active substances. Ideally, the polymer system and cross-linking agents should effectively release the active substance. To reduce the frequency of usage, the system should slowly release the active substance, equivalent to several desired applications. Different formulas provide different release profiles, and each component in the film contributes to this characteristic. In the formula, TGc, which only contains high molecular weight chitosan and glycerin as plasticizers, the release profile is lower than that of the formula using low molecular weight chitosan in the film (MGb). However, there was no significant difference between the two formulas. Consistent with the characteristics of absorption strength, this occurs because the glycerine component in MGb is less, but aided by the presence of low-molecular-weight chitosan. The medium used was a simulated tear fluid that mimicked the conditions of tear fluid, and its composition was almost the same as that of PBS buffer at pH 7.4. Drug release in high-molecular-weight chitosan alone with plasticizer tends to be less and slower owing to the solubility of chitosan. Chitosan solubility as a film base in the medium is relatively low because high-molecular-weight chitosan has good solubility in acidic environments. Components contributing to the release of this chitosan come from the acetic acid present in the film, which can solvate high-molecular-weight chitosan.

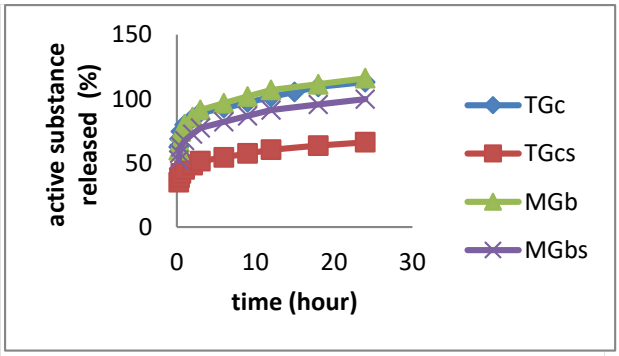

Figure 4. Graph of ofloxacin released from various ocular film

On the other hand, for films containing cross-linking agents, significant differences were observed between films with high molecular weight chitosan and those containing a combination of chitosan. This is because even though the glycerin composition decreases in MGbs, the effect of sodium tripolyphosphate is significant enough to retain the release of ofloxacin in the system. The best formula based on this parameter is MGbs, which can release almost all of the active substance within 24 h and is good for reducing usage frequency. However, the

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release profile for each formula was not as expected because the initial onset of the system was too large (burst effect). Therefore, other release control strategies are needed, such as encapsulation or nanoparticle delivery systems. The kinetic model is plotted against zero-order kinetics, first-order kinetics, or Korsmeyer-Peppas kinetics. None of these models showed that the 100% release profile followed a specific model. However, the closest model had zero-order kinetics in the second phase of each formula. The issue with this release is that the amount of drug initially released is too high and does not follow any kinetic model. Therefore, other kinetic model approaches are required to be more accurate. Table 7 lists the correlation coefficients of the applied kinetic models.

An approach to the Korsmeyer-Peppas kinetic model was also performed with an exponent value of n = 0.5, as the release mechanism is considered to follow Fickian diffusion. However, the Korsmeyer-Peppas constant values obtained were not constant at each sampling time for each formula. Thus, the release kinetics of the film system did not follow the Korsmeyer-Peppas kinetic model. The antimicrobial activity test was used to assess whether the matrix could release the active substance and act as a carrier for the antibiotic and to confirm the results of the release test. Ofloxacin is an antibiotic with a broad spectrum activity against Gram-negative and Gram-positive bacteria; therefore, two bacterial models were used: Escherichia coli and Staphylococcus aureus. Table 8 shows the data of the inhibition diameters in each formula used for the antimicrobial activity test using S. aureus and E. coli bacteria on Muller Hinton agar diffusion.

The highest inhibition was observed in the film formula that used a combination of chitosan as the main component of the film without sodium tripolyphosphate (MGbO), both against Staphylococcus aureus and Escherichia coli. This is consistent with the highest release profile produced. In almost all formulas using sodium tripolyphosphate as a cross-linking agent, a decrease in the inhibition diameter was observed. This was confirmed by the decrease in the capacity to release the active substance in the matrix due to cross-linking reactions through strong ionic bonds that prevent the active substance from exiting the matrix system, resulting in a decrease in the observed bacterial growth inhibition effect. The components of agar medium are water-soluble materials, and water is present in the medium, facilitating its release. In the MGbO formula, the presence of water-soluble low-molecular-weight chitosan components forms pores as pathways for more active substances to exit from the matrix system.

Table 7. Comparison of correlation coefficients for zero-order and first-order kinetics

Formula	r^2 zero order	r^2 first order
	Phase 1	Phase 2
HGc	0.728	0.974
HGcs	0.781	0.958
MGb	0.748	0.972
MGbs	0.786	0.974

Table 8. Inhibition diameter of film formulas containing ofloxacin

Formula	Inhibition Diameter (cm)
HgcO	5.5
HgcOs	5.5
MgbO	6.3
MgbOs	6

HGcO : High molecular weight chitosan +glycerine+ofloxacin
HGcOs :High molucular weight chitosan+glycerine+ofloxacin+STPP
MGcO : Mixed chitosan +glycerine+ofloxacin
MgcOs: Mixed chitosan+glycerine+ofloxacin+STPP

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CONCLUSION

Based on the evaluation results, the addition of low-molecular-weight chitosan and cross-linking agents improved the film characteristics. MGbs formula is one of the most suitable characteristics for ocular films, especially in terms of elasticity and release after initial onset. Optimizing the ratio of high- and low-molecular-weight chitosan and developing a formula with an encapsulation system are necessary for better release control.

AUTHOR CONTRIBUTIONS

Conceptualization, T.S.; Methodology, T.S.; Software, A.F.; Validation, E.J.; Formal Analysis, A.F.; Investigation, A.F.; Resources, T.S.; Data Curration, T.S.; Writing - Original Draft, A.F.; Writing - Review and Editing T.S.; Visualization, A.F.; Supervision, T.S.; Project administration, T.S.; Funding Acquisition, T.S.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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