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A REVIEW ON NANOPARTICLE CROSS-LINKED COLLAGEN SHIELD FOR SUSTAINED DELIVERY OF DRUG IN GLAUCOMA

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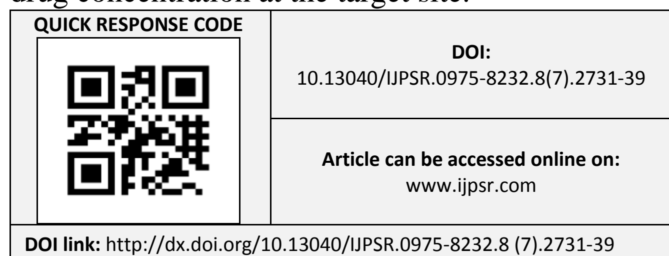
ABSTRACT: Glaucoma is a disease of the optic nerve. Progressive damage of the optic nerve will causes irreversible reduction in visual acuity; it leads to visual field loss and blindness. The main treatment of glaucoma is the reduction of intraocular pressure. Current therapy involves frequent administration of eye drops which results in poor patient adherence and therapeutic outcomes. This review is focusing on to overcome these limitation by developing a novel nanoparticle cross-linked collagen shield for sustained delivery of suitable drug. The drug is incorporate into the shield and cross-linked by cross-linking agent with the help of Ultraviolet irradiation. Metal oxide nanoparticles is most suitable as cross-linking agent.e.g-polyvinylpyrrolidone (PVP) capped zinc oxide (ZnO/PVP).There is some evaluation parameter for nanoparticle cross-linked collagen shield like cytotoxicity as well as shield transparency. Cross-linked collagen shields characterise for their mechanical strength, swelling capacity and bioadhesive properties, ZnO/PVP NP cross-linked shields is the most favourable characteristics compare to plain film. It release drug over a period of 14 days offering a promising sustained release treatment option for glaucoma. The purpose of this review article is to provide information about novel Nanoparticle cross-linked collagen shield for sustained delivery of drug.

INTRODUCTION: Glaucoma is a common progressive eye disorder which remains the second leading cause of blindness worldwide. The eye has become an important target for drug delivery. Topical administration of eye drops is the most common method for the treatment of anterior segment disorders and although the surface of the eye is readily accessible, drug molecules have to overcome several barriers to maintain an effective drug concentration at the target site.



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Current management mainly involves the application of intraocular pressure (IOP) lowering eye drops; however, due to their low bioavailability they are only effective when administered frequently. This results in poor patient adherence to therapy. Therefore, to overcome this limitation, advances in drug delivery aim at enhancing the bioavailability by increasing the drug retention time on the corneal surface [1].

The number of natural polymers is incorporated into drug delivery vehicle to create viscous eye drops or flexible films. Such polymers offer the advantage of being biodegradable, highly compatible and comprise similar mechanical properties to the body's natural tissues [2]. Collagen is a natural protein polymer which makes up the

majority of the cornea and has been used extensively for various ocular applications such as collagen shields [3]. Collagen can function as corneal bandage lenses for ocular surface protection following corneal surgery or trauma as well as drug delivery devices [4]. They have been successfully marketed in promoting wound healing after corneal procedures [5] and in relieving dry eye conditions [6]. Moreover, they have been investigated for drug delivery and when administered together with topical drug solutions have shown to increase the contact time between the drug solution and the corneal surface by serving as a reservoir. Shields loaded with steroids and antibiotics pre and post operatively have shown an equal or enhanced drug bioavailability in the anterior eye segment and a faster healing rate when compared to conventional formulations.

However, over the years the idea of using collagen shields in ocular drug delivery has become less popular due to various limitations including a reduction in visual acuity as they are not fully transparent as well as safety concerns with regards to the cross-linking agents. Moreover, they could only enhance drug bioavailability for brief periods of time. To overcome some of the limitations associated with conventional collagen shields this review focuses on to the use metal oxide nanoparticles (NPs) as cross-linking agents for collagen and evaluate the physicochemical properties and drug release characteristics of the shield. While replacing possibly toxic conventional cross-linking agents such as glutaraldehyde, these metal oxide NPs also offer broad spectrum antibacterial properties [7] and the ability to generate radical oxygen species when irradiated with blue light (400–470 nm) resulting in further cross-linking [8], thus making them excellent candidates for ocular device.

1. Collagen: Basically collagen is a naturally existing protein present in the animal body, fibrous in nature, and especially found in the connective tissue and flesh of mammals. Approximately 25%-35% of total body protein is comprised of collagen, in the form of elongated fibrils; collagen is abundantly present in fibrous tissue like bone, cartilage, tendons, blood vessels, ligament, skin, cornea, inter-vertebral disc and the gut. The synthesis of collagen in the body is made by fibroblast cells. Collagens possess good tensile strength, and found both outside and inside the body cells [9]. In combination with elastic, collagen provides support to body tissues and organs, basically collagen offers firmness and strength and elastic provides flexibility to body tissues. In fact gelatine which is used in food and pharmaceutical industries is collagen that has been hydrolysed irreversibly. Collagen is a natural protein polymer which makes up the majority of cornea. Collagen is the main structural protein in the extracellular space in the various connective tissues in animal bodies. As the main component of connective tissue, it is the most abundant protein in mammals.

1.1. The *in vivo* absorption of collagen is controlled by the use of cross-linking agents: [11]

- Glutaraldehyde
- Chromium tanning
- Formaldehyde
- Poly epoxy compounds
- Metal oxide
- Acylazide
- Carbodiimides
- Hexamethylenediisocyanate

Physical treatment, such as ultra-violet/gamma-ray irradiation and dehydrothermal treatments have been efficiently used for the introduction of cross links to the collagen matrix.[12]

1.2. Characteristics possessed by collagen: [11]

- Stretch-ability under stress condition collagen stretch rather than break.
- Strength.
- Biochemical compatibility.
- Several hydrogen bonds are present in collagen, on applying stress they can be wrecked and re-joined after removal of pressure.
- Collagen is biodegradable.
- Collagen show good absorption in-vivo.
- Collagen possesses weak antigenicity.
- Collagen having high ability to binds with drug.

1.3. Application of collagen for this system: [11] The primary reason for the usefulness of collagen in biomedical application is that collagen can form

fibres with extra strength and stability through its self-aggregation and cross-linking.

- Collagen shows better biocompatibility
- Collagen is a part of the body that's why it is nonantigenic
- Collagen is a non- toxic biopolymer
- Biodegradability of collagen can be controlled by cross-linking.

1.4. Disadvantages of collagen: [17]

- High cost of pure type I collagen; Variability of isolated collagen (*e.g.* crosslink density, fiber size, trace impurities, etc.).
- Hydrophilicity which leads to swelling and more rapid release.
- Variability in enzymatic degradation rate as compared with hydrolytic degradation;
- Complex handling properties.
- Side effects, such as bovine spongiform encephalopathy (BSF) and mineralization.

1.5. Structure of collagen: Basically collagen possesses a triple helix structure, which generally made up of two homologous chains (α-1) and one supplementary chain that varies slightly in its chemical composition (α-2). These chains are polypeptide in nature and coiled around one another in a cable form. Each has a distinct turn in the reverse direction, these chains are connected together chiefly by hydrogen bonds between nearby CO and NH groups [14]. The weight of collagen molecule is 300 k dal [13, 15] and its structure is rope shaped and having a length of 300 NM and a width of 1.5 NM. The major content of glycine and amino acid residue is affecting the helix formation [13]; in each of three chains of collagen molecule the amino acids are regularly arranged. The sequence of amino acids follows the pattern glycine-proline-X or glycine-X-hydroxyproline where X is the amino acid other than glycine, proline or hydroxyproline; glycines constitute about 1/3 of total sequence and proline or hydroxyproline accounting for the 1/6 of the sequence. This whole structure is joined with the help of hydrogen bonds and linking peptide bonds.

The unique physiological and biomaterial characteristics of collagen compared with most synthetic polymers derive from the structural complexity of the collagen molecule. The tertiary structure refers to the fundamental unit originally known as tropocollagen: three polypeptide chains intertwined to form a right-handed triple-helix with a pitch of approximately 8.6 nm. The rod-shaped triple helix has an average molecular weight of approximately 300 kDa, a length of 300 nm with a diameter of 1.5 nm [16].

This extreme ratio of the dimensions gives rise to high viscosity in solutions and high mobility in electrical fields. In addition, there are regions of 9–26 amino acids at the amino and carboxyl terminal chain ends of the molecule that is not incorporated into the helical structure. These non-helical regions are denoted as telopeptides. On the fourth level of order, the triple-helical molecules stagger longitudinally and bilaterally into fibrils with distinct periodicity. The collagen molecules aggregate through fibrillogenesis into microfibrils consisting of four to eight collagen molecules and further into fibrils. Those fibrils reach from 10 to 500 nm in diameter depending on tissue type and stage of development [16]. The triple-helices are staggered by 67 nm with an additional gap of 40 nm between succeeding molecules. These collagen fibrils organize into fibers, which on their part can form even larger fiber bundles.

2. Collagen shield: Collagen shields is also known as collagen corneal shield, they are newly developed, potentially versatile ophthalmic lens, which is made up of collagen, since collagen is a natural, commonly available protein involved in the support and protection of vital structures, many researchers have tried to use peripheral collagen to protect the surface of the eye. Generally collagen shields are manufactured from bovine or type 1 collagen. They are act as a short term bandage and allow sufficient oxygen transmission for essential metabolism occurring in eye cornea [18]. For the corneal surface lubrication these shields dissolve in collagen solution that minimize lids rubbing.

The collagen shield was originally designed for bandage contact lenses, which are gradually dissolved in cornea. The idea of using a shield or a hydrogel lens as a delivery device has led to the development of various drug delivery systems for

ophthalmic applications. One of the merits of the collagen-based drug delivery systems is the ease with which the formulation can be applied to the ocular surface and its potential for self-administration. The mechanical properties of the shield protect the healing corneal epithelium from the blinking action of the eyelids.

Drug delivery by collagen shields depends on loading and a subsequent release of medication by the shield. The collagen matrix acts as a reservoir and the drugs are entrapped in the interstices of the collagen matrix. As tears flush through the shield and the shield dissolves, it provides a layer of biologically compatible collagen solution that seems to lubricate the surface of the eye, minimize rubbing of the lids on the cornea, increase the contact time between the drug and the cornea, and faster epithelial healing. A bolus release of drug from the lenses was attributable to the enhanced drug effect.

Therefore, this system allows the higher corneal concentrations of drug, and the more sustained drug delivery into the cornea and the aqueous humor. Delivery of drugs through the collagen shield was more comfortable and reliable than frequent application of other conventional treatments, such as drops, ointment or daily subconjunctive injection. Modifications of collagen were made to simplify the application, to meet the highest compliance, to reduce blurring of vision, and to enhance the drug concentration and bioavailability of drugs in the cornea and aqueous humor. Collagen shields as a drug carrier for topical agents have many advantages. Experimental and clinical studies showed that the speed of epithelial healing is faster and more complete with the use of the collagen shield than conventional formulations. There was less stromal edema at the wound sites in collagen-treated corneas. The application of collagen shields for drug delivery is limited by several disadvantages, such as reducing visual activity, causing slight discomfort, and a short duration at the inserted site.

Collagen shield are shaped like a contact lens and are supplied in a dehydrated form, requiring rehydration prior to insertion. Variations in collagen crosslinking induced with ultraviolet light (UV) during manufacture dictate lens duration before dissolution. Three different collagen shields are currently available with dissolution times of 12, 24, and 72 hours. Corneal collagen shields have a diameter of 14.5–16.0 mm, a base curve of 9 mm, and a central thickness of 0.15–0.19 mm [19].

2.1. Marketed preparation of collagen shields: [11]

- Biocora®
- ProshieldO®
- MediLenso®
- Irvine®
- Chiron®

3. Cross-linking agent: A cross link is a bond that links one polymer chain to another. They can be covalent bonds or ionic bonds. “Polymer chain” can refer to synthetic polymer or natural polymers such as protein.

Cross-linking is the formation of chemical links between molecular chains to form a three-dimensional network of connected molecules. The vulcanization of rubber using elemental sulphur is an example of crosslinking, converting raw rubber from a weak plastic to a highly resilient elastomer. The strategy of covalent crosslinking is used in several other technologies of commercial and scientific interest to control and enhance the properties of the resulting polymer system or interface, such as thermosets and coatings. 1-3 Cross-linking has been employed in the synthesis of ion-exchange resins 4 and stimuli-responsive hydrogels 5 made from polymer molecules containing polar groups. As polyelectrolytes, hydrogels are inherently water soluble. To make them insoluble, they are chemically cross-linked during manufacture or by a second reaction following that of polymerization of the starting monomers. The degree of cross-linking, quantified in terms of the crosslink density, together with the details of the molecular structure, have a profound impact on the swelling characteristics of the cross-linked system. [20]

3.1. Commonly used cross-linking agent: [11]

- Glutaraldehyde
- Chromium tanning
- Formaldehyde
- Poly epoxy compounds

- Metal oxide
- Acylazide
- Carbodiimides
- Hexamethylenediisocyanate

3.2. Cross-linking agent are converted into nanotechnology: The nanoparticulate nature of the drug shows sustained release effect by increasing its residence time in the cul-de-sac. The nanoparticles protect the drug against agents which cause degradation [21]. The choice of matrix constituents can readily modulate the controlled release and particle degradation characteristics [22] to enhance the drug therapeutic efficacy along with reduction in side effects, nanoparticles sustain release of the drug at the site of localization, modifying drug's organ distribution followed by clearance of the drug. Development of a particular drug's pharmacokinetic release profile can be done by controlling both the architecture and particle size of nanoparticles. To achieve a constant therapeutic concentration at the site of delivery, zero-order kinetic drug release profile is required. Finally the present study is to design nanosuspension (NS) as a novel controlled dosage form that could release the drug in a controlled manner at the site to have better therapeutic efficiency at a much lower dose.

The nano and micro technology based drug delivery system have been investigated for transscleral sustained delivery of drug to retina. Nanoparticle is effective in sustained delivery compared to the drug in solution due to their high surface area. The materials on the Nano scale are being used to improve interaction with surrounding tissue and hence, improve bioavailability.

Nanosuspension had a quicker onset of action and enhanced dose proportionality [23] Nanosuspension also alter the pharmacokinetic parameters, improves the safety and efficacy of the drugs. Formulation of nanosuspension includes stabilizers like poloxamers, lecithins, povidones, polysorbates etc. Solvents used in formulation includes water miscible solvents like butyl acetate, benzyl alcohol, ethyl acetate and other pharmaceutically acceptable and less hazardous solvents. Tweens and spans are widely used as surfactants which are added the dispersion by reducing interfacial tension. Surfactants act as wetting or deflocculating agents. Ethanol, glycofurol, isopropanol etc can be used as co- surfactants, buffer salts, osmogent, cryoprotectants, polyols are used as additives in nanosuspension formulation [24].

Nanosuspension offers many advantages: firstly, the physical and chemical stability of drugs in the nanosuspension can be increased as they are actually in the solid state.; secondly, dose and toxicity can be reduced and the high drug loading can be achieved.; thirdly, It is valuable for those molecules which are insoluble in oils.; finally, nanosuspension can be used for the passive targeting [25].

3.3. Role of Cross-linking agent:

- Cross-linking agent cause formation of bond between drug and collagen.
- The bond between drug and collagen form a network like structure after irradiation of UV radiation.
- Because of this complex network matrix drug release slows down.
- Ultimately, the cross-linking agent (nanoparticle) is responsible for sustained delivery of drug.
- Metal oxide nanoparticles are plays important role in cross-linking with collagen when irradiate with UV radiation (400-470nm).

4. Method of Preparation of nanoparticles: On the basis of above information, this article focuses on various method used in preparation of nanoparticles for improvement of bioavailability.

1. Solvent displacement method.
2. Homogenization.
3. Ionic gelation.
4. Milling method.
5. Supercritical fluid method.

1. Solvent displacement method: It is also called as Nano precipitation method and has been widely used to prepare nanoparticles. The method is based on the precipitation of preformed polymer following displacement of a semi polar solvent miscible with water in the presence or absence of surfactant. The basic principle of this technique is similar to spontaneous emulsification of the organic phase containing drug and polymer into the external aqueous phase. In this method the polymer

and drug are dissolved in a water miscible organic solvent of intermediate polarity (*e.g.* acetone and ethanol). The resulting organic phase is injected into a stirred aqueous phase containing a surfactant as stabilizer. The nanoparticles are formed instantaneously during the rapid diffusion of the organic phase into the aqueous phase [26].

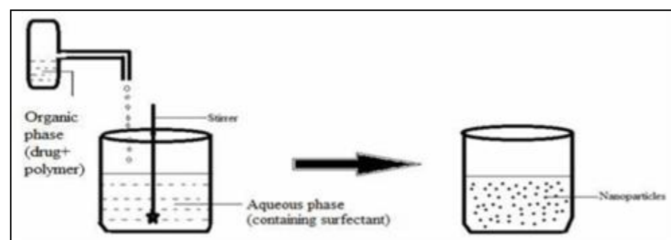

FIG. 1: SOLVENT DISPLACEMENT METHOD

2. Homogenization: This method is also used for preparation of nanosuspension. The process can be summarized into three steps: firstly, the presuspension is formed by dispersing the drug powders in a stabilizer solution; then pre-suspension formed was then homogenized by the high-pressure homogenizer at a low pressure for several times. It is also called as premilling, and finally the pre-milled suspension was homogenized at a high pressure for 10-25 cycles until the nanosuspensions with desired particle size were prepared [27].

Using high pressure homogenization method, nanosuspensions for ophthalmic delivery of practically insoluble glucocorticoid drugs like hydrocortisone, prednisolone and dexamethasone was prepared. Pluronic F68 (1%w/v) was used as surfactant. The effect of sub- micron and nanosized particles as well as effect of viscosity of the nanosuspension on ocular bioavailability was studied by measuring the intraocular pressure using Shioetz tonometer. From the mean particle diameter and particle size distribution of above glucocorticoid drugs analysed by laser diffractometer (LD) and photon correlation spectroscopy (PCS) were distinguished as micron size range and nano size range. After instillation of drug nanosuspension of different mean particle diameter, a mean percentage increase in intraocular pressure was calculated. It was concluded that, nanosuspensions of glucocorticoid drugs enhance the intensity of drug actions as well as rate and extent of ocular drug absorption. It was also observed that viscosity of nanosuspension increases that result, the duration of drug action was increased [28].

3. Ionic gelation: In the ionic gelation method, the positive or negative charge of the hydrophilic polymer is complexed with a multivalent cationic (*e.g.* calcium chloride) or polyanionic (*e.g.* sodium tripolyphosphate) to form highly viscous gel particles with a size in the range of a nanometer. Ionic gelation method was developed by Calvo and Co-workers for the preparation of chitosan nanoparticles. In this method polymer solutions and polyanionic solutions are mixed to form nanoparticles. The basic mechanism involved in the formation of nanoparticles is the electrostatic interactions between positively charged amino groups present in polymer and negatively charged anion. In other words it can be seen that in the ionic gelation method, due to interaction the material undergoes transition from liquid to gel phase. The obtained chitosan nanoparticles generally are of small size in the range of 200-500 nm [29].

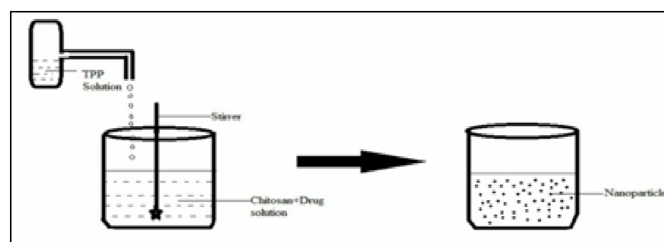

FIG. 2: IONIC GELATION

4. Milling method: High-shear media mills or pearl mills are used to prepare nanosuspensions. The media mill consists of three parts- a milling chamber, a milling shaft and a recirculation chamber. As a result of impaction of the milling media with the drug, high energy and shear forces are generated which provide the necessary energy to disintegrate the microparticulates drug into nanosized particles. The balls or milling media are ceramic-sintered aluminium oxide or zirconium oxide or highly cross-linked polystyrene resin and have high abrasion resistance. The size below 0.1 μm is achieved by planetary ball mills. In the media milling process, the milling chamber is charged with the milling media, water or suitable buffer, drug and stabilizer. Then milling media or pearls are rotated at a very high shear rate [30].

In this study cyclosporine A (CsA)-loaded nanosuspensions was prepared using a top-down media milling method. The effect on the particle size of the nanosuspension was investigated by studying the effect of bead material, polymer, milling time and milling speed. The nanosuspensions prepared with polyvinyl alcohol (PVA) were found to be stable as it showed no creaming or sedimentation phenomena and provide the smallest particles of about 530 nm. Nanosuspension having finer particles was obtained with zirconia beads of as compared to those with polystyrene beads.

This nanosuspension was physically and chemically stable for at least two months. Draize test and Schirmer tear test were performed to study ocular irritation. In Draize test, very slight ocular irritation was observed with both nanosuspension and commercial product. However in Schirmer tear test commercial product cause more ocular irritation the rabbits' eyes as compared to nanosuspension. The above prepared CsA-loaded nanosuspensions were found to be effective candidate for causing less ocular irritation [31].

5. Supercritical Fluid Method: Supercritical fluids are environmentally safe, so this technology have been investigated for the preparation of biodegradable micro and nanoparticles. Supercritical anti-solvent (SAS), rapid expansion of supercritical solution (RESS) and precipitation with compressed anti-solvent process (PCS) are commonly used methods using supercritical fluids. Supercritical anti-solvent process involves one liquid solvent and other is supercritical fluid. Both are entirely miscible with each other. As the solute is not soluble in the supercritical fluid, the formation of nanoparticles takes place as a result of the instantaneous precipitation of the solute formed by the extract of the liquid solvent by supercritical fluid. The SAS method can be used to prepare dexamethasone phosphate drug nanoparticles [32] (for microencapsulation) and griseofulvin nanoparticles. In RESS process, a supercritical fluid dissolves the solute and then the solvent power of supercritical fluid considerably falls and solute precipitates due to the quick extension of the solute through a tiny nozzle into a region lower pressure. This is the major point by which RESS differs from the SAS process [33].

5. Method of Preparation of collagen shield: Cross-linked collagen shield is prepared by using the solvent casting method. Collagen solution is pour into petri dish. Nanoparticle suspension is sonic ate for 10min prior to adding them to the collagen at different ratios petri dish is store at 4 °C to remove any trapped air bubbles and prepared shield is then left to overnight at room temperature. For the UV cross-linked shields the collagen-NP mixture is irradiate with blue light (400-470nm) for 20min *via* the laser lamp of a confocal laser scanning microscope. Drug loaded shields is prepare by adding a drug solution to the collagen-NP mixture pripor to casting resulting in cut film strips containig specific amount of drug [9].

6. Evaluation of Nanoparticles and Collagen Shield [9]:

6.1. Particle Size, Zeta Potential, Polydispersity Index (PDI) and Cytotoxicity of NPs: Nanoparticle size, zeta potential and PDI assess by light scattering spectroscopy using a Nanosized. The cytotoxicity of the NPs evaluate using the MTT assay on two cell lines; human corneal epithelial cells (HCECs) and human retinal pigment epithelium (ARPE-19) cells. Allow Cells to adhere for 24 h to a 96-well tissue culture plate at 5% CO_2 _ 95% atmospheric air at 37 °C. Then expose cells to varying concentrations of each NP solution ranging and culture for another 24 h before incubation with MTT for 4 h. measure the absorbance and the cell viability was calculated using the following equation:

Cell viability (%) = Absorbance (test)/Absorbance (control).

6.2. Transparency: The transparency of the shields determines using a UV–Vis spectrophotometer at 600 nm. Dis-tilled water use as the reference.

6.3. Swelling ratio: The swelling ratio investigate to determine the cross- linking density of the shields. Weigh Shields initially and then place into a petridish with simulated tear fluid (STF) at pH 7.4 [34], must maintain at 32 °C to mimic physiological conditions. After 6 h, when the equilibrium point reaches, retrieve shields and excess surface moisture carefully remove with a

filter paper. Swollen reweigh shields and their swelling ratio was calculated as follows:

Swelling ratio (%) = [(Wt -W0)/W0] x100

Where, W0 is the initial weight of the shield and Wt is the weight of the swollen shield at time t.

6.4. Mechanical properties: The tensile strength of the shields evaluates using a TA-XT plus texture analyser. Cut Shields into 30 x10 mm rectangular strips and held longitudinally between two clamps. Then pull at 2 mm/s and record force required to break the shields. Calculate the tensile strength was then as follows:

Tensile strength (g/mm2) = Breaking force of shield (g)/cross-sectional area of shield (mm2).

6.5. *Ex vivo* bio adhesion studies: The bioadhesive properties of the shields also evaluated on the TA-XT plus texture analyser using fresh bovine corneas. Cut Shields into 5 mm diameter circles and attach to the probe using double-sided adhesive tape. Secure the cornea on the tissue holder and lower the probe until the shield remain in contact with the cornea for 50 s. The bioadhesive strength of the shields determine by measuring the maximum force required to detach the shield from the corneal surface at a speed of 2 mm/s.

6.6. Thickness and surface pH of shields: The thickness of the cross-linked shields, with and without loaded drug, can be measure using a digital calliper at three random points around each shield. To measure the surface pH, placed shields into a petridish with STF (pH 7.4) and allowed to dissolve for 1 h at 32 °C. measure the pH of the solution using a pH meter.

6.7. *In vitro* release of Drug: The release of drug is determined by UV-visible in simulated tear fluid (STF). Cross-linked shields are cut into 15 x 15 mm strips, weigh and then placed into Append of tubes containing 2 mL of STF. Samples are placed into a Thermo mixer maintain at 32 °C under continuous shaking at 300 rpm. At set time intervals, 500 mL is withdrawn from each tube and replace with fresh STF. After sample removal take a UV measurement. Conduct the study for a period of 10 to 14 days.

CONCLUSION: Nanoparticle cross-linked collagen shield plays important role in the treatment of glaucoma. The shield is use as topical ocular drug delivery device. The cross linking agent is found to be the most important factor in sustained release. Collagen form matrix when nanoparticles induce crosslinking with the help of UV radiation. The cross-linked collagen shield is apply on cornea topically, it can sustained release of drug up to 14 days. This is significantly longer than with conventional eye drops which have to be applied at least twice daily and could thus enhance patient adherence to this system as well as clinical outcomes of glaucoma treatment.

Conflict of Interest: Certify that we have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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