DEVELOPMENT OF A NOVEL NANOTECHNOLOGY-BASED OPHTHALMIC DRUG DELIVERY DEVICE

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DRUG DELIVERY DEVICE

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TABLE OF CONTENTS

Chapter Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

The main objective of this project was to develop a drug delivery device that can supply medication to the eyes over an extended period without any side-effects.

The motivation to begin this project came when Dr. Jian-xing Ma, who is director of research for the section of Endocrinology at the University of Oklahoma Health Sciences Center, Oklahoma City, OK, expressed a need for such a device in order to deliver drugs to the eye. He is a pioneer in the development of angiogenic inhibitors that can be used for treatment of diabetic retinopathy and have therapeutic potential for ocular neovascular disorders. If ocular disorders like diabetic retinopathy and age-related macular degeneration are diagnosed early, then their progression can be prevented by supplying anti-angiogenic agents like Thalidomide to the retina of the eye. The retina lies so deep inside the eye that any drug supplied to the surface of the eye rarely cross all the layers of the ocular tissue to reach the retina. When the medication is supplied via eye-drops, it does not stay in contact with the surface of the eye long enough to transport across all the layers of the ocular tissue and reach retina.

Some highly invasive modes of drug delivery like ocular injection and ocular implants can be incorporated to deliver the drug to the retina, but due to their invasive nature, they suffer from patient incompliance. Some less invasive modes of drug delivery like ophthalmic gels, drug loaded liposomes and nanoparticles, and contact lenses soaked in drug solution, provide longer corneal residence time than eye-drops, but suffer from various side effects like blurring the vision, burst release of drug, low solubility of hydrophobic drugs, and systemic toxicity. This need of an ophthalmic drug delivery system that: (a) can deliver hydrophobic drugs, (b) does not blur vision, (c) releases drug slowly over an extended period, (d) does not let the drug be absorbed systemically, and (e) does not require surgery, became the motivation and focus of this research.

In the present model, the drug-loaded nanoparticles are incorporated inside a collagen-membrane, which a patient can wear in conjunction with any commercial contact lens. The lens system will provide a constant contact with the cornea, and the particles will supply a continuous release of drug, resulting in more drug reaching the target. Only biocompatible and biodegradable materials were used to synthesize the nanoparticles and collagenmembranes. The nanoparticles and the membranes were characterized for their physical and chemical properties and their suitability for the purpose of drug delivery.

CHAPTER II

REVIEW OF LITERATURE

2.1 Anatomy of the Eye:

As shown in figure 1, as light enters the eye, it first passes through a lubricating tear film that coats the cornea. The clear cornea covers the front of the eye and helps to focus incoming light. After light passes through the cornea it travels through a clear, watery fluid called the aqueous humor. The aqueous humor circulates throughout the front part of the eye, maintaining a constant pressure inside the eye. The iris is the colored part of the eye. As light conditions change, the iris may dilate to make the pupil bigger or constrict to make the pupil smaller. This allows more or less light into the eye. After light travels through the pupil, it must pass through the lens. The human lens, much like the lens of a camera, is responsible for focusing light. The lens can change its shape to focus on nearby and distant objects. After being focused by the lens, light passes through the center of the eye on its way to the retina. The eye is filled with a clear, jelly-like substance called the vitreous.

The retina is a thin, light-sensitive tissue lining the back of the eye that acts much like film in a camera. Light must be properly focused onto the retina, and the surface of the retina must be flat, smooth, and in good working order to produce a clear image. The center of the retina is called the macula. The macula contains a high concentration of photoreceptor cells which convert light into nerve signals. Because of the high concentration of photoreceptors, we are able to see fine details such as newsprint with the macula. At the very center of the macula is the fovea, the site of our sharpest vision. Behind the retina, a layer of blood vessels called the choroid supplies oxygen and nutrients to the outer layers of the retina. The white part of the eye is called the sclera. The sclera is composed of tough, fibrous tissue that protects the inner workings of the eye. The optic nerve is a bundle of nerve fibers which carries visual information from the eye to the brain.

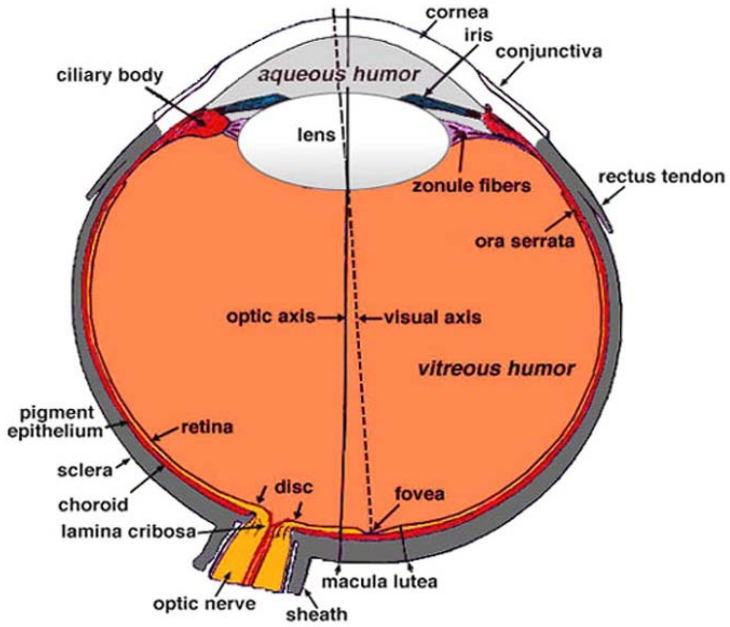


Figure 1. Anatomy of Eyeball [1]

2.2 Posterior Eye Diseases:

A diseased eye can have problems with anterior or posterior sections of the eye. Some of the diseases associated with the anterior section are conjunctivitis, keratitis and pterygium, whereas some of the diseases associated with the posterior section are diabetic retinopathy, age-related macular degeneration, and glaucoma. The anterior eye diseases are relatively easier to treat as compared with posterior eye diseases with the help of eye drops. The focus of this research is to supply enough drug to the posterior section of the eye in order to treat posterior eye diseases, especially the ones associated with retina.

A very common posterior eye disease is diabetic retinopathy. All diabetics are at risk for developing diabetic retinopathy, but not all diabetics do develop it. Chronic high blood glucose levels can damage blood vessels in the retina, and when they are damaged, they can leak fluid or bleed. This causes the retina to swell and form deposits. This is an early form of diabetic retinopathy called nonproliferative retinopathy. In a later stage, called proliferative retinopathy, new blood vessels grow on the surface of the retina. These new blood vessels can lead to serious vision problems because they can break and bleed into the vitreous.

Age-related Macular Degeneration is another posterior eye disease. It leads to loss of central vision. New blood vessels grow beneath the macular region of retina and leak blood and fluid. This leakage causes permanent damage to light-sensitive cells, which die off and create blind spots in the central vision.

2.3 Ophthalmic Drug Delivery Vehicles

Delivery of drug to the posterior section of the eye is one of the biggest challenge we face in treating posterior eye diseases. The most common mode of ophthalmic drug delivery i.e. eye-drops is ineffective in supplying desired amount of drug to the posterior section of the eye. Some uncommon modes like ophthalmic gels can blur the vision. Drug-loaded liposomes and nanoparticles give a burst release of drug requiring frequent applications. Inserts require surgery to be placed in the eye. Contact lenses and collagen membranes soaked in the drug solution could not be used effectively in the case of hydrophobic drugs, due to the low solubility of hydrophobic drugs in the lens matrix.

Of all the ophthalmic drug delivery systems, nanotechnology-based drug delivery system seems to be the most promising, owing to the following properties (a) the nanocarriers can protect the encapsulated drug molecule and simultaneously transport it to different compartments of the eye, (b) hydrophobic or hydrophilic drugs can be loaded into nanocarriers with equal ease by using compatible materials, and (c) the drug-release can be controlled by varying the method by which the drug is loaded into the nanocarriers and / or the material properties of the nanocarriers. In the following sub-sections some of the modes of drug delivery to eyes are explained in detail.

2.3.1 Eye-drops

Approximately, 90% of ophthalmic drugs are delivered by aqueous eye-drops [2]. There are many problems associated with using eye-drops as a mode of drug delivery. Due to tear drainage, there is a short residence time, requiring frequent applications, and

leading to only approximately 5% of the drug applied penetrating the outer layer of the eye and reaching the target [3]. The use of eye-drops can cause excess drug to be absorbed systemically, resulting in possible adverse side effects. For example, the delivery of Timolol Maleate, a drug often used to treat open-angle glaucoma, can cause cardiac arrhythmias if absorbed systemically [4]. Another limitation of using eye-drops as a drug delivery vehicle is the incompatibility of hydrophobic drugs in an aqueous medium; restricting the delivery of hydrophobic drugs through suspensions or emulsions.

2.3.2 Ophthalmic Gels

Ophthalmic gels are drug formulations with a high percentage of viscosity enhancers like cellulose, polyvinylalcohol, or polyacrylic acid. High viscosity of the gels increase the corneal contact time of the drug, and hence improve the bioavailability. Examples of ophthalmic gels are Pilopine HS gel, commercialized in 1986 by Alcon, and Timoptic-XE by Merck. On the downside, gels not only blur the vision, but can only improve the bioavailability to a limited extent and dosing can be decreased to once a day at most [5].

2.3.3 Drug Loaded Liposomes

Liposomes are microscopic bubbles, which are formed when phospholipids that have a hydrophilic head and a hydrophobic tail align themselves in such a way that a concentric bi-layer is formed, as shown in figure 2. These bubbles or vesicles are separated by water or aqueous buffer and range in diameter from 80 nm to 10 µm. Liposomes (also called phospholipid vesicles) were first described by Bangham et al [6]. These vesicles can entrap both hydrophobic and hydrophilic drug molecules. Some of the

advantages of using liposomes for ocular drug delivery are (i) hydrophobic drugs can be delivered, (ii) drug can be delivered in the form of liquid drops just like eye-drops, (iii) drug delivery is more controlled and prolonged than eye-drops, and (iv) drug metabolism by the enzymes present at the tear / corneal epithelial surface is prevented [7].

The major disadvantage of liposomal drug delivery is the burst release of drug that necessitates frequent applications. Liposomes can be modified in order to prolong the drug release. Two of the modifications that prolonged the delivery of acetazolamide to the eye were (a) introduction of multi layers of phospholipids, and (b) introduction of a charge density to the phospholipids [8]. In spite of the modifications, the drug delivery was prolonged only up to nine hours, indicating an opportunity to create a better mode of drug delivery that can deliver the drug for longer periods.

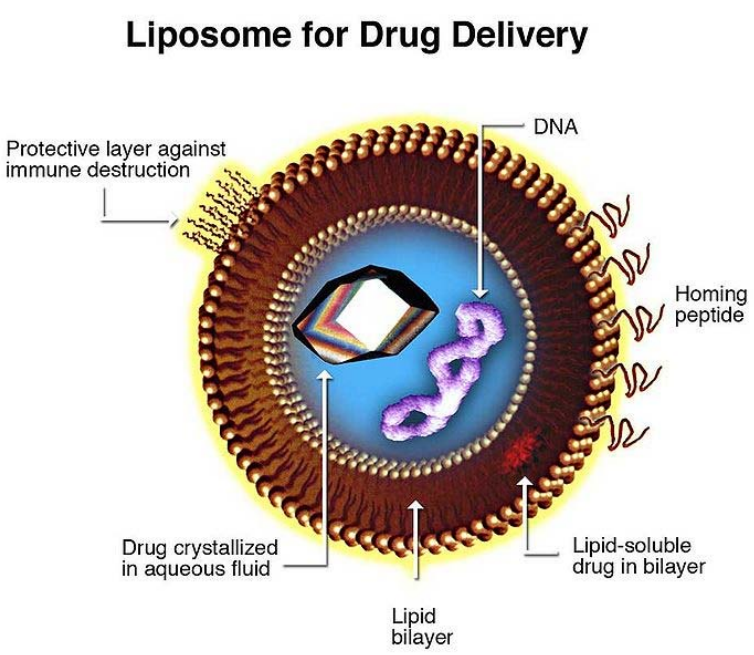


Figure 2. Structure of Liposome [9]

2.3.4 Drug Loaded Nanoparticles

Drug loaded nanoparticles are polymeric particles of diameter between 1 nm and 1000 nm that serves the purpose of delivering drug molecules to the body. Drug molecules can be small molecules like aspirin with a molecular weight of 180.2 Da or large molecules like avastin, a recombinant humanized monoclonal IgG1 antibody with molecular weight of 149 kDa. The drug molecules can be either contained within the particle or be conjugated to the surface of the particle. The drug molecules can be formulated at a nano-scale so as to function as their own "carriers" [10-14].

There are various ways to introduce drug-loaded nanoparticles into the body. When introduced intravenously, due to their small size, nanoparticles do not form an embolism even in the smallest capillaries, which are 5–6 μm in diameter [15]. In the case of oral drug delivery system, the main focus of research has been in lymphatic uptake of the nanoparticles by the Peyer's patches in the gut associated lymphoid tissue. The size and surface charge of the nanoparticles have been found to influence the uptake. The nanoparticles were found to disseminate systemically, whereas the microparticles were found to remain in the Peyer's patch [15-18]. In case of ocular drug delivery, mucoadhesive copolymeric micelle nanoparticles were also found to increase the bioavailability of some drugs by increasing the corneal contact time [19]. Although the use of nanoparticles for ocular drug delivery seems to be very promising, at present it needs improvement in a number of fronts including formulation stability, control of particle size, control of the rate of drug release, and large scale manufacturing of sterile preparations [20].

2.3.5 Ocular Inserts

Ocular inserts come in various sizes and shapes. Solid inserts like Ozurdex, an intravitreal implant can deliver an extended release of corticosteroid to the back of the eye [21]. It is administered via an intravitreal injection. It uses a biodegradable, solid polymer delivery system that is capable of maintaining a low level of dexamethasone over extended period thereby controlling edema and enhancing safety. Lacrisert, a small capsule used for dry-eye relief, can be placed in the lower eye lid of the patient by oneself [22]. Surodex, a dexamethasone delivery system that uses erodible-implant technology is approved in Singapore. It has an advantage of not requiring surgical excision after its contents are exhausted [23]. Some other types of inserts are membrane-controlled diffusional inserts, hydrophilic but water-insoluble inserts, and erodible inserts [24]. Owing to their strategic location, i.e. inside the eye, inserts can be used for controlled delivery of the drug. In spite of this advantage, patient compliance towards ocular inserts have always been low due to the surgery involved in placing the insert, difficulty with self insertion, and the feeling of a foreign body in the eye. Examples of some of the implants are shown in figure 3.

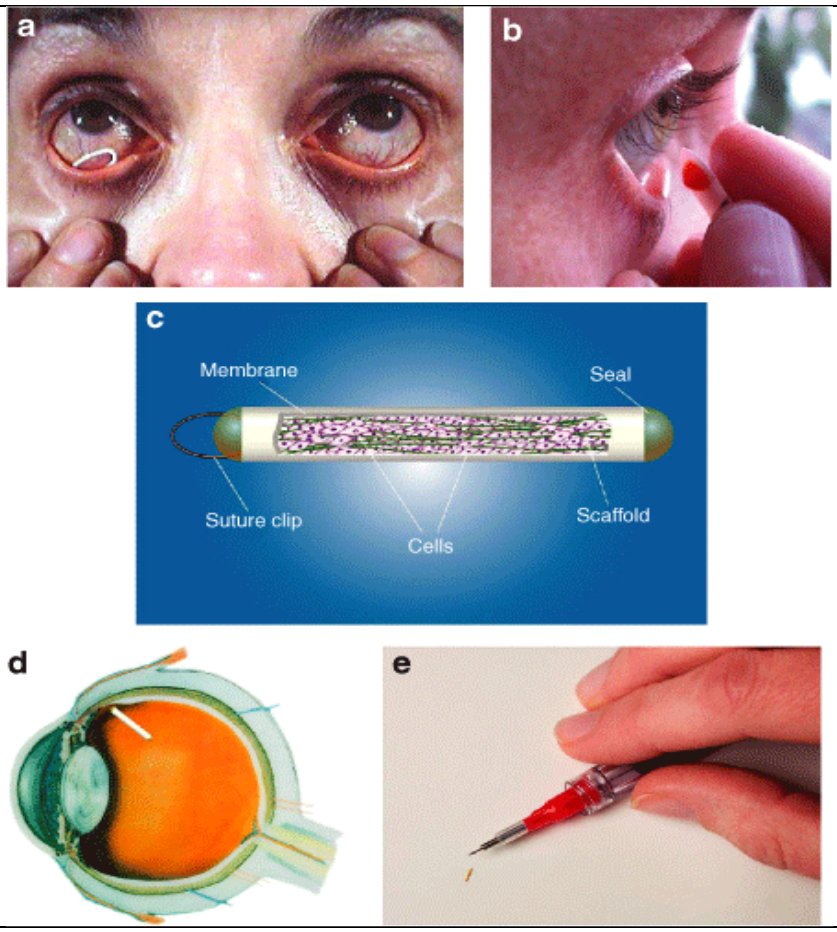


Figure 3. Examples of ophthalmic delivery systems [23]. (**a**) Photograph of patient with Ocusert (pilocarpine) in place in lower cul-de-sac of right eye. (**b**) Photograph of use of fluorescein lyophilized on Teflon strips. Photograph courtesy of Prof. Michael Diestelhorst. (**c**) Encapsulated cell technology: schematic diagram of implant. (**d**) Encapsulated cell technology: schematic diagram of implant placed in vitreous cavity of human eye. Photograph courtesy of Neurotech, Inc. (**e**) Iluvien fluocinolone acetonide implant. Photograph courtesy of Alimera Sciences.

2.3.6 Contact Lenses Soaked in Drug Solution

A number of attempts have been carried out to deliver drug to the eyes by entrapping the drug on to soft contact lenses via soaking it in drug solution followed by insertion into the eye [25-27]. Contact lenses soaked in drug solution, when worn by a patient, marginally increase the corneal contact time. Hehm, EM et al. compared *in vivo* release of five different aminoglycosides and fluoroquinolones through contact lenses

soaked in drug solution with their release through eye drops [28]. They concluded that the amount of drug released by the lenses is lower, or of the same order of magnitude, as the amount of drug released by eye-drops. There are some major limitations that contact lenses soaked in drug solution suffer. After placing the drug soaked lens into the eye, all the drug diffuses into the eye within a few hours, rendering it inapplicable for controlled release. After placing the lens in aqueous drug solution, it takes a few hours for the drug to load on to the lens and the large fraction of the drug that is left in the solution is wasted. The drug loading of the lens depends upon the solubility of the drug into aqueous media, rendering this technology inapplicable to hydrophobic drugs.

2.3.7 Contact Lenses Embedded with Drug-Loaded Nanoparticles

Gulsen and Chauhan developed a contact lens embedded with drug-loaded nanoparticles as shown in figure 4. They used lidocaine as a model drug and prepared nanoparticles with OTMS (octadecyltrimethoxysilane). They synthesized the particle loaded p-HEMA hydrogels by free radical solution polymerization of the monomer with chemical initiation. *In vitro* drug release experiments were carried out in water at pH of 6.5. It was concluded from the drug release studies that 1-mm thick hydrogels loaded with nanoparticles can deliver hydrophobic drugs for three to four days. The main drawback of this delivery system was that the drug release rate decreased exponentially after the initial burst release [29]. An improved system would include (a) minimal burst release, (b) sustained drug release rates, and (c) focusing the drug-release only to the inside i.e. towards the cornea, instead of releasing the drug to the conjunctiva. This would lead to (a) a higher concentration gradient at the cornea-lens interface resulting in higher drug diffusion into the eye (b) a reduction of any drug-drainage into the lachrymal duct,

and hence reducing any side-effects due to systemic absorbance of the drug, and (c) an increase in the overall release time.

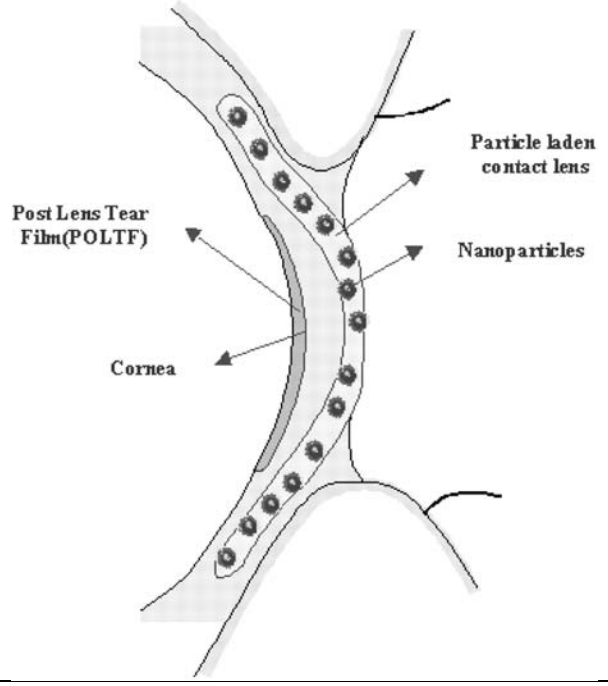


Figure 4. Contact Lens Embedded with Drug-Loaded Nanoparticles [29]

2.4 Development of a Novel Nanotechnology Based Ophthalmic Drug Delivery Device

For this study, we investigated a design composed of lidocaine-loaded, PLGA poly(lactic-co-glycolic acid) nanoparticles suspended within a transparent, ultrathin collagen membrane that can be attached to a standard commercial-grade contact lens for support, as shown in figure 5. The lens system will provide constant contact with the cornea, and the particles will supply a continuous release of drug, resulting in more drug

reaching the target. Lidocaine was selected as the model drug, since it is a hydrophobic drug that has extensively been investigated in other ophthalmic drug delivery systems [29]. The nanoparticles were synthesized with PLGA polymer, due to its biodegradable and biocompatible nature, and the fact that it has already been used to deliver a variety of therapeutic agents by targeted and/or sustained delivery.[30-32]. Collagen – type 1, the most abundant collagen of the human body which is found in tendons, skin, artery walls, and the organic part of bones and teeth was selected due to the following properties: transparent, malleable, high tensile strength, biocompatible, and biodegradable. Moreover, after drying, it can be rehydrated just by adding equal amount of water, making it suitable for dry storage.

Using various analytical techniques, the characterization of the nanoparticles was carried out by examining the morphology, size, and drug-loading. The collagen membranes were characterized by examining the morphology, mechanical strength, and light transmittance. Drug release rate of the complete lens system was characterized and compared to drug-infused nanoparticles and drug-infused collagen membranes.

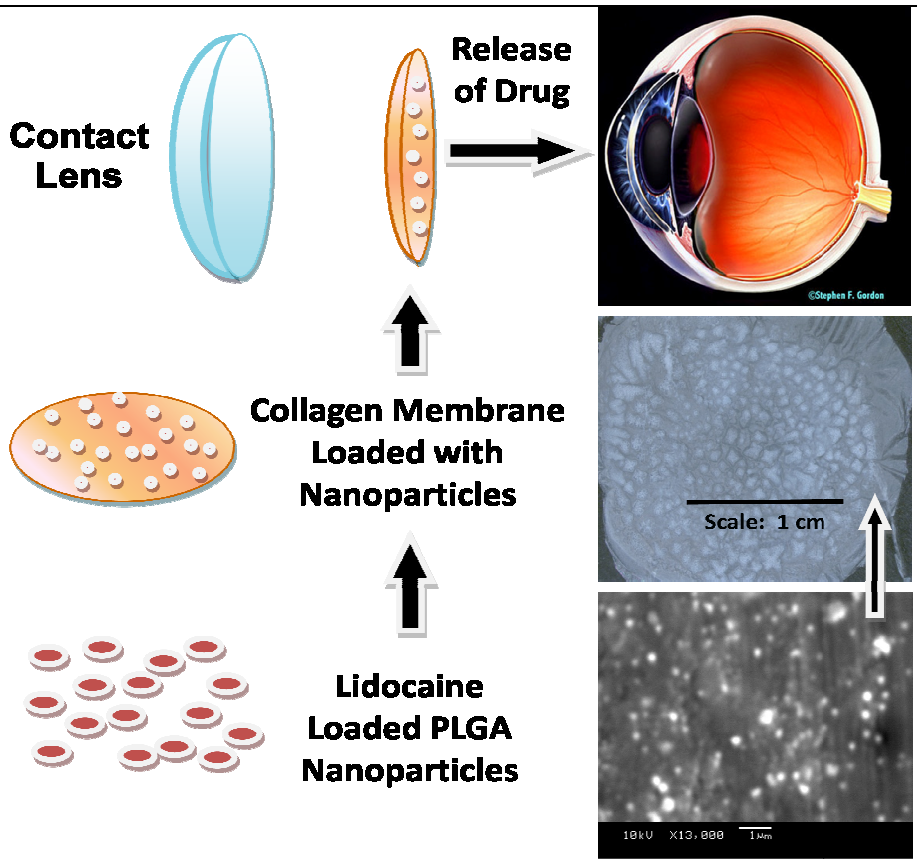


Figure 5. Details of Novel Ophthalmic Drug Delivery Device

2.5 Model Drug Selection

The model drug, lidocaine was selected based on its physical and chemical properties. Its empirical molecular formula is $C_{14}H_{12}N_2O$, molecular weight is 234.34 Da, and molecular structure is as shown in figure 6. Its use as a local anesthetic agent in ophthalmology, specifically during ocular surgery has been widely studied and proved [33]. It is an important class 1B antiarrhythmic drug regularly used for the treatment of ventricular arrhythmias. It has also proved effective in treating jellyfish stings [34]. Its use in humans has been very well studied.

Most of the ophthalmic drugs like metipranolol, epinephrine, dichlorphenamide, and lidocaine are hydrophobic in nature i.e. they are either insoluble or sparingly soluble in water, which makes them hard to be delivered via aqueous eye-drops. By conjugating these molecules with an acid improved their solubility in water. For example metipranolol hydrochloride, epinephrine d-bitartrate, and lidocaine hydrochloride are hydrophilic and therefore are soluble in water. In some cases where conjugation is not possible or where the efficacy of drug is compromised due to conjugation it is imperative to utilize a non-conventional mode of drug delivery to deliver the drug in its original form. In this study a hydrophobic drug, lidocaine was loaded into nanoparticles that can be delivered to the eyes *via* a collagen membrane.

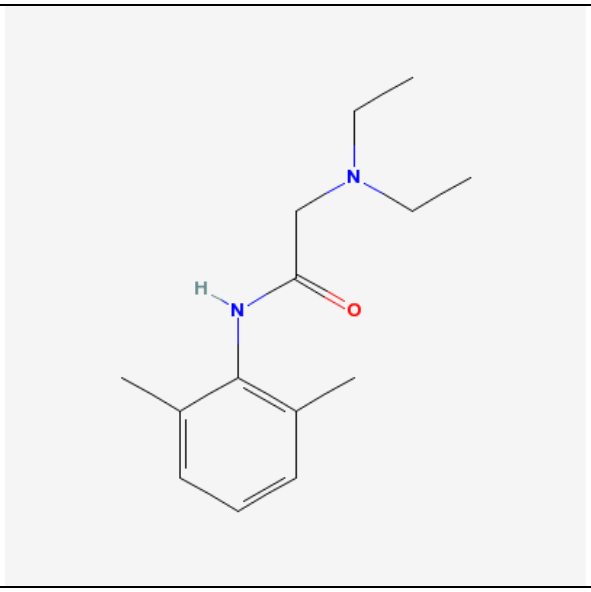


Figure 6. Structure of Lidocaine Molecule [35]

2.6 Drug Stability

The stability of the drug is of concern during the synthesis process and during the drug-release study. If the drug decomposes or metabolizes during either of these steps, the results of this study would be inconclusive. The drug molecules undergo temperature changes and interact with organic solvents, which might affect the stability of the drug.

A couple of experiments were conducted in order to establish stability of lidocaine in the present matrix and under the elevated temperature and pH conditions. The stability of lidocaine in various other matrices that consisted of excipients, fillers and drugs have been studied and proved [36].

CHAPTER III

MATERIALS AND METHODS

3.1 Reagents

Poly(lactide-co-g lycolide) Acid (PLGA) (50:50) and Poly(vinyl alcohol) (PVA), 87-90% hydrolyzed, MW 30k – 70k were purchased from Sigma-Aldrich (St. Louis, MO). Lidocaine U.S.P. was purchased from Spectrum (Gardena, CA). Ethyl Acetate (HPLC-UV grade) was purchased from Pharmco (Brookfield, CT). Purified Bovine Collagen solution (type 1) was purchased from Advanced Biomatrix (San Diego, CA). PBS (Phosphate Buffer Saline) solution, pH 7.4 was prepared from salt that that was purchased from Invitrogen. Ethyl Alcohol was purchased from Pharmco (Brookfield, CT). All other chemicals and solvents used were purchased from local suppliers and of analytical grade unless specified.

3.2 Synthesis of Lidocaine Loaded Nanoparticles

All the procedures were carried out in a well ventilated hood. PLGA (50:50) was removed from cold storage at 4 °C and let it sit for approximately one hour to come to room temperature. This was done to avoid any moisture that might accumulate on the cold powder when the bottle is opened. A 1.0% (w/v) PVA solution (MW 30k – 70k, 87 – 90% hydrolyzed) was prepared in a 250 ml beaker by dissolving 1500 mg of PVA in 150 ml water and mixing at 200 rpm on a stir plate for 2 hours. Inside a 100 ml sample bottle with cap, 1500 mg PLGA (50:50) was dissolved in 50 ml ethyl acetate. Inside a 50 ml sample bottle, 750 mg lidocaine was dissolved in 25 ml ethyl acetate. Finally the contents of 50 ml sample bottle were added to 100 ml sample bottle and gently shaken in order to mix. The PVA solution was placed on an ice bath and the tip of sonicator probe (Fisher Scientific Sonic Dismembrator Model 500) was inserted to a depth of half the height of the solution. With sonication at 75% of total intensity, the organic solution from the previous step was slowly added using a 1 ml pipette. Within 10 minutes, all the organic mixture was added to the aqueous solution leading to the formation of nanoparticles (figure 7a). The contents were transferred to a 1000 ml beaker and stirred at 200 rpm on a magnetic stir plate for 10 hours, to evaporate the organic solvent i.e. ethyl acetate (figure 7b). After 10 hours, 200 ml of DI water was added to the contents and stirred at 200 rpm on magnetic stir plate for 5 minutes. The contents were filtered through a 1.2 μm filter (Fisherbrand G4) using a vacuum filtration unit.

The filtered solution was collected in eight 50 ml centrifuge tubes (Nalgene Oak Ridge Centrifuge tube, PPCO) and as suggested by T Gorner et al. [37], it was centrifuged at 16,000 g and 4 °C for 25 minutes with a high speed centrifuge (Sorvall

Instruments, Dupont, RC5C, figure 7c). The supernatant was collected in a separate container leaving 10 ml solution behind. This solution was re-suspended in 40 ml fresh DI water. The steps of centrifugation and re-suspension were repeated until the supernatant became transparent, indicating the removal of PVA. One milliliter of the supernatant that was collected above was withdrawn, stored and later, analyzed for the amount of lidocaine using gas chromatography. This helped in assessing the drug loss during the synthesis process.

After the last centrifugation the particles were re-suspended in 6 ml water and agitated to get an even suspension. By this step the PVA and ethyl acetate had been completely removed from the nanoparticles. One ml of suspension was added to six pre-weighed and pre-marked 2 ml vials. These vials were placed inside an isopropyl alcohol-jacketed container (Nalgene Cryo 1° C Freezing Container) placed in a -80 °C freezer for 10 hours. This container is used to freeze the suspension slowly at a rate of minus 1 °C per minute, prior to freeze drying. Slow cooling of the suspension prevents unwanted agglomeration of the particles that might occur if cooled too quickly. The frozen suspension of nanoparticles was lyophilized (freeze dried) for 12 hours (figure 7d). The dried nanoparticles in the vials were re-weighed and used for characterization studies and for the synthesis of nanoparticle-loaded collagen shields.

			
a. Ethyl acetate containing PLGA : lidocaine (2:1) was added dropwise to 1% PVA solution while sonicating.	b. The suspension was stirred on a magnetic stir plate for 10 hours in order to evaporate the ethyl acetate.	c. The nanoparticle suspension was washed and concentrated by centrifuging at 4 ˚C and 16,000 g for 25 minutes, and repeated several times.	d. The concentrated suspension was lyophilized for 12 hours to obtain the nanoparticles in dry powder form.

Figure 7. Steps Involved in the Synthesis of Lidocaine-Loaded Nanoparticles

3.3 Synthesis of Collagen Membranes

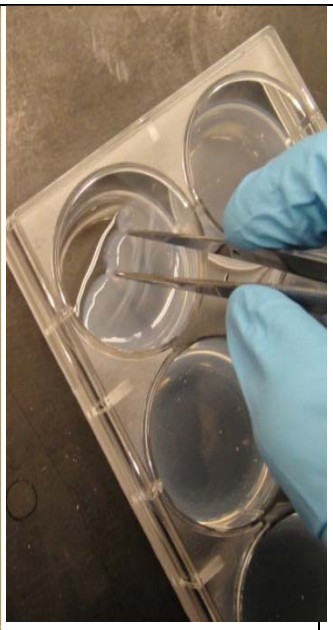
Under a class II A2 biological safety cabinet, a collagen solution for preparation of the membranes was prepared by combining of purified bovine collagen solution (3mg/ml), 10x PBS solution, and 0.1 N sodium hydroxide at 57.1% : 7.2% : 35.7% by volume, respectively. The collagen solution was mixed with nanoparticles to make the final concentration of 1.5 mg nanoparticles per ml of collagen solution (figure 8a). The remaining solution was used to make collagen shields without nanoparticles. Both solutions with and without nanoparticles were poured into wells of two separate six well plates at 1502 µL per well. Both plates were covered and incubated at 37 °C in a CO_2 incubator for one hour to gel the collagen (figure 8b). With the help of a pair of tweezers,

the gels were removed from each well, spread on a Teflon sheet, and left for 10 hours to dry under room temperature in a biological safety cabinet (figure 8c and 8d). After drying, the collagen membranes were removed carefully from the Teflon sheet, weighed and stored inside 2 ml vials, until further analysis.

Lidocaine-loaded collagen membranes were prepared by adding 0.07765 mg lidocaine per ml collagen solution instead of loading nanoparticles. The 0.07765 mg / ml is equivalent to the amount of lidocaine loaded on nanoparticle-loaded membranes, which contain 1.5 mg nanoparticles per ml of collagen solution and the average lidocaine loading on the nanoparticles is 5.18%.

a. Collagen (type 1 bovine) was mixed with a fixed amount of drug loaded-nanoparticles.

b. Collagen suspension (1.502 ml) was added to each well of a six-well plate and incubated at 37 °C for 1 h to gel.



c. The collagen gels were carefully removed from the wells.

d. The gels were spread on a Teflon-sheet and allowed to dry overnight at room temperature.

Figure 8. Steps Involved in Synthesis of Collagen Membranes

3.4 Size and Morphology

The size of the nanoparticles was measured by running a 1 % (wt/vol) suspension of nanoparticles in water through a dynamic light scattering machine, Malvern HPPS – 5001 Particle-sizer.

The morphology of nanoparticles and collagen membranes was analyzed by using a scanning electron microscope (SEM; Quanta 600F, FEI, Hillsboro, OR). The nanoparticle samples for SEM analysis were prepared by drying a drop of nanoparticle suspension on a metal stub and sputter coating a thin layer of gold onto the sample for 30 seconds. The collagen membrane samples were prepared by spreading the membrane over a carbon coated stub and sputter coating with gold. The morphology of the collagen membranes embedded with nanoparticles was compared with those that were synthesized without nanoparticles by using SEM. The nanoparticle size distribution was also calculated by analyzing the SEM pictures by an image analysis software called ImageJ.

The morphology of the membranes was also studied by taking full view pictures of the dry and wet membranes with a 10X digital microscope (Olympus SZX9, Tokyo, Japan). The membranes were sandwiched between two glass cover slips, the distance of microscope lens was adjusted to bring the picture in focus, and the pictures were taken by a computer-controlled camera that was attached to the microscope.

3.5 Visible Light Transmittance

Since the proposed drug delivery system is designed as a lens system for the eye, it must not interfere with the normal vision of the eye. In order to evaluate the transparency of the membranes, an analysis was conducted to determine the amount of

visible light transmitted through the collagen membranes. Collagen membranes were immersed in 1 ml PBS and added to a 24-well plate. The optical density of the samples was measured at several wavelengths by using a spectrophotometer (SpectraCount, Packard). Optical density was measured for the following samples: (1) a collagen membrane loaded with nanoparticles that has been in PBS solution for five minutes, (2) a collagen membrane loaded with nanoparticles that has been in PBS solution for fifteen days: to investigate the effect of wetting with PBS over time, (3) a collagen membrane without nanoparticles that has been in PBS solution for five minutes: to investigate the effect of the presence of the nanoparticles, (4) a blank 1 ml PBS solution, and (5) a commercially available contact lens (BC: 8.6, DIA : 14.2, PWR : -0.25, Proclear, CooperVision, Scottsville, NY): to be able to compare the transmittance of light through a standard device.

3.6 Tensile Strength

A Tensile Strength test was conducted on collagen membranes in order to quantify the mechanical strength of the membranes. The mechanical strength can be related to the structural integrity of the membrane, which is also tied to the degradation of the membrane. The membrane was cut into 1 cm x 2 cm rectangular piece and clamped onto the tensile testing machine (Instron 5542, Norwood, MA). Tensile stress in MPa was plotted against percent strain when an elongation of 1 mm per minute was applied to the sample. The tensile strength of a collagen membrane containing nanoparticles was compared with one without nanoparticles, both with and without wetting in PBS for fifteen days.

3.7 Chromatographic Analysis of Lidocaine

The drug-loading and drug-release samples were analyzed for lidocaine by gas chromatography. For drug-release, the standards were prepared in 50% ethanol solution, and the samples were collected from PBS solution. For drug-loading, the standards and samples were both prepared in ethyl acetate. The following GC method was developed on Agilent 6890 GC machine based on work of Mohammed Abdel-Rahim et al [38].

a) Injector: Temperature = 250 °C,
b) Splitless mode with purge activation time at 1 min
c) FID Detector: Temperature = 305 °C
d) GC Column: SPB-50 Supleco 30 m * 0.25 mm * 0.25 μm
e) Oven Temp Gradient:
    - Start - 100 °C – 1 min
    - 50 °C/min – 200 °C – 0 min
    - 20 °C/min – 295 °C – 3 min
f) Injection Volume: 0.5 μL
g) Flow rate of carrier gas through column: 2 ml/min

A standard linearity curve between area under the peak and concentration in μg/ml, with R-square value of more than 0.99 was drawn by running 40, 100, and 200 μg/ml standards. Concentration of the drug in the samples was calculated by interpolation.

3.8 Drug Stability

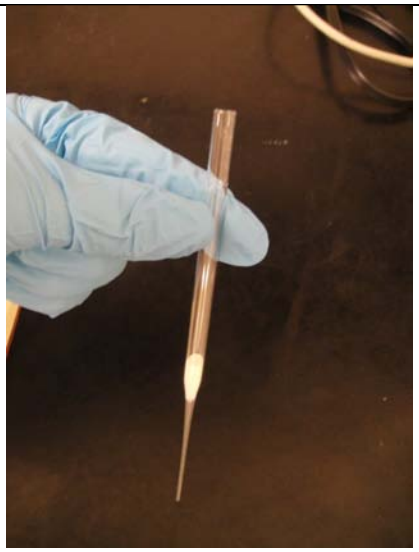
The stability of the drug, lidocaine was investigated in two scenarios: first, thermal stability at 37 °C and pH 7.4, and second, compatibility with ethyl acetate.

The drug-release study is carried out at 37 °C, hence any thermal instability of the drug molecules would render the study-results inconclusive. The thermal stability of lidocaine was assessed by carrying out an experiment in which 100 mg of lidocaine was dissolved in 2 ml ethanol and diluted to 100 ml with PBS solution. One ml of this lidocaine solution was poured into 24 vials each and stored at -20 °C. At each time point: 0, 14, 28, 42, 56, and 70 days, three vials were withdrawn and mounted on a rotary shaker at 37 °C. At the end of 70 days the concentration of lidocaine was measured in all the vials using gas chromatography.

The process of nanoparticle-synthesis involves dissolving the drug in an organic solvent, ethyl acetate. An experiment was conducted to check the stability of lidocaine during its interaction with ethyl acetate. A 1% solution of Sigma reference standard lidocaine in ethyl acetate was prepared and then ethyl acetate was removed by evaporation. IR spectra were measured on Scimitar Series model Varian 800 FTIR spectrometer in the wave number region 400-4000 cm-1. All IR measurements were carried out at room temperature using the KBr pellet technique. The IR spectrum of residual lidocaine was produced and compared with the standard lidocaine using “compare” function of the machine.

3.9 Drug Loading

Approximately 10 mg of nanoparticles were dissolved in 3 ml ethyl acetate and analyzed by GC for lidocaine concentration. The sample solutions were passed through a 1 cm column of activated basic Aluminum Oxide in order to remove PLGA, and hence prevent the deposition of PLGA polymer inside the GC column (figure 9a and 9b). A standard linearity curve between area under the peak and concentration in μg/ml, with R-square value of more than 0.99 was drawn by running 40, 100, and 200 μg/ml standards. Concentration of the drug in the samples was calculated by interpolation. Drug-loading was calculated by factoring the dilution of the sample into the concentration of the drug from the standard curve.



a. 1 cm column of activated basic Aluminum Oxide

b. Drug-solution collected in 1.5 ml vial

Figure 9: PLGA Removal from Drug-Solution.

3.10 *In vitro* Release of Lidocaine

To measure the release of lidocaine from the nanoparticles, 10 mg of nanoparticles were suspended in 1.5 ml of PBS solution and shaken (Hand-motion

shaker; Scinics, Tokyo, Japan) at 60 rpm at 37 °C, in order to mimic physiological conditions. At the following time points, a 30 μL sample was withdrawn and 30 μL of fresh PBS was added: 1, 3, 6, 12, 24, 48, 168, and 408 hours. The samples were filtered through a 0.1 μm filter to remove any nanoparticles and thereby avoid any drug contribution from the nanoparticles. All the samples were stored at -20 °C until ready for analysis.

To measure the release of lidocaine from collagen membranes, a membrane was placed inside a vial containing 1.5 ml PBS and shaken at 60 rpm at 37 °C. At the following time points, a 30 μL sample was withdrawn and 30 μL of fresh PBS was added: 1, 3, 6, 12, 24, 48, 168, and 408 hours. The samples were filtered through a 0.1 μm filter to remove any nanoparticles and thereby avoid any drug contribution from the nanoparticles. All the samples were stored at -20 °C until ready for analysis. Three batches of membranes, each consisting of three membranes containing a single batch of nanoparticles were simultaneously tested against one batch of three membranes that contained an equivalent amount of drug, but no nanoparticles. All the samples collected were stored at -20 °C until ready for analysis.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Size and Morphology:

SEM pictures of nanoparticles showed an irregular shape and mean particle diameter of approximately 247 nm as calculated by the image analysis software ImageJ (http://rsbweb.nih.gov/ij/download.html). An SEM image of nanoparticles like the one shown in figure 10a is cropped and inverted by ImageJ as shown in figure 10b. Scale is set based on SEM image, sphericity is set to one, threshold is adjusted accordingly and the particles are analyzed for their size and shape by drawing their perimeter as shown in figure 10c. By using the "Particle Analysis" function of ImageJ, the size distribution of the particles in a spreadsheet format was also obtained as shown in appendix A.

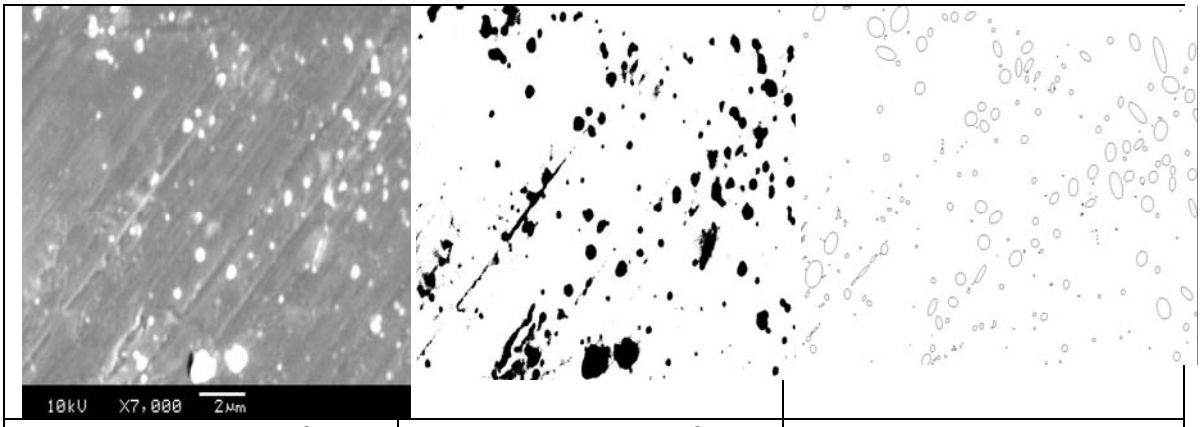
The average size of nanoparticles as measured by Malvern HPPS – 5001, an instrument based on dynamic light scattering, was found to be in the range of 150 nm and 300 nm. As shown in figure 11a, the vital parameters of the system were chosen to be the following: water as dispersant with refractive index 1.330 and viscosity 0.8872 cP, PLGA as the nanoparticle-material with refractive index 1.45, and temperature as 25 °C. A logarithmic size-distribution curve and a histogram showing the particle size distribution was generated by the machine (figure 11b and 11c). The DLS results for all the three batches are shown in appendix A. The mean particle diameter measured by SEM was within range of the size distribution measured by dynamic light scattering, confirming the mean particle size.

Nanoparticle-loaded collagen membranes are white to colorless, ultrathin, circular, sheets of collagen of approximately 1 cm in diameter. Some characterization techniques required larger samples for analysis. Therefore, the diameter of the membranes that were prepared for the drug-release study and tensile strength was 2.5 cm.

The SEM pictures of the nanoparticle-loaded membranes showed an uneven distribution of particles on the membrane-surface, forming some regions with higher density of particles than others (figure 12a and 12b). In the regions of low particle density, collagen fibers were very prominent and looked quite similar to the ones in the nanoparticle-free membranes (figure 12c). In the regions of high particle density, the nanoparticles were more prominent on the surface and collagen fiber structures were hidden below. As shown in appendix B, assuming sphericity of the particles as one, the average size of nanoparticles measured by ImageJ from these pictures was 56.89 ± 1.36 nm. One possibility is that the larger diameter particles were embedded within the

membrane, while the smaller diameter particles remained on the surface of the membrane.

The pictures of collagen membranes taken by a stereomicroscope at 10x revealed that on wetting, the membranes rearranged their fibers in such a way that they become transparent again (figures 13a and 13b). The thickness of the membranes was found to be in the order of 50 microns.



a. SEM image of nanoparticles	b. SEM image of nanoparticles after processing by ImageJ	c. Perimeter representation of the particles by ImageJ

Figure 10. Nanoparticle Size Measurement via SEM

File Name:	RM-174-TTA-nC60-1.dts	**Dispersant Name:**	Water
Record Number:	2	**Dispersant RI:**	1.330
Material RI:	1.45	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.01	**Measurement Date and Time:**	Friday, January 15, 2010 4:1...

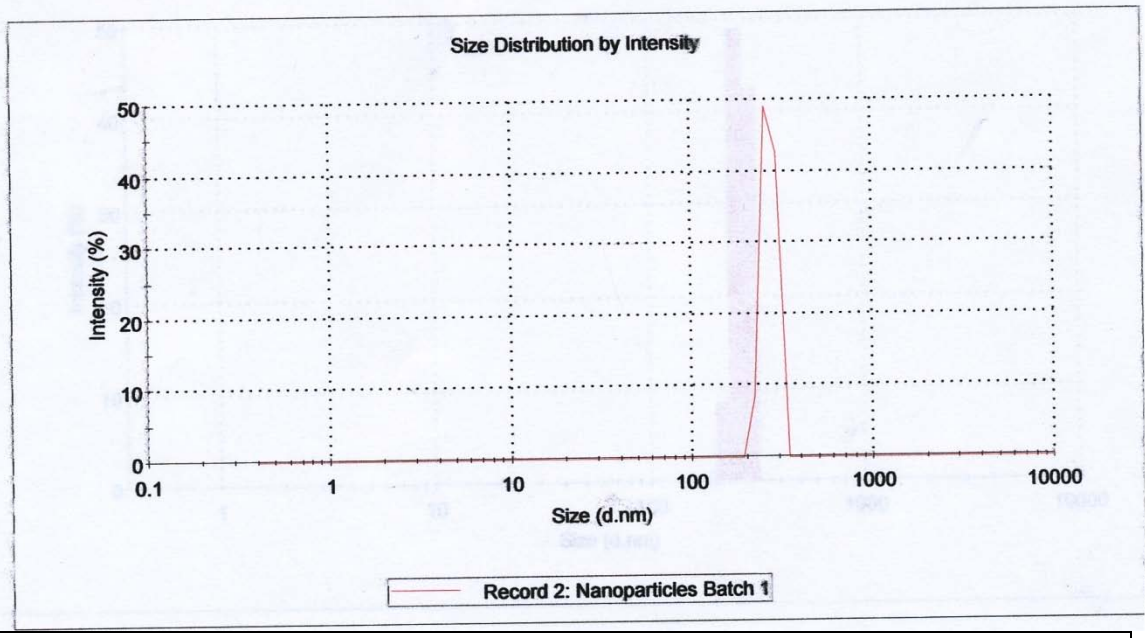
System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	334.4	**Measurement Position (mm):**	2.00
Cell Description:	Disposable sizing cuvette	**Attenuator:**	7

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	1520	**Peak 1:**	269	100.0	24.3
PdI:	1.000	**Peak 2:**	0.00	0.0	0.00
Intercept:	0.940	**Peak 3:**	0.00	0.0	0.00

a. System parameters



b. Logarithmic size distribution curve

SOP Name: Manual measurement settings

Measurement Date and Time: Friday, January 15, 2010 4:10:01 PM

Z-Average (nm): 1515.04

Standard Deviation: 0

%Std Deviation: 0

Variance: 0

Derived Count Rate (kcps): 29300.5840415...

Standard Deviation: 0

%Std Deviation: 0

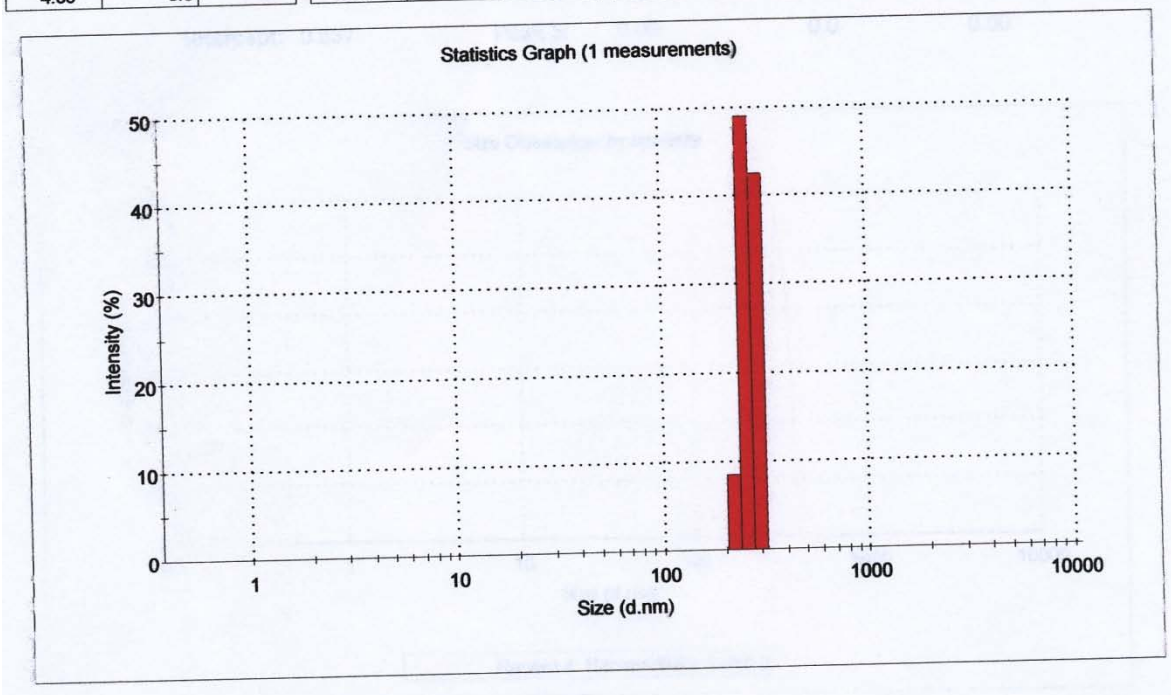
Variance: 0

Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0	
0.463	0.0	
0.536	0.0	
0.621	0.0	
0.719	0.0	
0.833	0.0	
0.965	0.0	
1.12	0.0	
1.29	0.0	
1.50	0.0	
1.74	0.0	
2.01	0.0	
2.33	0.0	
2.70	0.0	
3.12	0.0	
3.62	0.0	
4.19	0.0	
4.85	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
5.61	0.0	
6.50	0.0	
7.53	0.0	
8.72	0.0	
10.1	0.0	
11.7	0.0	
13.5	0.0	
15.7	0.0	
18.2	0.0	
21.0	0.0	
24.4	0.0	
28.2	0.0	
32.7	0.0	
37.8	0.0	
43.8	0.0	
50.7	0.0	
58.8	0.0	
68.1	0.0	

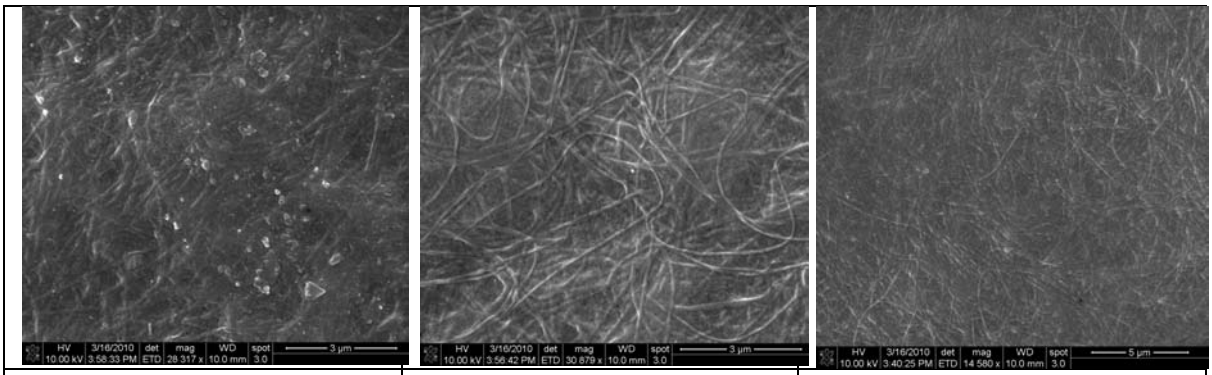
Size d.nm	Mean Intensity %	Std Dev Intensity %
78.8	0.0	
91.3	0.0	
106	0.0	
122	0.0	
142	0.0	
164	0.0	
190	0.0	
220	8.4	
255	49.0	
295	42.6	
342	0.0	
396	0.0	
459	0.0	
531	0.0	
615	0.0	
712	0.0	
825	0.0	
955	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
1110	0.0	
1280	0.0	
1480	0.0	
1720	0.0	
1990	0.0	
2300	0.0	
2670	0.0	
3090	0.0	
3580	0.0	
4150	0.0	
4800	0.0	
5560	0.0	
6440	0.0	
7460	0.0	
8630	0.0	
1.00e4	0.0	



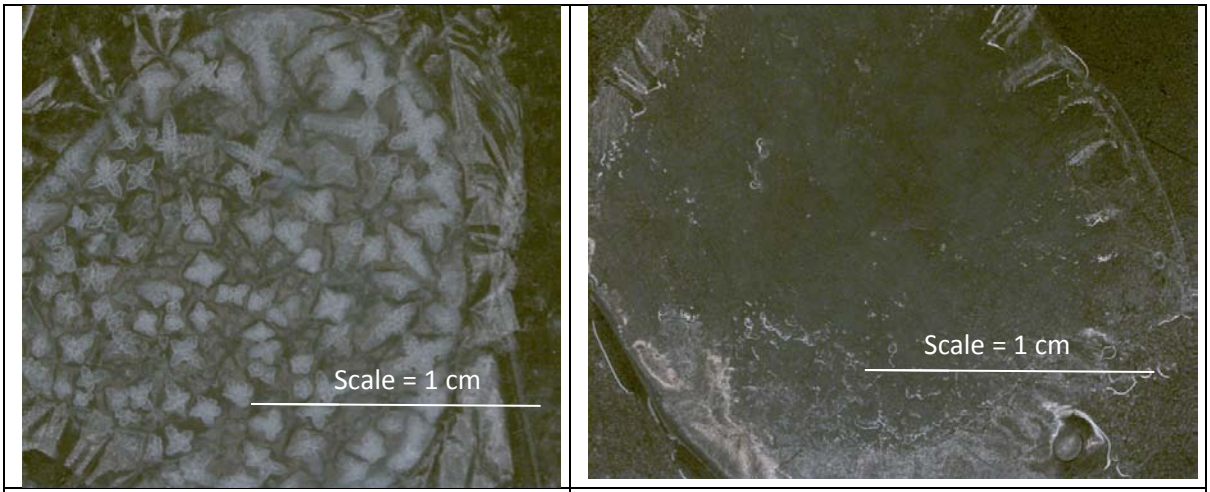
c. Statistical analysis of nanoparticle size

Figure 11. Results of Dynamic Light Scattering



a. A region of a collagen membrane containing numerous nanoparticles on the surface	b. A region of the same collagen membrane containing very few nanoparticles on the surface	c. Collagen fibers of a nanoparticle-free membrane

Figure 12. SEM Images of Collagen Fibers of a Membrane With (a, b) and Without (c) Nanoparticles



a. Ordered structures of collagen fibers in a dry membrane	b. Structures of collagen fibers disappear when membrane is wetted

Figure 13. Pictures of Collagen Membranes with 10 X Camera

4.2 Transparency of Membranes

Figure 14 shows the results of the visible light transmittance of the samples tested, as measured by UV-Vis spectrophotometer. The mean % transmittance of light through the membranes with nanoparticles decreased from 88 to 81 over a period of 15 days. The slight decrease in transmittance could be due to the membrane degrading, resulting in

realignment of the collagen fibers, which could block the transmittance of light. Mean % transmittance of light through membranes without nanoparticles is 91, which is slightly higher than those with nanoparticles. This shows that nanoparticles slightly decreased the transparency of the membranes. Proclear (Omafilcon A), a commercially available contact lens, has a % transmittance of 96, which is almost equal to the % transmittance through the blank sample (1 ml PBS).

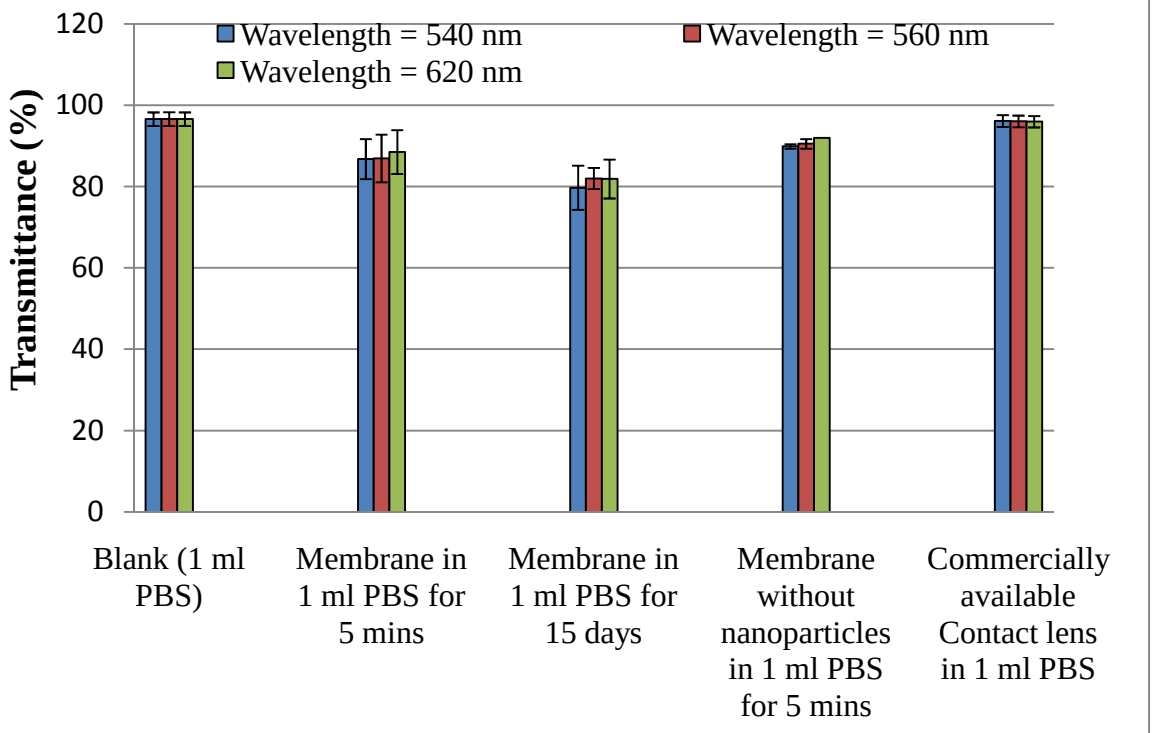
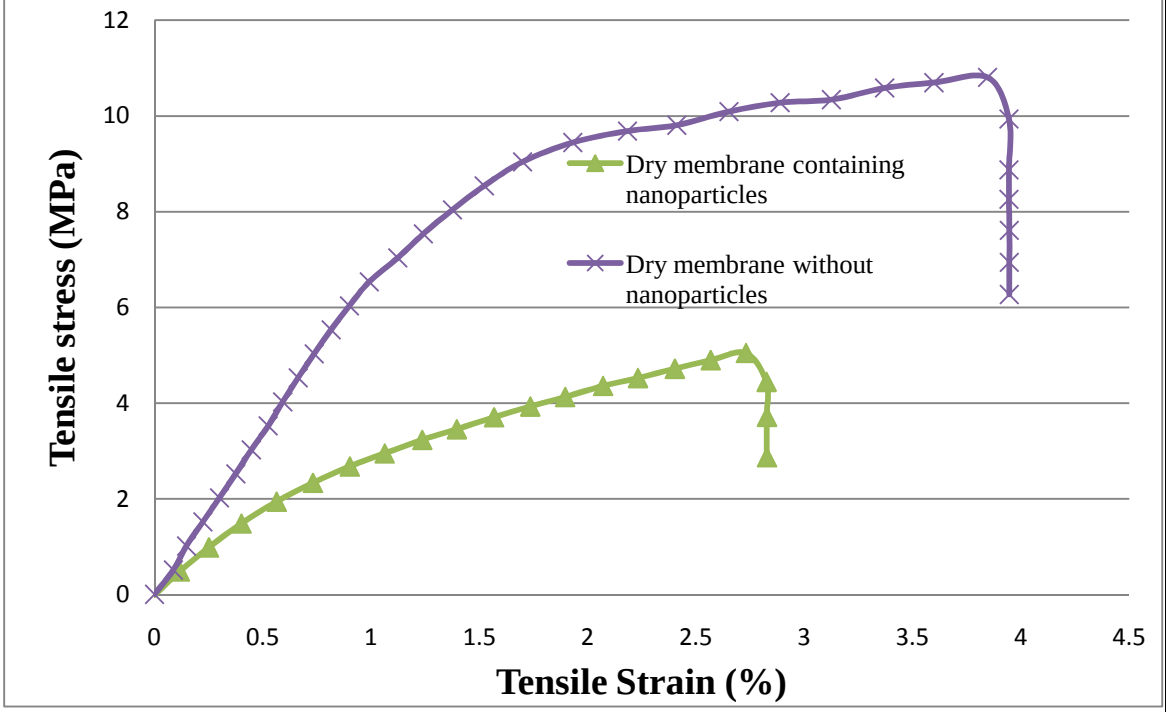


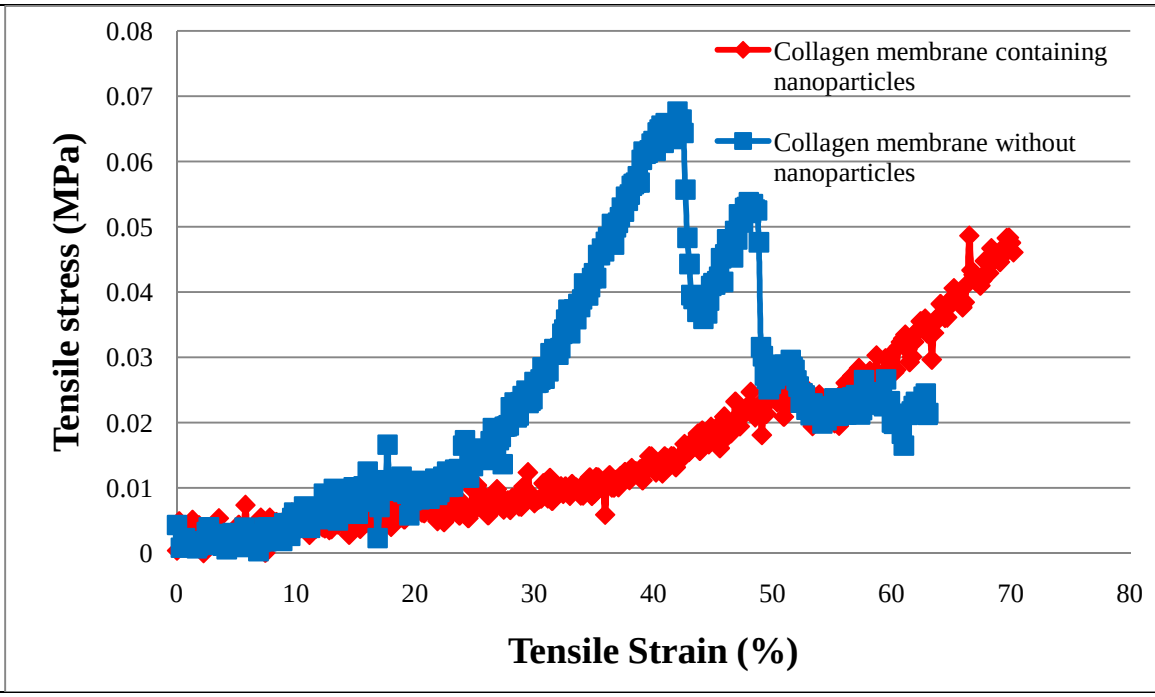
Figure 14. Transparency of Membranes With and Without Nanoparticles, Compared to the Control Samples of PBS and a Commercially Available Contact Lens. Data shown are mean values ± sd; n=3.

4.3 Tensile Strength

Tensile strength of collagen membranes containing nanoparticles was compared with membranes without nanoparticles, both without wetting and after wetting in PBS for 12 days. For without wetting, until fracture point is reached, the amount of strain was higher for collagen membranes loaded with nanoparticles than the membranes without nanoparticles, when same amount of stress was applied on membranes (figure 15a). The membranes without nanoparticles could bear more mechanical stress (10.8 MPa as compared to 5.1 MPa) as well as more strain (3.8% as compared to 2.7%) before breaking. An explanation of this behavior could be that in the presence of nanoparticles the collagen fibers are not able to fully align themselves when under stress, resulting in lower tensile strength. On the other hand, when the membranes were wetted with PBS for 12 days, their mechanical strength was reduced considerably (figure 15b). Both the membranes with nanoparticles and those without nanoparticles behaved identically, and could not bear a tensile stress of more than 0.07 MPa. The reduction in mechanical strength of the membranes after 12 days shows that the membranes are degrading to some degree.



a. Tensile strength of dry collagen membranes



b. Tensile strength of collagen membranes wetted in PBS for 12 days

Figure 15. Tensile Strength of Collagen Membranes

4.4 Chromatographic Analysis of Lidocaine

As shown in figure 16, a standard linearity curve between area under the peak and concentration of lidocaine in µg/ml, with R-square value of more than 0.99 was drawn by running 40, 100, and 200 µg/ml standards. Concentration of the drug in the samples was calculated by interpolation from this standard curve.

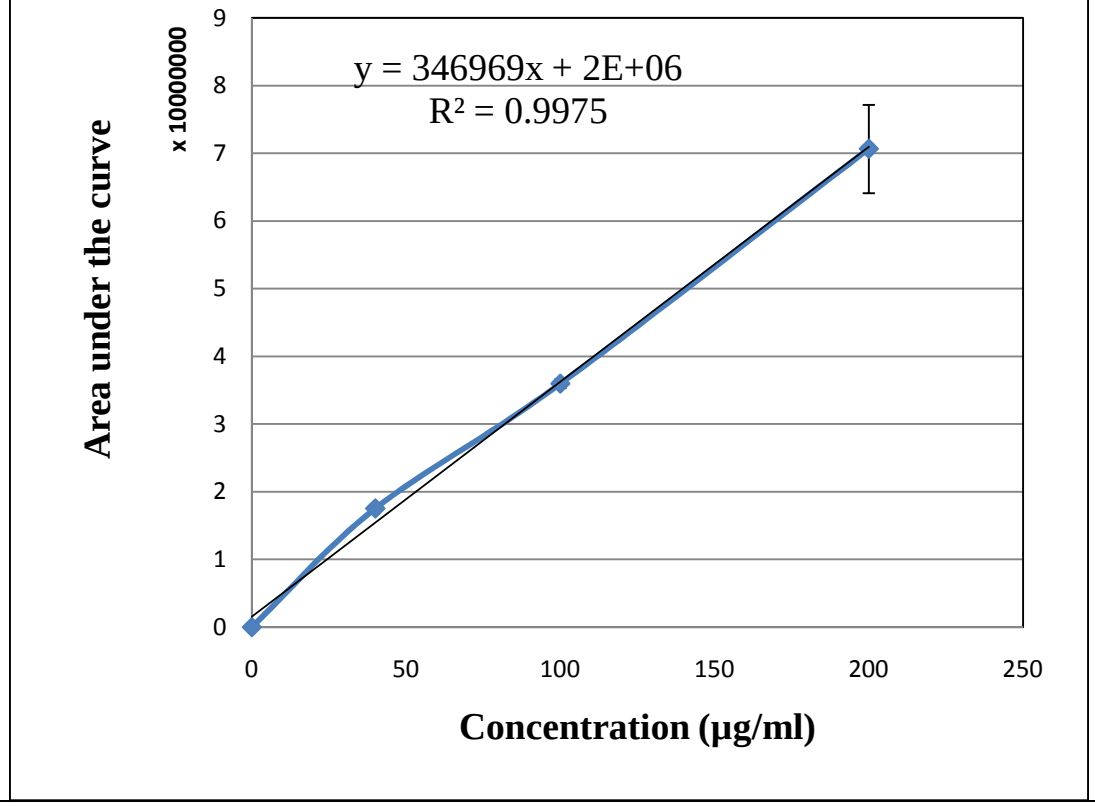


Figure 16. Standard Linearity Curve for Lidocaine Analyzed on GC (Agilent 6890). Data shown are mean values ± sd; n=3

4.5 Drug Stability

The drug, lidocaine, was found to be stable in both the scenarios tested: (a) at 37 °C in the presence of PBS and (b) after interaction with ethyl acetate. The results of the thermal stability test, as shown in figure 17, indicate that at an elevated temperature of 37 °C and in the presence of PBS solution, lidocaine does not degrade or decompose. At all time points, the concentration of lidocaine remained 1000 µg/ml ± 8%, which was within the experimental error range.

The FTIR spectrum produced from ethyl acetate-treated lidocaine showed identical peaks as untreated lidocaine. There was a slight peak mismatch at wavenumber of 2360, but it is believed to be from CO_2 contamination from air. This is shown in figure 18a, which is an overlap spectrum achieved by using "compare" function of the Perkin Elmer's FTIR machine. The overall match between the two spectra from the "compare" function was higher than 95%. This proved that the lidocaine molecule does not change structurally after treatment with ethyl acetate. It also confirmed that the results of the drug release study are not affected by the stability of the drug. The spectra in figures18b and 18c represent background from KBr pellet and lidocaine reference standard respectively.

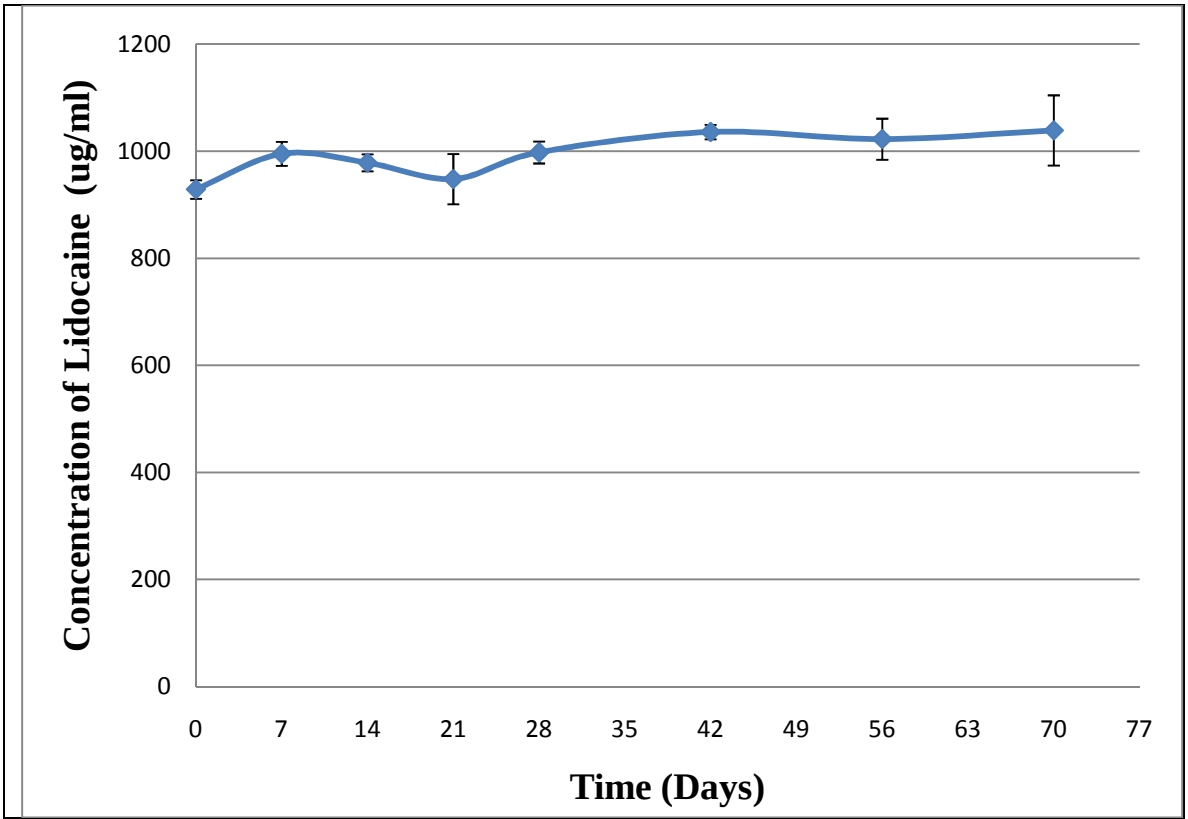
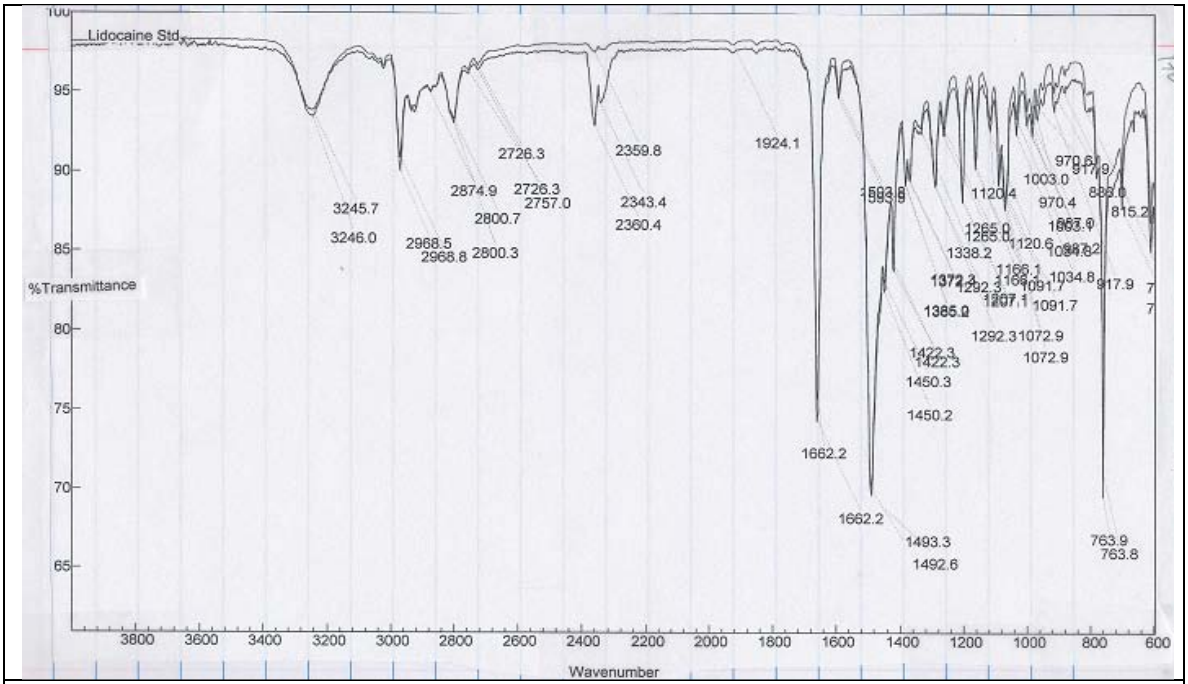
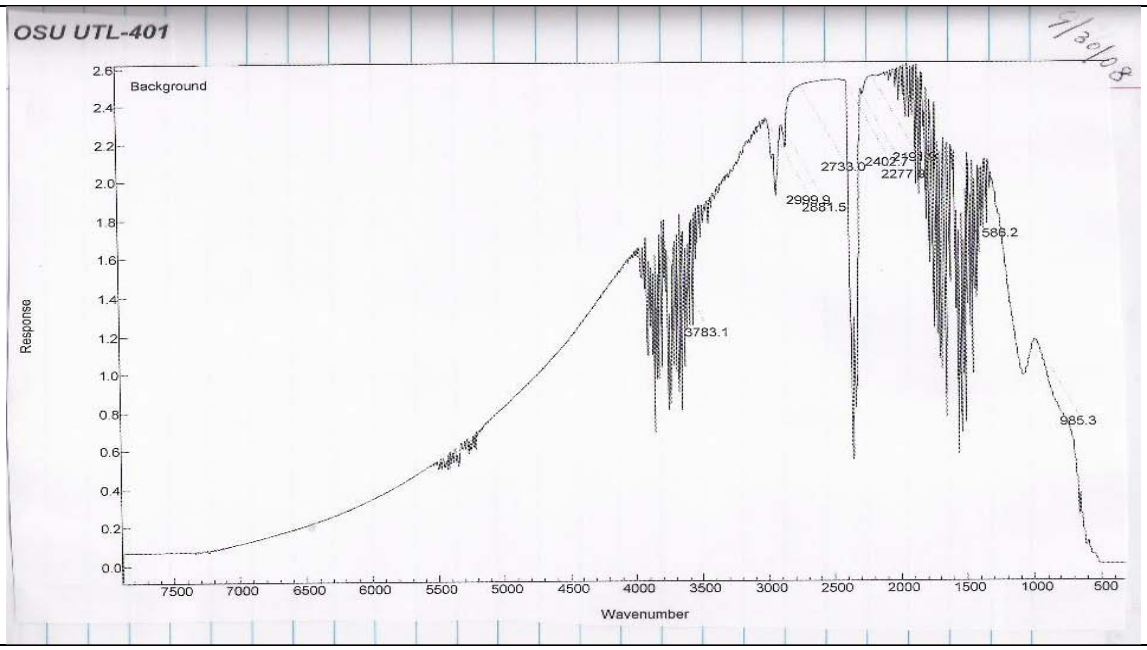


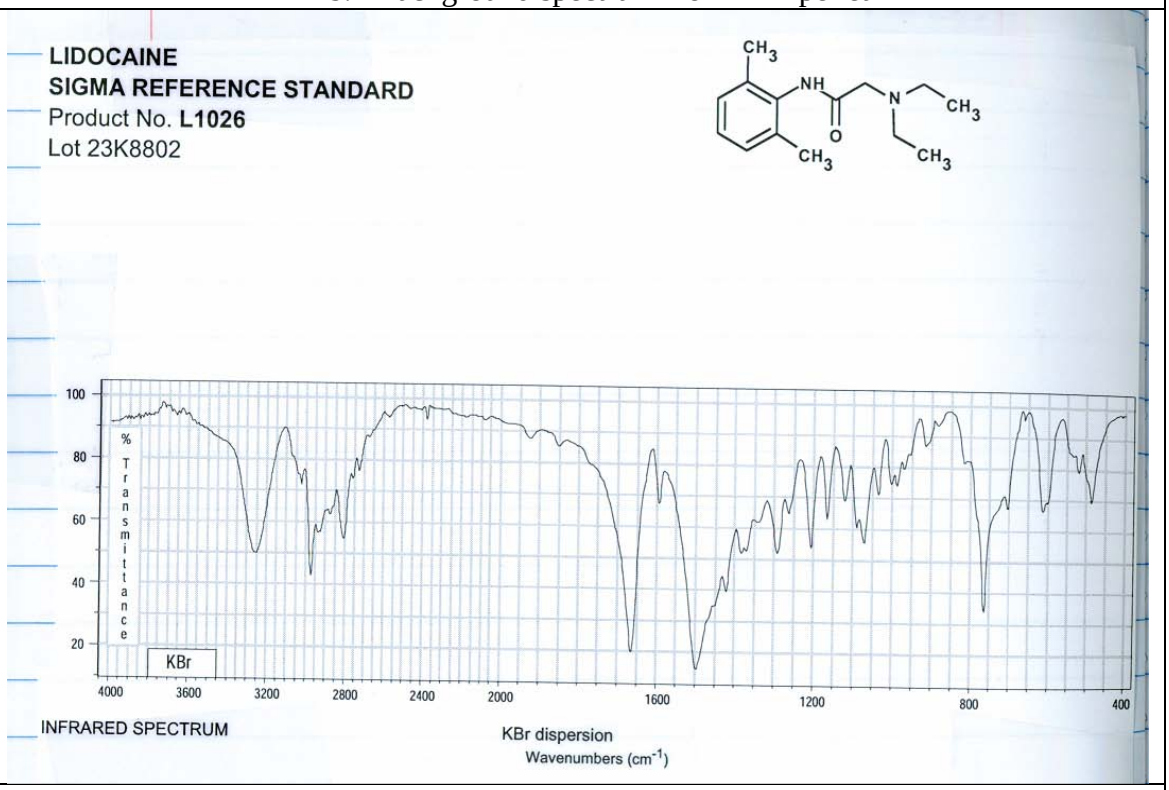
Figure 17. Drug Stability in PBS at 37 °C. Data shown are mean values ± sd; n=3



a. Comparison of IR spectra of standard lidocaine with ethyl acetate treated lidocaine



b. Background spectrum from KBr pellet



c. Lidocaine reference standard from Sigma Aldrich

Figure 18. FTIR Spectrum of (a) Lidocaine Sample Compared to Ethyl Acetate-Treated Lidocaine Sample, (b) KBr Background Sample, and (c) Lidocaine Reference Standard from Sigma-Aldrich.

4.6 Drug Loading

The percent (weight of drug / weight of nanoparticles) of lidocaine loaded in the nanoparticles, was measured to be 5.17% ± 0.88%. Based on 2.253 mg of nanoparticles per collagen membrane, the concentration of drug per membrane, in the units of µg of drug / membrane, was theoretically calculated to be 116.63 ± 17.12.

4.7 Drug Release

The *in vitro* drug release from nanoparticles and collagen membranes was studied by monitoring the increase in the concentration of the drug in a constantly stirred beaker over time. The drug release profile from collagen membranes containing nanoparticles was compared to nanoparticles only and to collagen membranes containing an equivalent amount of drug but no nanoparticles (figure 19). In the case of nanoparticles only, a burst release of drug was noted. Within one hour of the release experiment, all the drug adsorbed to the surface of nanoparticles was released and percent drug release rose to 41.8%. Within 12 hours of the release experiment, the percent drug release rose to 53.6 % and remained at that level until 48 hours. A possible explanation could be that the drug reached its solubility limits in aqueous media and could not diffuse any more. After that the nanoparticles seem to erode and the process of dissolution took over diffusion, and the concentration rose to 64% at 168 hours time point. In case of membranes containing drug but no nanoparticles, the percent drug release rose to only 3.1% in the first three hours and reached a maximum of 8.6% in 24 hours. Such a low drug release could be attributed to the drug interacting either physically or chemically with the collagen membrane, thus preventing it from being released into the aqueous environment. The

drug release from collagen membrane containing drug-loaded nanoparticles reached 16.2 % in one hour and then steadily increased to 23.4% over the next seven days. This kind of drug release profile is highly desired from any sustained release drug delivery device because it helps in quickly reaching the therapeutically effective level and then sustaining it for extended period.

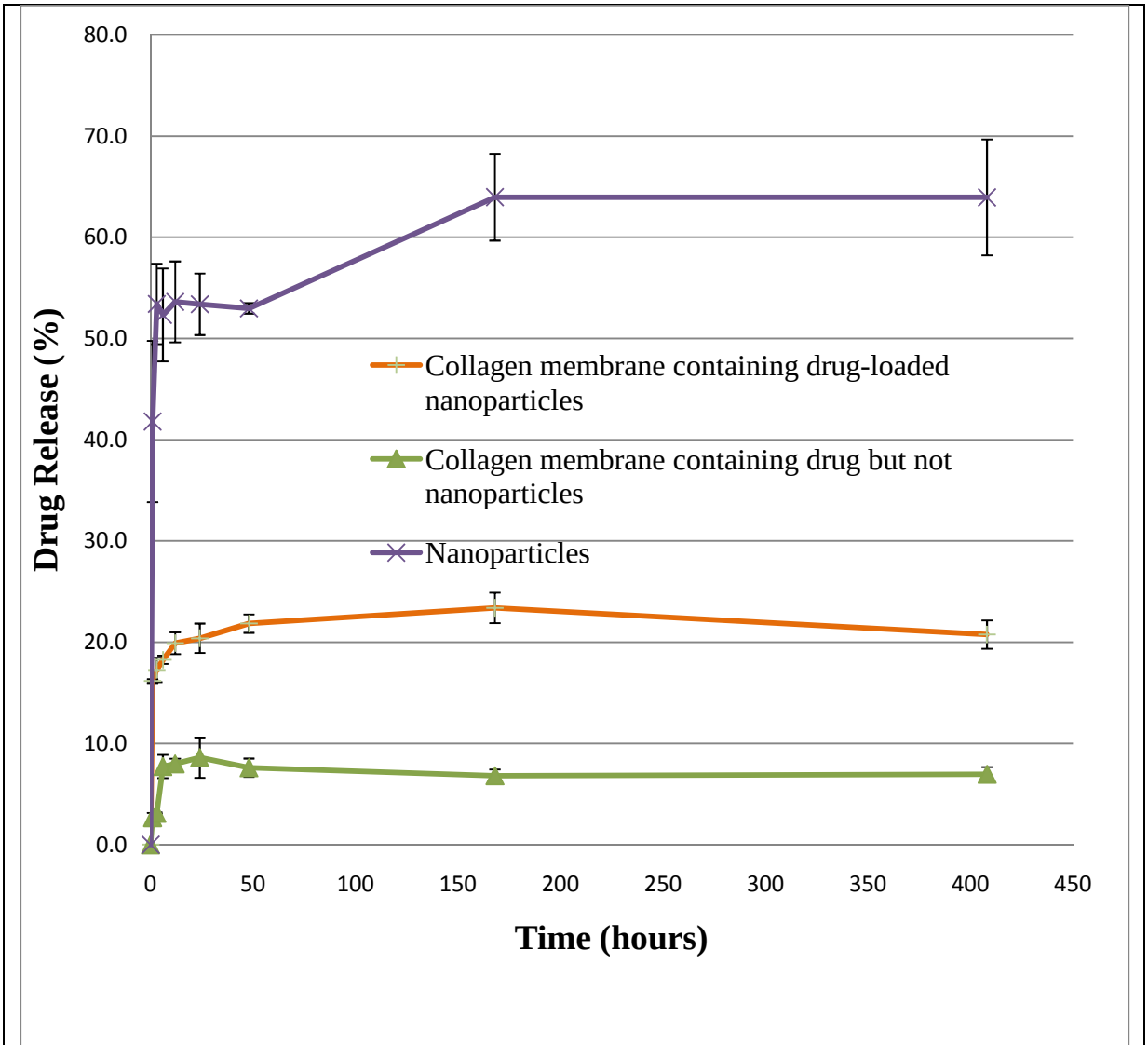


Figure 19. Comparison of Drug Release Profiles from Three Different Systems. Data shown are mean values ± sd; n=3.

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTIONS

5.1 CONCLUSIONS

The main objective of this research was to develop an ocular drug delivery device that is capable of consistently delivering the drug over an extended period. The proposed design consisted of a collagen membrane embedded with lidocaine-loaded PLGA nanoparticles. The drug release profile of nanoparticles only, showed a burst release of drug. The drug release profile of a collagen membrane loaded with drug showed a low level of the drug released over the time period tested. Only the collagen membranes loaded with nanoparticles showed a significant release of drug without the large initial burst release.

The physical characteristics of the device indicate a feasible commercialization of the device in the future. The device can be loaded with a hydrophobic or hydrophilic drug with equal ease. It can be stored in a dry place and wetted just before insertion into the eye. Although, the tensile strength of the membrane seems to be high enough to withstand the shear stress due to eye-lid movement, it will only be accurately determined after *in vivo* studies. The light transparency of the membrane was found to be slightly lower than commonly used contact lens but whether or not this transparency is acceptable to patients will only be determined after *in vivo* experiments. To some extent transparency can be controlled by controlling the number of nanoparticles per collagen membrane. The diameter of the membrane can be varied according to the need by varying the amount of collagen.

In comparison with other already existing technologies, this device can be expected to deliver the drug for a longer period without any invasive procedure and with minimal drug wastage. Collagen membrane embedded with nanoparticles not only overcomes all the limitations of eye-drops, but also inherits minimal side effects that are associated with other drug delivery devices. Collagen membranes do not blur the vision like ophthalmic gels do. They can be used to deliver hydrophobic or hydrophilic drugs with equal ease. They can be stored in a dry environment for a long time, unlike some nanoparticle formulations. They do not require a surgery to be placed inside the eye like most of the ocular inserts.

5.2 Future Directions

A study to determine drug-loading of collagen membranes containing nanoparticles and and of collagen membranes containing equivalent amount of drug would be highly recommended in the future. It will increase our confidence in the percent drug release results. In the present study the percent drug release results were based on theoretical drug loading, which was calculated from the amount of nanoparticles loaded onto collagen shield and drug loading onto nanoparticles.

In the future, *in vivo* testing of this device will help in predicting (a) the *in vivo* drug release profile (b) the level of eye-irritation after wearing the device (c) the acceptability of compromised light- transparency (d) the oxygen and carbon dioxide permeability through the lens-membrane system. Developing a mathematical model to predict the drug release through these membranes will help in optimizing some of the key parameters like size of nanoparticles, number of nanoparticles per membrane etc. The drug release model could be based on dissolution, diffusion, or both.

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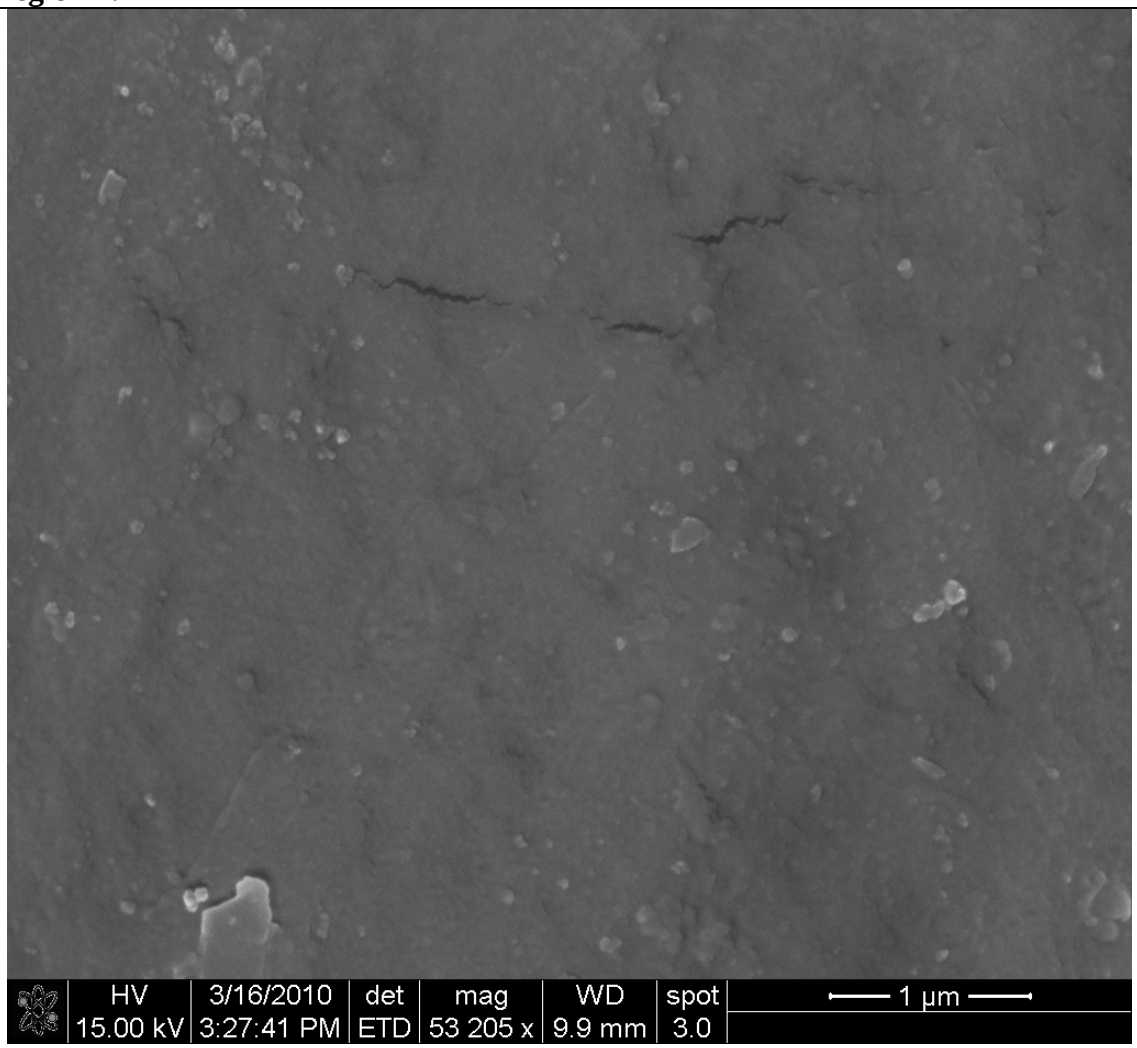
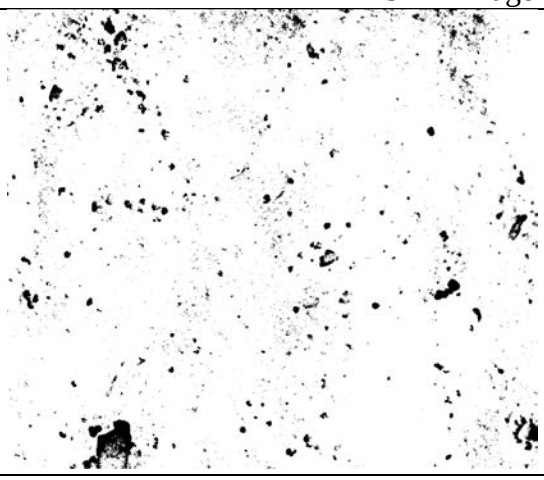
Appendix A

SIZE DISTRIBUTION REPORT FROM IMAGEJ

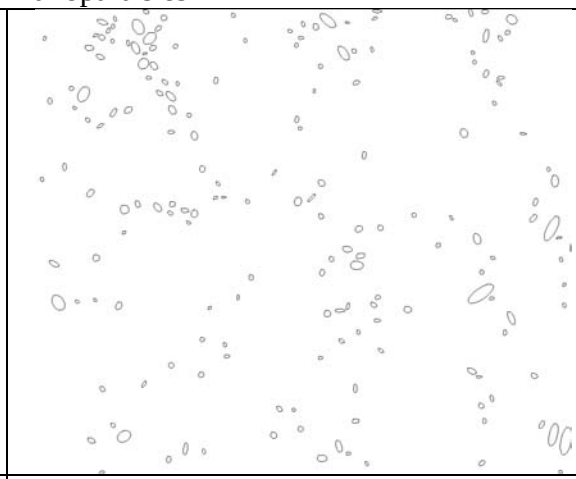
Region 1.



SEM image of nanoparticles



SEM image of nanoparticles after processing by ImageJ



Perimeter representation of the particles by ImageJ

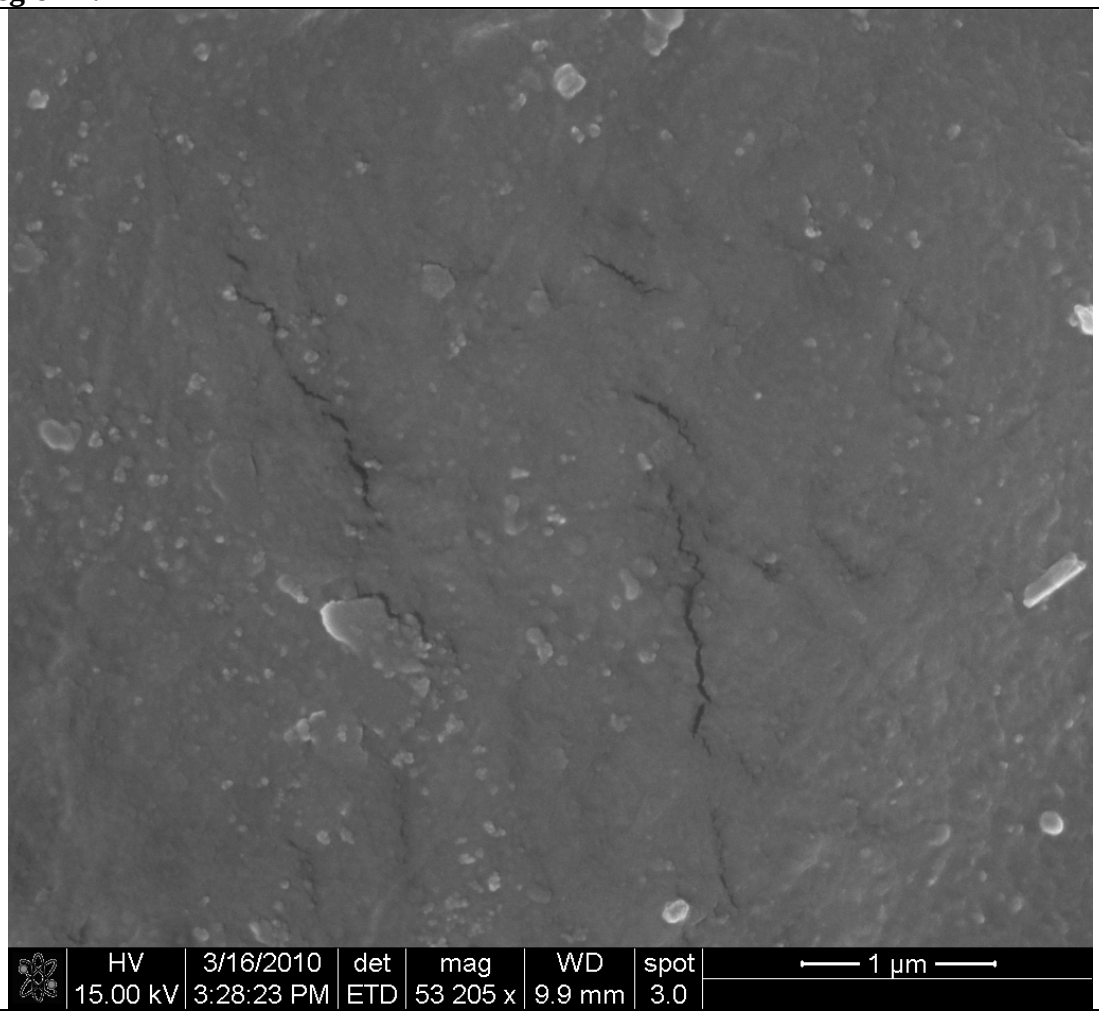
Region 1 Contd…

Size Distribution of nanoparticles:

Area	Dia	Count	Dia * Count
1023	36	94	3384
2394	55	39	2145
3765	69	21	1449
5136	81	7	567
6507	91	4	364
7878	100	2	200
9249	109	2	218
10620	116	1	116
11991	124	2	248
13362	130	1	130
14733	137	2	274
16104	143	2	286
17475	149	0	0
18847	155	0	0
20218	160	0	0
21589	166	1	166
22960	171	0	0
24331	176	1	176
25702	181	0	0
27073	186	1	186
	Total =	180	9909

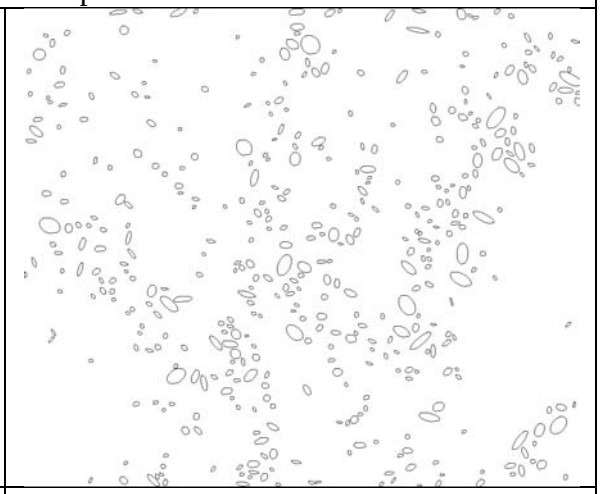
Mean Diameter = 55.05

Region 2.



SEM image of nanoparticles

SEM image of nanoparticles after processing by ImageJ



Perimeter representation of the particles by ImageJ

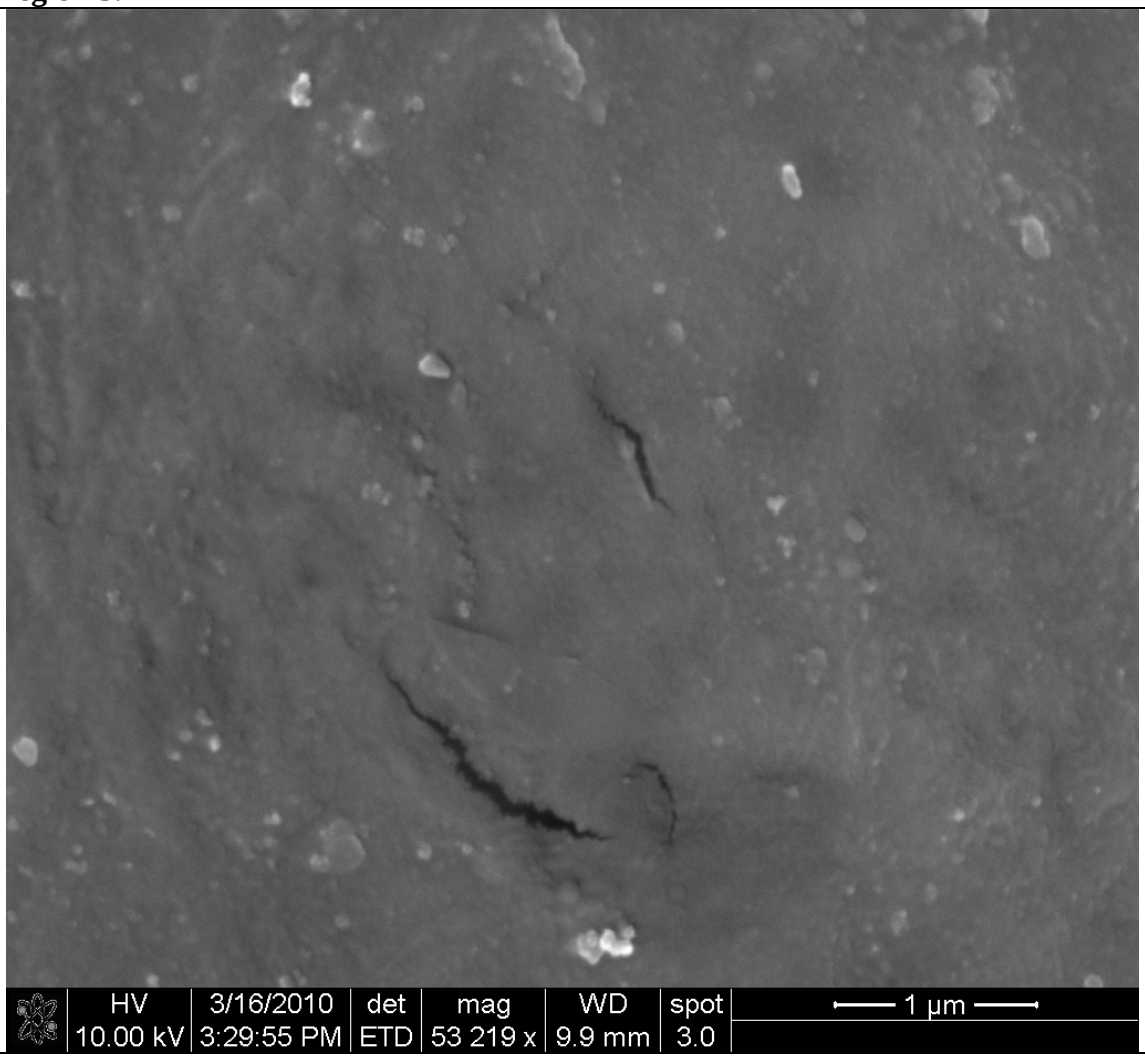
Region 2. Contd…

Size Distribution of nanoparticles:

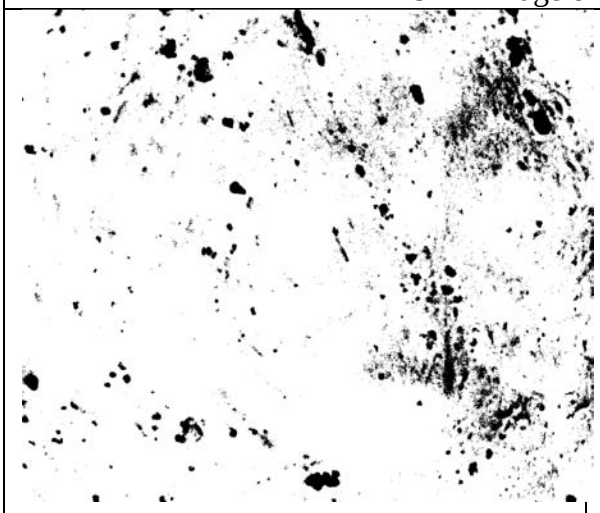
Area	Dia	Count	Dia * Count
1023	36	235	8460
2423	56	78	4368
3824	70	38	2660
5224	82	22	1804
6625	92	19	1748
8025	101	12	1212
9426	110	8	880
10826	117	5	585
12227	125	4	500
13627	132	2	264
15028	138	2	276
16428	145	0	0
17829	151	0	0
19229	157	3	471
20630	162	4	648
22030	168	1	168
23431	173	0	0
24831	178	2	356
26232	183	1	183
27632	188	1	188
	Total =	437	24771

Mean Diameter = 56.68421

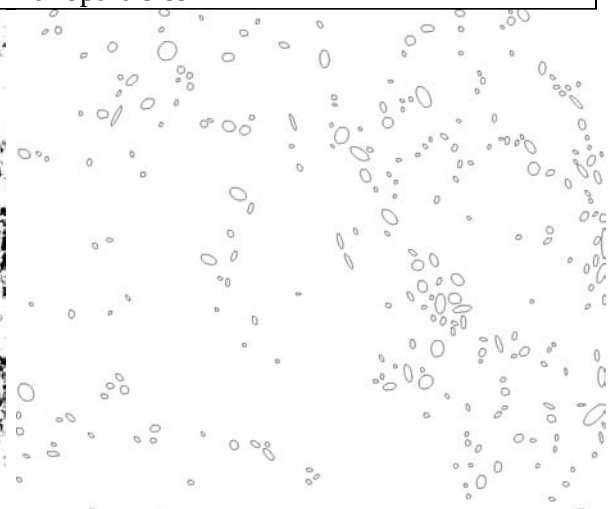
Region 3.



SEM image of nanoparticles



SEM image of nanoparticles after processing by ImageJ



Perimeter representation of the particles by ImageJ

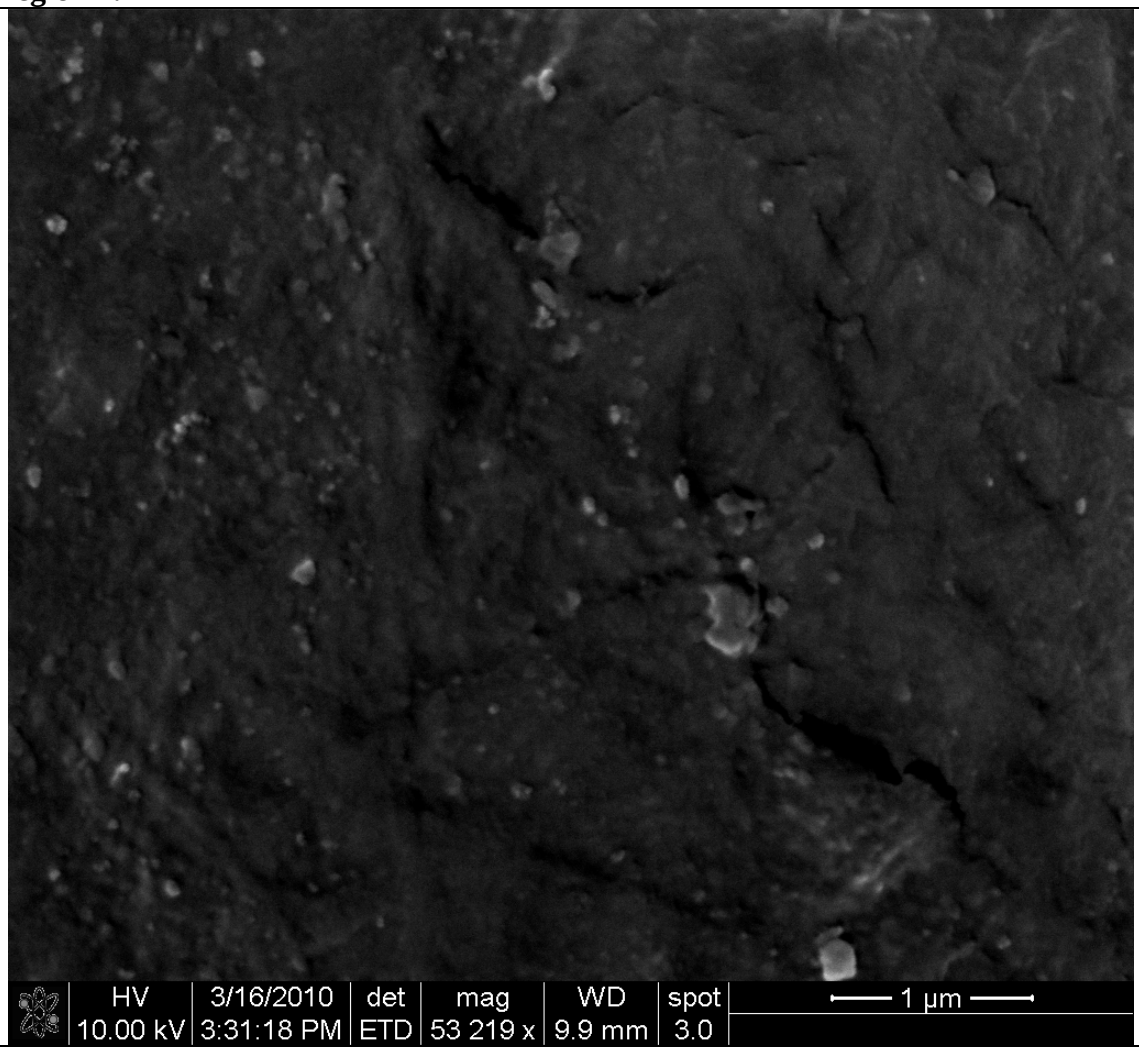
Region 3. Contd…

Size Distribution of nanoparticles:

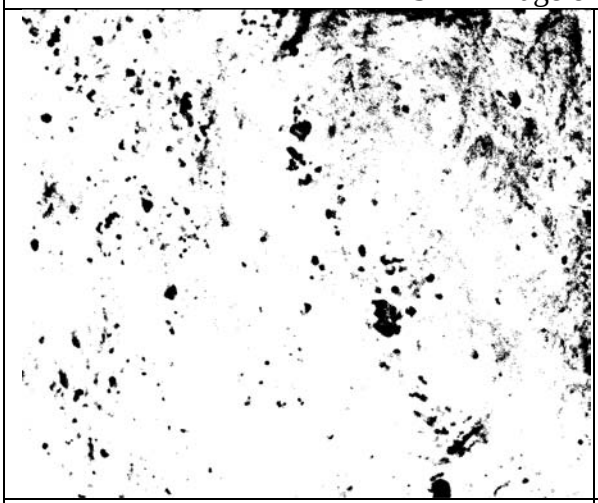
Area	Dia	Count	Dia * Count
1023	36	128	4608
2229	53	40	2120
3436	66	25	1650
4643	77	16	1232
5850	86	12	1032
7057	95	7	665
8264	103	7	721
9471	110	7	770
10677	117	3	351
11884	123	1	123
13091	129	2	258
14298	135	3	405
15505	141	1	141
16712	146	1	146
17919	151	0	0
19125	156	2	312
20332	161	0	0
21539	166	0	0
22746	170	1	170
23953	175	1	175
	Total =	257	14879

Mean Diameter = 57.89494

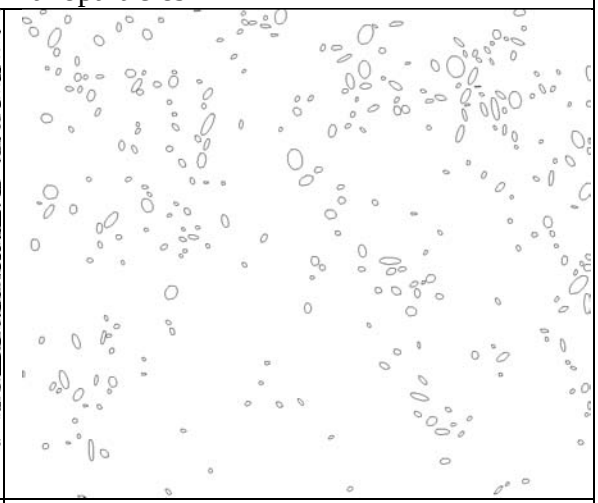
Region 4.



SEM image of nanoparticles



SEM image of nanoparticles after processing by ImageJ



Perimeter representation of the particles by ImageJ

Region 4. Contd…

Size Distribution of nanoparticles:

Area	Dia	Count	Dia * Count
1023	36	147	5292
2437	56	54	3024
3851	70	28	1960
5266	82	18	1476
6680	92	15	1380
8095	102	10	1020
9509	110	3	330
10924	118	5	590
12338	125	1	125
13753	132	3	396
15167	139	5	695
16582	145	0	0
17996	151	0	0
19410	157	0	0
20825	163	1	163
22239	168	1	168
23654	174	1	174
25068	179	0	0
26483	184	0	0
27897	189	1	189
	Total =	293	16982

Mean Diameter = 57.95904

Appendix B

SIZE DISTRIBUTION REPORT FROM DYNAMIC LIGHT SCATTERING

Batch 1.

Size Distribution Report by Intensity

Sample Details

Sample Name: Nanoparticles Batch 1
SOP Name: Manual measurement settings
General Notes: nanoparticles mixed in water

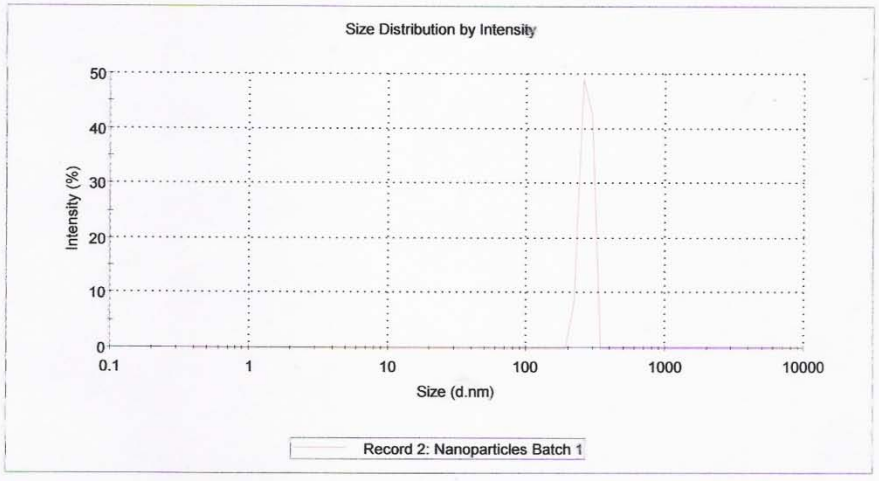
File Name: RM-174-TTA-nC60-1.dts
Record Number: 2
Material RI: 1.45
Material Absorbtion: 0.01
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Friday, January 15, 2010 4:1...

System

Temperature (°C): 25.0
Count Rate (kcps): 334.4
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 2.00
Attenuator: 7

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 1520	Peak 1:	269	100.0	24.3
PdI: 1.000	Peak 2:	0.00	0.0	0.00
Intercept: 0.940	Peak 3:	0.00	0.0	0.00



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Serial Number : MAL060051

File name: RM-174-TTA-nC60-1.dts
Record Number: 2
15 Jan 2010 4:13:56 PM

Batch 1 contd…

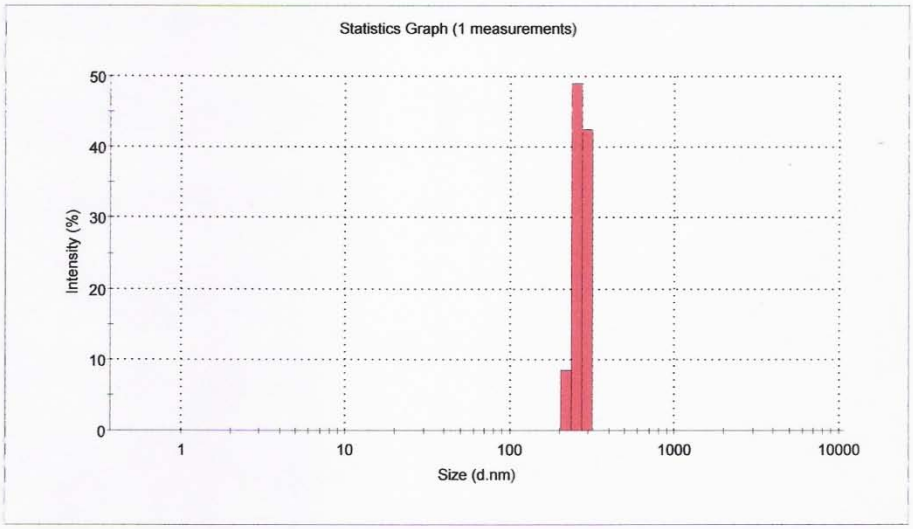
Size Statistics Report by Intensity

Sample Details

Sample Name: Nanoparticles Batch 1
File Name: RM-174-TTA-nC60-1.dts
SOP Name: Manual measurement settings
Measurement Date and Time: Friday, January 15, 2010 4:10:01 PM

Z-Average (nm):	1515.04	Derived Count Rate (kcps):	29300.5840415...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0		5.61	0.0		78.8	0.0		1110	0.0	
0.463	0.0		6.50	0.0		91.3	0.0		1280	0.0	
0.536	0.0		7.53	0.0		106	0.0		1480	0.0	
0.621	0.0		8.72	0.0		122	0.0		1720	0.0	
0.719	0.0		10.1	0.0		142	0.0		1990	0.0	
0.833	0.0		11.7	0.0		164	0.0		2300	0.0	
0.965	0.0		13.5	0.0		190	0.0		2670	0.0	
1.12	0.0		15.7	0.0		220	8.4		3090	0.0	
1.29	0.0		18.2	0.0		255	49.0		3580	0.0	
1.50	0.0		21.0	0.0		295	42.6		4150	0.0	
1.74	0.0		24.4	0.0		342	0.0		4800	0.0	
2.01	0.0		28.2	0.0		396	0.0		5560	0.0	
2.33	0.0		32.7	0.0		459	0.0		6440	0.0	
2.70	0.0		37.8	0.0		531	0.0		7460	0.0	
3.12	0.0		43.8	0.0		615	0.0		8630	0.0	
3.62	0.0		50.7	0.0		712	0.0		1.00e4	0.0	
4.19	0.0		58.8	0.0		825	0.0				
4.85	0.0		68.1	0.0		955	0.0				



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Serial Number : MAL060051

File name: RM-174-TTA-nC60-1.dts
Record Number: 2
15 Jan 2010 4:14:37 PM

Batch 2.

Size Distribution Report by Intensity

Sample Details

Sample Name: Nanoparticles Batch 2
SOP Name: Manual measurement settings
General Notes: nanoparticles mixed in water

File Name: RM-174-TTA-nC60-1.dts
Record Number: 4
Material RI: 1.45
Material Absorbtion: 0.01
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Friday, January 15, 2010 4:3...

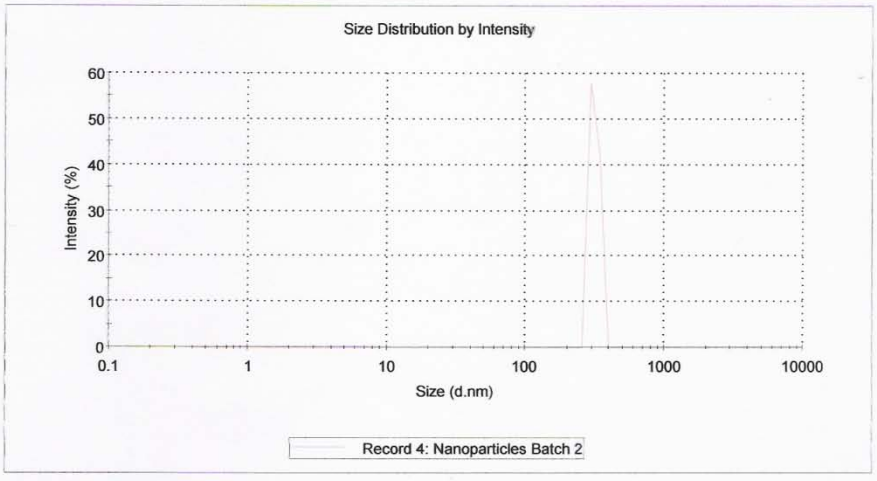
System

Temperature (°C): 25.0
Count Rate (kcps): 412.0
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 2.00
Attenuator: 8

Results

Z-Average (d.nm): 7060
PdI: 0.704
Intercept: 0.837

	Diam. (nm)	% Intensity	Width (nm)
Peak 1:	315	100.0	23.1
Peak 2:	0.00	0.0	0.00
Peak 3:	0.00	0.0	0.00



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DTS Ver. 4.20
Serial Number : MAL060051

File name: RM-174-TTA-nC60-1.dts
Record Number: 4
15 Jan 2010 4:37:25 PM

Batch 2 Contd…

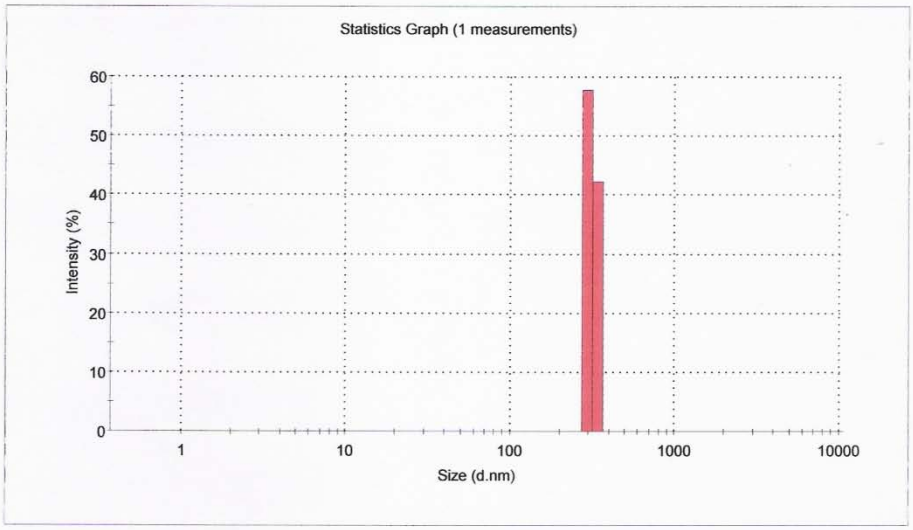
Size Statistics Report by Intensity

Sample Details

Sample Name: Nanoparticles Batch 2
File Name: RM-174-TTA-nC60-1.dts
SOP Name: Manual measurement settings
Measurement Date and Time: Friday, January 15, 2010 4:33:38 PM

Z-Average (nm):	7061.495	Derived Count Rate (kcps):	13615.4138858...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0		5.61	0.0		78.8	0.0		1110	0.0	
0.463	0.0		6.50	0.0		91.3	0.0		1280	0.0	
0.536	0.0		7.53	0.0		106	0.0		1480	0.0	
0.621	0.0		8.72	0.0		122	0.0		1720	0.0	
0.719	0.0		10.1	0.0		142	0.0		1990	0.0	
0.833	0.0		11.7	0.0		164	0.0		2300	0.0	
0.965	0.0		13.5	0.0		190	0.0		2670	0.0	
1.12	0.0		15.7	0.0		220	0.0		3090	0.0	
1.29	0.0		18.2	0.0		255	0.0		3580	0.0	
1.50	0.0		21.0	0.0		295	57.8		4150	0.0	
1.74	0.0		24.4	0.0		342	42.2		4800	0.0	
2.01	0.0		28.2	0.0		396	0.0		5560	0.0	
2.33	0.0		32.7	0.0		459	0.0		6440	0.0	
2.70	0.0		37.8	0.0		531	0.0		7460	0.0	
3.12	0.0		43.8	0.0		615	0.0		8630	0.0	
3.62	0.0		50.7	0.0		712	0.0		1.00e4	0.0	
4.19	0.0		58.8	0.0		825	0.0				
4.85	0.0		68.1	0.0		955	0.0				



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DTS Ver. 4.20
Serial Number : MAL060051

File name: RM-174-TTA-nC60-1.dts
Record Number: 4
15 Jan 2010 4:38:00 PM

Batch 3.

Size Distribution Report by Intensity

Sample Details

Sample Name: Nanoparticles Batch 3
SOP Name: Manual measurement settings
General Notes: nanoparticles mixed in water

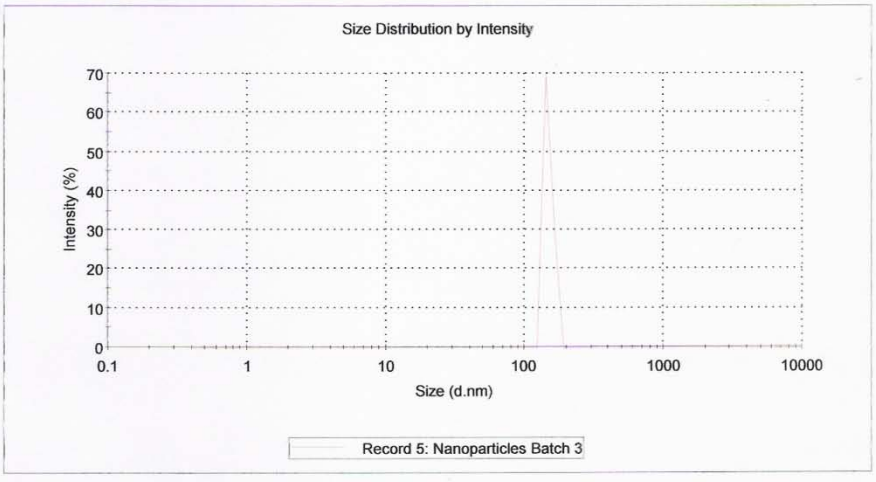
File Name: RM-174-TTA-nC60-1.dts
Record Number: 5
Material RI: 1.45
Material Absorbtion: 0.01
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Friday, January 15, 2010 4:4...

System

Temperature (°C): 25.0
Count Rate (kcps): 162.6
Cell Description: Disposable sizing cuvette
Duration Used (s): 70
Measurement Position (mm): 2.00
Attenuator: 8

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 6160	Peak 1:	149	100.0	10.3
PdI: 1.000	Peak 2:	0.00	0.0	0.00
Intercept: 0.930	Peak 3:	0.00	0.0	0.00



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DTS Ver. 4.20
Serial Number : MAL060051

File name: RM-174-TTA-nC60-1.dts
Record Number: 5
15 Jan 2010 4:46:48 PM

Batch 3 Contd…

Size Statistics Report by Intensity

Sample Details

Sample Name: Nanoparticles Batch 3
File Name: RM-174-TTA-nC60-1.dts
SOP Name: Manual measurement settings
Measurement Date and Time: Friday, January 15, 2010 4:46:03 PM

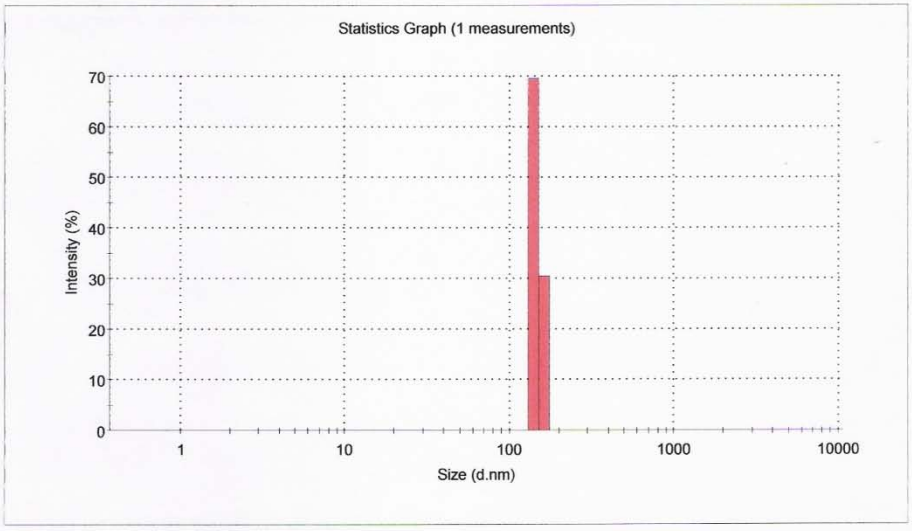
Z-Average (nm):	6161.682	Derived Count Rate (kcps):	5372.18680350...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0	
0.463	0.0	
0.536	0.0	
0.621	0.0	
0.719	0.0	
0.833	0.0	
0.965	0.0	
1.12	0.0	
1.29	0.0	
1.50	0.0	
1.74	0.0	
2.01	0.0	
2.33	0.0	
2.70	0.0	
3.12	0.0	
3.62	0.0	
4.19	0.0	
4.85	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
5.61	0.0	
6.50	0.0	
7.53	0.0	
8.72	0.0	
10.1	0.0	
11.7	0.0	
13.5	0.0	
15.7	0.0	
18.2	0.0	
21.0	0.0	
24.4	0.0	
28.2	0.0	
32.7	0.0	
37.8	0.0	
43.8	0.0	
50.7	0.0	
58.8	0.0	
68.1	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
78.8	0.0	
91.3	0.0	
106	0.0	
122	0.0	
142	69.6	
164	30.4	
190	0.0	
220	0.0	
255	0.0	
295	0.0	
342	0.0	
396	0.0	
459	0.0	
531	0.0	
615	0.0	
712	0.0	
825	0.0	
955	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
1110	0.0	
1280	0.0	
1480	0.0	
1720	0.0	
1990	0.0	
2300	0.0	
2670	0.0	
3090	0.0	
3580	0.0	
4150	0.0	
4800	0.0	
5560	0.0	
6440	0.0	
7460	0.0	
8630	0.0	
1.00e4	0.0	



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DTS Ver. 4.20
Serial Number : MAL060051

File name: RM-174-TTA-nC60-1.dts
Record Number: 5
15 Jan 2010 4:46:41 PM

VITA

Munish Sharma

Candidate for the Degree of

Master of Science

Thesis: DEVELOPMENT OF A NOVEL NANOTECHNOLOGY-BASED OPHTHALMIC DRUG DELIVERY DEVICE

Major Field: Chemical Engineering

Biographical:

Citizenship: India

Education:

Completed the requirements for the Master of Science in Chemical Engineering at Oklahoma State University, Stillwater, Oklahoma in May 2010.

Completed the requirements for the Bachelors of Technology in Chemical Engineering at Punjab Technical University, Punjab, India in 2000.

Experience:

- Design experiments to be conducted at various stages of the research i.e.
  - Stage 1: Synthesis of drug-loaded nanoparticles and collagen matrix,
  - Stage 2: Characterization of nanoparticles, and collagen membranes for drug release, drug loading, particle size and morphology, tensile strength, and light absorbance.
- Troubleshoot and Brainstorm to overcome the obstacles by reviewing the Literature and works of other scientists working on the same the topic.
- Write protocols to synthesize and characterize the nanoparticles, and collagen membranes.
- Set up, install and troubleshoot a number of analytical and formulation equipment like HPLC, GC, GC-MS, Lyophilizer, Centrifuge, and Ultra-Sonifier.
- Develop and validate methods to run analytical equipment like GC, GC-MS, and SEM.
- Organize and compile experimental data for advisor review and formal publication submission.

Professional Memberships:

Member, American Institute of Chemical Engineers (AIChE).

Name: Munish Sharma Date of Degree: July 2010

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: DEVELOPMENT OF A NOVEL NANOTECHNOLOGY-BASED OPHTHALMIC DRUG DELIVERY DEVICE

Pages in Study: 64 Candidate for the degree of Master of Science

Major Field: Chemical Engineering

Topical delivery by eye drops, which accounts for approximately 90% of all ophthalmic formulations, is extremely inefficient. Only approximately 5% of the drug applied as drops penetrates the outer layer of the eye and reaches the target, while the rest is lost due to tear drainage. A number of conditions like glaucoma, conjunctivitis, and proliferative retinopathy need a sustained release of drug inside the eye for drug to be therapeutically effective. In addition to eye drops, other ocular drug delivery systems include drug-loaded liposomes or nanoparticles and drug-loaded contact lenses (hydrogels). Particle drug delivery systems also have difficulty penetrating the outer layer of the eye and can be lost before the drug can be released. Contact lens delivery systems often show a burst release with poor release over long periods of time.

To improve sustain delivery of drugs to the eye, we have designed a novel system that includes drug-loaded nanoparticles suspended within a thin, membrane that can be attached to a standard commercial-grade contact lens for support. The lens system will provide constant contact with the eye's surface and the particles will supply a continuous release of medication, resulting in more drug reaching the target. First, PLGA nanoparticles, synthesized by the emulsion solvent evaporation technique, are loaded with the hydrophobic drug lidocaine. Next, the nanoparticles are loaded within a thin, collagen membrane that can be stored dried, and later rehydrated and attached to a contact lens for delivery to the eye.

Drug loading and drug release rates must be controlled within the novel delivery system in order to ensure that therapeutic levels of the drug are delivered safely to the eye. The PLGA nanoparticles were found to be irregular in shape, 247 nm in diameter, and contained 5.17 wt% of lidocaine. The nanoparticles loaded into the collagen membrane did not have a significant effect on light transmittance through the membrane, compared to a commercially available contact lens. For initial drug release in the first 48 hours, the complete lens system, nanoparticles only, and collagen membrane only released 21.8%, 53.0% and 7.6% of the initial drug loading, respectively. For the first 400 hours tested, the complete lens system, nanoparticles only, and collagen membrane only released 20.8%, 63.9 and 7.0% of the initial drug loading, respectively.

ADVISER'S APPROVAL: Dr. Heather Fahlenkamp