NOVEL CONTACT LENS SURFACE DEPOSITION USING CYSTEINE GRAPHENE OXIDE & SILVER NANOCLUSTERS

A Thesis submitted during 2025 to the university of Hyderabad in partial fulfilment of the award of

Doctor of Philosophy in Optometry and Vision Sciences.

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CERTIFICATE

This is to certify that the thesis entitled **"Novel contact lens surface deposition using cysteine graphene oxide & silver nanoclusters"** submitted by **Ms. Mukkaragari Krupa, Registration number: 17MOPH02** to University of Hyderabad, Hyderabad, towards award of the **Degree of Doctor of Philosophy** from University of Hyderabad, is a Bonafede work carried out by him under my guidance. The contents in this thesis in full or in part have not been submitted to any other institute or University for award of any degree or diploma.

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DECLARATION

I, **Ms. Mukkaragari Krupa**, hereby declare that this thesis entitled "**Novel contact lens surface deposition using cysteine graphene oxide & silver nanoclusters"** submitted by me under the guidance and supervision of **Dr. Nagaraju Konda, Associate Professor** is an original and independent research work. I also declare that it has not been submitted previously in part or in full to this University or Institution for the award of any degree or diploma.

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1. ***Krupa Mukkaragari***, Konda Nagaraju, Pratap Kollu; Synthesis and Characterization of Cysteine Graphene Oxide for antimicrobial studies. *Invest. Ophthalmol. Vis. Sci.* 2023;64(8):1342.

B. Presented in the following conferences:

1. Presented poster titled "Synthesis of cysteine graphene oxide" at International Conference on Frontier Areas of Science and Technology (ICFAST-2022) conference at University of Hyderabad, Hyderabad.
2. Presented a poster titled "Synthesis and deposition of silver nanoclusters on contact lenses" at Frontiers in Physics – 2023 conference at University of Hyderabad, Hyderabad.
3. Oral presentation of "Synthesis and characterisation of cysteine graphene oxide for antimicrobial studies" at The Association for Research in Vision and Ophthalmology-(ARVO-2023) conference, New Orleans, USA. (International travel grant received from ARVO and IoE)

Further, the student has passed the following courses towards the fulfilment of the coursework requirement for Ph.D:

Serial number	**Course code**	**Subjects**	**Credits**	**Pass/fail**
1	OV801	Research methodology including epidemiology.	6	Pass
2	OV802	An overview of biostatistics.	1	Pass
3	OV803	Health communication and introduction.	1	Pass
4	OV804	Qualitative research in health sciences.	2	Pass

5	OV805	Bio and research ethics	1	Pass
6	OV806	Research in special circumstances.	1	Pass

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THIS THESIS IS DEDICATED

TO

GOD, MY FAMILY

AND

MY SUPERVISOR

ACKNOWLEDGEMENT

Research is an exciting trip to discover new knowledge in which we explore at the ever-shifting borders of the known and unknown. Like with any other endeavours, this cannot be completed fruitfully unless we are constantly guided and encouraged. In my opinion, one's ambitions are more likely to be realized with the support and prayers of those closest to him as well as with his own determination, perseverance, hard work and desire. In this moment, when I am so close to accomplishing a landmark milestone, I want to take a moment to express my gratitude to the countless people who have played a role in my success. Because I was so excited and passionate throughout this journey, I was blessed with a lot of amazing and enlightening moments. Now that I've reached the finish line, it feels nice to look back on the journey and express my sincere gratitude to everyone who assisted me by making the experience one I will never forget.

I would like to convey my heartfelt gratitude and admiration to my research supervisor, **Dr. Nagaraju Konda,** for his persistent support, thoughtful direction, attentive advice, and warm encouragement during my research; without all of these, I would not have been able to attain my goal. His enormous scientific intuition, passion for science, and focus on research have made him an oasis of innovative ideas, which drove us and individually inspired me as a student, researcher, and better human being I aspire to be. He has not only taught me the value of accuracy in scientific observation, but also fostered in me the need for discipline, balance, and a scientific orientation. I've always attempted to follow in his excellent organization, meticulous planning, and perseverance, as well as her dedication to science. The opportunity you provided me to join your research work is quite fortunate for me. Thank you, sir, for your patience with my inquiries and mistakes and for providing me with a pleasant environment and first-rate facilities so that I could finish the current research. Right now, I feel incredibly privileged and blessed to have the opportunity to train with and learn from you.

Sir, I am motivated by your enthusiasm, dedication to science, vigor, and velocity in creating and putting scientific techniques into practice—all of which underpin our work.

I would like to thank my co-supervisor, **Dr. Rishi Bhardwaj (late)** for providing constant support and encouragement in a laboratory work space, and insightful suggestions whenever I have required.

I am grateful to my Research Advisory committee members, **Dr. Pratap Kollu, Dr. Rajanikanth, and Dr. Tushar Jana** for their perceptive comments and recommendations throughout my doctoral research. I would like to express my sincere thanks to Dr. Pratap, for taking the time to carefully educating me with the synthesis techniques in your lab, starting from scratch. I am grateful to Dr. Rajanikanth for the collaboration, resources and the valuable inputs he has provided.

I would like to thank **Prof., Geeta Kashyap Vemuganti, Dean, and entire faculty in School of Medical Sciences, Prof. BR Shamanna, Dr. Athar Siddiqui, Dr. Ajitha K, Dr. CT Anita, Dr. M Varalakshmi, Dr. Mahadev Kalyankar, Dr, Surya, Dr. Shiva Ram and Dr. Roopa Reddy** for all the stimulating multidisciplinary discussions and contributions to my research. I am appreciative of the assistance from the Medical Sciences non-teaching staff, including **Mr. Murthy, Mrs. Beena, Mr. Jayanth, Mr. Shivakumar, Ms. Meher, Mrs. Karimunissa, and Mr. Mallesh**, who kindly assisted me with all the formalities throughout the program and the laboratory staff who assisted with the upkeep of the instruments and facilities.

I want to express my gratitude to **Prof. BJ Rao,** Vice chancellor and **Prof. Apparao Podile** former Vice chancellor of University of Hyderabad, for all the assistance provided to me as I pursued my doctoral studies there. I am very much grateful to the staff of the administration

office, controller of examination, eGov and library for facilitating a smooth processing of all the formalities of the programme.

I am extremely grateful to ESI medical college for providing me the opportunity to learn the microbiology techniques and I extend my gratitude to **Dr. Joveeta Joseph** for providing microbial cultures from the microbiology department, L.V. Prasad Eye Institute for allowing me to use their resources.

I am immensely grateful to my esteemed PhD colleagues- **Pravalika, Venkat, Naresh, Annapurna and Narayana** who have been an indispensable source of support and encouragement throughout this arduous journey. Their unwavering dedication, camaraderie, and collaboration have made this pursuit of knowledge truly fulfilling. Further, I am very much thankful to my lab colleagues- **Dr. Asra, Dr. Ananth, Sony and Vennela** for their support and camaraderie throughout my doctoral journey. Their presence has made the research lab feel like a second home. I would like to extend my heartfelt thanks to my wonderful friends in Medical Sciences, Physics and life sciences -**Navya, Dinesh, Karthik, Sharon, Sasirekha, Shenoy, Vidya, David, Ramitha, Pranav and Sandhya -** for being an amazing source of fun and joy throughout my academic journey. I am grateful for the laughter, humour, and shared moments of relaxation that we experienced together. Amidst the pressures of our academic pursuits, your friendship has been a refreshing escape, allowing us to unwind and find solace in each other's company.

I extend my heartfelt appreciation to the funding agencies- Australian Alumni Grant Scheme-2022, for their generous financial assistance and Institute of Eminence, University of Hyderabad, ARVO international travel grant and IoE international travel grant which helped me in travelling to USA to present my work in a prestigious conference like The Association for Research in Vision and Ophthalmology (ARVO). Their belief in the importance of research

and investment in my work have empowered me to undertake meaningful studies and undertake projects that have the potential to bring about positive change.

Last but not least, I want to express my deepest gratitude to my loving family (**Mom, Dad and Brother**). Throughout my PhD journey, they have been my unwavering source of support and love.

Mukkaragari Krupa

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LIST OF ABBREVIATIONS AND SYMBOLS

Mg: Milligrams

mL: Milli litres

M: Moles

Cm: Centimetres

%: Percentage

µm: Micro metre

ºC: Degree centigrade

D: Dimension

µL: Micro litres

G: Gram

CHAPTER 1

INTRODUCTION

Contact lenses (CL) are used by about 140 million people worldwide to treat refractive abnormalities such as astigmatism, hyperopia, and myopia[i & ii]. Contact lenses are also used for therapeutic, and cosmetic purposes[iii & iv]. The Food, and Drug Administration (FDA) in the United States of America (USA) regulates contact lenses as optical devices[v]. The end user of the lenses demands comfortable wearing, durability, ease of handling, vision stability, and no complications, etc. It guides the researchers to focus on improving lens materials and surface coatings for better performance. Researchers focus on modifying contact lens materials that pave the way for new lenses, innovations, and applications[vi]. In recent times, graphene materials like graphite oxide (GtO), graphene oxide (GO), reduced graphene oxides (rGO), and also silver particles which are widely used in human health monitoring applications, specifically as wearable and implantable devices[vii & viii].

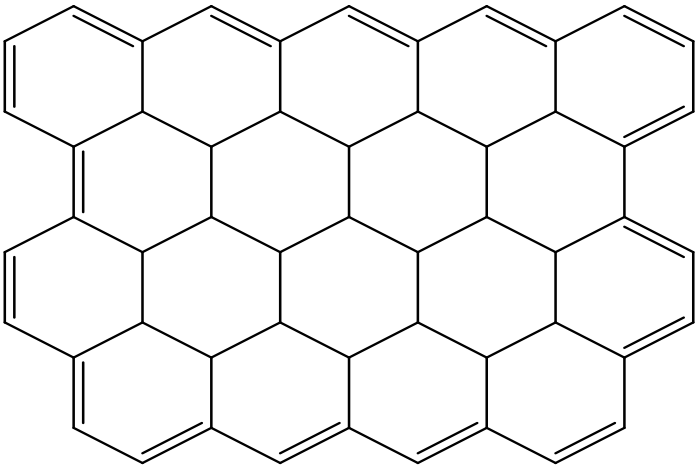
1. GRAPHENE BASED MATERIALS:

A 2D substance called graphene has hexagonal carbon atoms arranged in a honeycomb pattern. Each carbon atom in graphene has one pure Pz electron and experiences sp2 hybridization. Because of this, it has remarkable electrical qualities and is the thinnest substance ever discovered on earth[ix & x]. Geim and colleagues won the Nobel Prize in 2010 for developing graphene, a 2D sheet-like substance that demonstrated the significance of carbon nanomaterials' transformative potential[xi]. Graphene material has received great interest because of its outstanding physicochemical and structural properties[xii & xiii]. Graphene has properties that make it attractive for applications, particularly for its transparency, high carrier mobility, flexibility, and strength, which shows that graphene has potential for implementation in a range of functional sensors, optoelectronics, composites and so on ix.

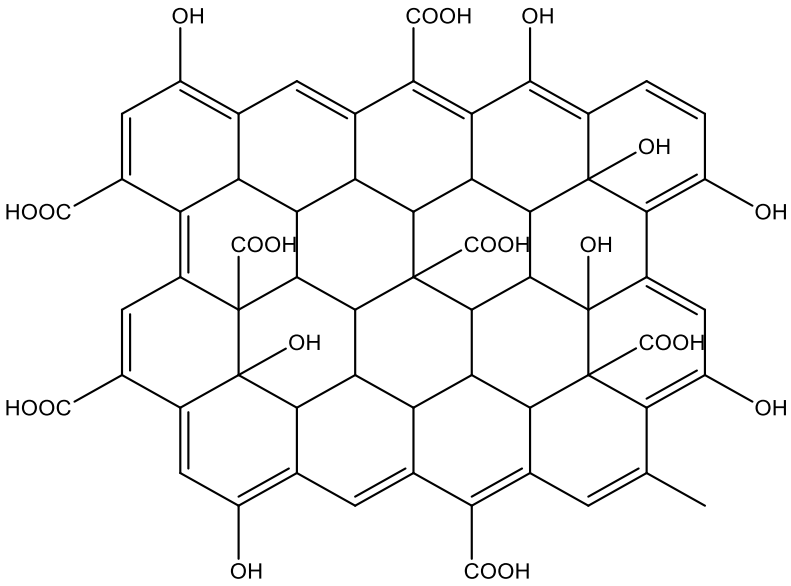
Graphene and its derivatives (fig.1(a-c)), such as graphite oxide, graphene oxide, and reduced graphene oxide have significantly increased the utilization of carbon-based compounds in the biomedical field. The distinctive chemistry of graphene derivatives includes the presence of functional groups like hydroxyls (OH), carboxylic acids (COOH), and epoxides (COC) on its surface. Graphene materials may be coupled with a variety of biomolecules, particularly reduced-graphene oxide and graphene oxide, to expands its uses in biomedical research[xiv].

Because of their large π-conjugated aromatic structure and high specific surface area, GBMs could be employed in ocular applications, particularly in sensors and ocular administration systems[xv]. Recently, with advances in micro/nanofabrication electronics, and information technology research in wearable devices has emerged and been studied for the past few years, these advancements resulted in the practical applications including smart watches[xvi & xvii], fitness trackers[xviii & xix], augmented reality (AR) glasses[xx & xxi] and health care monitoring applications[xxii & xxiii]. Due to its ultrathin layers and mechanical flexibility of carbon materials and they are used in sensors for human health monitoring, especially as implantable devices and wearable sensors[xxiv]. In addition, it achieved a conformal and intimate contact with organs like the brain, skin and eyes which is important in obtaining high quality signals without causing contamination, irritation and motion artifacts[xxv & xxvi & xxvii]. For a variety of uses, including wireless wearable technology for the diagnosis and treatment of diabetes and intraocular pressure (IOP), graphene materials have been integrated into contact lens materials[xxviii & xxix].

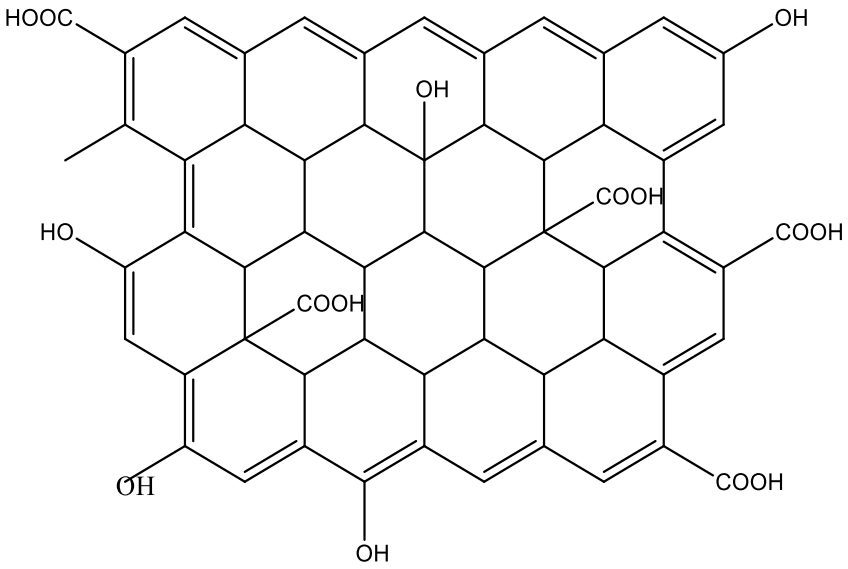
Fig.1 (a) Graphene



(b) Graphene oxide



(c) Reduced graphene oxide (Source: Chem Draw software).



The suitable physicochemical properties of carbon derivatives can be advantageous for biomedical applications such as drug delivery, biosensing, and imaging[xxx & xxxi]. Graphene-based materials have gained great interest in ocular therapeutic delivery and targeting[xxxii & xxxiii].

1.1. OCULAR APPLICATIONS:

Graphene oxide laden CL have been used for controlled release of bimatoprost and cyclosporine with the improvement in the swelling property (107.9 ± 1.0 % and 124.5 ± 3.9 %) as the key aspect which may impact comfort while wearing contact lenses is the percentage of swelling or the water content of the lenses. Furthermore, any change to contact lenses' swelling characteristics may have an impact on their wettability as well as their permeability to ions and oxygen[xxxiv & xxxv]. Graphene oxide contact lenses, silver nanoparticles, and quaternized chitosan (HTCC) have demonstrated antifungal action against fungal keratitis. The

in vitro antimicrobial activity of HTCC/Ag/GO/Vor hydrogel matrix has shown higher antimicrobial activity compared to antibiotics used alone against *E. coli, S. aureus, F. solani* and *A. fumigatus*. HTCC/ Ag/GO CLs did not inhibit the corneal epithelial cells growth, and reduced the infiltered inflammatory cells[xxxvi]. Graphene oxide loaded onto contact lens with hyaluronic acid (HA) has shown high HA tear fluid concentration and increased tear fluid volume when compared to the eye drop solution[xxxvii].

Polydimethylsiloxane (PDMS) elastomer loaded with graphene oxide has shown significant improvement in elastic module and hydrophilicity with the growth of the retinal cells and tight junction formations[xxxviii]. Graphene transferred onto contact lenses enhances electromagnetic wave (EMW) absorption and reduces dehydration[xxxix].

Therefore, graphene-based materials demonstrate excellent potential for drug delivery, electromagnetic wave absorption, and dehydration protection while maintaining good biocompatibility in combination with contact lens materials. However, further optimization of the concentration of GBMs were needed. Summary of therapeutic applications of all the graphene-based structures in contact lens materials are given in table .1

Table. 1 Therapeutic application of graphene structures contact lenses.

Graphene based materials and drug loaded	**Contact lens material**	**Functions**	**References**
GO +bimatoprost	HEMA	Controlled bimatoprost release	xxxiv

GO+ cyclosporine	HEMA	Controlled release of cyclosporine	xxxv
GO+HTCC+Ag+ Vor	PHEMA	Sustained release of Vor and efficacy in antifungal activity	xxxvi
GO+HA	HEMA	Corneal epithelial healing	xxxvii
GO	PDMS	Retinal pigment epithelial cells growth	xxxviii
Graphene	PMMA	Electromagnetic wave (EMW) absorption and dehydration	xxxix

Glaucoma is a chronic ocular disease condition which progressively damages the optic nerve. It is the second leading cause of blindness in the world[xl]. The currently used instrument for the recording of IOP is the Goldman applanation tonometer. It only gives temporal values that do not follow up IOP variations over. For early detection of IOP, continuous monitoring for 24 hrs is necessary. So, there is a need for a simple, low-cost, and continuous monitoring device for IOP[xli].

Graphene as a sensor on PDMS-parylene-based contact lens substrate was used for IOP detection, with a slight reduction in the transparency of 5%[xlii]. graphene woven fabric (GWF) employing contact lens tonometer have more than 80% transparency[xliii].

These graphene materials incorporated contact lens IOP sensors shown good transparency and sensitivity in detecting IOP variations, however, these contact lens sensors are not free of limitations, so there is a need for more clinical trials on humans. Summary of graphene and graphene-based structures in contact lens materials in diagnostic applications are given in table. 2

Table.2. Graphene based CLs in IOP detection.

Graphene based structures	**Contact lens material**	**Functions**	**References**
Graphene	PDMS	IOP monitoring	[xlii]
Graphene nano walls	PDMS	IOP monitoring	[xliii] & [xliv]
Graphene woven fabric (GWF)	CL	IOP monitoring	[xlv]
Graphene+AgNWs	PDMS	Glucose and IOP detection	[xlvi]
Graphene	ELASTOFILCON A	Glucose detection from tears	[xlvii]

1.2.OTHER APPLICATIONS:

Soft graphene contact lens electrodes (GRACEs) have been applied in full cornea recording of electroretinography, demonstrating good optical transparency, and have shown to have a highly conformal and tighter interface with the cornea[xlviii]. Graphene coated textile electrodes for electrooculogram acquisition[xlix]. Graphene cortisol CL sensor cortisol concentration range in human tears is (1 to 40 ng/ml)[l]. Lee, M. J., *et al*. manufactured an ophthalmic lens containing 2-hydroxy ethyl methacrylate (HEMA), poly vinyl pyrrolidone (PVP), Bis-GMA, GO nanoparticles and ethylene glycol dimethacrylate (EGDMA) in the presence of azobisisobutyronitrile (AIBN) were copolymerized with the the addition of the GO the wettability has increased because of the hydrogen bonding between the GO and water molecules with the increase in the amount amount of GO the water content decreased slightly but did not differ significantly and there is a decrease in the light transmittance in the blue light range and also there is a decrease in the contact angle, therefore there is a need to study the optimal amount of concentration of GO[li].

Graphene oxide also exhibits hydrophilic properties, it is also highly flexible[lii & liii & liv]. Functionalization of graphene oxide is a simple technique that can be employed to improve its application and minimize its detrimental consequences[lv]. It is readily dispersed and effectively functionalized[lvi]. It is simpler to functionalize graphene oxide using micromolecules as opposed to macromolecules, and the functionalized molecules usually preserve the beneficial characteristics of the unique nanomaterial that makes their use easier[lvii]. Therefore, scientists are developing methods for functionalising graphene-based materials with biocompatible moieties like poly (acrylic acid), dextran, and polyethene glycol (PEG). These surface alterations make it safer to use graphenic materials in medical applications[lviii]. Explicitly, in ocular research, GO, in combination with a few biomolecules, has shown remarkable results.

2. L – CYSTEINE HYDROCHLORIDE MONOHYDRATE:

The semi-essential amino acid, L-cysteine is produced by organisms, it is affordable, readily available, and biocompatible[lix & lx]. As a reducing and bridging agent, l-cysteine functionalizes graphene oxide, increasing the product's conductivity and improving it by four to five orders of magnitude[lxi]. When added to graphene oxide layers, the functionalized l-cysteine acts as a dispersion, reducing graphene oxide layer-stacking[lxii].

The biocompatibility study of functionalized l-cysteine-graphene oxide was investigated to assess the development of zebrafish embryos. When compared to embryos exposed to graphene oxide alone, the results demonstrated that cysteine graphene oxide (CysGO) did not cause any malformations during embryonic development. CysGO have also effectively protected embryos from arsenic poisoning which can be used as a breathable coating for biomedical application[lxiii]. There is no sufficient data regarding the biocompatibility of l-cysteine – graphene oxide and no studies on antimicrobial activity of l-cysteine- graphene oxide composite but the antimicrobial activity of graphene oxide studies alone.

3. MICROBIAL KERATITIS:

Microbial keratitis is one such type that occurs when microbes invade the cornea; these microbes are bacteria, fungi, viruses, parasites or amoeba[lxiv]. The causes of contact lens-related infections are by using extended-wear contact lenses, sleeping with contact lenses, not maintaining hygiene of the lenses or lens cases or reusing the lenses[lxv]. The types of microbial keratitis are bacterial keratitis, fungal keratitis, amoebic keratitis, viral keratitis[lxvi].

Bacterial keratitis is an ocular infection that leads to severe visual impairment. About 90% of infection is microbial keratitis and is associated with bacterial keratitis. The predominant risk factors of bacterial keratitis among CLs wearers are due to sleeping with CLs[lxvii] and formation of bacterial biofilm on CLs and CLs storage cases[lxviii]. The important clinical features reported

in bacterial keratitis are redness, eye pain, decreased visual acuity and stromal infiltration[lxix & lxx]. Organisms causing contact lens associated bacterial keratitis are, the gram-negative bacteria include *pseudomonas aeruginosa, serratia marcescens, serratia liquefasciens, stenotrophomonas maltophilia, alcaligenes xylosidans, shewanella putrefasciens, klebsiella oxytoca enterobacter cloacae etc*[lxxi & lxxii], and the gram-positive bacteria includes *staphylococcus aureus, streptococcus pneumoniae, staphylococcus pyogenes etc*[lxxiii]. Bacterial keratitis in contact lens wearers is mostly associated with gram negative bacteria than gram positive bacteria. The most affecting gram-negative bacteria are pseudomonas, Serratia, Acenetobacter Klebsiella spp and other species, and also the most affecting gram-positive bacteria are *staphylococcus, streptococcus spp* and others[lxxiv].

The contact lens related infections are prevented by proper handling, frequent cleaning of the lens and lens case with multipurpose contact lens cleaning solutions, by maintaining proper hygiene and wearing the CL for a prescribed period. Currently available treatment modalities for contact lens related microbial keratitis (MK) are topical application of antibiotic combinations are considered as gold standard in treating bacterial keratitis[lxxv & lxxvi]. Quinolonies and erythromycin are used in the treatment of gram-negative bacteria associated bacterial keratitis and aminoglycosides and erythromycin combination is used for treating gram positive bacteria associated bacterial keratitis[lxxvii]. Other drugs also used in treating bacterial keratitis are fluoroquinolones, chloramphenicol, ofloxacin, tobramycin, clindamycin and vacomycin [lxxviii & lxxix & lxxx]. Fungal keratitis is often treated with a combination of topical and systemic antifungal drugs such as topical amphotericin B solution[lxxxi], voriconazole[lxxxii], and antibiotics are aminoglycosides, cephalosporins[lxxxiii], natamycin, amphotericin B[lxxxiv], econazole, itraconazole and amphotericine[lxxxv]. Among the medications used to treat Acanthamoeba keratitis include metronidazole eye drops, topical neomycin-polymyxin B, oral ketoconazole[lxxxvi], moxifloxacin hydrochloride drops, amphotericin B drops, vancomycin

drops, propamidine isethionate ointment, amikacin drops[lxxxvii], prednisolone[lxxxviii] and other antibiotics such as polyhexamethylenebiguanide, clotrimazole and chlorhexidine[lxxxix] and benzalkonium chloride preserved saline and solutions containing thimerosal with adentate[xc]. Antiviral agents which are used in the treatment of viral keratitis are acyclovir, trifluridine, idoxuridine, vidarabine, bromovinyl deoxyuridine, foscarnet, and ganciclovir[xci].

These currently available treatments can be taken after discontinuation of the contact lens: orally, in the form of eye drops or ointment. The most popular way of preventing contact lens infections is by maintaining proper hygiene and frequent cleaning as we mentioned previously, due to their busy lives or laziness, people ignore the cleaning of the lenses and hygiene maintenance of the lenses and end up with severe ocular infections, therefore, this lead for the development of antimicrobial contact lens surface.

4. SILVER NANOPARTICLES:

Because of their special physical and chemical characteristics, silver nanoparticles (AgNPs) have found use in a variety of industries, including food, medicine, and industry[xcii & xciii & xciv]. AgNPs have been used in many applications like consumer products, medical device coating, cosmetics, optical sensors, antibacterial agents, diagnostics, and drug delivery[xcv]. AgNPs were utilized in ophthalmology to protect against perioperative and early postoperative infections by preventing the formation of biofilms of Pseudomonas aeruginosa and Staphylococcus aureus in corneal prosthetic devices (KPros)[xcvi]. There have been reports of using silver-amplified immunochromatography to identify adenoviral conjunctivitis[xcvii]. After trabeculectomy, AgNPs may be used as an adjuvant therapy to treat glaucoma in order to decrease fibroblast proliferation and bleb fibrosis[xcviii]. In rodent eye models of age-related macular degeneration and proliferative diabetic retinopathy. In order to prevent further angiogenesis pathways, silver nanoparticles have been shown to be an efficient therapeutic

agent against vascular endothelial growth factor (VEGF)[xcix & c & ci]. The incorporation of silver nanoparticles into silicone hydrogel (SiHy) lenses showed significant inhibition of biofilm formation[cii & ciii].

Need of the study:

Hence there is a need of such functionalized materials which are biocompatible and have excellent antimicrobial activity together for contact lens surface to protect the ocular surface from infections and causing no harm to the eye. Therefore, we have synthesised the material composites to enhance the contact lens properties and have addressed the infections caused by the contact lens wear.

Objectives:

In order to address the need, we have framed the following objectives

1. Synthesis and characterization of L cysteine-graphene oxide coated contact lenses.
2. Synthesis and characterization of silver nanoclusters deposited contact lenses.
3. Antimicrobial effect of synthesised and deposited L cysteine-graphene oxide and silver nanoclusters on contact lens surface.

CHAPTER 2

THE SYNTHESIS AND CHARCTERIZATION OF L-CYSTEINE GRAPHENE OXIDE DEPOSITED CONTACT LENSES.

1. INTRODUCTION

The two-dimensional substance makes up graphene is composed of a single thick atomic sheet of sp2 hybridization with hexagonal carbon atoms arranged in a honeycomb pattern[civ]. In the field of biomedicine, the use of graphene derivatives like graphene oxide, reduced-graphene oxide, and graphite oxide (GtO) has increased. Specifically, GO and rGO help graphene couple to different biomolecules, which increases its application in biological research[cv & cvi]. The GBMs have the potential to be used in ocular applications, particularly in sensors and drug delivery systems[cvii]. The GO application has been expanded to contact lens materials to track glucose levels and intraocular pressure (IOP)[cviii & cix]. GO has also shown enhanced drug loading and controlled release with improved lens swelling and surface contact angle due to its capacity to hold water[cx]. It has also recently demonstrated antibacterial efficacy when mixed with chitosan and silver nanoparticles in a hydrogel matrix than commercial antibiotics[cxi]. Graphene oxide can be functionalised more easily with micro molecules than with macromolecules. The beneficial qualities of the original nanomaterial are usually retained by the functionalized molecules, allowing for their use[cxii & cxiii & cxiv & cxv & cxvi]. Therefore, researchers are focusing on graphene-based materials functionalisation with biocompatible molecules for safer use in biomedical research.

The semi-essential amino acid L-cysteine is synthesized by organisms and is easily accessible, reasonably priced, and biocompatible[cxvii & cxviii]. The functionalized L-cysteine functions as a dispersion when it is incorporated into graphene oxide layers[cxix]. because L-cysteine functionalizes graphene oxide by acting as a reducing and bridging agent[cxx & cxxi]. L-cysteine reduces the layers of graphene oxide from stacking across each other. L-cysteine's approach is

extended to ocular applications as well, when administered orally has significantly healed corneal epithelium after excimer photoablation in mice model and showed faster corneal re-epithelization by reducing corneal haze in myopic patients subjected to photorefractive keratectomy (PRK) in the long-term recovery. For applications involving the human eye, such materials are therefore necessary[cxxii & cxxiii].

Contact lenses are used for vision correction, therapeutic, and cosmetic purposes[cxxiv & cxxv]. The end user of the lenses demands comfortable wearing, durability, ease of handling, vision stability, and no complications, etc. Researchers focus on modifying contact lens materials that pave the way for new lenses, innovations, and applications. Therefore, to investigate the effects of these biocompatible materials on the lenses for future uses, L-cysteine was immobilised on GO and deposited on the most commonly prescribed contact lens materials, such as hydrogel and silicone hydrogel lenses.

2. MATERIALS AND METHODS:

2.1. MATERIAL: single layer graphene oxide (SLGO) was purchased from Tokyo chemical industry co, LTD. Japan**,** L-Cysteine Hydrochloride Monohydrate was purchased from Sigma Aldrich, chloroacetic acid, sodium Hydroxide **(**NaOH**),** 1-ethyl-3- (3-dimethylaminopropyl)-carbodiimide (EDAC), N-hydroxysuccinimide (NHS), phosphate buffer solution (PBS), sodium acetate buffer, and sodium chloride (NaOH) were purchased from Sisco research laboratories Pvt. Ltd, Comfilcon A lenses (Biofinity Brand) and Etafilcon A contact lenses (Acuvue Brand) were used in this study.

2.2. METHODS

2.2.1. SYNTHESIS OF CYSGO: Using the following methodology, CysGO was synthesised. The carboxylic groups on GO are preferred for the immobilisation of L-cysteine. The method used to convert hydroxyl groups into carboxylic groups was modified from Hu X et al. work.

GO was suspended in water (2 mg/mL) and sonicated till one hour to get a homogenous suspension. To form carboxylic groups on GO nanosheets 3 g of NaOH and 2 g of chloroacetic acid was mixed with 100 mL of GO solution and it is sonicated for 2 hours. The NHS (0.5 M) and EDAC (0.2 M) were used to activate the carboxylic groups of GO (0.2 mg/mL) and sonicated for one hour. L-cysteine was mixed with GO suspension with a mass ratio of 1.0 of cysteine to GO for the immobilisation of L- cysteine and it is incubated overnight. The CysGO suspension was washed to remove unbound L-cysteine. Finally, the prepared CysGO suspension was lyophilized.

2.2.2. CHARACTERIZATION OF CYSTEINE GRAPHENE OXIDE

To characterise the synthesised CysGO and CysGO deposited CL's and control lenses the following techniques were used:

Field Emission Scanning Electron Microscope **(**FE-SEM): To assess the surface morphology the FE-SEM (Carl Zeiss AG - ultra-55, Germany,) measurements were performed; with system vacuum 2.64e-006mbar and gun vacuum 6.78e-010mbar and the beam energies used were SE2 and InLens. The lyophilized GO, cysteine, and the synthesised CysGO were sprinkled on the carbon tape which is covered over stubs for the imaging, and the CysGO deposited CL's and control lenses were cut into four pieces and placed on the carbon tape-covered stubs and sputter coated with gold for 2-3 mins to increase the conductivity and the chemical stability of the sample and then FE-SEM images of CL's were captured.

Fourier Transform Infrared spectroscopy (FTIR)**:** The FTIR (ID7 ATR, Nicolet IS5, Thermo Scientific; USA), was used to measure the infrared spectrum of the absorbance over the range of 500-3500 $cm^{-1}$with 32 Scans and 8 resolutions. The background data subtraction was applied in the FTIR instrument itself. To measure the spectra a small amount of GO, cysteine, and synthesised CysGO powder was placed on the sample holder.

Raman Spectroscopy (RS): To analyse the structural alterations the measurements were carried out on Raman Spectroscope (RS; HORIBA HR800, France), with 512nm excitation laser, 5 sec acquisition time, 2 sec accumulation time, and 1 sec real-time display. The lyophilized GO, cysteine, and synthesised CysGO were placed on the glass slide for the Raman spectra.

X-ray photoelectron spectroscopy (XPS): Chemical binding energy measurement was conducted on XPS (Model: AXIS SUPRA, C332905/01, UK). The XPS spectra was analysed using Casa-XPS V2.3.13 software, and Gaussian components were used to deconvolute the peaks following Shirley background subtraction. To run the spectra the synthesised CysGO powder was placed on the carbon tape covered over the stubs.

Ultraviolet-Visible- Near-Infrared Spectroscopy (UV-vis- NIR): The transmittance percentages were studied by UV-vis- NIR; Agilent Technologies, model: carry 5000, in the 200-800nm wavelength range. Baseline line spectra were taken on the blank lenses placed in a cuvette filled with saline and then subtracted from the deposited lens spectra. The CysGO deposited CL's were collected by placing them in a saline-filled cuvette.

Swelling percentage: To measure the swelling percentage, the CysGO deposited contact lenses (W_2) and a control contact lens were weighed (W_1). The Swelling percentage of the CL's were calculated by the following equation:

$$\text{Swelling } \% = \frac{W_2 - W_1}{W_1} \times 100$$

W_1 is the weight before deposition with CysGO, and W_2 is the weight after deposition with CysGO.

2.2.3. STATISTICAL ANALYSIS

The statistical test employed was the paired two sample t-test (assuming equal variance) using Excel.

2.2.4. METHOD FOR DEVELOPING CYSTEINE GRAPHENE OXIDE CONTACT LENSES

Comfilcon A & Etafilcon A CLs were used in this study. CL rinsed twice in a glass vial using 2 ml of sodium acetate buffer (pH 5.0) and once more using 2 ml of 1 x PBS (pH 7.4). After that, the CL was once again washed three times in a glass vial using two ml of PBS after being soaked in one ml of the final CysGO desired concentration. In 2ml of 10% NACL CL was soaked overnight.

The following day, the deposited CysGO contact lenses were cleaned in a glass vial using 2 ml of 1 x PBS (pH 7.4), and the CL was soaked in a glass vial using 3 ml of 1 x PBS (pH 7.4). The glass vial was then sealed with a silicone stopper, autoclaved (sterilized) in a glass beaker using aluminium foil wrap, and the glass vial was kept at room temperature.

3. RESULTS

3.1 RESULTS OF CysGO

FE-SEM was used to assess the morphology of the compounds, where Figure 1a shows the morphology of single flakes of GO, a sheet structure with a relatively large surface that is interlinked and well-defined. It has a porous network that resembles a loose sponge-like structure. The L-cysteine (Fig 1b) shows crystal structures, whereas synthesized CysGO (Fig 1c) shows the morphology of a GO sheet and has an additional irregular structure, indicating the surface characteristics of a synthesized CysGO.

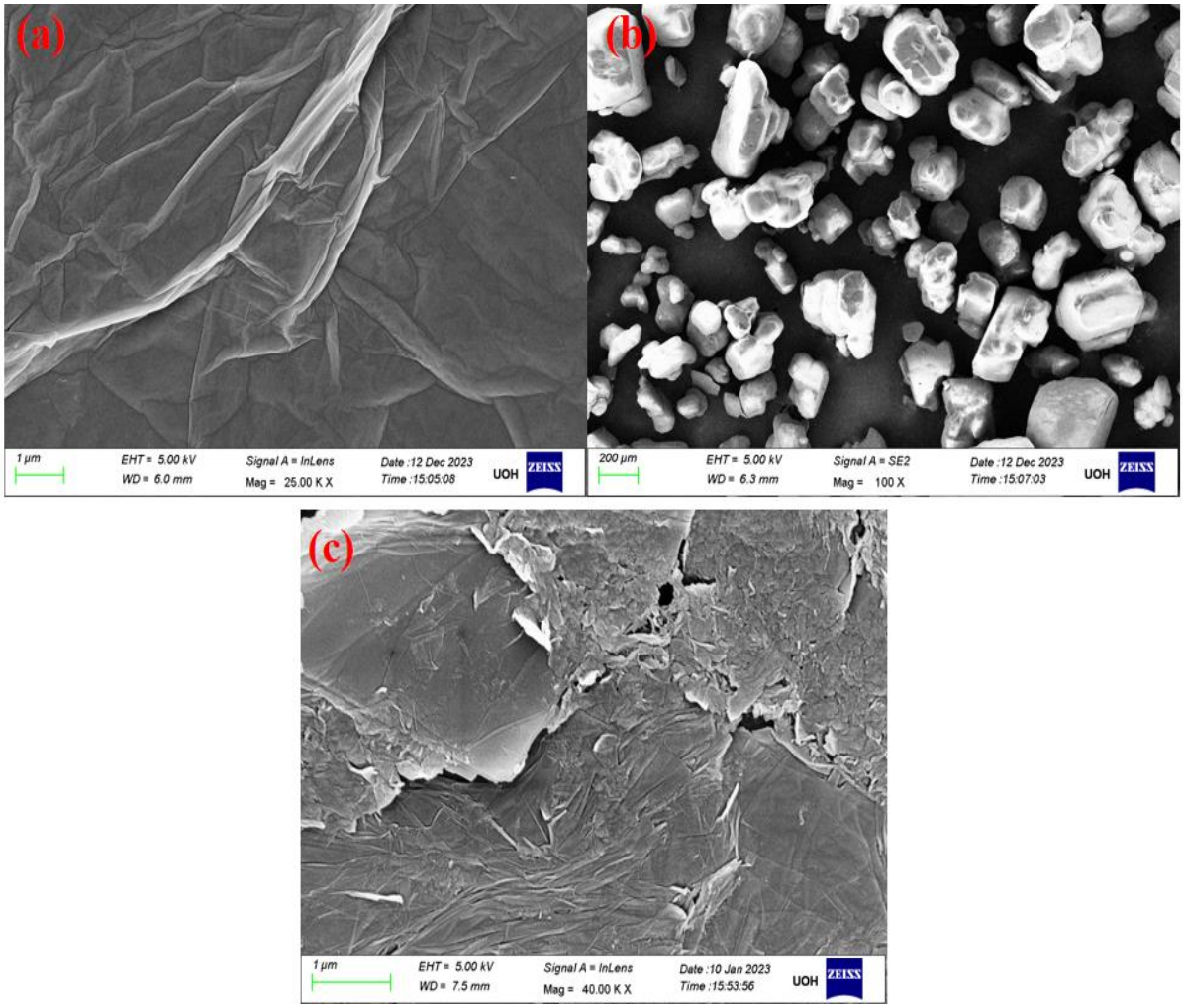


Fig 1. . The FE-SEM images of (a). Sheet structured Graphene Oxide, (b). Crystal structured L-cysteine, and (c). Irregular sheet structured synthesized L-cysteine graphene oxide.

The Raman spectroscopy fig 2 (a, b, c) was performed to analyse the structural alterations of L-Cysteine hydrochloride monohydrate, GO, and Cysteine graphene oxide (1:1) in the range of 100-3500cm^{-1}. The usual D and G bands of graphene oxide (fig 1. a) are visible at 1351 and 1594cm$^{-1.}$ The Raman spectra of L-Cysteine shown peak at 941cm^{-1} represents S-H peak, a group of C-H modes were seen at 616, 660, and 678 cm^{-1}. The S-S stretching modes at 498, 512 and 529 cm^{-1} were noticed. The disulphide bonds are visible at 512 and 529 cm^{-1}.

The G band in CysGO was at 1589 cm^{-1} there is a 17cm^{-1} shift to a lower frequency when compared to graphene oxide. CysGO (fig 2.c) presented slightly larger ratio of 2.5 than did GO whereas GO shown 2.25 I_d/I_g ratio proving that the layer-stacking of GO was lessened by the functionalization of L-cysteine.

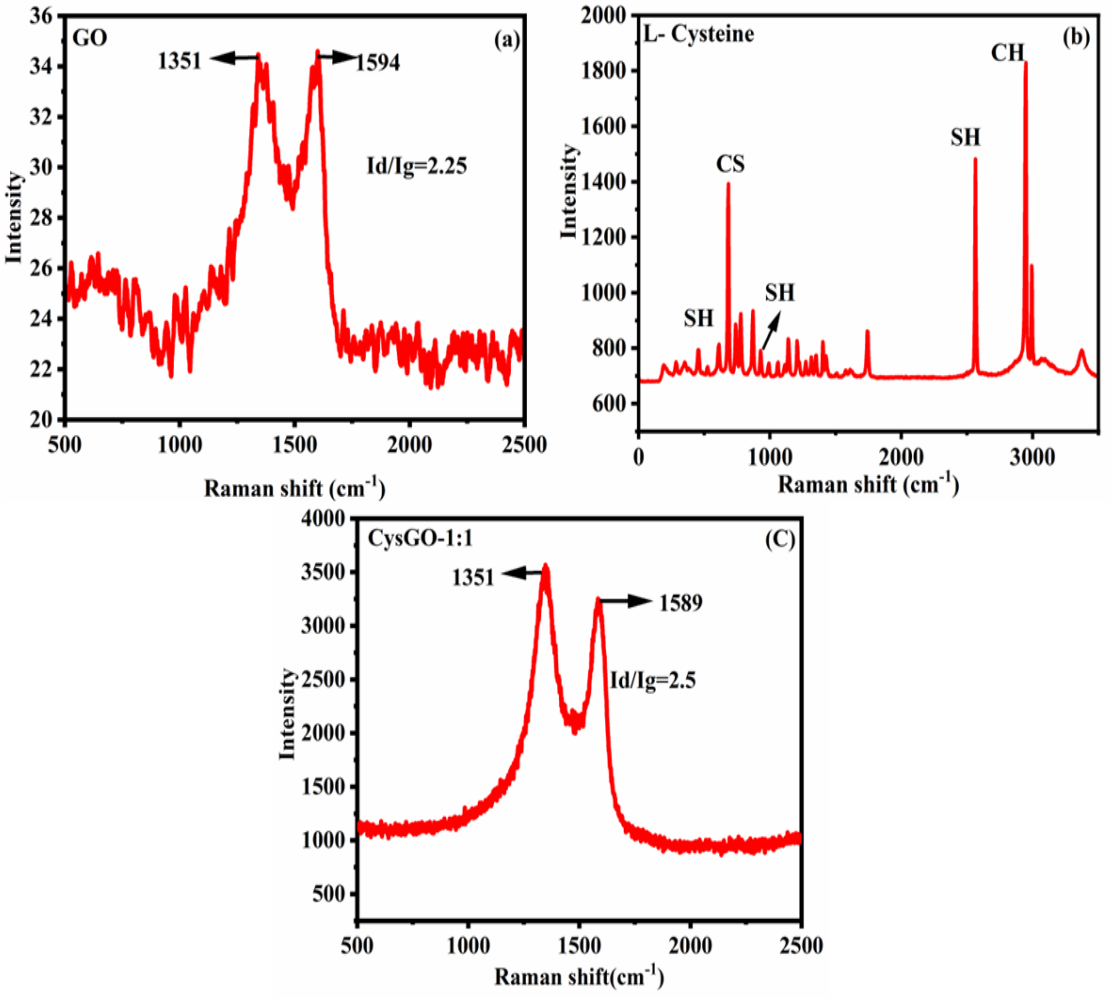


Fig 2. The Raman spectroscopy images shown the structural fingerprints of (a). Graphene oxide, (b). L-cysteine hydrochloride monohydrate, and (c). Structural alterations in L-cysteine graphene oxide.

In FTIR spectra, the peak of GO (fig 3.a) was found at 3384 cm^{-1} indicate O-H stretching vibration. A small peak at 1725 cm^{-1} may be attributed to C=O stretching of carbonyl or carboxyl group. The peak detected at 1630 cm^{-1} is ascribed of aromatic C=C stretching. Peaks

at 1030 cm^{-1} correspond to the C-O-C (epoxy) vibrational stretching respectively. On the other hand, the cysteine spectrum shown a broad band between 3000-3500, a weak signal at 2560 cm^{-1}, and sharp bands at 1740 and 1514 cm^{-1}. Whereas the peak at 1640 cm^{-1} in CysGO is assigned to amide group and the region between the 3100–3500 cm^{-1} is due to a wide N-H stretching band (fig 3. c).

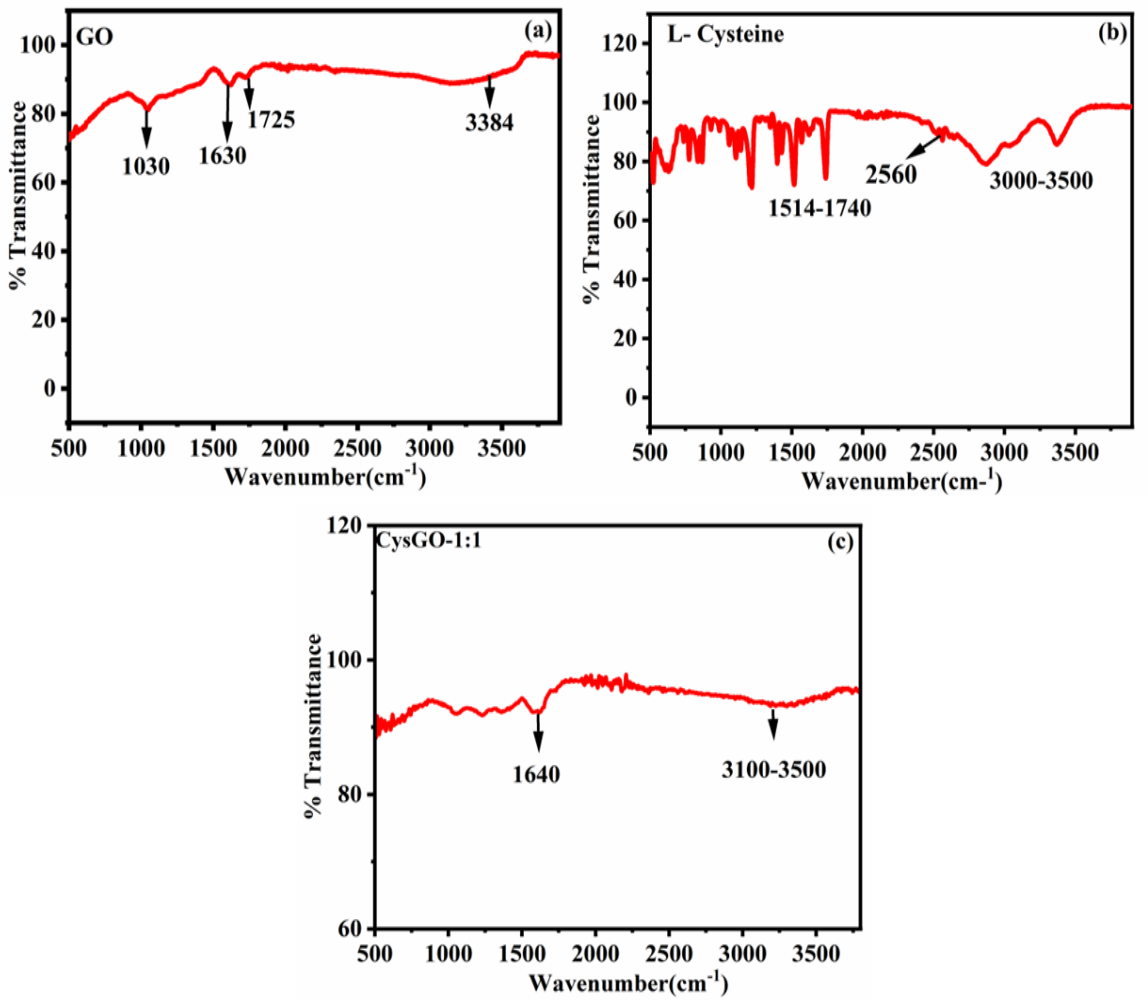


Fig 3. FTIR spectra shows the percentage of transmittance of (a). Graphene oxide, (b). L-cysteine hydrochloride monohydrate, and (c). L-cysteine graphene oxide.

The XPS spectra (figure 4.a, b, c, and d) showed deconvoluted peaks of C1s, O1s, N1s, and S2p. C-C, C-H, C-O-C, C=O, N-C, and N=C were the elements of GO, whilst C-C, C-OH, C-O-C, O-C, pyridinic-N, graphitic-N, pyrrolic-N, S-H, inorganic S, and organic S were the

components of CysGO [20]. The S2p spectra was only found in the spectra of CysGO. Three peaks emerged from the deconvoluted S2p spectra; the primary peak, which may be attributed to S-H, was found at 164 eV.

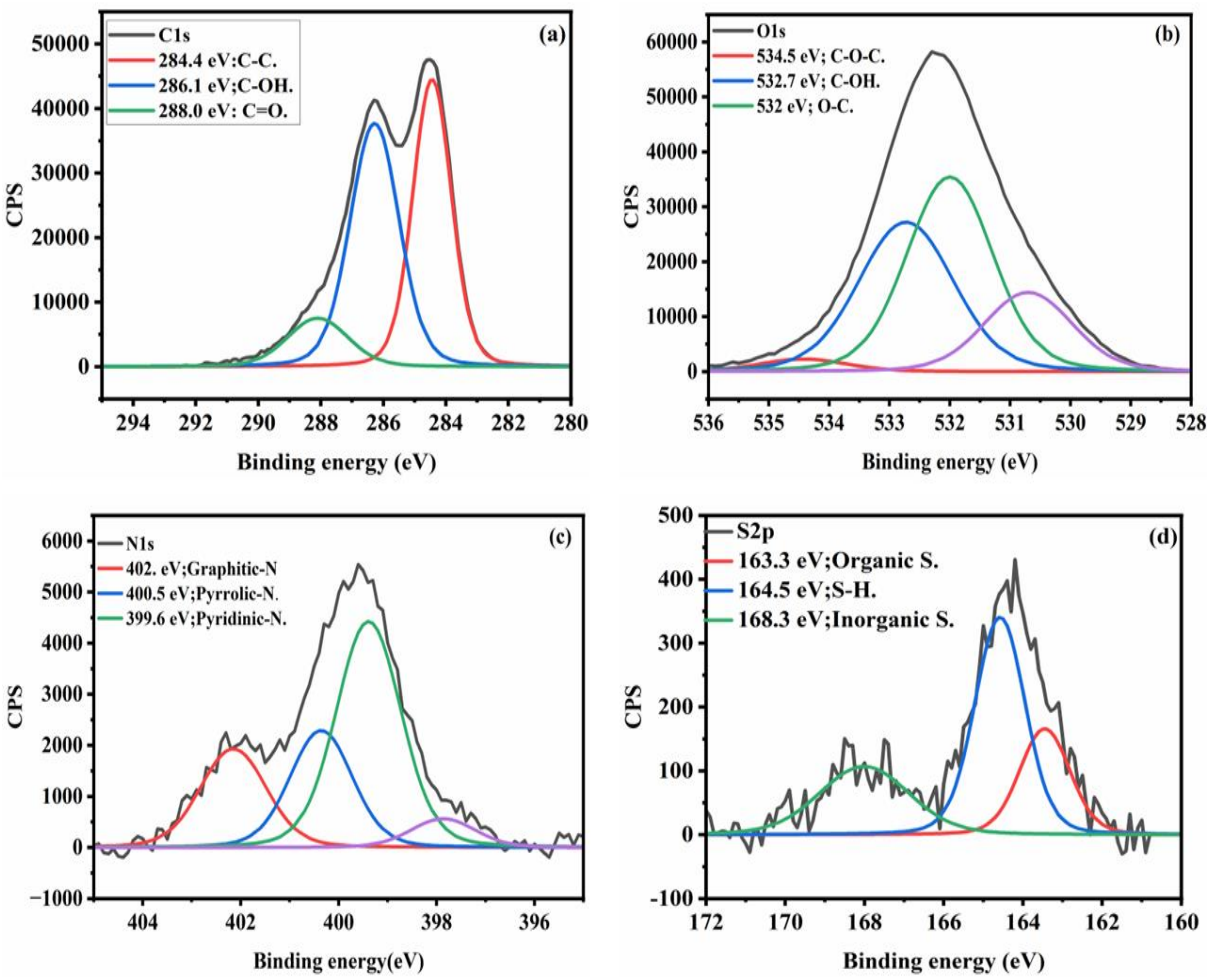


Fig 4. CysGO's XPS spectra (a). C1s, (b). O1s, (c). N1s and (d). S2p.

respectively. Following Shirley's background subtraction, and by using the Gaussian components the peaks were deconvoluted.

3.2 RESULTS OF CysGO DEPOSITED ON CONTACT LENS

The FESEM had been employed to characterize the synthesized CysGO deposited and control lenses. The deposited lens displayed consistent CysGO deposition (Figures 5 and 6), whereas the control lenses' images display a plane surface. Etafilcon A lenses exhibit dense deposition as opposed to Comfilcon A lenses.

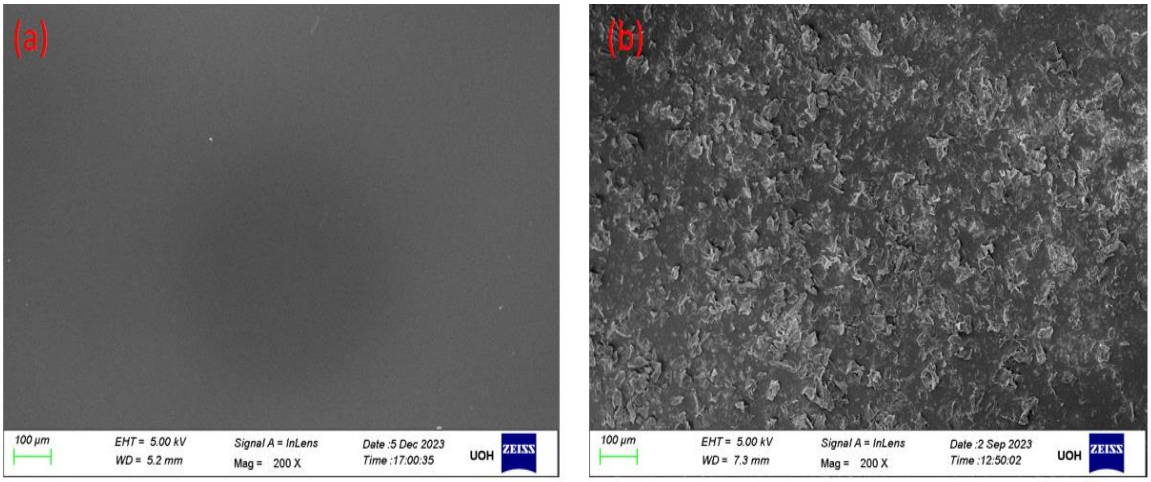


Fig 5. FE-SEM images of (a). Control lens, and (b)**.** Deposited Etafilcon A lens.

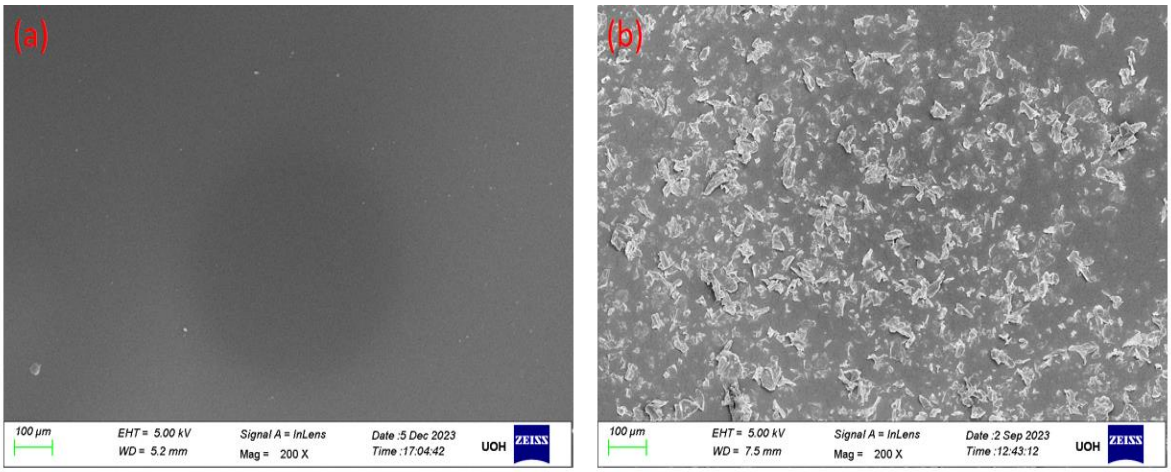


Fig 6. FE-SEM images of (a). Control lens, and (b). Deposited Comfilcon A lens.

To investigate the effect of CysGO deposition on swelling percentages of the lenses we have conducted a two-sample t- test between comfilcon A control lenses (94.325 ± 0.30) and CysGO deposited comfilcon A lenses (98.35 ± 1.01) (Fig.7a). There was a significant increase in lens swelling after deposition $t(2) = 6.0$, $p < 0.001$ in comparison with the control lenses. The swelling effect was also investigated on CysGO deposited Etafilcon A (97.9 ± 0.74) and control lenses (94.3 ± 0.29) (Fig. 7b), there was a significant rise in lens swelling following the deposition ($t(2.4) = 8.8$, $p < 0.001$) as compared to the control lenses.

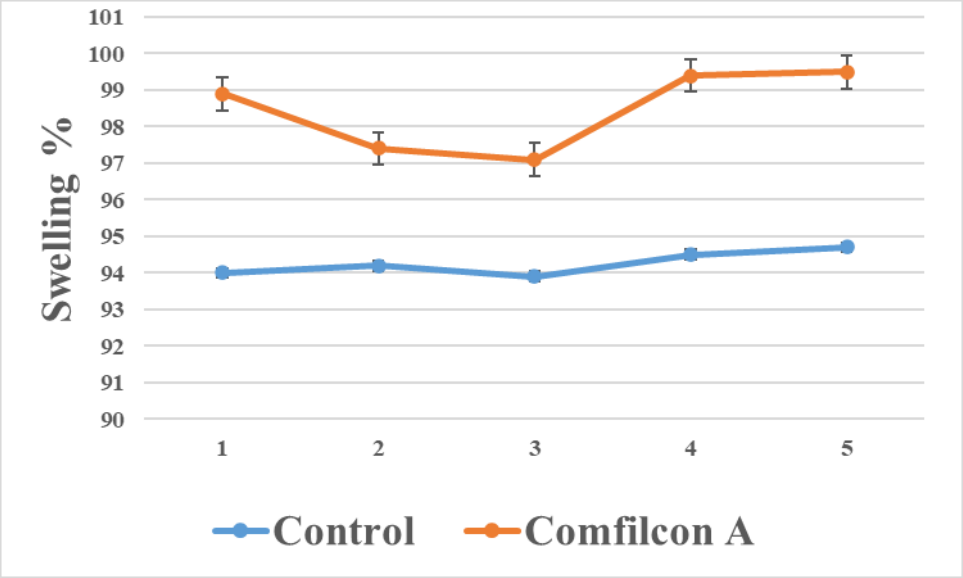


Fig 7a. The line diagram shows the swelling percentages of (a). Control and CysGO deposited Comfilcon A contact lenses.

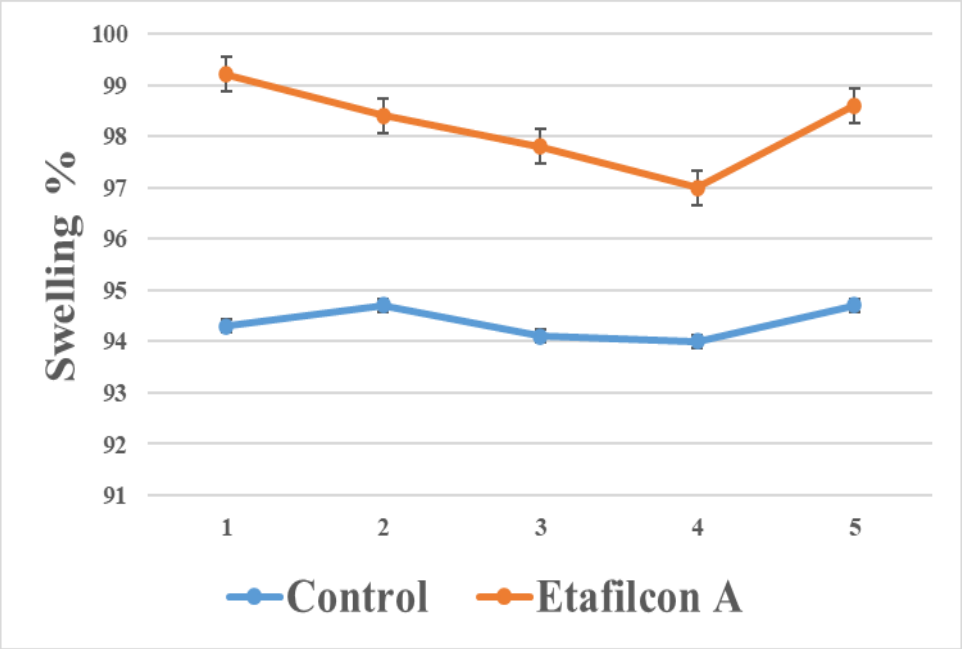


Fig 7b. The line diagram shows the swelling percentages of Control and CysGO deposited Etafilcon A lenses.

Furthermore, the effect of CysGO deposition on the transmittance percentages of the control and deposited lenses was assessed. The deposited Comfilcon A lens shown 99% while control lens shown 97% (Fig 8. a) hence there is 2% increase in the transmittance percentage in the deposited lens whereas the control lens shown 98.45% and the deposited Etafilcon A lens shown 99.57% hence there is a 1% increase in the transmittance (Fig 8. b).

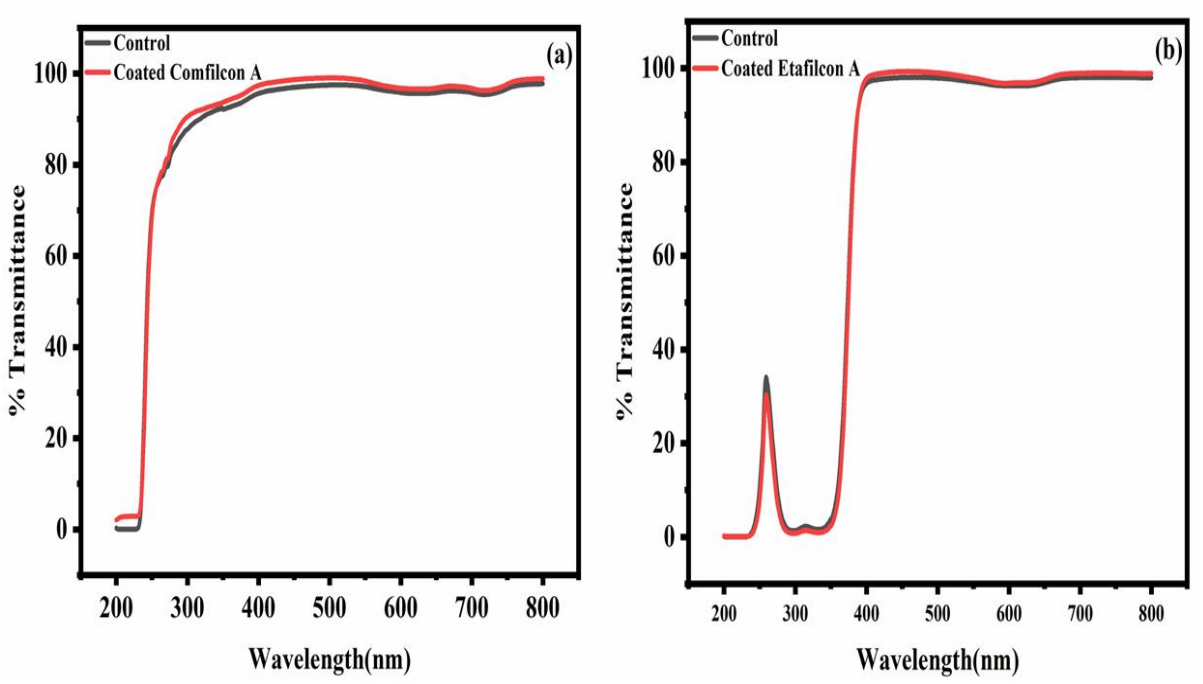


Fig 8. Transmittance percentages of (a)**.** Control and deposited Comfilcon A lenses, and (b). Control deposited Etafilcon A lenses.

4. DISCUSSION:

This study demonstrated the deposition of hydrogel and silicone hydrogel contact lens with L-cysteine graphene oxide. CysGO was successfully synthesised confirmed its synthesis with the FESEM, Raman spectroscopy, FTIR, and XPS. This study's depiction of the GO sheet structure is consistent with previous studies[cxxvi]. The current study also depicted the L- cysteine morphology where it shown crystal structures, this study's findings on the irregular structure of CysGO are consistent with those of Mu, L., et al lxiii. The graphene oxide's classical D band and G band, which are observed at 1351 cm-1 and 1594 cm-1[cxxvii], are as reported and the G band was located in CysGO at 1589 cm-1. The shift in the G band could have been caused by the reduction of double bonds after the immobilisation of L-cysteine[cxxviii]. L-cysteine acts as a dispersant when added to GO layers, which could explain this behaviour, resulting in slightly elevated Id/Ig ratios. Since it is anticipated that when cysteine is altered, the modes in L-

cysteine will alter significantly[cxxix]. FTIR analysis of GO demonstrates that the amide group is formed in the CysGO by the removal of water during the condensation process[cxxx]. Whereas, the broad band in the cysteine spectrum between 3000 and 3500 cm-1 attributes to NH3 stretching, the weak signal at 2560 cm-1 is corresponding with S-H stretching, and the sharp bands at 1740 and 1514 cm-1 are caused by the COOH group's C=O stretching modes[cxxxi]. The COOH sites of GO are interacted with the NH2 groups, amide groups are created (fig 3.b). The s2p peak in XPS is exclusively seen in spectra of CysGO. The primary peak of the N2s spectra, located at 399.6 eV, is in line with results from earlier investigations on the deposition of L-cysteine on flat surfaces[cxxxii].

The surface morphology of CysGO on both lens materials has been reported by us. Where the shape of deposited Etafilcon A lenses slightly differs. The greater percentage of swelling on deposited contact lenses is probably due to the capacity of GO's carboxylic and hydroxyl groups to form hydrogen bonds with water molecules[cxxxiii]. Research on GO in contact lenses or GO in combination with other materials on contact lenses that are already available have demonstrated comparable increases in swelling percentages. Comparing deposited lenses to control lenses, there is no discernible variation in the transmission percentages. Similarly, contact lenses treated with GO in conjunction to other medications have not demonstrated a discernible decline in transmittance[cxxxiv]. Moreover, beyond the aforementioned attributes, additional properties of the synthesized lenses can be investigated for prospective applications in the future.

5. **CONCLUSION:**

FESEM, Raman spectroscopy, FTIR & XPS confirmed the synthesis of CysGO. This study demonstrated the deposition of L-cysteine graphene oxide on silicone hydrogel and hydrogel contact lenses. This deposition would be advantageous for the lenses' homogeneity, enhanced

swelling, and lack of discernible variation in transmittance percentages. For its most comprehensive applications, its other properties can be investigated.

CHAPTER 3

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOCLUSTERS DEPOSITED CONTACT LENSES

1. INTRODUCTION:

Globally, there are approximately 140 million Contact lens (CL) wearers, with an increase of 6% every year[cxxxv & cxxxvi & cxxxvii]. CLs are exclusively used to correct ametropia to provide clear vision and also for therapeutic and cosmetic purposes[cxxxviii]. Currently, due to many advances in technologies, CLs are promising as diagnostic devices and drug delivery systems[cxxxix & cxl]. However, the CLs are not free of complications like hypoxic, mechanical, immunologic, hypersensitive reactions and infectious keratitis[cxli] such as microbial keratitis (MK)[cxlii].

Nanotechnology has rapidly evolved into a diverse and attractive area of scientific study that includes many biological investigations and applications[cxliii & cxliv]. In recent years, there has been a lot of interest in and investment in the special biological, chemical, and physical characteristics of nanoparticles made from noble metals like gold and silver. As a result, their potential applications in the biological field have been reevaluated and reconsidered[cxlv & cxlvi]. Because of their distinctive physical and chemical characteristics, silver nanoparticles (AgNPs) have found use in a variety of industries, including food, medicine, and industry[cxlvii & cxlviii & cxlix]. AgNPs have been used in many applications like consumer products, medical device coating, cosmetics, optical sensors, antibacterial agents, diagnostics, and drug delivery[cl]. Ag NPs, have generated a lot of interest among researchers and academic scientists due to their distinct antimicrobial properties and the biological assessment of their potential uses.

In ophthalmology, the AgNPs were used for corneal prosthetic devices (KPros) for the protection against perioperative and early postoperative infections[cli] and for the detection of adenoviral conjunctivitis[clii]. Biofilm generation was significantly inhibited when silver

nanoparticles (AgNPs) were deposited on hard lenses and incorporated into silicone hydrogel lenses[cliii & cliv]. Silver is also impregnated into CLs cases for controlling the infection[clv]. The silver nanoparticle CLs have shown better colour distinction for blue-yellow colour vision deficiency patients, intraocular pressure monitoring, blue light protection, corneal wound healing and drug release[clvi & clvii & clviii & clix].

However, the available methods for the preparation of silver CLs are: commercially available AgNPs or synthesised silver nanoparticles with different synthesis methods were added during the polymerisation process[clx & clxi & clxii & clxiii & clxiv]or soaking of the dried polymers[clxv & clxvi & clxvii]or commercially available contact lenses or the deposition of the silver nanoclusters through nanocluster deposition system[clxviii], in which the cost of the commercially purchased silver nanoparticles and the instrument used are very expensive. Therefore, in this study the silver nanoclusters were synthesised by Tollen's reagent method and deposited on contact lenses, which is the cost effective and easiest method reported till date.

2. MATERIALS AND METHODS:

2.1. MATERIALS: silver nitrate (AgNO3), sodium hydroxide (NaOH), D-glucose (C6H12O6) and ammonium hydroxide (NH4OH) were purchased from Sigma Aldrich Co. USA. Rigid gas permeable (RGP) lens buttons (product code: 4190071) were purchased from CONTAMAC LTD, UK. Soft lenses (Etafilcon A) were purchased from ACUVUE, Johnson & Johnson Vision Care.

2.2. METHODS:

2.2.1. SYNTHESIS OF SILVER NANOCLUSTERS BY TOLLEN'S REAGENT:

Silver nanoclusters were synthesised using the Tollens Reagent method. The Tollens test, also referred to as the silver-mirror test, is a qualitative laboratory procedure used to differentiate between ketone and aldehyde. It exploits the fact that ketones are not oxidized whereas

aldehydes are readily oxidized. The fabrication details are as follows: Firstly, Tollens' reagent was freshly prepared by uniformly dissolving 0.3 molL-1 silver nitrate (AgNO3) in water. Then, 1.25 molL-1 sodium hydroxide (NaOH) was added to the AgNO3 solution, which resulted in a brown precipitate of Ag2 O. To obtain a transparent solution, ammonium hydroxide (NH_4OH) solution was then added to this solution drop by drop until the precipitated Ag2O was completely dissolved. This ammoniacal solution contains Ag (NH3)2OH (silver diamine hydroxide, the silver complex), When the Tollens' reagent reacts with a formyl group-containing compound such as D-glucose on the contact lens surface, silver ions (Ag^+) are reduced to metallic silver (Ag), forming a coating on the CL surface.

2.2.2. CHARACTERIZATION OF SYNTHESIZED SILVER NANOCLUSTERS:

To characterise the synthesised silver nanoclusters and silver nanoclusters deposited CL's the following techniques were used to measure the chemical and physical properties of the synthesised compound.

2.2.2.1. FIELD EMISSION SCANNING ELECTRON MICROSCOPE (FE-SEM)

The FE-SEM photographs were taken to evaluate the surface morphology on a FESEM (Carl Zeiss AG - ultra-55, Germany), with system vacuum 2.64e-006 mbar and gun vacuum 6.78e-010 mbar and the beam energy used was InLens. The synthesised silver nanoclusters were placed on the glass slide, also the silver nanoclusters deposited CL's and the control CLs were placed on the carbon tape-covered stubs and sputter coated with gold for 2-3 mins to increase the conductivity and the chemical stability of the sample and then the FE-SEM images of silver nanoclusters, deposited CL's and the control CLs were captured.

2.2.2.2. ATOMIC FORCE MICROSCOPY (AFM)

Surface topography and roughness was assessed by AFM, Hitachi company, scanning probe microscopy 4000, with dynamic force microscopy tip, non-contact mode was used. the silver nanoclusters deposited CL's were mounted on the sample holder to capture the images.

2.2.2.3. RAMAN SPECTROSCOPY (RS)

To analyse the structural alterations, the measurements were carried out on RS; HORIBA HR800, France, with 512nm excitation laser, 5 sec acquisition time, 2 sec accumulation time, and 1 sec real-time display. The synthesised silver nanoclusters on a glass slide and CLs were placed on the sample stage for the Raman spectra.

2.2.3. DEPOSITION OF SILVER NANOCLUSTERS ON CONTACT LENSES:

In the Tollens' test, the Tollens' reagent (Ag (NH3)2OH) reduces to form a continuous Ag film on the contact lens. However, by carefully regulating two reduction parameters—the temperature (25 °C) and the reduction period (2–3 minutes)—Ag nanoclusters rather than a continuous Ag thin layer can form on the contact lens and using a laminar flow of deionized (DI) water to wash the Ag nanoclusters that were deposited on the CL surface. The ideal moment to quickly halt the Tollens' reagent reaction will make it easier to interrupt the normal 3D film growth, including that along the CL surface plane.

2.2.4. CHARACTERIZATION OF SILVER NANOCLUSTERS DEPOSITED CONTACT LENSES

To evaluate the surface morphology of the silver nanocluster-deposited Gas Permeable Contact Lenses (GPCLs), the field emission scanning electron microscopy (FESEM), the Carl Zeiss ultra-55, INCAx-act were used, to assess the topography the atomic force microscopy (AFM)

is used and the Raman spectroscopy (RS), HORIBA HR800 with 532 nm laser was performed to study its fingerprints.

3. RESULTS

3.1. CHARACTERIZATION OF SYNTHESISED SILVER NANOCLUSTERS:

Synthesised silver nanoclusters on a glass slide were characterized by FESEM, which showed the morphology of the synthesised spherical structured silver Nanoclusters (fig. 1a) and the Raman spectra showed a peak at 233 cm-1 (fig. 1b), which resembles the Ag-O stretching mode which confirms the synthesis of silver nanoclsuters.

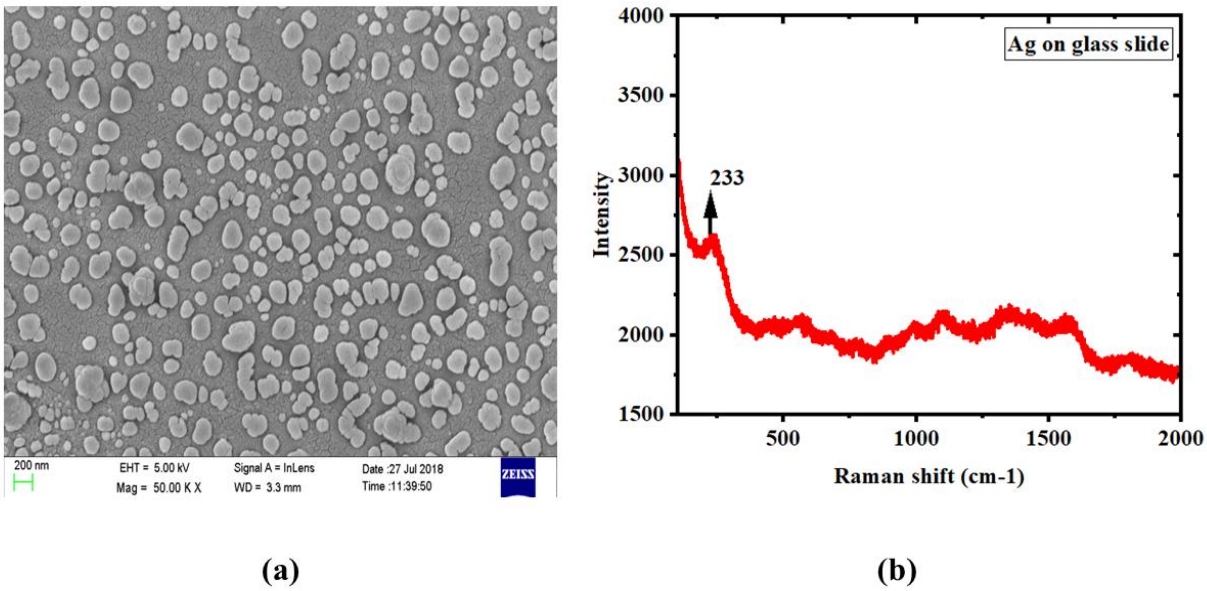


Fig .1. (a) FESEM image of silver nanoclusters on a glass slide. (b) Raman spectroscopy of silver nanoclusters on a glass slide.

3.2. CHARACTERIZATION OF SILVER DEPOSITED CONTACT LENSES:

FESEM images (fig. 2.a and fig. 3.a) of silver deposited on soft contact lenses shown uniform spherical clusters on the contact lenses, and also the atomic force microscopic image of Rigid gas permeable lenses (fig. 2.b) shown the spherical shaped topography with the average particle

size distribution of approximately 10nm. In fig. 2.c and fig. 3.b a peak at 233 cm^{-1} is noticed because of the Ag-O stretching mode of Raman spectroscopy from which the silver deposition is confirmed on both RGP and soft contact lens surfaces.

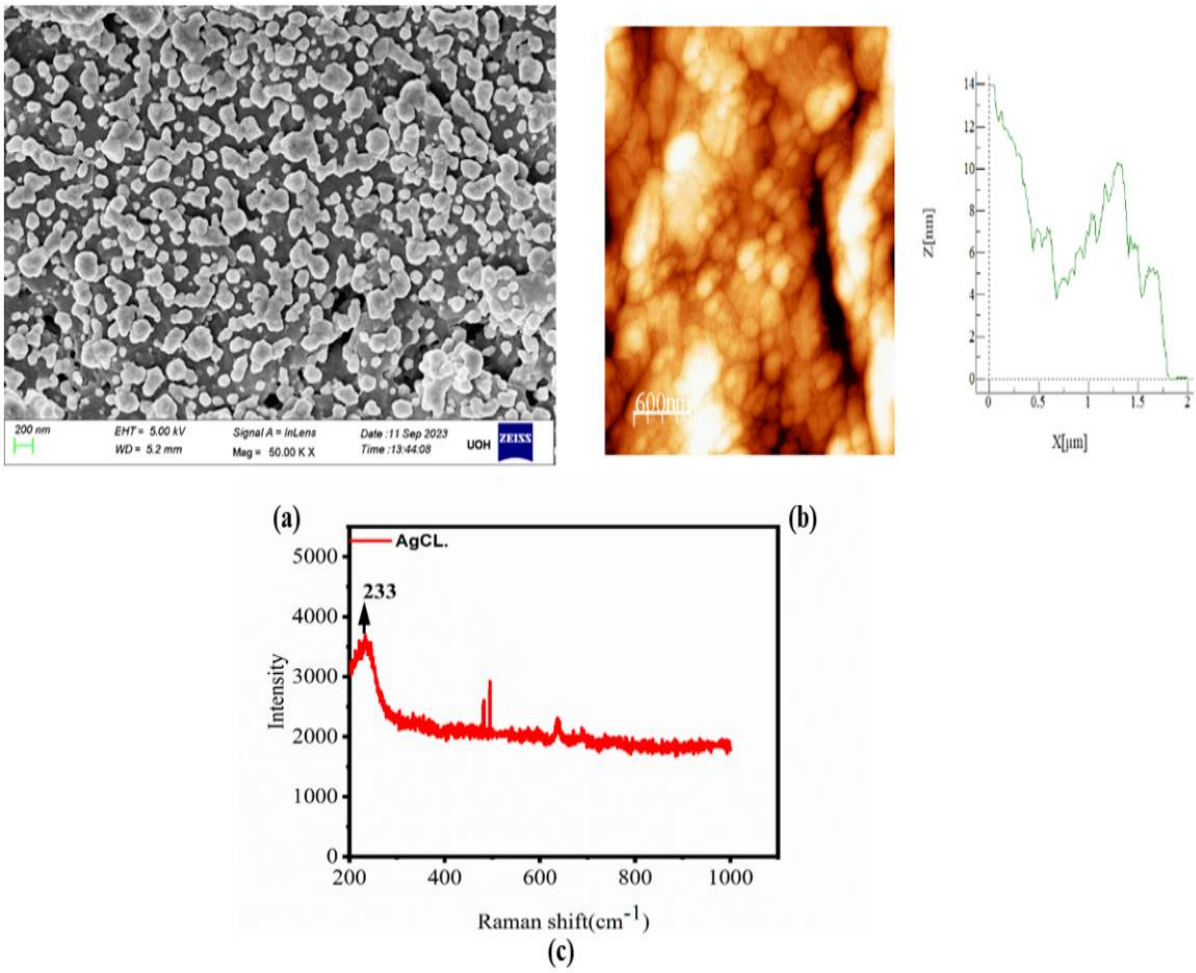


Fig. 2. (a) FESEM image of silver deposited RGP lens. (b) AFM of silver deposited RGP lens. (c) Raman spectra of silver deposited RGP lens.

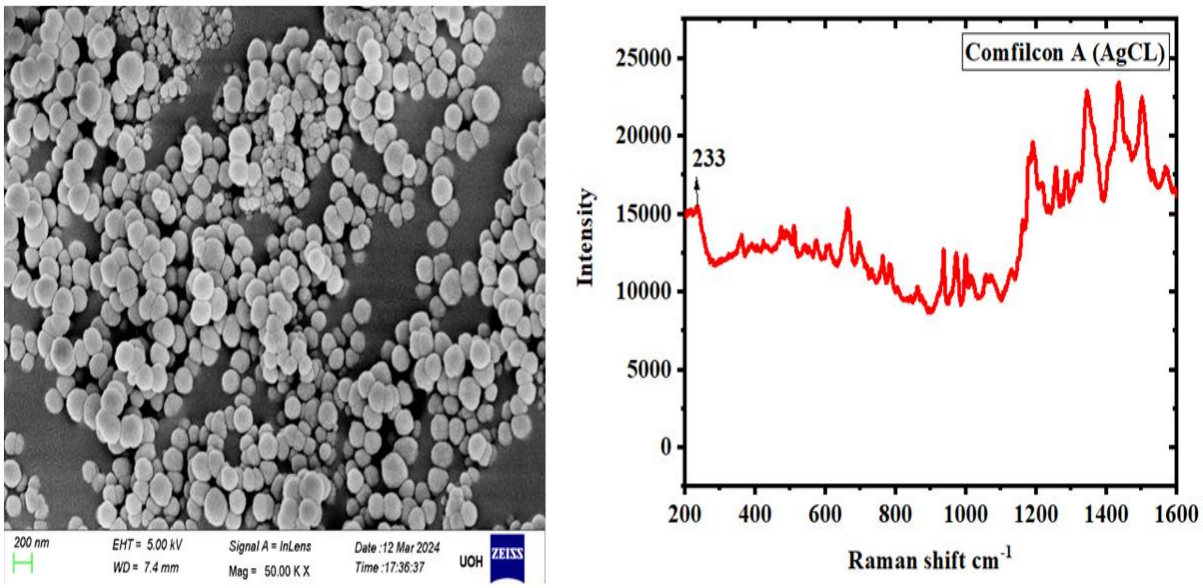


Fig. 3. (a) FESEM image of silver deposited soft CL. (b) Raman spectra of soft AgCL.

4. DISCUSSION:

The silver nanoclusters were successfully synthesised by well-known Tollens' method. confirms its synthesis by physical and chemical measurements like FESEM and Raman spectroscopy the deposition on the contact lenses were confirmed by FESEM, AFM and Raman spectroscopy. A continuous Ag film was formed on a glass slide by the reduction of Tollens' reagent (Ag (NH3)2OH)[clxix]. Instead of a continuous thin film, Ag nanoclusters are formed on the glass slide by closely regulating two reduction parameters: temperature (25°C) and time (2–3 min). The time duration has optimized to cease the Tollens' reaction helped to facilitate the normal 3D film growth, including the one along the glass surface plane was reported in our previous study. The morphology of the silver is seen as spherical clusters by FESEM analysis which is also reported in our previous report[clxx] and the peak at 233 cm^{-1} which corresponds to Ag-O mode in the Raman spectroscopy from which we can confirm the synthesis of silver nanoclusters[clxxi].

we have also successfully deposited the silver nanoclusters on the contact lens surface for the first time by confirming its deposition by FESEM, AFM and Raman spectroscopy. The spherical clusters of the silver on both the lens materials were seen in FESEM images which is in terms with the morphology of the silver deposited on the glass slide and on the hard lens surface when deposited with nanocluster deposition system clxviii. First time we have reported the spherical clusters of silver on the RGP lens button with atomic force microscopy with an average particle size of ~10nm which is similar to the reports on silver nanoclusters on thin films[clxxii], we could not perform AFM analysis on the soft lenses due to its shape but can be performed on the soft lens button. We have also reported the Raman peak at 233 cm^{-1}on both the contact lens surfaces which indicates the Ag-O stretching mode clxxi by which the deposition of the silver nanoclusters on the contact lens surfaces was confirmed. However, by varying the deposition time the silver nanoclusters deposition would vary on lens surface. Moreover, additional characterizations on the deposited lens surface can be explored for the future applications.

5. CONCLUSION:

In this study, we have deposited the silver nanoclusters onto the contact lens surface by controlling the reduction parameters of the Tollens' method, which is confirmed by FESEM, AFM and Raman spectroscopy. It is a novel, cost-effective method where many contact lenses can be coated simultaneously-with less cost. Hence, it would be a beneficial coating for further ocular applications.

CHAPTER 4

ANTIMICROBIAL EFFECT OF SYNTHESISED AND DEPOSITED SILVER NANOCLUSTERS ON CONTACT LENS SURFACE

1.INTRODUCTION

Bacterial keratitis is a deadly ocular infection that leads to a profound visual impairment related to contact lens wear[clxxiii]. About 90% of infection is contact lens-related microbial keratitis and is associated with bacterial keratitis[clxxiv]. The predominant risk factors of bacterial keratitis among CLs wearers are wearing daily wear lenses overnight[clxxv & clxxvi], using lenses for longer duration[clxxvii], being of male gender[clxxviii], improper hygiene of the contact lenses[clxxix], and poor contact lens storage case cleaning[clxxx & clxxxi]. Eye discomfort, redness, reduced visual acuity, and stromal infiltration are the key clinical characteristics associated with bacterial keratitis[clxxxii & clxxxiii]. Organisms causing contact lens-associated bacterial keratitis are, the gram positive and negative bacteria predominantly. Bacterial keratitis in contact lens wearers is mostly associated with gram negative bacteria than gram positive bacteria, the most effecting strains of gram-negative bacteria are *pseudomonas, serratia, acenetobacter klebsiella spp* and other species and also the most effecting gram-positive bacteria are *staphylococcus, streptococcus spp* and others[clxxxiv]. The contact lens related infections are reduced by proper handling of the lens and by using recommended contact lens solution, rubbing and rinsing lenses with disinfecting solution[clxxxv]. Apart from this, current treatment modalities for contact lens related ocular infectious keratitis are the topical application of gentamicin and cephazolin antibiotic combinations are considered as the gold standard in treating bacterial keratitis[clxxxvi]. These currently available treatments can be taken after discontinuation of the contact lens, in the form of eye drops or ointment. Hence there is a need for the antimicrobial surface on the lens which will not interrupt the clear vision and control the ocular related infections therefore, this leads for the development of antimicrobial contact lens surface.

Graphene related materials toxicity studies are currently available, it was reported recently that graphene oxide and reduced graphene oxide exhibit strong antibacterial activity by causing membrane and oxidation stress[clxxxvii & clxxxviii & clxxxix]. A number of possible hypotheses have been proposed till now to explain the antibacterial effect including contact with sharp edges, cell wrapping[cxc], destructive extraction of phospholipids[cxci] and oxidative stress[cxcii]. However, there is no single unifying model till date to explain the possible toxicity of graphene oxide and also there is no consistent data on the bacterial viability. One possible explanation for this would be the unavailability of the consistent reports on the source of the GO and the process from which it is fabricated. At the same time its biological effects are still debated. To realise the graphene-based materials potentials on health and environment their impact has to be thoroughly evaluated.

In ophthalmology, the AgNPs were used for corneal prosthetic devices to prevent *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm formation to protect against the perioperative and early postoperative infections[cxciii]. There have been reports of using silver-amplified immunochromatography to identify adenoviral conjunctivitis[cxciv].

Biofilm development was significantly inhibited when silicone hydrogel lenses were infused with silver nanoparticles[cxcv & cxcvi]. Hence, there is a need of such materials which are cost effective, biocompatible and have excellent antimicrobial activity together for contact lens surface to protect the ocular surface from infections. Therefore, in the current study we have assessed the antimicrobial activity of synthesised cost-effective silver nano clusters and AgNPs deposited contact lenses on ocular infections causing microorganisms.

2. MATERIALS AND METHODS

2.1. MATERIALS: SLGO was purchased from Tokyo chemical industry co., LTD. Japan**,** L-Cysteine Hydrochloride Monohydrate and silver nitrate (AgNO3), were purchased from Sigma

Aldrich Co. USA and Soft lenses (Etafilcon A) were purchased from ACUVUE, Johnson & Johnson Vision Care.Trypto soy agar (TSA), purchased from Sisco research laboratories Pvt. Ltd, India. *Staphylococcus aureus (L-4454/24) and Pseudomonas aeruginosa (L-4404/24)* bacterial strains were collected from LV Prasad Eye Institute. (GRAMMATICAL issues, please fix)

2.2. METHOD:

2.2.1. ANTIMICROBIAL ACTIVITY OF SYNTHESISED CysGO AND SILVER NANOCLUSTERS:

The agar well diffusion method[cxcvii] was also used to evaluate the produced silver nanoclusters' antibacterial efficacy. Trypto soy agar (TSA) plates were taken for the bacterial cultures and incubated overnight at 37 ºC. The plates were cultured with gram positive and negative bacteria such as Staphylococcus *aureus and Pseudomonas aeruginosa* strains which were collected from LV Prasad Eye Institute. The synthesized 1:1 concentration of CysGO suspension and 1gm of $AgNO_3$ was used for well diffusion method. The 50 µL of the suspensions (CysGO & AgNPs) were filled in the wells prepared in culture plate. After 24 hrs, the zone of inhibition was measured. That is the measurement of bacterial growth inhibition by the CysGO and silver nanoclusters.

2.2.2. ANTIMICROBIAL ACTIVITY OF SILVER NANOCLUSTERS DEPOSITED CL:

The antibacterial activity of the synthesised silver nanoclusters deposited CLs were assessed by agar well diffusion method. Trypto soy agar (TSA) plates were taken for the bacterial cultures and incubated overnight at 37 ºC. The media was cultured with gram positive and negative bacteria such as *Staphylococcus aureus and Pseudomonas aeruginosa*, these strains were collected from LV Prasad Eye Institute. The silver nanoclusters deposited CLs were

vortexed in Milli Q water for 10 min with a maximum speed the silver nanoclusters detached from the CLs were used for well diffusion method. The 50 μL of the AgNPs solution was filled in the wells prepared in culture plate. By using the disc diffusion method, the silver nanocluster deposited CLs were placed on the bacterial culture media. Following 24 hrs of incubation, the zone of inhibition was measured. That is the measurement of inhibiting the growth of bacteria by the silver nanoclusters deposited CL's.

3. RESULTS

3.1. ANTIMICROBIAL ACTIVITY OF CysGO AND SILVER NANOCLUSTERS:

Following 24 hours of incubation with 50 μL of the synthesized 1:1 concentration of CysGO and 1 g of AgNO3, the zone of inhibition was measured for both Gram-positive (Staphylococcus aureus) and Gram-negative (Pseudomonas aeruginosa) bacteria. The results indicated that CysGO exhibited no observable zone of inhibition, as shown in figure 1 (a & b). Please follow more formal phrasing with standard time notation. The synthesised silver nanoclusters shown inhibition zone of 3.3cm for *Staphylococcus aureus* and 3.0 cm for *Pseudomonas aeruginosa* respectively seen in figure 2 (a & b).

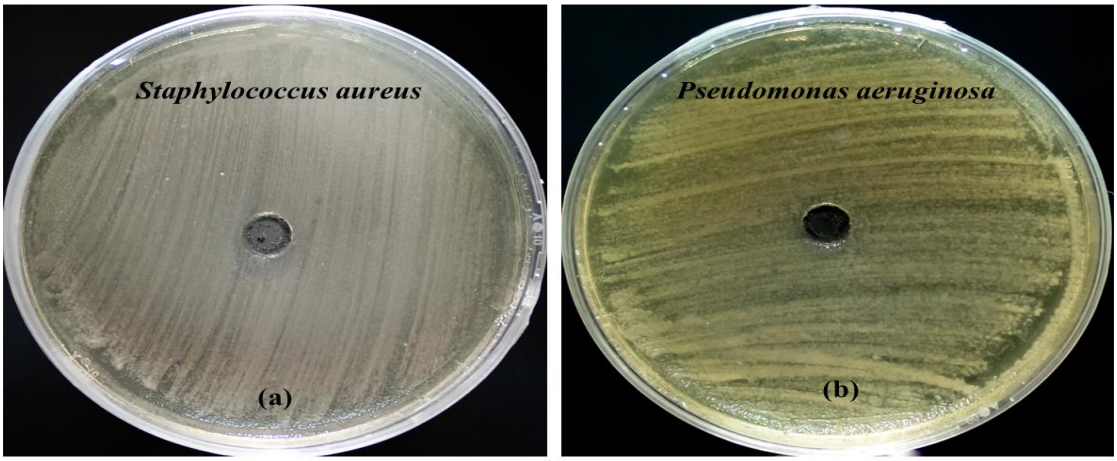


Fig.1. Well diffusion method of cysteine graphene oxide with (a). *Staphylococcus aureus* and (b). *Pseudomonas aeruginosa.*

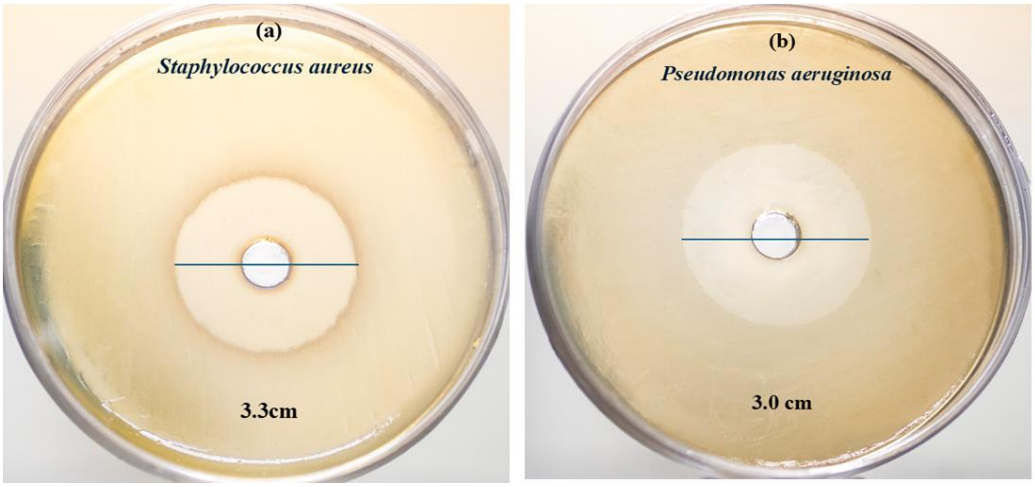


Fig. 2. Inhibition zones seen for silver nanoclusters with (a). *Staphylococcus aureus*, and (b). *Pseudomonas aeruginosa*

3.2. ANTIMICROBIAL ACTIVITY OF SILVER NANOCLUSTERS DEPOSITED CONTACT LENSES:

Following 24hrs of incubation with the 50 μL silver nanoclusters which are detached from contact lenses, for 1gm of $AgNO_3$ concentration the bacterial inhibition was measured. For both gram-positive and gram-negative bacteria, the zone of inhibition was assessed. The inhibition zone for *Staphylococcus aureus* is 1.8 cm and *Pseudomonas aeruginosa* is 1.4 cm respectively which is seen in fig.3 (a & b). The antimicrobial activity can be assessed with different concentrations deposited on the contact lenses.

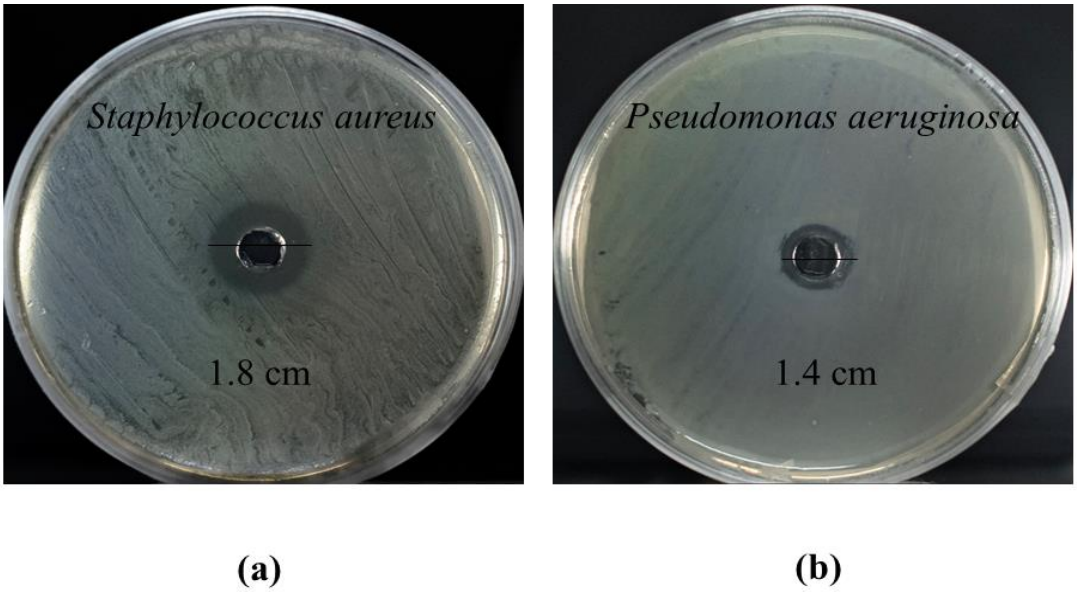


Fig.3. Inhibition zones of (a). *Staphylococcus aureus,* and (b). *Pseudomonas aeruginosa* with silver nanoclusters detached from CLs.

Following 24 hours of incubation, the silver nanoclusters deposited CLs antimicrobial activity was assessed with disc diffusion method. The zone of inhibition was measured for both gram-positive and gram-negative bacteria. The inhibition zone for *Staphylococcus aureus* is 1.8 cm and *Pseudomonas aeruginosa* is 1.7 cm respectively which is seen in fig.4. The antimicrobial activity can be assessed with different concentrations deposited on the contact lenses.

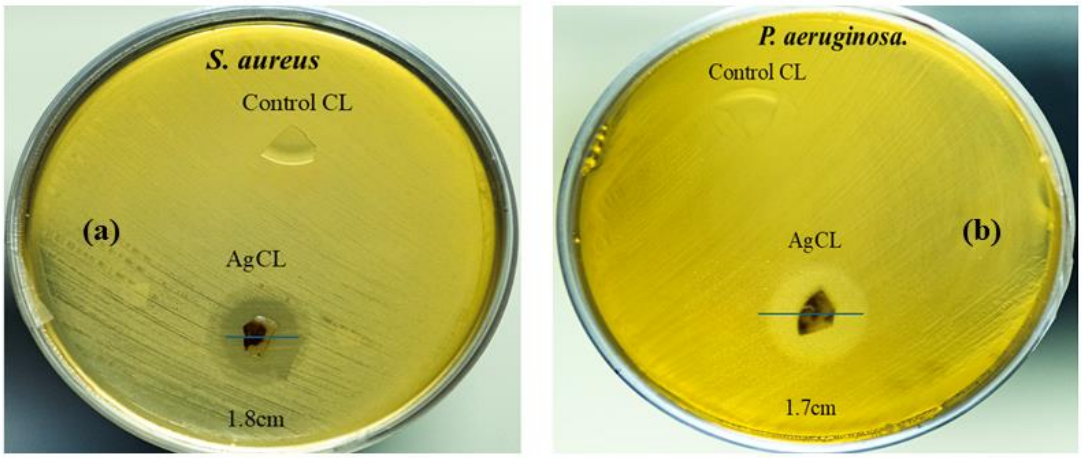


Fig.4. Inhibition zones of (a). *Staphylococcus aureus,* and (b). *Pseudomonas aeruginosa*.

4. DISCUSSION

The antimicrobial activity of the synthesised CysGO, silver nanoclusters and the Ag nanoclusters deposited on the soft contact lens surface was assessed. The detailed synthesis process of the CysGO and the silver nanoclusters were explained in chapter 2 & 3.

The synthesised CysGO suspension shown no inhibition zone, one possible explanation is that the graphene oxide used in the study was commercially purchased with 99 % purity, the antibacterial activity of the GO depends on the purity of the compound which is also reported in Barbolina *et al* study, the pure GO do not have antimicrobial activity[cxcviii]. The debris or the unbound chemicals on the surface of the GO are responsible for the antibacterial activity of the GO, therefore the immobilisation of the L-Cysteine on to the GO did not affect the pure GO properties. According to the Li et al. study, pure graphene oxide's antibacterial properties can be strengthened by hydrating it[cxcix]. Hence in this study, the pure GO did not have any effect on either the gram positive or negative bacteria.

Here, we have reported the antimicrobial activity of the synthesised silver nanoclusters for the first time by arresting the tollens reagent method and the silver nanoclusters deposited contact lens surface. The antibacterial activity of both gram positive (Staphylococcus aureus) and gram negative (Pseudomonas aeruginosa) bacterial strains was assessed using the well diffusion and disc diffusion techniques. There is an effective antimicrobial activity of synthesised silver nanoclusters on both the strains. In our study we could find that antibacterial activity is more towards gram-positive bacteria, *Staphylococcus aureus* than *Pseudomonas aeruginosa* which is similar to the Willcox MD et al study[cc]. Unlike other studies where the silver effect is more on gram-negative bacteria, *Pseudomonas aeruginosa*[cci & ccii]. However, the detailed GO to cell interactions need to be studied for the better understanding of this cause. Synthesised silver

nanoclusters concentration increase effect on the antibacterial activity need to be studied. Moreover, antibacterial activity of the different concentrations of the silver nanoclusters deposited on the CL surface can be explored for the minimum concentration required to reduce the adverse effects on the ocular surface.

There are many reports available on the antimicrobial activity of the silver nanoparticles but were synthesised by various methods, it has been used as a coating on medical devices like endotracheal tubes[cciii], catheters[cciv], contact lenses, and contact lens storage cases with different coating methods[ccv]. In our study, we have exclusively reported the antibacterial activity of the silver nanoclusters synthesised by arresting the tollens reagent method which is cost effective and easiest method reported till date. Hence the synthesised cost-effective silver nanoclusters would be a beneficial coating on medical devices especially on the contact lens surface.

5. CONCLUSION

The silver nanoclusters deposited on contact lenses by arresting Tollens' reagent method have shown antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* the most common bacterial strains causing microbial keratitis on ocular surface. The deposition of silver nanoclusters on the other contact lens surface can also be done and their antimicrobial activity can also be tested for holistic understanding and approach. This is an effective coating on the contact lens surface which will control the ocular infections and also will be a beneficial coating on the medical devices to kill the infection-causing microorganisms.

CHAPTER 5

DISCUSSION AND CONCLUSION

1. DISCUSSION

The synthesis, deposition and characterisation of cysteine graphene oxide (CysGO), onto the contact lens surface to assess the lens parameters was discussed in the chapter 2. To achieve this objective, we have used hydrogel contact lens materials, viz., Etafilcon A and silicone hydrogel contact lens material viz., Comfilcon A. The L-cysteine was immobilized onto GO and deposited on hydrogel and silicone hydrogel lenses to study the surface characterisation and its effect on the lenses for further ocular applications. The Field Emission Scanning Electron Microscope **(**FE-SEM), Fourier Transform Infrared spectroscopy (FTIR), Raman Spectroscopy (RS), X-ray photoelectron spectroscopy (XPS), Ultraviolet-Visible- Near-Infrared Spectroscopy (UV-vis- NIR) and Swelling percentage were used to characterise the synthesised CysGO and CysGO deposited CL's and control lenses. Synthesized CysGO shown the morphology of a GO sheet and has an additional irregular structure, indicating the surface characteristics of a synthesized CysGO. A single layer of GO, a sheet structure with a relatively large surface that is well-defined and interlinked was noticed and the crystal structures were seen from L-cysteine[ccvi & ccvii]. The Raman spectra shown usual D and G bands of graphene oxide are seen at 1351 and 1594cm^{-1}, respectively[ccviii]. The L-cysteine shown CH stretching modes at 2982 cm^{-1} and 2953 cm^{1} and a peak at 941 cm-1, representing the S-H peak. A group of C-S stretching modes were seen at 616, and 678 cm^{-1} and S-S stretching modes at 498, 512, and 529 cm^{-1}. The modes at 512 and 529 cm^{-1} are due to disulphide bond[ccix]. The CysGO presented a slightly larger ratio of 2.5 than GO, whereas GO showed a 2.25 I_d/I_g ratio, proving that the functionalization of L-cysteine lessened the layer stacking of GO. In the FTIR spectra, the peak of GO was found at 3384 cm^{-1}, indicating O-H stretching vibration. A small peak at 1725 cm^{-1} may be attributed to the C=O stretching of the carbonyl or carboxyl group. The peak detected at 1630 cm^{-1} is ascribed to aromatic C=C stretching. Peaks at 1030 cm^{-1} correspond

to C-O-C (epoxy) vibrational stretching, respectively[ccx]. On the other hand, The L-cysteine spectrum displayed strong bands at 1740 and 1514 cm−1, a weak signal at 2560 cm−1, and a broad band between 3000 and 3500 cm−1[ccxi]. Where the peak at 1640 cm^{-1} in CysGO is ascribed to the amide group, and the region between the 3100–3500 cm^{-1} is due to an extensive N-H stretching band[ccxii]. The C1s, O1s, N1s, and S2p peaks of CysGO in the XPS spectra were deconvoluted. The components of GO were C-C, C-H, C-O-C, C=O, N-C, and N=C, whereas the components of CysGO were C-C, C-OH, C-O-C, O-C, pyridinic-N, graphitic-N, pyrrolic-N, S-H, inorganic S, and organic S. S2p was only detected in CysGO's spectrum. Three peaks emerged from the deconvoluted S2p spectra; the main peak, which may be attributed to S-H, was found at 164 eV[ccxiii].

The deposited lens had homogeneous CysGO deposition, whereas the control lenses' FE-SEM photographs revealed a plane surface. Dense deposition is observed in Etafilcon A lenses as opposed to Comfilcon This could be because of the hydrogel lenses' ionic nature. The swelling percentages of CysGO deposited Comfilcon A and Etafilcon A contact lens material was investigated. An increase in lens swelling was noted in Comfilcon A (98.35 ± 1.01) after deposition in comparison with the control lenses (94.325 ± 0.30), this was significant ($p < 0.001$). A similar trend was noticed between deposited (97.9 ± 0.74) and control Etafilcon A (94.3 ± 0.29) material ($p < 0.001$). Consequently, the ability of GO's carboxylic and hydroxyl groups to create hydrogen bonds with water molecules is most likely the cause of the rise in the percentage of contact lens swelling[ccxiv]. The effect of CysGO deposition on the transmittance percentages at 480 nm (blue light range) wavelength of control and deposited lenses was assessed. The deposited Comfilcon A lens showed 99%, while the control lens showed 97%. In contrast, the control Etafilcon A lens showed 98.45%, and the deposited Etafilcon A lens showed 99.57%.; Hence, there is approximately a 3% increase in the

transmittance. Similarly, contact lenses treated with GO in conjunction to other medications have not demonstrated a discernible decline in transmittance[ccxv].

The synthesis of the silver nanoclusters by arresting the Tollen's reagent method and deposition onto the contact lens surface was discussed in the chapter 3, which is a cost-effective, and easiest method. Synthesized silver nanoclusters on a glass slide were characterized by FE-SEM which shown the morphology of spherical structured silver nanoparticles[ccxvi] and RS shown peak at 233 cm^{-1} which resembles the Ag-O stretching mode[ccxvii]. FE-SEM images of silver-deposited contact lenses shown uniform clusters on contact lenses, and the atomic force microscopy (AFM) image of rigid gas permeable (RGP) lenses shown the spherical shaped topography which confirms the FE-SEM images and the average particle size distribution which is approximately 10µm[ccxviii]. A peak at 233 cm^{-1} is noticed due to the Ag-O stretching mode in Raman spectroscopy from which silver deposition is confirmed on both RGP and soft contact lens surfaces. Therefore, it is a novel, cost-effective method where many contact lenses can be coated at a time with less cost. Hence would be a beneficial coating for further medical applications.

The antimicrobial activity of synthesized CysGO and silver nanoclusters deposited contact lenses was assessed and discussed in the chapter 4. Following 24 hrs of incubation with the 50 µL of CysGO (1:1) suspension, the zone of inhibition was measured for both the gram-positive and gram-negative bacteria like *Staphylococcus aureus & Pseudomonas aeruginosa*. No zone of inhibition was seen because highly purified GO and has no antibacterial properties against both the gram (positive and negative) bacterial strains. In contrast, GO which is not sufficiently purified can act as an antibacterial material due to soluble acidic impurities and also the hydrated pure graphene oxide is also responsible for the antimicrobial activity[ccxix & ccxx]. The inhibition zones were assessed for the first time for the silver nanocluster which were synthesised by arresting the Tollens reagent method. For *Staphylococcus aureus* the inhibition

zone was 3.3 cms and for *Pseudomonas aeruginosa* the zone of inhibition was 3.0 cms respectively for 1 gram of silver nitrate concentration.

The antimicrobial activity of silver-deposited contact lenses was assessed by disc diffusion method. Following 24 hrs of incubation with the silver nanoclusters deposited contact lenses, the zone of inhibition was measured for both the gram-positive and gram-negative bacteria like *Staphylococcus aureus & Pseudomonas aeruginosa* the most common bacterial strains causing microbial keratitis on ocular surface. The inhibition zone for *Staphylococcus aureus* is 1.8 cm and *Pseudomonas aeruginosa* is 1.7 cm respectively. In our study we could find that antibacterial activity is more towards gram-positive bacteria, *Staphylococcus aureus* than *Pseudomonas aeruginosa* which is similar to the Willcox MD et al study[ccxxi]. Unlike other studies where the silver effect is more on gram-negative bacteria, *Pseudomonas aeruginosa*[ccxxii & ccxxiii]. There are many reports available on the antibacterial activity of the silver nanoparticles but were synthesised by various methods. In our study, we have exclusively reported the antimicrobial activity of the silver nanoclusters synthesised by arresting the tollens reagent method which is cost effective and easiest method reported till date. However, the detailed GO to cell interactions need to be studied for the better understanding of this cause. Synthesised silver nanoclusters concentration increase effect on the antibacterial activity need to be studied.

The deposition of silver nanoclusters on the contact lens surface like RGP lenses and hydrogel lenses can also be done and their antimicrobial activity can also be tested for holistic understanding and approach. This is an effective coating on the contact lens surface which will control the ocular infections and also will be a beneficial coating on medical devices to kill the infection-causing microorganisms.

2. LIMITATIONS

We were unable to use XPS on a coated cysteine graphene oxide contact lens in objective 1 in order to determine the binding energy of CysGO on the lens surface, which would have allowed us to determine the binding energy of the depositions to the lens surface. Additionally, because to the lack of the instrument's facilities, we were unable to use AFM to assess the surface roughness and average particle size of the deposition on the coated and uncoated contact lens using CysGO. In addition, the wettability of the lenses needs to be assessed. In objective 2, we could not assess surface roughness on both silicone hydrogel and hydrogel lenses (coated and uncoated) due to the lens state. We could not evaluate the wettability and the binding energy of silver nanoclusters on the contact lens surface. However, by including these characterizations, we might have a better understanding of the above-mentioned material's deposition on the contact lens surface. In objective 3, we could not check the antimicrobial activity of the lens coated with different deposition times and concentrations of silver nanoclusters due to time constraints.

2. FUTURE SCOPE

To meet the end users demands, the enhancement of the lens characteristics might focus on the additional lens properties of the deposited lenses such as wettability, oxygen permeability, lens thickness and mechanical properties. The forementioned properties will result in the comfort, wearing time, easy handling and durability without effecting the vision clarity. The antimicrobial activity of the both the coatings can be explored more in terms of the minimum concentrations and employment of different characterisation techniques, therefore can be applied to other medical devices as well.

3. SUMMARY

The synthesis of the cysteine graphene oxide was confirmed by characterisation techniques like FE-SEM, FTIR, Raman spectroscopy, and XPS. The deposition of the CysGO on both the contact lens surfaces was confirmed by the FE-SEM. The effect of CysGO deposition on CL's was evaluated by UV spectroscopy and swelling percentage, there is an increase in the transmittance percentage of the deposited lenses by approximately 3% and with a significant increase in the swelling percentage ($p < 0.001$). The silver nanoclusters were synthesised by arresting Tollens' reagent method and were confirmed by FE-SEM and Raman spectroscopy. The deposition of the silver nanoclusters on the contact lens surface was confirmed by FE-SEM, AFM, and Raman spectroscopy with uniform distribution on the lens surface with a $233cm^{-1}$ peak in Raman spectra resembling Ag-O vibrational mode. The antimicrobial activity of the silver-deposited contact lens surface showed clear inhibition zones for both gram-positive and gram-negative bacterial strains, the inhibition zone for *Staphylococcus aureus* is 1.8 cm and *Pseudomonas aeruginosa* is 1.4 cm respectively. The synthesis of these novel materials and deposition on the contact lens surface will enhance the lens parameters and reduce adverse effects like microbial keratitis caused by contact lens use. The cost-effective and easiest method used to synthesize silver nanoclusters will also be a beneficial coating on medical devices for infection control.

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