ABSTRACT

Protein folding is a post-translational process where a newly synthesized polypeptide chain adopts a stable and functional three-dimensional structure through intermediate stages. Errors in this process can result in misfolded proteins, which are usually eliminated by the cell. However, when the removal system is overwhelmed, these proteins accumulate, forming aggregates. These aggregates can be amorphous or have organized amyloid-like β-sheet structures. These aggregates are linked to over 30 diseases, including cataracts caused by crystallin protein aggregates. Cataracts are a leading cause of blindness worldwide, predominantly affecting the elderly. Studies suggest that long-living individuals face a high risk of developing cataracts.

Crystallins are classified into three groups—α, β, and γ—each with subclasses. Among these, human γD-crystallin is associated with cataracts and plays a crucial role in the disease's progression. Factors such as aging, mutations, and environmental stress cause γD-crystallin to aggregate in lens fiber cells. These aggregates scatter light, impairing the lens's ability to focus it on the retina, leading to poor vision.

Currently, the only effective treatment is surgical replacement of the affected lens with an artificial one. However, challenges such as surgical side effects, limited access to facilities for surgery and costs associated with it, especially in developing countries, highlight the need for affordable and accessible alternatives. Developing a drug that can prevent or delay crystallin aggregation could be a potential solution. While some small molecules have shown promise *in vitro*, none have advanced to clinical trials. Similar approaches have been explored for other amyloid diseases, with some drugs undergoing clinical trials. Curcumin, quercetin and baicalein act as potent antiamyloidogenic and fibril destabilizing agents for SOD1 fibrils, and few others. Quercetin, baicalein, curcumin belongs to group of class known as polyphenols.

Polyphenols are natural occurring compounds which have antioxidant, anti-inflammatory properties. Due to these properties, polyphenols can act as potential drug for aggregation prone disease like cataract. Despite these properties, polyphenols have limitations such as less water solubility and prone to degradation and aggregation. These limitations can be overcome by encapsulation systems made up of biodegradable polymers like chitosan, TPP and PLGA.

This thesis entitled as "**Polyphenol-Based Strategies to Inhibit γD-Crystallin Aggregation and their Encapsulation for Improved Delivery**"

The research aims to look at the aggregation of γD-crystallin established under different conditions, uncover a potential polyphenol for its inhibition followed by encapsulation of the polyphenol in biodegradable nanoparticles and finally using polyphenol loaded biodegradable nanoparticles as potential drug for disease like cataract. The thesis is organized into seven chapters, each addressing these objectives.

Chapter 1: This chapter presents a review of the existing literature on protein and diseases caused by aggregation of proteins. It then discusses cataracts, with a specific focus on the role of crystallin protein aggregation in their development. It systematically explores the various factors contributing to crystallin aggregation while providing a detailed overview of the eye's components, including the structure, stability, and functions of different crystallin protein types. The chapter further discusses the encapsulation of polyphenols in chitosan-based nanoparticles.

Finally, the chapter concludes by outlining the hypothesis and research objectives aimed at optimizing various condition for aggregation of γD-crystallin. Investigating a potential polyphenol for inhibition of aggregation of γD-crystallin and finally fabrication of polyphenol loaded nanoparticles. Study of these nano complex was done in a elaborate manner. At the end of this chapter,

the origin of the scientific problem, objectives designed for this thesis, and outline of the research work performed were discussed.

Chapter 2: This chapter details the chemicals procured for protein expression and purification aggregation-related experiments, emphasizing their relevance and application in the study. It also discusses the principles underlying the use of experimental and computational techniques employed in the research. Furthermore, the chapter provides comprehensive information about the various analyses performed, offering insights into the methodologies and their significance in finding a potential drug (polyphenol).

Chapters 3-7 present all the experimental work done in this thesis.

Chapter 3: In this chapter, fabrication and characterization of chitosan-based nanoparticles is discussed. Two set of nanoparticles CS-TPP NPs and CS-PLGA NPs were fabricated, and their characterization was done by biophysical techniques like TEM, DLS, SEM and few others. Parameters like encapsulation efficiency and loading efficiency was also calculated for both polyphenol loaded nanopartilces.

Chapter 4: In the current work, we carried out an in-depth investigation of the effect of polyphenol loaded nano-formulations on the aggregation of γD-crystallin. At first, the protein was allowed to form amorphous aggregates under denaturating conditions. Several polyphenols were then tried to inhibit the aggregation of the protein. Amongst the polyphenols tested, most effective polyphenol are found to be resveratrol and quercetin. Since polyphenols are prone to degradation, they were encapsulated in chitosan nanoparticles in order to provide an ambient condition for them to function effectively. The loading efficiency and release kinetics of polyphenols were subsequently tested. Finally, the efficacy of resveratrol/quercetin loaded chitosan nanoparticles as inhibitors for γ-D-crystallin aggregation were confirmed in a series of experiments demonstrating the potency of the system in the prospective therapeutic intervention of eye ailments associated with self-

assembly of γ-D-crystallin proteins.

Chapter 5: Human eyes get exposed to UV light which can be caused by environmental changes like ozone depletion and global warming. Exposure to UV light can be a cause of cataract, one of the ocular diseases which may cause vision impairment. In our present study, we have carried out an extensive examination of polyphenols as inhibitors for UV-induced aggregation of γD-crystallin. On exposure to UV-C light, γD-crystallin forms fibrils instead of amorphous aggregates. Various polyphenols were tested as inhibitors, out of these quercetin, baicalein, and caffeic acid were found to be effective. As polyphenols are insoluble in water, nanoencapsulation was tried to enhance their bioavailability. CS-TPP and CS-PLGA encapsulating systems were considered as they form biodegradable nano-capsules. Out of three polyphenols (quercetin, baicalein, and caffeic acid), quercetin forms nanocarriers of smaller sizes, a must for crossing corneal/retinal barrier. Quercetin nanocarriers were considered as an effective system that could be used for therapeutic application. For these nanocarriers, encapsulation efficiency, and polyphenol release kinetics were studied. CS-PLGA NPs were found to have better loading efficiency for quercetin than CS-TPP NPs.

Chapter 6: It has been found that in cataractous lens, concentration of metal ions like copper is increased significantly as compared to a healthy lens. γ-D crystallin is one of the more abundant proteins in the core of the lens. Experimentally, it has been found that metal ions like Cu^{2+} causes nonamyloid aggregation of γ-D crystallin. Using techniques like turbidity assay, CD spectroscopy, ANS binding study and microscopic techniques like TEM , we show here that Cu^{+2} form nonamyloid aggregates. Polyphenols, like quercetin, can act as potential inhibitor for these metal-based aggregates. However, polyphenols are associated with a drawback, that is, less solubility and stability in water. To overcome this drawback, nano carriers loaded with quercetin were employed to act as inhibitors.

Finally, **Chapter 7, Future aspects and conclusions,**

In this chapter briefly future aspects and conclusions from research carried out during this thesis are discussed. Future aspects involve toxicity studies, crossing of retinal barriers and few other cell culture studies.