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Title of Project: The Potential of Curcumin as Antioxidant for Diabetic Cataract Prevention

Project Abstract:

Curcumin's role as an antioxidant has been studied in numerous studies and has shown potentials as a glycation inhibitor. This study aimed to study curcumin's ability to inhibit glycation of α-crystallin to find out its potentials as an antioxidant for preventing diabetic cataract. The first experiment looked at the difference between the sample containing curcumin and the sample without curcumin without any incubation period. The Fluorescence detection was set at at 37 degrees Celsius for wavelengths of 325/434 nm, 295/340 nm, 330/415 nm and 370/440 nm measuring for N-formylkynurenine, tryptophan, dityrosine and MGO respectively. There was no significant difference between the sample containing curcumin and the sample without curcumin. The results were the same in the experiment set at same conditions but with 24-hours pre-incubation period. The control experiment which was done in the same conditions except with a known glycation inhibitor, aminoguanidine hydrochloride. With aminoguanidine hydrochloride, there was a significant decrease in the fluorescence compared to the control. Although there were no experiments directly with α-crystallin, the experiments with curcumin and aminoguanidine hydrochloride provided evidence that curcumin failed to inhibit glycation of BSA and has low probability with successful inhibition of α-crystallin glycation.

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Applicant Signature: Nawon Lee

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Faculty Sponsor Signature: Myoung Lee

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Nawon Lee Research Grant/Capstone Project Final Paper

Title

The Potential of Curcumin as Antioxidant for Diabetic Cataract Prevention

Abstract

Curcumin's role as an antioxidant has been studied in numerous studies and has shown potentials as a glycation inhibitor. This study aimed to study curcumin's ability to inhibit glycation of α-crystallin to find out its potentials as an antioxidant for preventing diabetic cataract. The first experiment looked at the difference between the sample containing curcumin and the sample without curcumin without any incubation period. The fluorescence detection was set at at 37 degrees Celsius for wavelengths of 325/434 nm, 295/340 nm, 330/415 nm and 370/440 nm measuring for N-formylkynurenine, tryptophan, dityrosine and MGO respectively. There was no significant difference between the sample containing curcumin and the sample without curcumin. The results were the same in the experiment set at same conditions but with 24-hours incubation period. The control experiment which was done in the same conditions except with a known glycation inhibitor, aminoguanidine hydrochloride. With aminoguanidine hydrochloride, there was a significant decrease in the fluorescence compared to its absence. Although there were no experiments directly with α-crystallin, the test-run experiments with curcumin and aminoguanidine hydrochloride provides evidence that curcumin failed to inhibit glycation of BSA and has low probability with successful inhibition of α-crystallin glycation.

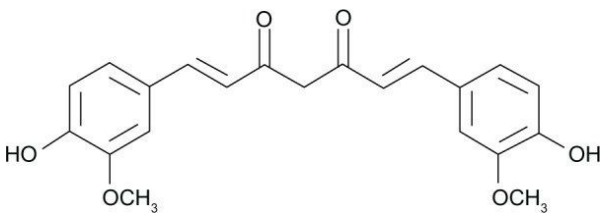
Introduction

Advanced glycation end products are the result of protein glycation and often lead to development of several complications in diabetic patients. Early onset of cataracts is one of many complications involved.[1] The development of cataracts is common in people of age 65 or

older but diabetes can hasten the process through glycation of lens proteins.[2] When cataracts develop and begin to disrupt eyesight, most people residing in United States can easily remove it through surgery since cataract surgery is one of the most common surgery in US with an overall success rate of 98% or higher.[3] However, many people in developing countries still cannot afford cataract surgery and live with the discomfort until blindness sets on because there are no medications available for the disease. To find an alternative to surgery for treating cataracts, many different methods have been studied. Among several potential treatment methods, antioxidants that inhibit advanced glycation end products have been studied as a way to prevent cataracts. Although this is only preventive, it may still be helpful for diabetic patients who do not expect to get the surgery to delay the early onset of cataracts. In this research, curcumin, an antioxidant, will be studied for its effectiveness in preventing development of cataracts due to advanced glycation.

Aggregation of advanced glycation end products is stimulated by oxidative stress and hyperglycemia with diabetes and this aggregation of advanced glycation end products is what leads to complications such as cataracts. Curcumin has been studied in the past with diabetic rats for the relationship it may have with the advanced glycation end product levels. The study showed that the oxidative stress and cross-linking of collagen decreased and glycation was prevented in these diabetic rats.[4] In this study, the focus is put on the glycation of lens crystallin and the role of curcumin as an antioxidant to inhibit the glycation of this protein found in the eye that leads to diabetic cataracts.

Figure 1: Structure of curcumin



Specifically, the glycation of α-crystallin found in the lens is common because α-crystallin has a relatively long half-life and hence more prone to posttranslational modifications such as glycation.[5] Glycation of α-crystallin results in several problems as it loses its anti-apoptotic activities and its function as chaperone protein leading to other protein aggregates.[6] Curcumin which has been found to reduce glycation in diabetic rats will be used with of α-crystallin and D-glucose to measure its effectiveness in inhibiting glycation of lens crystallin to prevent cataracts.

As a control experiment, aminoguanidine hydrochloride will be used in place of curcumin. Aminoguanidine hydrochloride is a known glycation inhibitor due to its ability to lower the effective concentration of glucose or MGO to slow down the glycation. Trial experiments will first be conducted with bovine serum albumin (BSA) instead of α-crystallin. This is done to attain the correct experimental parameters for α-crystallin and prevent wasting the limited resources.

In addition, as an alternative for when D-glucose is not significantly glycated in a given amount of time, MGO will be used to replace it. MGO is a more powerful glycating agent than glucose.

Figure 2: Structure of aminoguanidine hydrochloride

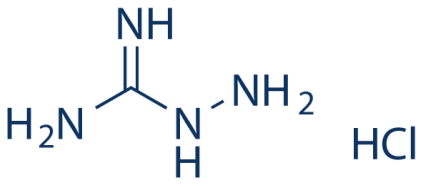


Figure 3: Structure of D-glucose

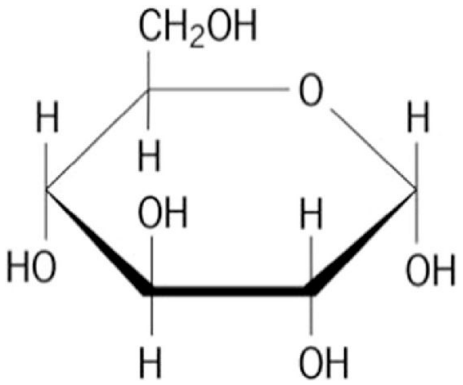
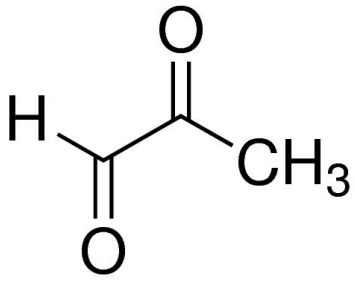


Figure 4: Structure of MGO (Methylglyoxal)



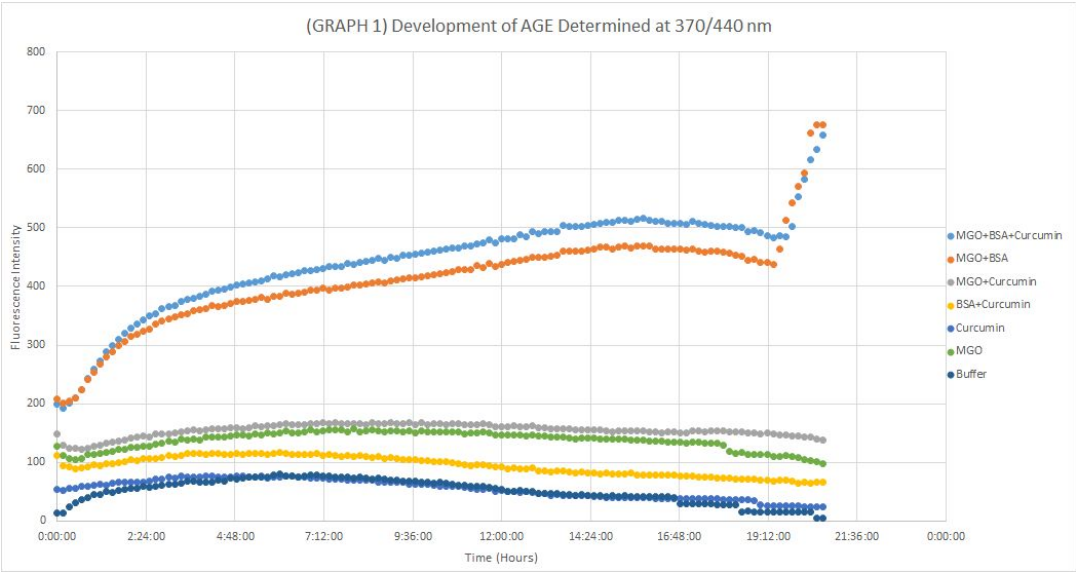
Materials and Methods

Curcumin from *Curcuma longa* (Turmeric), MGO, BSA and DMSO were the main materials used in determining the influence of curcumin in inhibiting glycation. All materials were purchased from Sigma Aldrich. To create an MGO solution with a concentration of 50mM, 77 µL of filter-sterilized MGO was mixed with 10 mL of PBS (pH 7.4) which acted as a buffer. For the protein solution with a concentration of 5 mg/mL, 50 mg of BSA was mixed in 10 mL of buffer. Lastly, 0.0037g of curcumin was mixed with 1 mL of DMSO before adding 0.1 mL of this solution to 29.9 mL of buffer to create a concentration of 0.05 mM. The purpose of mixing curcumin with DMSO first was to aid in better resolution of curcumin in buffer. Different

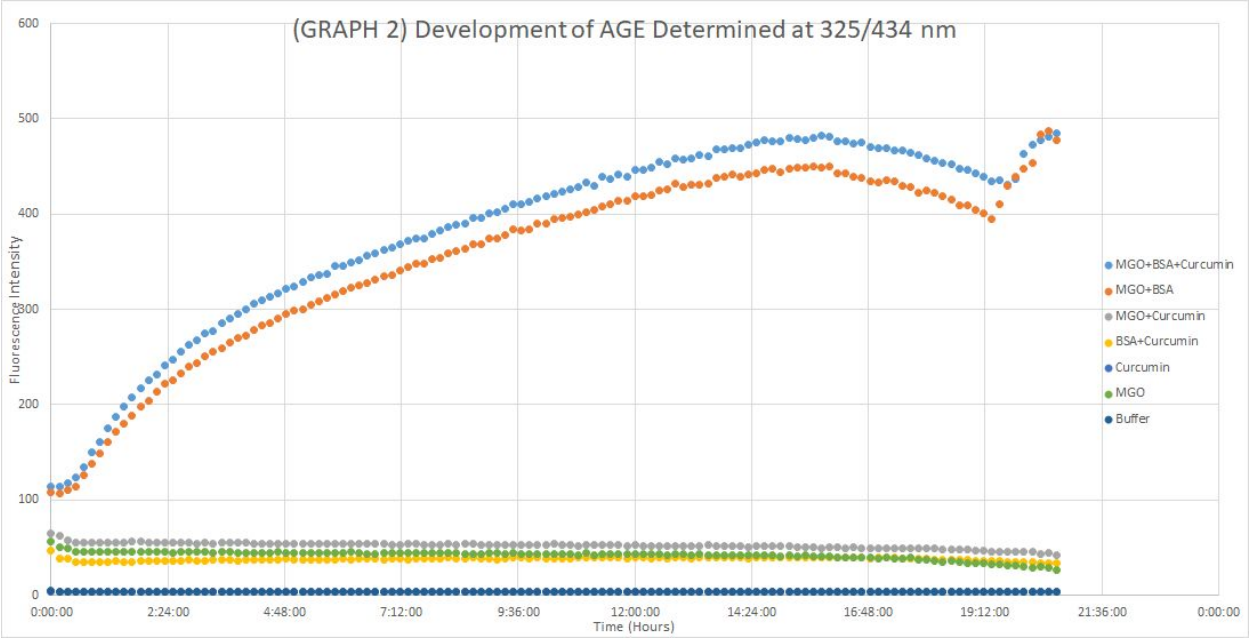
combinations of these prepared solutions were pipetted into the microplate to create 6 controls and 1 experimental condition. For the experimental solution, 0.1 mL of MGO, 0.1 mL of BSA and 0.01 mL of curcumin was pipetted into each well in triplicates. The rest of the 6 control samples were missing either BSA, curcumin, MGO, both BSA and MGO, both BSA and curcumin or all BSA, MGO and curcumin. All the controls were pipetted in triplicates. In the first experiment, the samples were incubated for 24 hours at 21 degrees Celsius and the incubation was excluded in the second experiment. To read and measure glycation-related chemical and conformational changes, the microplate reader was set at 37 degrees Celsius for 325/434 nm (N-formylkynurenine), 295/340 nm (Tryptophan), 330/415 nm (Dityronsine) and 370/440 nm (formation of advanced glycation end product). To test what effects were brought by curcumin, all conditions were kept the same in the third experiment with 1 mM of aminoguanidine hydrochloride replacing the curcumin solution. Aminoguanidine hydrochloride is a known glycation inhibitor.

Results and Discussion

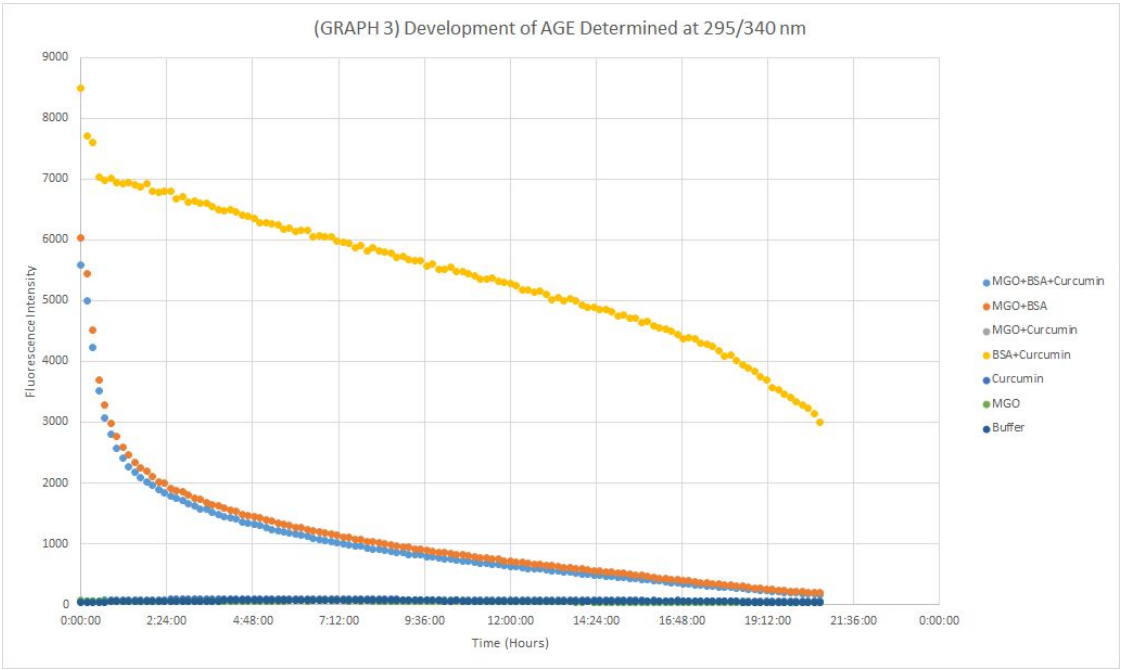
Graph 1: Glycation progression without incubation in the presence of curcumin measured at 370/440 nm.



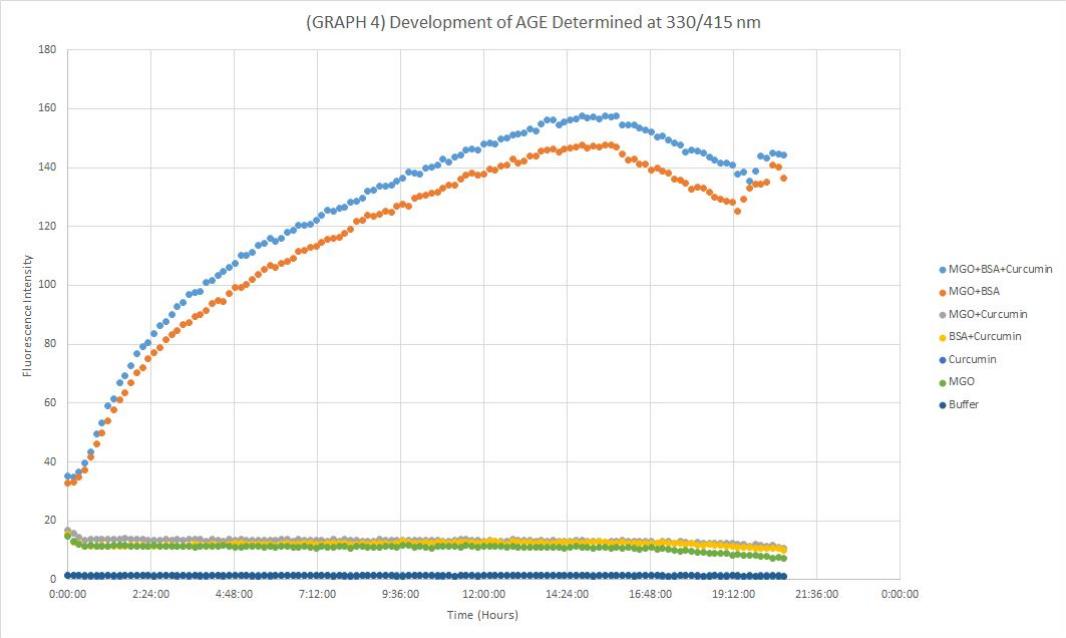
Graph 2: Glycation progression without incubation in the presence of curcumin measured at 325/434 nm.



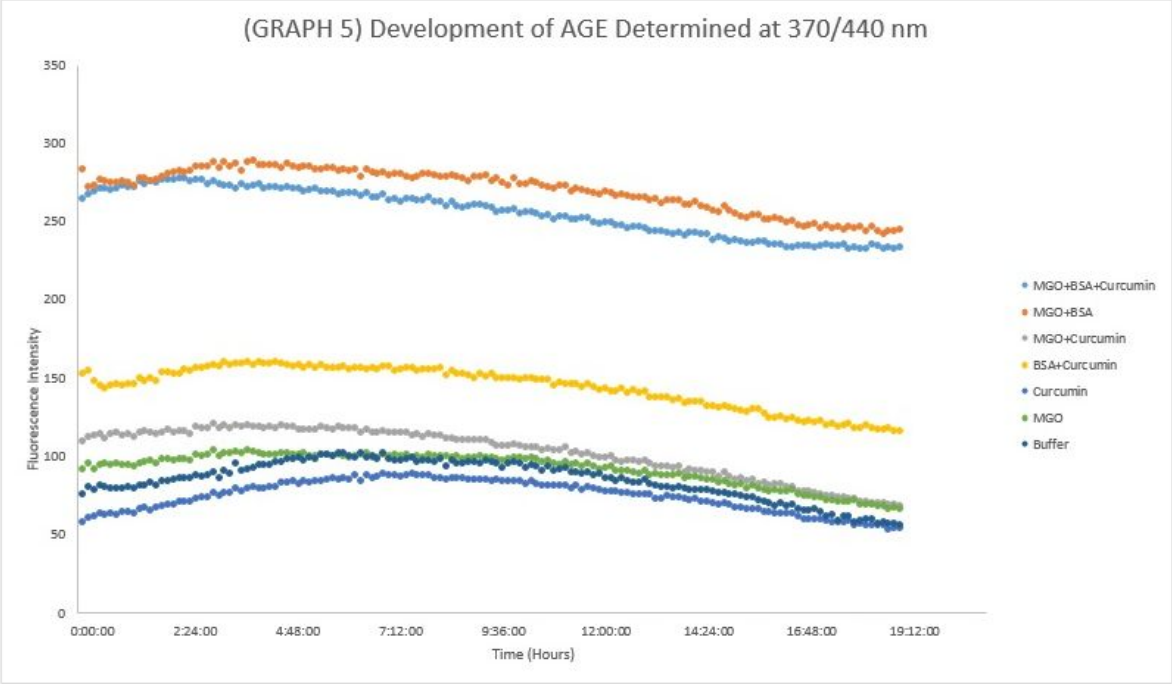
Graph 3: Glycation progression without incubation in the presence of curcumin measured at 295/340 nm.



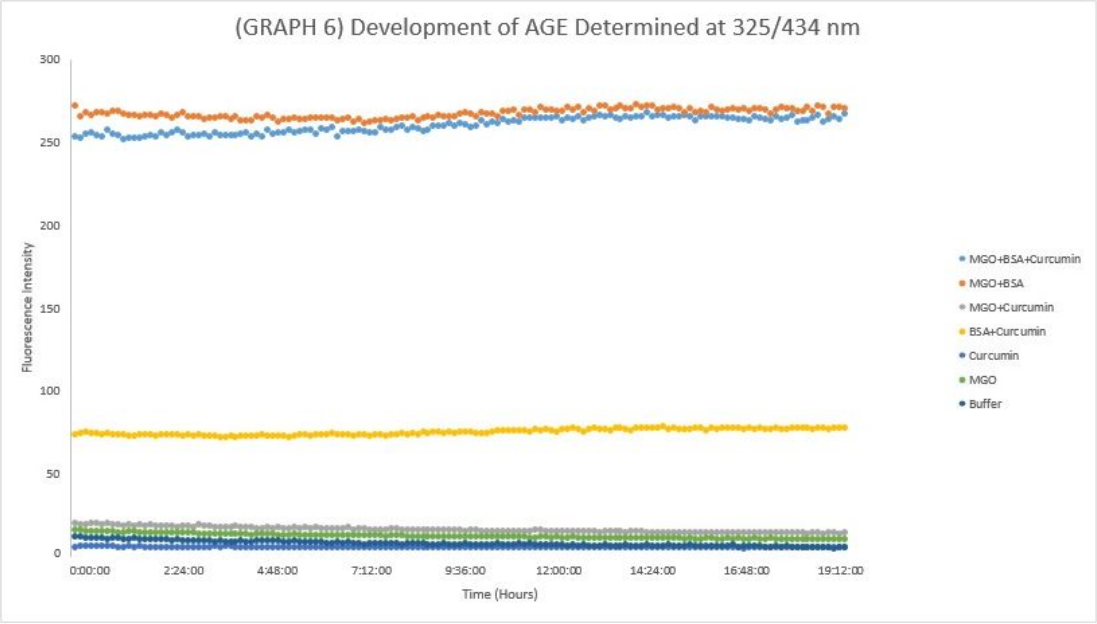
Graph 4: Glycation progression without incubation in the presence of curcumin measured at 330/415 nm.



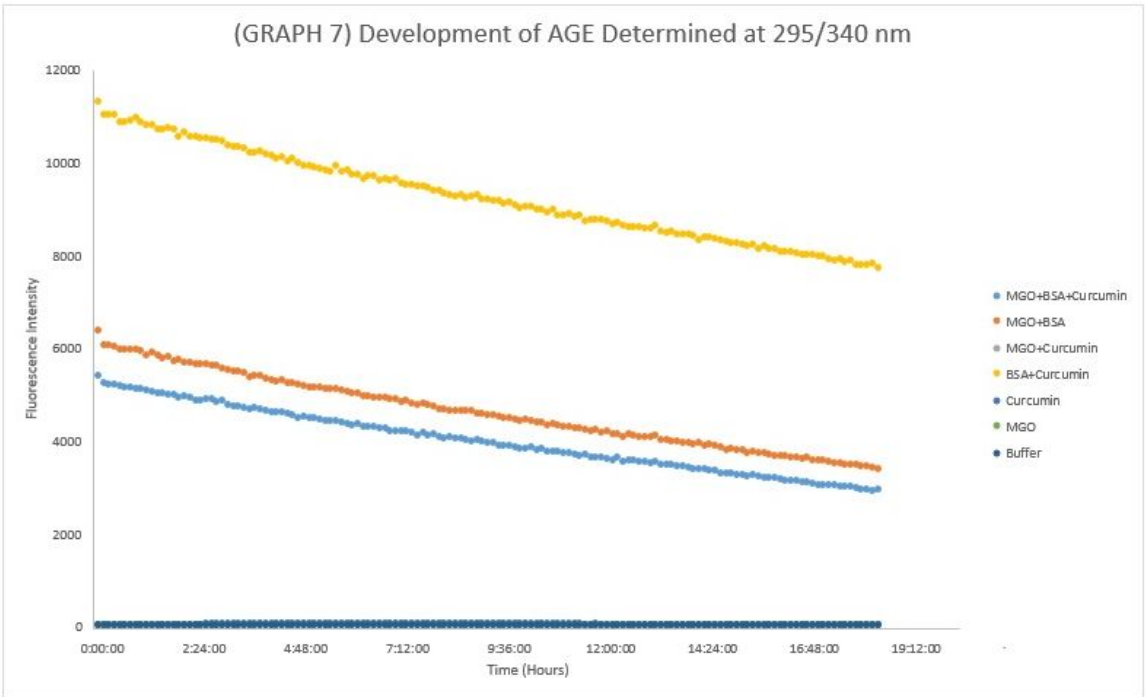
Graph 5: Glycation progression after incubation in the presence of curcumin measured at 370/440 nm.



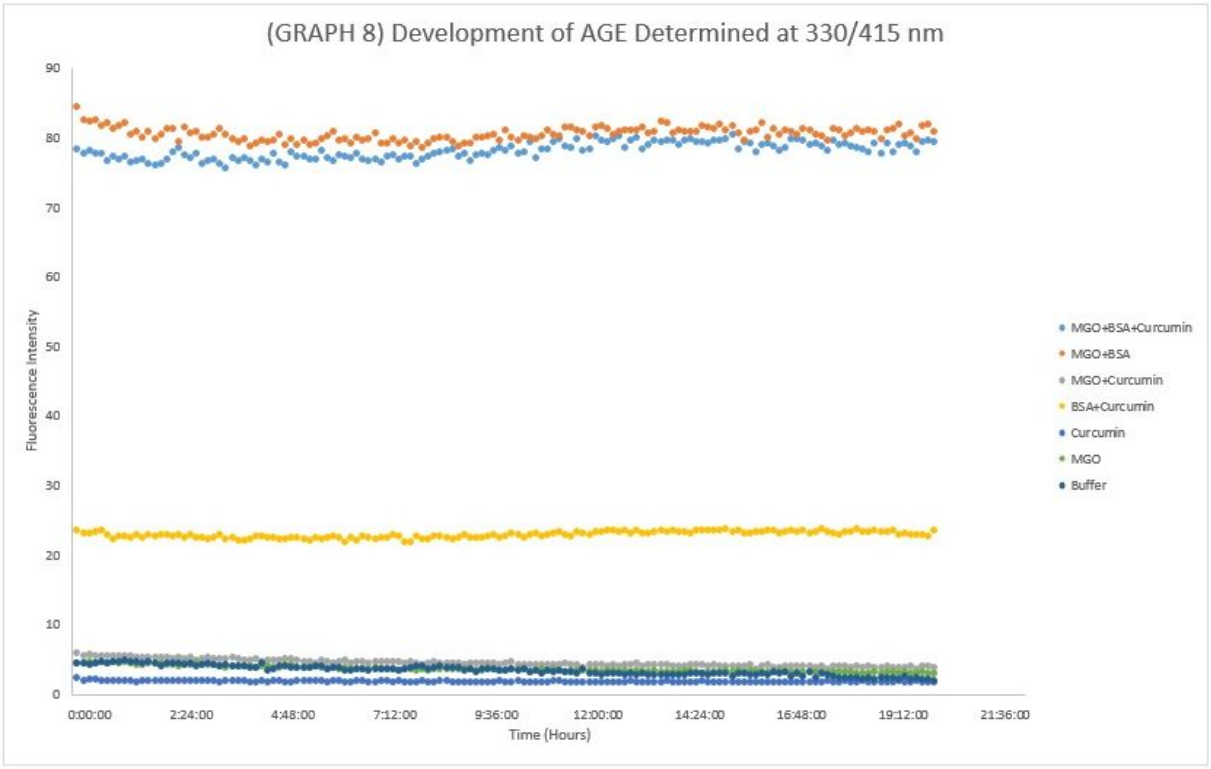
Graph 6: Glycation progression after incubation in the presence of curcumin measured at 325/434 nm.



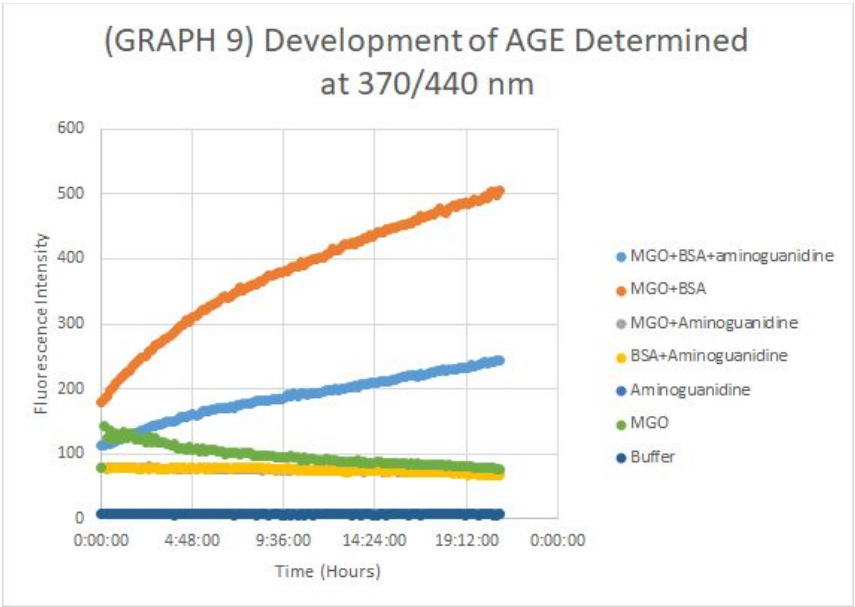
Graph 7: Glycation progression after incubation in the presence of curcumin measured at 295/340 nm.



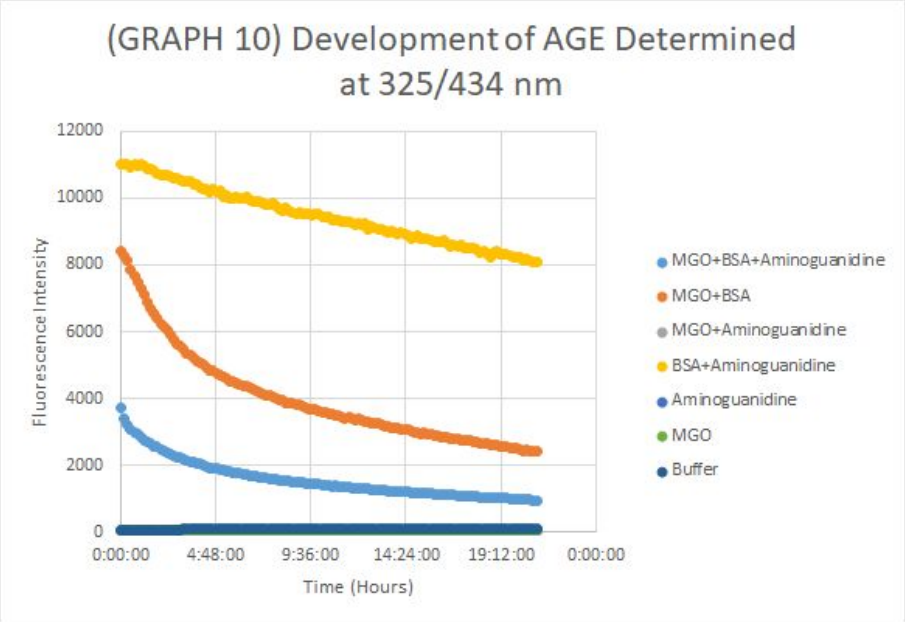
Graph 8: Glycation progression after incubation in the presence of curcumin measured at 330/415 nm.



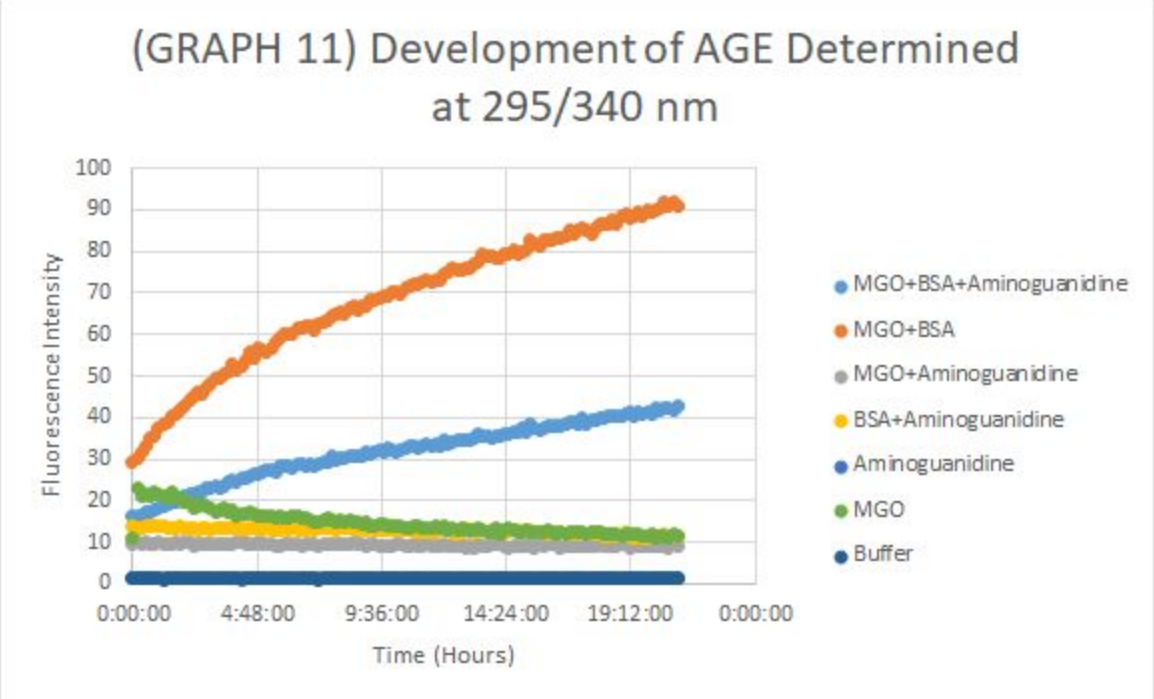
Graph 9: Glycation progression after incubation in the presence of aminoguanidine hydrochloride measured at 370/440 nm.



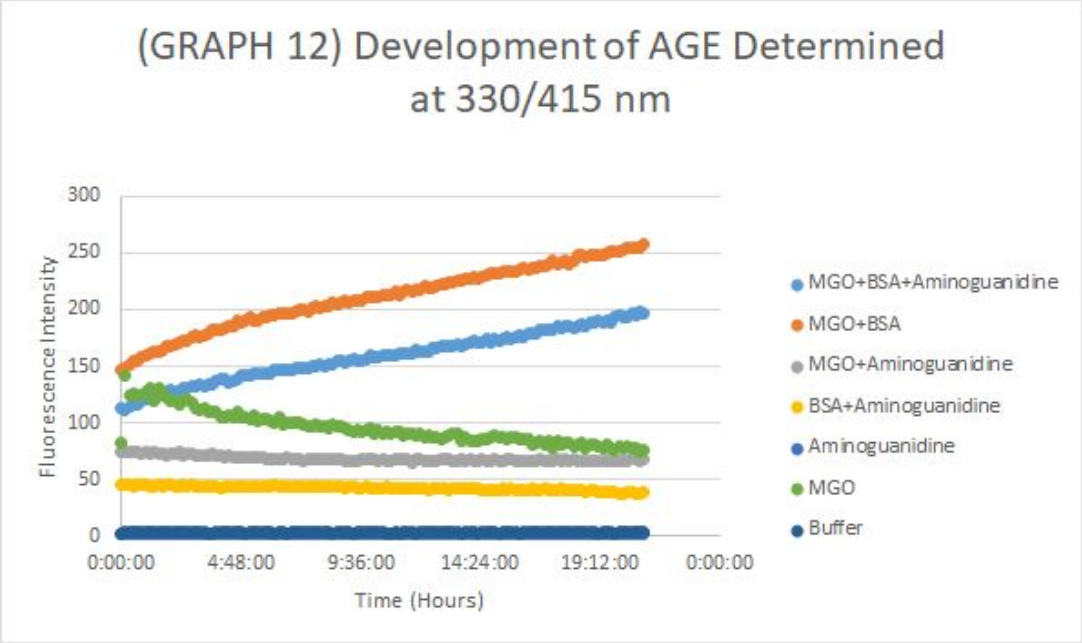
Graph 10: Glycation progression after incubation in the presence of aminoguanidine hydrochloride measured at 325/434 nm.



Graph 11: Glycation progression after incubation in the presence of aminoguanidine hydrochloride measured at 295/340 nm.



Graph 12: Glycation progression after incubation in the presence of aminoguanidine hydrochloride measured at 330/415 nm.



The initial design for this research was to find the glycation inhibition potential of curcumin with α-crystallin. The first few experiments with BSA was going to be test runs to find out the optimal concentration, incubation period and wavelength for the experiment with α-crystallin. However, these experiments with BSA failed to demonstrate curcumin as a glycation inhibitor.

Graphs 1, 2, 3, 4 show insignificant difference in the rate of AGE production between the experimental sample containing all MGO, BSA and curcumin and the control sample containing just MGO and BSA. As graphs 5, 6, 7, 8 indicate, a 24 hour incubation period did not make much difference between the control and experimental samples. Still, a 24 hour incubation seem to be effective with detecting a more stable glycation progression as illustrated by the relatively constant fluorescence in graphs 5, 6, 7, 8 compared to graphs 1, 2, 3, 4.

The control experiment with a known glycation inhibitor, aminoguanidine hydrochloride, shows a significant difference in the rate of AGE production between the experimental and control samples. Graphs 9, 10, 11, 12 show a significantly lower fluorescence at every wavelength for the sample containing aminoguanidine hydrochloride compared to the sample not containing it. This control experiment supports the failure of inhibiting glycation with curcumin in the previous experiments.

Conclusion

This series of experiments failed to support or reject the hypothesis that curcumin inhibits the glycation of α-crystallin. This is because there were no actual experiments with curcumin and α-crystallin to draw conclusions from. However, experiments with curcumin and BSA which were supposed to be test-runs provided enough evidence that there is a low probability that curcumin will prevent glycation of α-crystallin. There were no significant decrease in the fluorescence for the sample containing curcumin compared to the sample without curcumin. The results remained unchanged even after a 24 hour incubation.

The experiment under same conditions with aminoguanidine hydrochloride as the glycation inhibitor showed a significant decrease in the fluorescence for the sample containing aminoguanidine hydrochloride compared to the sample without it. This helped determine that it was curcumin that failed to cause decrease AGE production.

Acknowledgements

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