An Investigation of Pyclen Metal Chelator Release on the Aggregation of Amyloid-Beta

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I. Introduction

Alzheimer's Disease (AD) – Potential therapies. Previous Research links Alzheimer's Disease (AD) with likely aggregation of Amyloid-beta-40 (Aβ40) in the brain, which creates neurotoxic plaques, causing further development of AD[1]. Metal Chelation Therapy is a form of potential treatment for AD, whereby the scavenging of metal ions inhibits Aβ40 aggregation.

Motivation for Choosing Pyclen Macrocycles. A series of pyclens (Fig. 1) are being evaluated for this purpose, each possessing different pyridyl moieties and ring substituents.

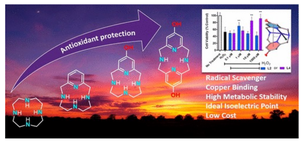


Figure 1: Antioxidant capabilities of pyclen[5]

- Pyclens are strong antioxidants and copper binding ligands, and their properties may be tuned as a function of size and outer rim chemical functional group, especially with regard to release from $pSiO_2$ (Fig. 2).
- These macrocycles were loaded into mesoporous silica via incipient loading protocols, and subsequently released into HEPES buffer at a physiological temperature of 37°C as measured via UV-VIS spectroscopy.

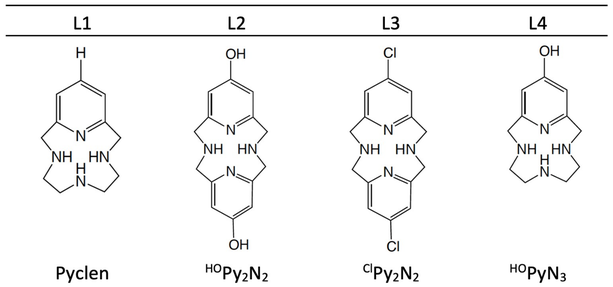


Figure 2: The structures of L1, L2, L3, and L4 chelating agents

Motivation for Pyclen loading / release from a mesoporous carrier. Three primary reasons:

1) A potential for achieving a diffusion-limited sustained release profile, with the capacity to remain in an optimal therapeutic concentration range longer than a conventional release profile release (Fig. 3)[3].

2) Mesoporous silica is selected as a delivery system for these pyclen derivatives due to its biocompatibility and its large surface area allowing for large capacity loading[4].

3) While previous research has investigated the possibility of covalent attachment of pyclen-like molecules to mesoporous silica, this project explores the potential benefits of incipient loading techniques to aid pyclen molecules in crossing the Blood-Brain Barrier[1] (Fig. 4).

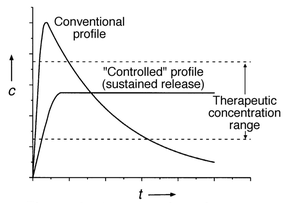


Figure 3: A comparison between controlled and conventional release profiles[3]

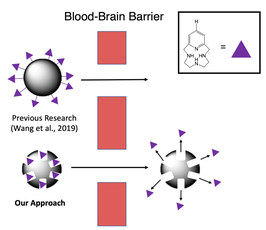
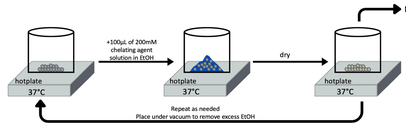


Figure 4: Illustration of covalent bonding vs porous particle release crossing the Blood-Brain Barrier

II. Loading / Release of Pyclens from Mesoporous Silica

A. *Incipient Loading of Pyclen Derivatives into Mesoporous Silica*



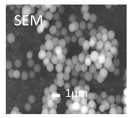
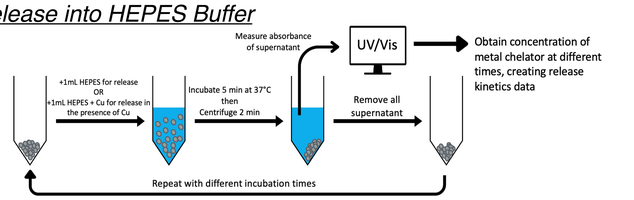


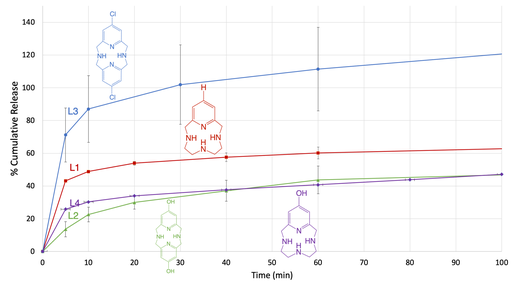
Figure 5: SEM image of 1µm mesoporous silica used as the delivery system

B. *Release into HEPES Buffer*



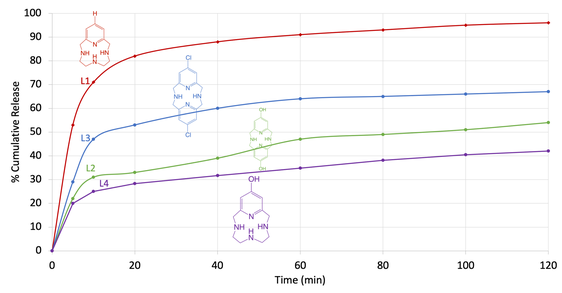
- The release is done into both HEPES and HEPES + Cu(II) ion solution (4 mM) to test whether complex ion formation affects the release profile from the porous matrix.

C. *Figure 6: Release of Pyclen Derivatives into HEPES buffer*



- All four chelating agents achieve sustained release by 20-minutes. L1 had a high burst effect relative to its maximum release. Both L2 and L4 have a slower initial release, but both continue to steadily release over time.
- Cumulative release values for L3 >100% are the result of $pSiO_2$ trapping EtOH in its pores.

D. *Figure 7: Release of Pyclen Derivatives into HEPES Buffer & Cu(II)*



- In the presence of copper ions, L1 achieved a much higher cumulative release.
- L2 and L3 slower releases in the presence of copper, but L2 had a faster initial burst in copper than in HEPES buffer alone. The steric hindrance and present hydroxyl groups on L2 seem to slow down its release much more than L1 and L3.

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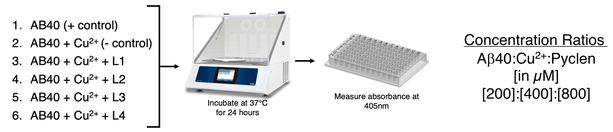
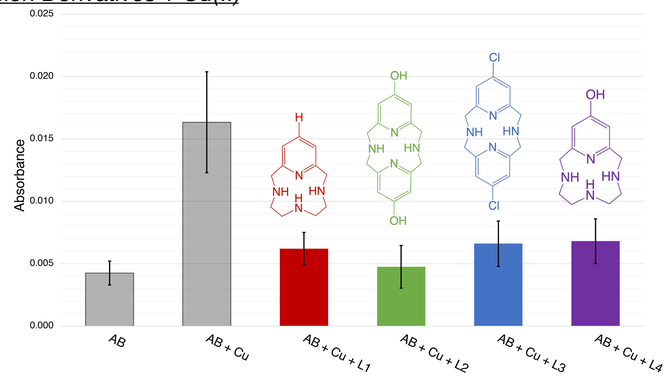
III. Protein Aggregation Study

A. *Protein Aggregate Turbidity Assay & Analysis*



- To observe the preventative effects that pyclen metal chelators may have against metal ion-induced aggregation, pyclen solutions were added to a given Aβ40 solution at room temperature, left to incubate for 5 min, then a given Cu(II) solution was added.

B. *Figure. 8: Turbidity Assays of Aβ 40 in the Presence of Selected Pyclen Derivatives + Cu(II)*



- The solution that did not have any chelators present in the presence of copper had the highest absorbance values, indicating increased protein aggregation
- Solutions with metal chelators were able to prevent protein aggregation. All of these groups were significantly less turbid than solution without metal chelator present (AB + Cu)
- No significant difference was found between the solutions containing L1-L4

IV. Conclusion

- All four chelating agents were able to achieve sustained release.
- Hydroxyl groups of L2 and L4 decreased both the encapsulation efficiencies and the %-cumulative release of these two molecules.
- This may be a result of hydroxyl groups interacting with the surface of the $pSiO_2$ more strongly than the pyridyl moieties alone in L1 or the Cl^- groups present in L3.
- Turbidity assays showed successful prevention of protein aggregation with all four metal chelators.

Future studies hope to find a reliable and consistent quantitative assay to quantify protein concentrations after incubation periods. Release studies can also be expanded upon by conducting these experiments on more authentic BBB models.

V. References

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VI. Acknowledgements

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