Pyclen Macrocycle Release from Mesoporous Silica as a Drug Carrier and Impact on Amyloid-Beta Peptide Aggregation

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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 (AB40) 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 AB40 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.

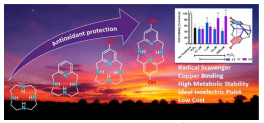


Figure 1: Antioxidant capabilities of pyclen[5]

- Pyclens are strong antioxidants and copper binding ligands, and their properties may be tuned as a function as size and outer rim chemical functional group, especially with regard to release from $pSiO_2$ (Fig. 2)
- These macrocycles were loaded into mesoporous silica either possessing 2-nm or 4-nm average pore diameters via incipient loading protocols, and subsequently released into HEPES buffer at a physiological temperature of 37°C as measured via UV-VIS spectroscopy.

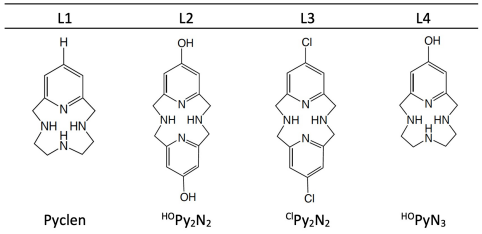


Figure 2: The structures of L1, L2, L3, and L4 chelating agents

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].

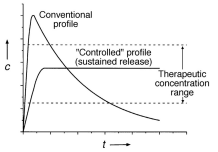


Figure 3: A comparison between Controlled and Conventional release profiles[3]

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).

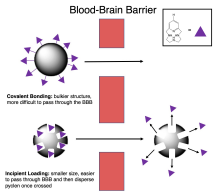


Figure 4: Illustration of covalent bonding vs porous particle release crossing the Blood-Brain Barrier

II. Experimental

A. Incipient Loading of Pyclen Derivatives into Mesoporous Silica

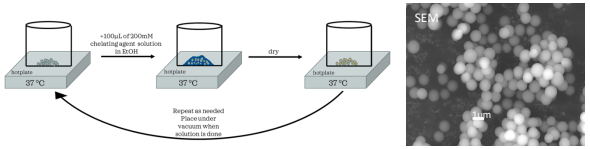
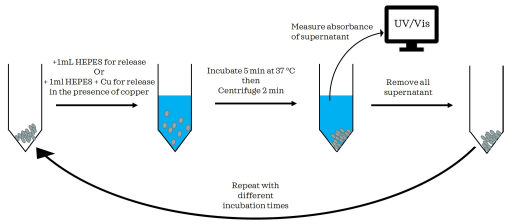


Figure 5: SEM image of 1µm mesoporous silica used as the delivery system

B. Release into HEPES Buffer



- The release is done into both HEPES and HEPES + Cu(II) ion solution (4mM) to test whether complex ion formation affects the rate of release

III. Results

A. Encapsulation Efficiency

Pyclen Molecule	Encapsulation Efficiency
L1	17%
L2	10%
L3	16%
L4	9%

Table 1: Encapsulation Efficiency measurements

- Encapsulation efficiency quantifies how much pyclen was loaded into a given sample of $pSiO_2$. A sample of loaded $pSiO_2$ is placed into solution with DMSO and is subjected to sonification and vortex to draw out all present pyclen.
- Calibration Curves were constructed from UV—Vis spectra to determine pyclen concentration in HEPES
- $\frac{\text{mass of pyclen}}{\text{mass of pSiO}_2} \times 100 = \text{encapsulation efficiency}$

- Encapsulation efficiency between all four molecules ranges between 9-17%. Both L2 and L4 have lower encapsulation efficiencies than L1 and L3, suggesting that the presence of hydroxyl groups may interact with $pSiO_2$ in a way that decreases its ability to load efficiently into $pSiO_2$.

B. Representative Spectra of L4 released in the presence of Cu(II)

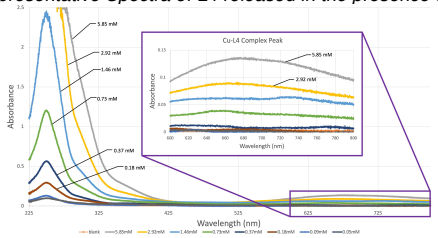


Figure 6: Spectrum of L4 released in the presence of Cu(II) ions and HEPES buffer

- The spectra in Figure 6 shows evidence of ligand-field transition interactions occurring between Cu and pyclen macrocycles, as seen by the broad peak around 700nm.
- Interaction between the pyclen and copper ions impacted release of pyclen from HEPES

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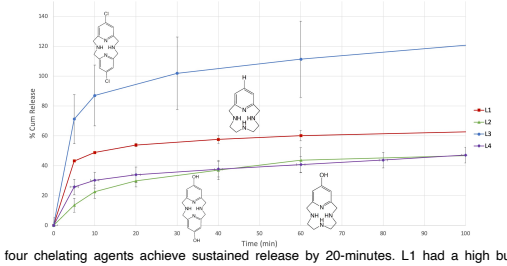
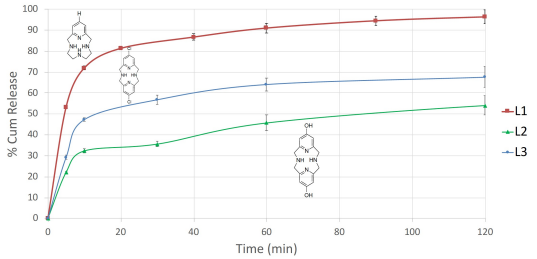
III. Results Cont.

C. Release of Pyclen molecules into HEPES Buffer Results



- 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.
- Measurement of L3 over 100% are the result of $pSiO_2$ trapping loading solvent, EtOH, in its pores.

D. Release of Pyclen molecules into HEPES Buffer & Cu(II) ions Results



- 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 seems to slow down its release much more than L1 and L3.

IV. Conclusions

- 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 $pSiO_2$ and the pores of $pSiO_2$ more strongly than just the pyridine group present in L1 or the Cl^- groups present in L3.

Future studies include studies of additional pyclen derivatives possessing different steric electronic factors that may affect release and encapsulation. The effect of the above pyclen molecules on copper-ion induced AB40 aggregation will be assessed using solubility assays with protein.

V. References

1. Wang et al., *RSC Adv.* **2019,** 9, 14126-14131.
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4. Kalluri et al., *ACS Omega.* **2019**, 4(5), 8359-8364.
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6. Youanna Ibrahim, Honors Thesis, John V. Roach Honors College, TCU, **2022**

VI. Acknowledgements

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