



Cu-Al Layered Double Hydroxide Synthesis and Loading as Drug Adjuvant

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Objective

To evaluate the encapsulation efficiency and loading capacity of Cu-Al Layered Double Hydroxide (LDH) nanoparticles as a promising candidate for vaccine delivery through optimized synthesis parameters, such as temperature, pH, nucleation time, and reactant concentrations.

Background

- One of the pressing needs in vaccine development and immunotherapy is the enhancement of vaccine efficacy and safety.
- Traditional vaccine adjuvants, such as alum, have limitations in terms of eliciting robust immune responses and ensuring sustained immunity.
- To address these challenges, researchers have turned to novel platforms like LDH nanoparticles for their ability to stimulate comprehensive immune responses.
- The development of nanopatforms based on LDH represents a significant advancement in personalized medicine, allowing for real-time monitoring of treatment response and precise delivery of therapeutic agents to disease sites.

Methods

Synthesis of LDH Nanoparticles:

- Nucleation at cold temperature followed by hydrothermal growth of precipitated nuclei was employed. The pH of the solution at nucleation will be adjusted to 8 with 2M NaOH and HNO₃
- The hydrothermal treatment ranged from temperatures of 100 °C, 120 °C, and 140 °C, and times ranged from 1 hour, 1.5 hour and 2 hour

Characterization of Cu-Al LDH:

- Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) were utilized to analyze the size, morphology, and composition of the Cu-Al LDHs synthesized.

Ovalbumin Loading and Encapsulation:

- LDH to OVA solution at a concentration of 1 mg/mL for both in a 4:1 ratio was mixed. The mixture was agitated, and centrifuged. Then, the supernatant was collected to determine the unbound OVA. The BCA Assay was done to determine the encapsulation and loading efficiency of the particles using the given formula:
- %EE = (Actual Antigen Added- Conc. in Supernatant)/ (Actual Antigen Concentration) X 100
- %LE = (Weight of antigen entrapped) / Weight of Nanoparticle X 100

Ovalbumin Release over Time:

- Suspending Ova-loaded pellets in PBS and subjecting them to continuous shaking at 200 rpm. At intervals of 1, 2, 3, 6, and 12 hours, supernatant was collected to determine amount released.

Results

LDH Characterization:

- Morphology from SEM showed platelet (small, disc-shaped cell fragments) particles, indicating that our procedures were correct and successful.
- Lower temperatures showed more variability in sizes while high temperatures were more uniform.
- XRD showed crystalline structures, exhibiting sharp and symmetric peaks at the lower angles and broad and asymmetric ones at higher angles.

Table 1: Encapsulation and loading efficiency for Alum and Cu-Al LDH samples.

Particle	Encapsulation Efficiency (%) (n=3)	Loading Efficiency (%) #1 (n=3)
Alum Standard	65.6	15.5
Cu-Al 120 C 1 hour	83.8	19.6
Cu-Al 120 C 1.5 hours	67.9	15.4
Cu-Al 120 C 2 hours	75.7	15.6
Cu-Al 100 C 1 hour	70.7	15.7
Cu-Al 100 C 1.5 hours	46.6	12.0
Cu-Al 100 C 2 hours	44.1	9.5
Cu-Al 140 C 1 hours	63.8	12.2
Cu-Al 140 C 1.5 hours	67.8	15.2
Cu-Al 140 C 2 hours	45.9	10.1

Encapsulation & Loading Capacity:

- Highest encapsulation efficiency (EE) and loading efficiency (LE) found in LDH nucleated at 120 C for 1 hour and the lowest at 100 C for 2 hours.
- When nucleation time was over 1 hour, the EE and LE decreased possibly due to prolonged exposure of LDH to the oven heat that adversely affects the nanoparticle morphology.

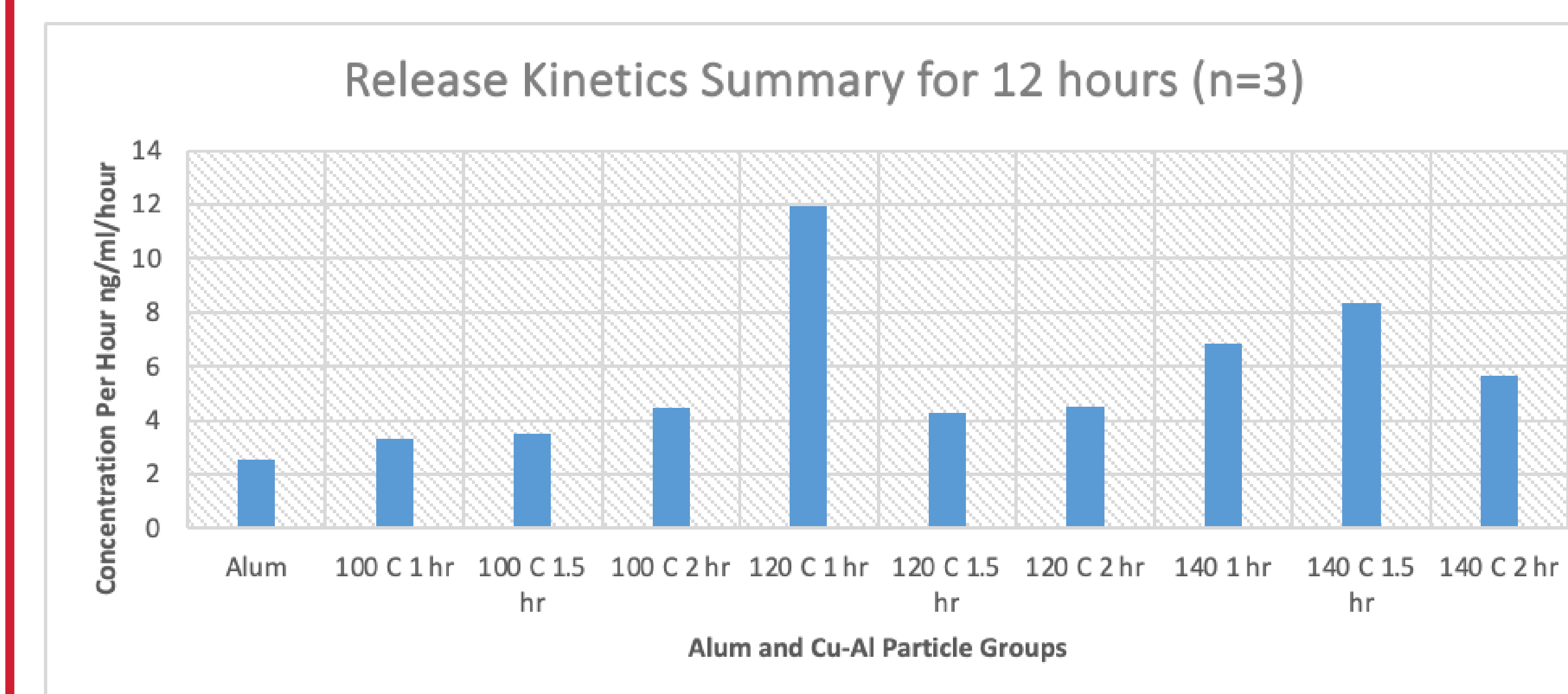


Figure 1: BCA Assay results for Alum and Cu-Al LDH samples from 0-12 hours

- The highest release is at 120 C for 1 hour, followed by 140 C 1.5 hours.
- The lowest release is at 100 C 1 hour, with 100 C 1.5 hour at the second lowest
- All versions released more than Alum, proving the LDH has a better performance compared to Alum.

Conclusion

This project focuses on Cu-Al LDH nanoparticles as a vaccine delivery platform. Based on our results, Cu-Al synthesized at 120 C at 1 hour is able to showcase significantly better adjuvanticity than industry standard, Alum. However, further testing for longer duration needs to be conducted to determine the best synthesis parameters and validate results.

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