Polyphenol-based Creatine-loaded Nanoparticles: A Potential Therapeutic Avenue for Creatine Transporter Deficiency

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Introduction

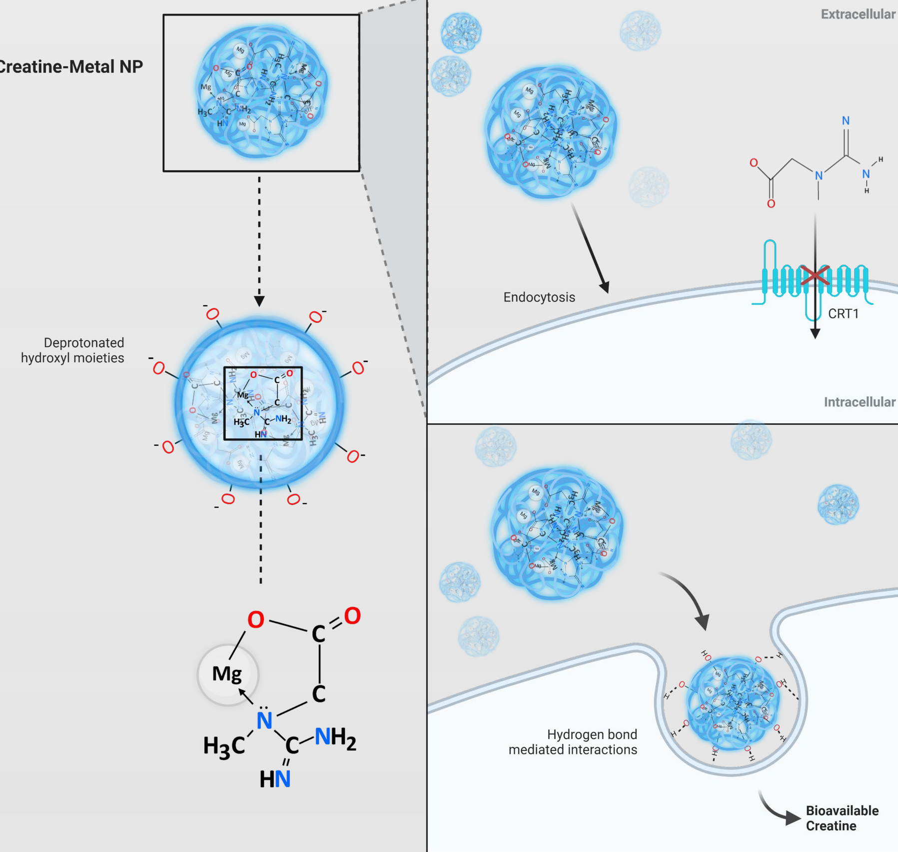
Creatine Transporter Deficiency (CTD) is an X-linked disorder arising from mutations in the SLC6A8 gene, resulting in diminished brain creatine levels and associated cognitive and behavioral impairments. Existing treatments are inadequate, highlighting the urgent need for innovative therapeutic solutions. This research investigates the potential of a polyphenol-based polymer particle system as a novel drug excipient for creatin. Renowned for their antioxidant and neuroprotective properties, polyphenols offer a promising non-toxic profile and the ability to complex with metal ions and interact with other compounds through hydrogen bonds and π-π stacking. The polymer of choice is synthetic but GRAS, ensuring it is not mutagenic and safe for consumption.

Our particles exhibit an impressive loading capacity exceeding 50% and demonstrate no discernible toxicity towards mature hiPSC cortical neurons at concentrations below 150 ppm of creatine or 100 ppm of polyphenol.

Material analysis and characterization employing LC-MS, FTIR, UV-visible spectroscopy, fluorescence spectrophotometry, and Dynamic Light Scattering highlights the distinctive properties of this innovative formulation, laying a solid foundation for its further advancement. FTIR spectroscopy analysis reveals no alterations to creatine, suggesting its loading mechanism relies on Van der Waals forces rather than permanent chemical conjugation. Further analysis demonstrates an ability for surface modification including surface coating with PEG, chitosan, and PEI, though these alterations reduce stability over time.

In-vitro testing on fibroblasts expressing a CTD variant gene shows increased intracellular creatine levels after 24 hours. Moreover, CTD-positive fibroblasts treated with CrNPs demonstrated significantly greater survival during 5 hours of glucose starvation.

Polyphenol-based, Creatine-loaded Polymer Matrices



Supplementary figure: variations of creatine-metal complexes demonstrate their unique appearances. Unloaded without metal (left-most). The particles above include varieties synthesized with and without creatine with metals such as Iron, Gold, Calcium, Copper, Selenium, and Zinc. Sizes range from 200nm to as much as 3 microns depending on the ratio and metal utilized.

Results

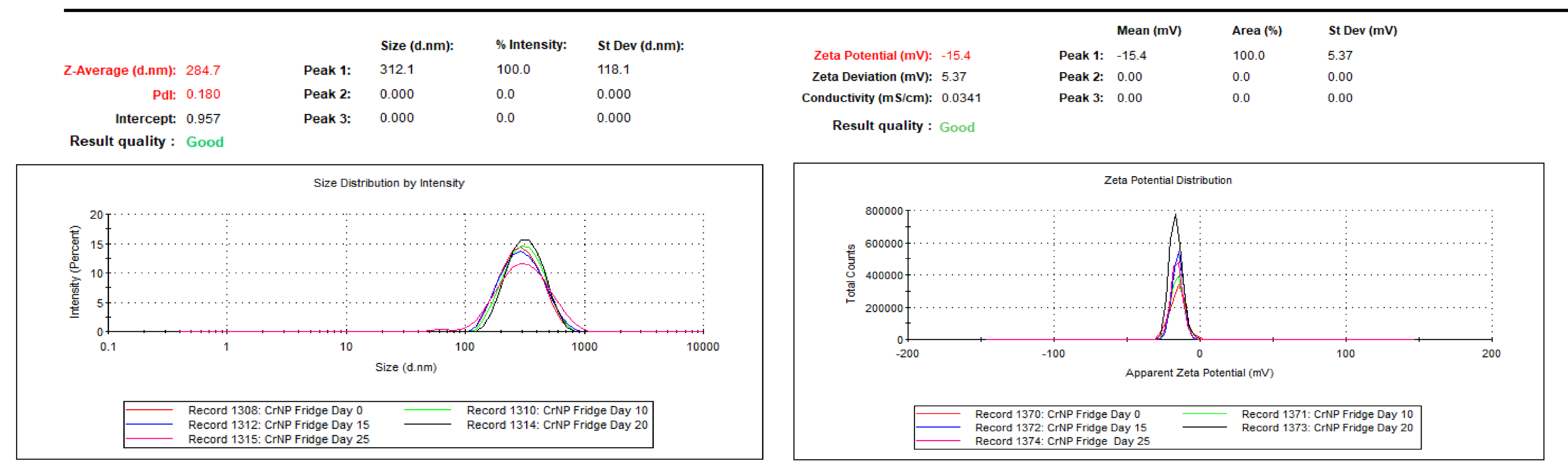


Figure 1: Particle hydrodynamic diameter (left) and zeta-potential (right) after washing step across 25 days in deionized water solution. Samples kept at room temperature and refrigerator showed no significant difference over 25 days.

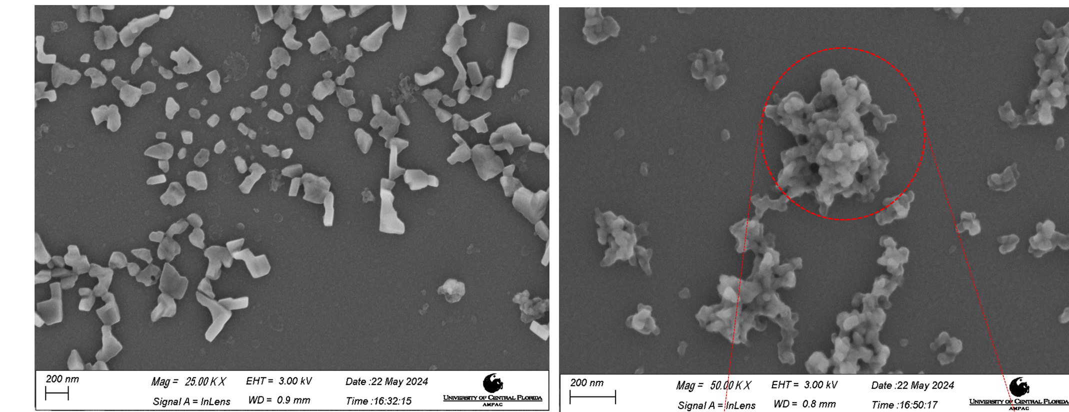


Figure 2: Scanning Electron Microscopy images of Creatine-loaded nanoparticles (above) and unloaded nanoparticles (right). Significant morphology change was observed with creatine-loaded samples, likely due to crystallization when dried.

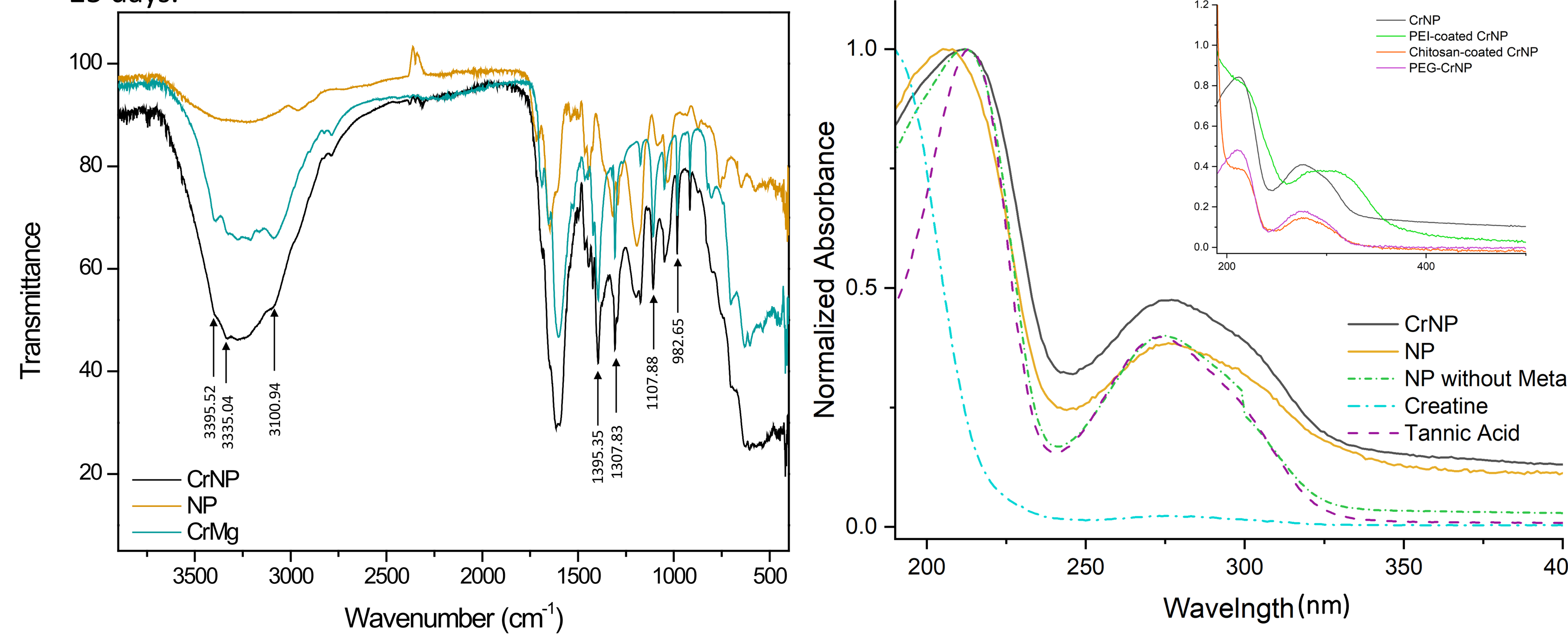


Figure 3: FTIR Spectroscopy (left) of Creatine-loaded nanoparticles (black), unloaded nanoparticles (gold), and creatine-magnesium complex. All samples were analyzed after lyophilization. UV-Visible Spectroscopy (right) of particle components and surface coatings.

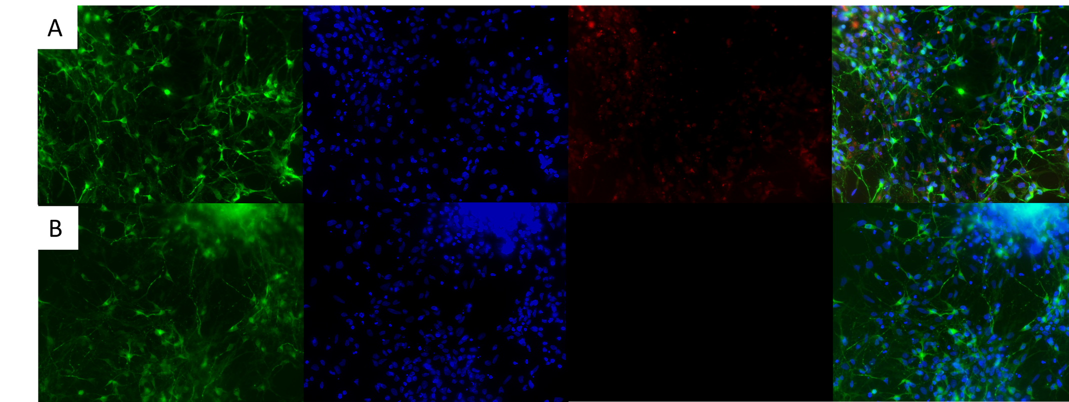


Figure 4: Fluoresce-based images taken on a Keyence BZ-X800 microscope of WT-Cortical neurons treated with DAPI stain (blue), MAP2K (green), and R6G-CrNPs (red). The top row (A) is the treated group while the bottom row (B) is untreated.

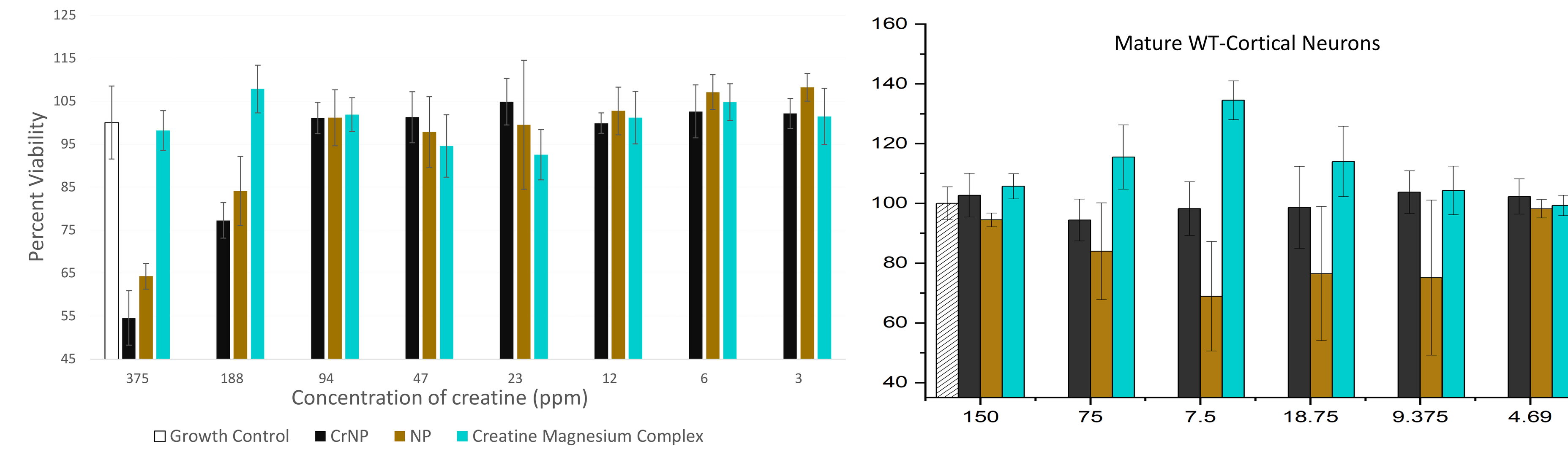
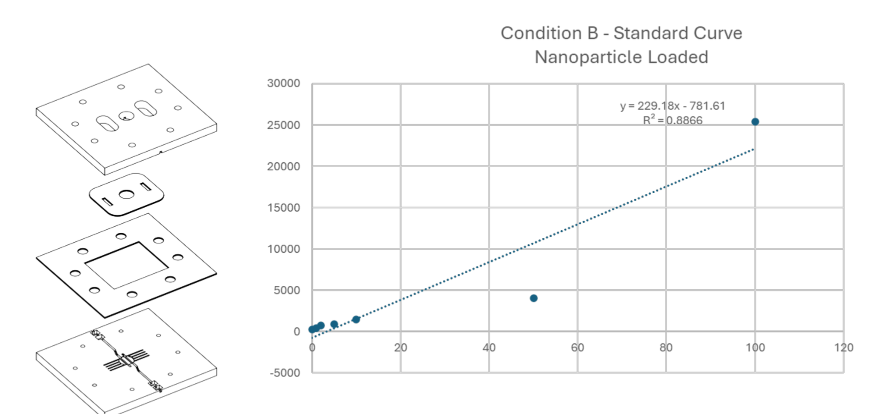


Figure 5: Alamar Blue cell viability assays performed on J774 Murine Monocytes (left) and iPSC Wild-Type (WT) Cortical Neurons (right). Toxicity is seen at concentrations above 150 ppm of creatine, 100 ppm of Tannic Acid, and 100 ppm of PVP, well above physiological normal content of creatine per cells.



Average TEER = 1575.5

	Average	St. Dev
CNS Side	24.82922	5.150347
Blood Side	5.823414	0.701874
Acellular CNS Side	20.95344	4.003403
Acellular Blood Side	10.75404	2.919103

Figure 6: In-vitro Blood-Brain Barrier on-a-chip assay performed using CrNPs fluorescently tagged with Rhodamine 6G. LCMS data from similar tests corroborate poor passage through the BBB in vitro.

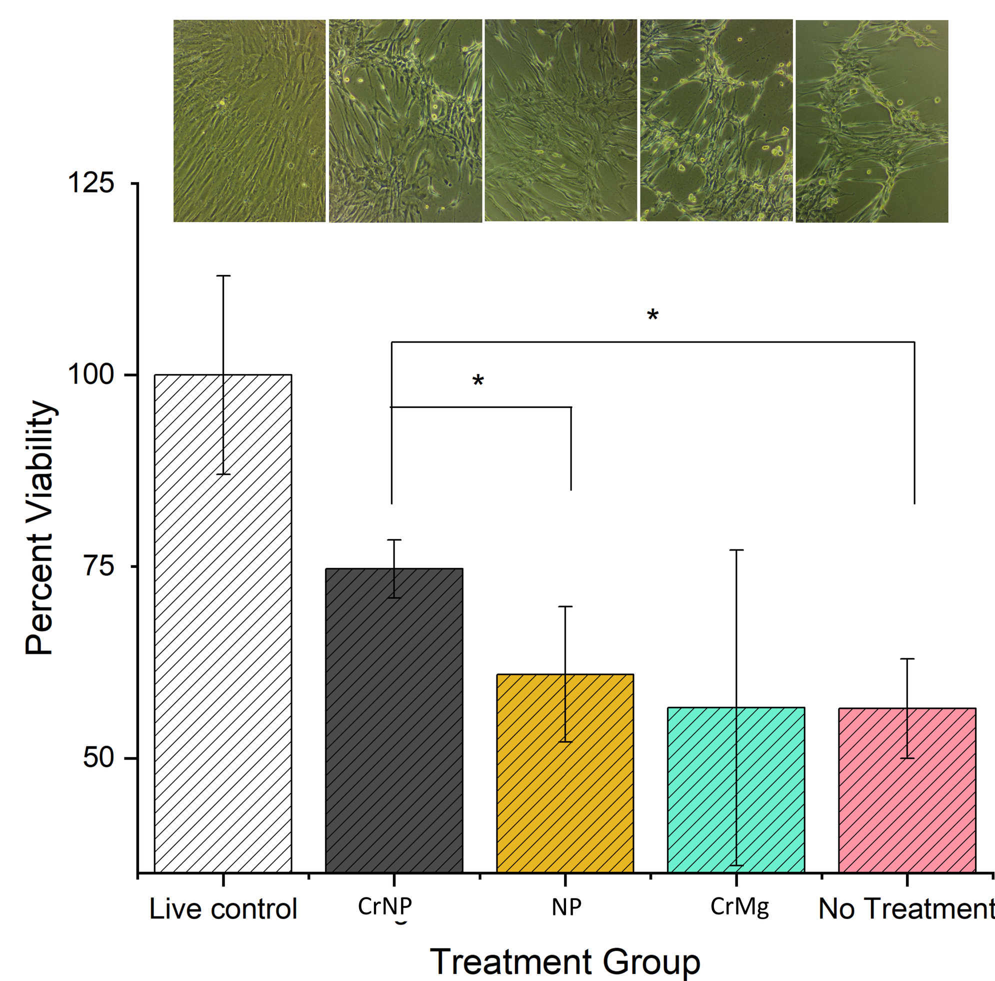


Figure 7: Alamar Blue cell viability assay of CTD-positive fibroblasts after 12 hours of treatment and 5 hours of media starvation.

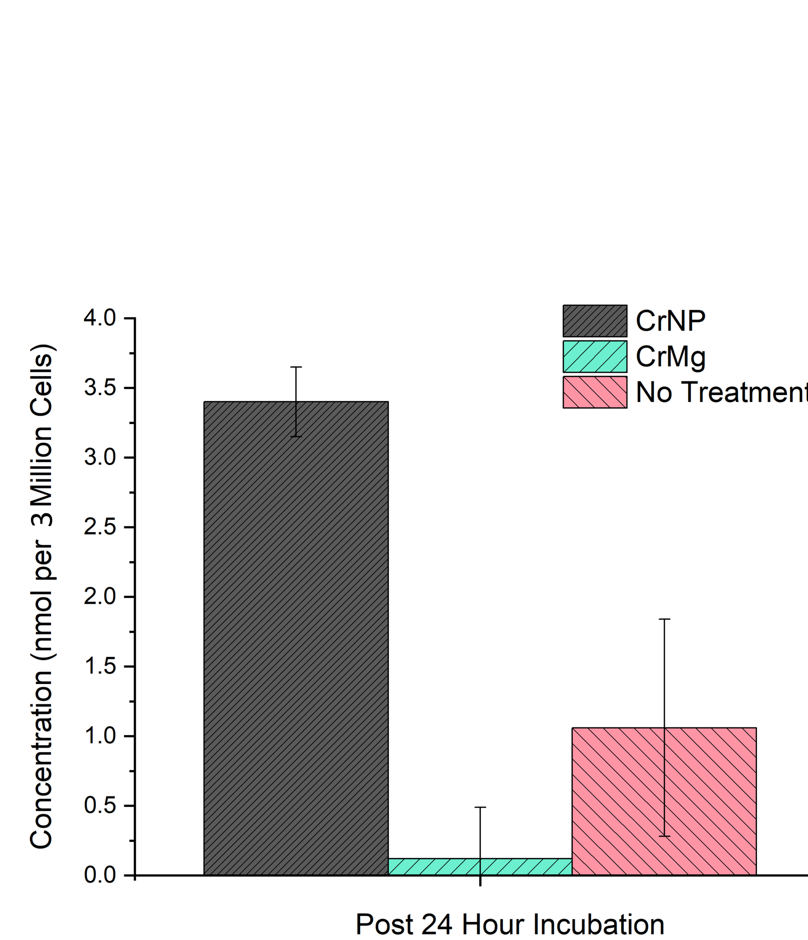


Figure 8: Fluorescence-based Creatine Detection Assay on CTD-positive fibroblasts 24 hours post-treatment shows clearly that creatine enters fibroblasts with a mutated SLC6A8 gene and remains after 24 hours.

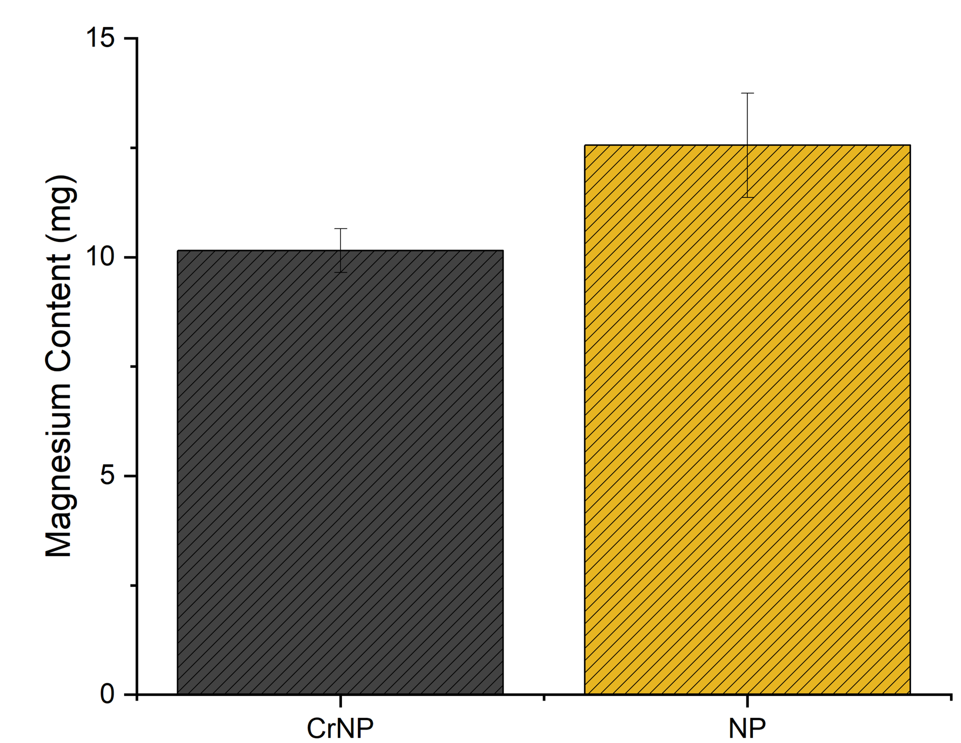


Figure 9: AAS quantification of Magnesium within CrNPs and unloaded NPs. Interestingly, creatine presence reduces the efficiency of Magnesium complexation with Tannic Acid.

Discussion

Creatine-loaded particles:

- Synthesized and characterized nanoparticles loading creatine with a loading capacity of 50%.
- Measured their stability in storage conditions over 25 days demonstrating robust stability.
- Screened for toxicity at ranges above physiological normal.
- Investigated the potential of other metals.

Enhanced survival:

- Mammalian and mouse cells had significantly improved survival rates as compared to controls when treated with NPs
- Effect attributed to anti-apoptotic properties of creatine and tannic acid, aiding ATP production and free-radical scavenging.

Potential applications:

- Enhanced cell survival under low energy conditions suggests applications in head trauma, stroke, neurodegenerative diseases, and continued development for CTD.

Challenges:

- Particle size is a barrier, especially for crossing the Blood-Brain Barrier.
- Alternative delivery methods like intranasal administration might offer better results.
- Need to optimize particle size for improved efficacy.

Creatine Detection:

- Creatine detected in CTD-positive fibroblasts, but in lower quantities than expected.
  - Potential reasons: early utilization or need for shorter exposure times/particle size optimization.
- Average particle diameter: 260-300nm, with many particles below 200nm.
- Smaller particles may enter cells more efficiently; larger ones may be washed away during assays.

Surface modification:

- Particle system can undergo surface modifications with fluorescent markers and secondary polymers.
- Stability concerns with different surface coatings:
  - PEI Coating: Particle size ~240nm; zeta change from -18 mV to +33 mV; enhanced uptake but reduced stability.
  - Chitosan Coating: Sporadic size distribution; significant zeta potential change.
  - PEG Coating: Particle disruption at high concentrations; reduced stability at all tested concentrations.

ACD ASSOCIATION FOR CREATINE DEFICIENCIES

Poster 4

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