Toxicity assay of green synthesized Iron oxide Nanoparticles on *Caenorhabditis elegans*

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Abstract

In this study, iron oxide nanoparticles (INP) were synthesized using cilantro leaf extract and ferrous sulfate salt. The synthesized INP were characterized by Fourier-transform infrared spectroscopy (FTIR), Ultraviolet-visible spectroscopy (UV-Vis) and X-ray diffraction (XRD) techniques. XRD showed crystalline structure with a crystallite size of 42.75 nm while Scanning Electron Microscopy (SEM) revealed a particle size of 68 nm along with clusters of particles up to 192 nm.

The potential of INPs for treating neurodegenerative diseases was tested using a worm model for Parkinson's disease (WLZ3) using Caenorhabditis elegans (C. elegans) . The worms were exposed to four different concentrations of INPs mixed with DI water: 0.5 mg, 1 mg, 2 mg and 5 mg/ml. After 48 hours of exposure, the survival rate of wild type (N2) worms were 50%, 70%, 20% and 0% respectively. For the WLZ3 strains the survival rate was 30%, 30%, 0% and 0% respectively.

When the nanoparticles were coated with curcumin, an antioxidant known for its effectiveness against neurodegenerative diseases and anti-inflammatory properties, the survival rate of the WLZ3 strain was 61%, 79%, 64%, and 9% respectively. The data suggests that there is a significant toxicity when the concentration of INPs is greater than 1 mg/ml concentration and a significant boost in effectiveness when adding curcumin to the nanoparticles.

Introduction

The BBB is a semi-permeable membrane made of endothelial cells that protects the central nervous system (CNS) from harmful substances. Since neurological illnesses are not uncommon, the BBB has become a target for therapeutics and drug delivery. Nanoparticles, with their small size, are an emerging field of novel treatments due to its ability of crossing the BBB.

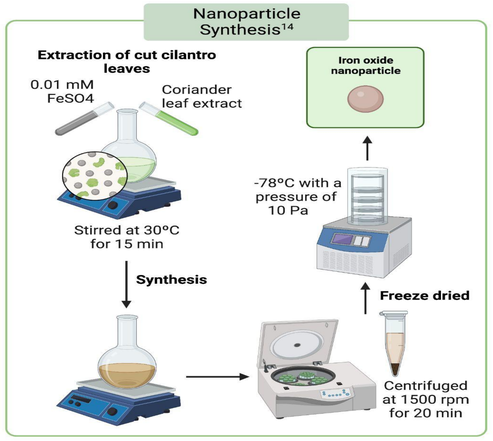
Drugs alone are too large to pass the barrier, but they can permeate the barrier when attached to small nanoparticles. In particular, metal nanoparticles are a preferred method of use as they can be produced in various shapes and sizes. Linked with a drug, these metals reach the CNS, typically binding with receptors on the exterior surface of the BBB. Then they are ejected to the interior through vesicles. Metals play an important role in the brain in the regulation of synaptic plasticity, myelination, and as cofactors in the production of neurotransmitters. A potential drawback of nanoparticle usage is an increase in permeability of the BBB, which could cause toxic particles to damage neural cells.

Iron oxide nanoparticles can cross the BBB and be synthesized using cilantro plant extract. Cilantro leaves consist of reducing and capping agents which are incorporated as a feature in the nanoparticle. Phytochemicals are present, consisting of ketones, aldehydes, flavones, and amides. Iron is one of the most targeted delivery drugs and biocompatibility is also present in the plants through vivo studies of polysaccharides.

Curcumin coated nanoparticles may be another root to effective drug delivery. Curcumin has a variety of health benefits because it is naturally anti-inflammatory and is a natural antioxidant. Curcumin has been seen to have positive effects when dealing with neurodegenerative diseases. Curcumin coated nanoparticles may lead to higher success rates.

To test out the toxicity and effectiveness of the nanoparticles, we are using *Caenorhabditis elegans*, a transparent nematode that has 60-80% genetic homology to humans. Additionally, it has a simple, transparent CNS which can be visualized under a microscope. *C. elegans* can be genetically modified to produce strains to test different experimental conditions. The WLZ3 strain is a good model to study Parkinson's disease, while the wild type is used as the control to test the toxicity and effectiveness of synthesized iron oxide nanoparticles. By using the lifespan assay, we are testing for an appropriate dosage that *C. elegans* could withstand without the nanoparticles being toxic based on how many of the nematodes were able to survive.

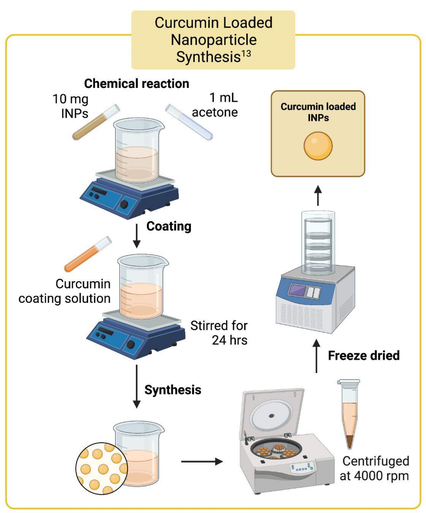
Methods



Biorender. BioRender. (n.d.). https://biorender.com/

Lifespan Assay[16] of *C. elegans*

1. Transfer L4 larva to seeded FUDR plates. For each strain or condition being tested, it is typical to set up 2-3 plates with 25-30 worms per plate.
2. Place Amp/FUDR plates at 20 °C for 24 hours. After 24 hours, visually assess worms, media, and bacteria.
3. Ensure that all worms on the plate are not adults and have not laid eggs or are dead.
4. Record the date and the number of worms that are alive and dead after analysis of worms.
   - Note: Worms are dead if they don't respond to external stimuli such as tapping using a dissecting scope. If the worms are dead, then remove from the plate.
5. Return plates to 20 °C.



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Results

Concentration of INP (in mg/ml)	N2 (wild type) Survival rate	WLZ3 strain Survival rate	WLZ3 strain Curcumin coated Survival rate
0.5	52%	30%	61%
1	67%	30%	79%
2	25%	0%	64%
5	0%	0%	9%

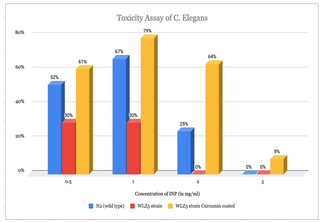
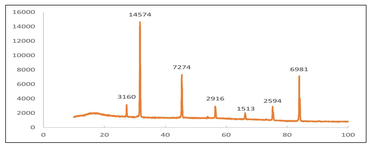


Table and bar chart illustrating the survival rate of wild type and WLZ3 strain of *C. elegans* after exposure to different concentrations of Iron nanoparticles



Results of X-Ray Diffraction (XRD) analysis

Photomicrograph from toxicity assay illustrating the *C. elegans* (arrows): Left (wild type) and Right (WLZ3) after 48 hour exposure to 1 mg/ml concentration of Iron nanoparticles

Interpretation

In the characterization of the iron oxide nanoparticles (INPs), the X-ray diffraction (XRD) analysis showed a faint signal, indicating a crystalline structure with size of 42.75 nm using the Scherrer Formula and the highest peak of 14574. The size was similar to previous studies with Cu Kalpha is 1.75 angstroms, 2θ is 31.6°, and the β is 0.004.

In the toxicity assay, C. elegans were exposed to four different concentrations of INPs dissolved in DI water: 0.5 mg, 1 mg, 2 mg and 5 mg/ml. The use of DI water as a solvent was found to be less toxic to the worms compared to ethanol. After 48 hours of exposure, the survival rate of the wild type (N2) worms was 58.84%, 70%, 20% and 0% at the respective concentrations. For the WLZ3 strain, a model for Parkinson's disease, the survival rate was 30%, 30%, 0% and 0%. These results indicate that there is a significant toxicity starting at 1 mg/ml and this difference in toxicity was seen more in the WLZ3 strain.

In addition, the toxicity assay was also performed on the WLZ3 strain using curcumin coated iron nanoparticles and the survival rate was found to be 61%, 79%, 64%, and 9%, respectively, suggesting that the curcumin coating on iron nanoparticles can have a positive effect on reducing toxicity, also curcumin is a known anti-inflammatory and antioxidant agent, which can help to mitigate the negative effects of iron overload in C. elegans and may also have potential applications in human medicine.

Overall, the results of this study indicate that the use of curcumin coated iron nanoparticles may be an optimal approach for reducing toxicity in drug delivery. Further studies are needed to optimize the concentration of curcumin and iron in the nanoparticles for maximum efficacy. Significant toxicity can be observed after 1 mg/ml in all types of particles and strains.

Conclusion

In our study, we were able to synthesize iron oxide nanoparticles using an environmentally friendly method with the usage of cilantro extract. The synthesized iron oxide nanoparticle measured a crystalline size of 42.75 nm. Further, based on toxicity assay on *Caenorhabditis elegans*, we report that iron nanoparticles at 1 mg/ml concentration showed the least toxicity and can possibly be a viable concentration for future research involving development of iron oxide nanoparticles for therapeutic application. We hope to look more into using curcumin in our green synthesis method for iron oxide nanoparticles and to test its effects on *C. elegans.*

Future studies

We are planning on doing a few more trials of the toxicity assay to see which concentration would work best for our future research. As our study of the green synthesis of nanoparticles is still developing, we hope to bring in compounds that have anti-inflammatory and anti-cancer effects into our research. One compound is called curcumin. Prior studies[12] have shown that using curcumin in the synthesization of nanoparticles reduces the production of toxic waste. Curcumin plays a dual role in the process by acting as a capping agent and as a reducing agent. We hope to synthesize the INPs through utilizing curcumin to see if there is any significant difference in the size, shape, and form of nanoparticles. We plan to do a toxicity assay with curcumin based nanoparticles to determine if using curcumin would provide us with more significant results in terms of whether utilizing it to make nanoparticles is a viable approach towards drug delivery. We also plan on doing a basal slowing assay or bioluminescent assay with both types of particles to see its effects through another lens.

Bibliography

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