

Tannic-acid Assisted Synthesis of Tin (IV) Oxide Nanoparticles Encapsulated in Calcium Alginate Matrix as a reusable adsorbent for the removal of heavy metal ions, dyes, and inhibition of bacteria in wastewater

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Abstract

Wastewater discharged from industrial and commercial sources that contain heavy metal ions, dyes and bacteria affect and pose a serious threat to the environment and our health. Our experiment focused on the synthesis of calcium alginate-tin (IV) oxide nanoparticle composite and evaluating its adsorption capabilities and versatilities in the adsorption of heavy metal ions (Cu^{2+} , Fe^{3+} , Zn^{2+}) as well as cationic and anionic dyes (methylene blue, brilliant green, direct red) and investigating its antibacterial abilities. In this study, 0.5g of adsorbent was left in 25 cm^3 of 20ppm heavy metal ion solution, 0.1g of the adsorbent was left in 4 cm^3 of 25ppm dye solution, left in an orbital shaker overnight and the concentration of the remaining solution was evaluated. Results shows that the percentage removal of Cu^{2+} , Fe^{3+} ions, methylene blue and brilliant green was more than 70% and that tin (IV) oxide generally enhanced the adsorption capabilities of calcium alginate beads, with the exception of adsorption removal for Zn^{2+} ions and direct red dye. Antibacterial tests against *Escherichia Coli* (*E. coli*) were conducted; however due to the insufficient amount of data that was collected, antibacterial results were inconclusive. The composite bead's overall adsorption capability and versatility is higher than that of the solely calcium alginate beads. Overall, composite beads that were highly effective at adsorbing Fe^{3+} ions and methylene blue and relatively effective at adsorbing Cu^{2+} ions and brilliant green have been produced.

1. Introduction

Water pollution is a major problem in today's world, threatening the freshwater sources humans rely for drinking and other critical needs. Main water pollutants include heavy metals, chemical wastes like volatile organic compounds, pesticides, herbicides and pathogenic bacteria that cause waterborne diseases (Muralikrishna & Manickam, 2017). Water borne pathogens [*Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio* spp., and protozoa (*Cryptosporidium*)] contamination in water resources can cause and spread diseases (i.e., diarrhoea, gastrointestinal illness) (Lukhele et al., 2010). Drinking water that has been polluted by microbial contamination increases the risk of certain diseases like typhoid, dysentery,

diarrhoea, hepatitis A and hepatitis B (Shar et al., 2007). Heavy metals such as Pb, Zn, Cu, Hg, etc. could pose severe threats to the health of humans because they can be accumulated biologically in the food chain (Cheraghi, Lorestani & Yousefi, 2009). According to the World Health Organization, more than 3.4 million people die as a result of water related diseases every year, making it the leading cause of disease and death around the world (Berman, 2009).

Over the years, metal nanoparticles have been widely researched due to their strong antibacterial activity (Slavin, Asnis, Häfeli & Bach, 2017), as well as their effectiveness for the adsorption of metal ions (Singh, Barick & Bahadur, 2011). As many bacteria species form biofilms responsible for causing diseases, and there is growing resistance in biofilm resistance against chemical disinfection, metal nanoparticles show promising results as antibiofilm agents due to their many antibacterial properties and mechanisms (Wolska, Grudniak, Kamiński & Markowska, 2015).

Furthermore, heavy metals, organic pollutants, inorganic anions, and bacteria have been reported to be successfully removed by various kinds of nanomaterials. (Lu et al., 2016).

However, most nanomaterials are unstable and tend to aggregate, thus reducing their removal capacity. Additionally, it is usually difficult to separate the nanomaterials from the aqueous solution swiftly and efficiently due to their nanoscale size. The synthesis, as well as operating costs of nanomaterials should also be optimized for the sake of the economy and the production of these nanomaterials should meet the requirements of green chemistry. The impacts and toxicities of nanoparticles towards both the environment and human beings should be taken into consideration. (Yang et al., 2019)

The alginate gel cluster is able to act as a connection that binds the nanoparticles together. In a study conducted by Bezbaruah, Krajangpan, Chisholm, Khan, & Bermudez, 2009, the entrapment of iron-based nanoparticles by alginate does not impair the removal rates of nitrate, which illustrates that such nanoparticles can be successfully entrapped in the alginate beads without reducing their reactivity. Furthermore, the nanoparticles are able to be reused again after regeneration experiments with HNO₃ as the desorbent (Bezbaruah et al., 2009). In contrast to metal nanoparticles, alginate particles can be modified either before or after their synthesis. In the former case, bulk alginate is modified and then used to prepare the particles. This way is usually preferable from the two options because it avoids the leak or destruction of encapsulated substance during the modification (Pestovsky, 2019). The use of calcium alginate

matrix as a carrier of metal nanoparticles improved the reutilization of the nanoparticles as well as the usage of the nanoparticles for simultaneous and continuous processes. In a study by Kuang et al., 2015, calcium alginate was used to encapsulate Nickel and Iron nanoparticles for the simultaneous removal of Copper II and monochlorobenzene. The study also showed that the encapsulation process improved the simultaneous removal efficiency of Copper (II) and monochlorobenzene.

Tannic acid is a biocompatible natural polyphenol that is not only a non-surfactant template, but also a reducing agent, making the process of synthesising SnO₂ nanoparticles environmentally benign (Suvith, Devu & Philip, 201) allowing the synthesised SnO₂ nanoparticles to be eco-friendly and biocompatible (Zhang et al., 2020).

Therefore, we aim to synthesise tin IV oxide nanoparticles with tannic acid and encapsulate them in calcium alginate for reusable and simultaneous removal of heavy metal ions and inhibition of bacteria in water in this project.

2. Objectives and Hypotheses

The objectives of this experiment are to investigate the adsorption capability and versatility, as well as antibacterial properties of a calcium alginate-tin (IV) oxide nanoparticle composite. It is hypothesised that the calcium alginate-tin (IV) oxide nanoparticle composite has a high degree of adsorption capability and versatility when compared to pure alginate beads, that the composite demonstrates significant antibacterial properties and that the calcium alginate matrix enhances the reusability of tin (IV) oxide nanoparticles.

3. Materials

Chemicals

The chemicals used in our experiment are as listed: tin (IV) oxide, tannic acid, copper (II) sulfate, zinc nitrate, iron (III) nitrate, methylene blue anhydrous, brilliant green anhydrous, direct red anhydrous, sodium carbonate, sodium alginate, calcium chloride, nutrient agar, nutrient broth, *Escherichia Coli* ATCC 25922, deionised water

Apparatus and Equipment

The apparatus and equipment used in our experiment are as listed: volumetric flasks, colorimeter vials, centrifuge tubes, sieve, measuring cylinder, funnel, beaker, dropper, petri dish,

micropipette, UV-Vis spectrophotometer, magnetic stirrer, DR/890 colorimeter, centrifuge, electronic balance, orbital shaker, lysogeny broth, colony counter, agar plates and oven.

4. Experimental Procedures

Synthesis of Tin (IV) Oxide Nanoparticles

20 cm³ of 0.1 M of aqueous tannic acid solution at different pH (adjusted by adding Na₂CO₃) was prepared in a beaker. 20 cm³ of 0.3 M tin (IV) oxide was then added into the tannic acid solution. The solution underwent vigorous magnetic stirring for 5h at room temperature, in the absence of light. The obtained reaction mixture was centrifuged at 10000rpm for 5 minutes. The supernatant was decanted, leaving the precipitate in the centrifuge tube. The precipitate was washed with deionized water and placed back in the centrifuge. This procedure was repeated until the solution has pH 7.

Characterisation of Tin (IV) oxide nanoparticles

SnO₂ NPs were sent for SEM imaging to analyse the morphology of the composite.

Synthesis of Calcium Alginate encapsulated Tin (IV) Oxide Nanoparticles Composite

2g of sodium alginate was dissolved in 100 cm³ deoxygenated deionized water at room temperature. The solution was then stirred until complete dissolution was achieved and left at room temperature for around 30 minutes. 40cm³ of sodium alginate solution was mixed with tin (IV) oxide nanoparticles in a beaker and stirred using a glass rod. The mixture is then drawn into a micropipette and slowly dripped into a beaker of CaCl₂ solution. Composite beads were retained in a 4% w/v CaCl₂ solution overnight for hardening and then washed with deionized water.

Investigating the adsorption of heavy metal ions

25 cm³ of a 20ppm solution of CuSO₄, Fe(NO₃)₃ and Zn(NO₃)₂ was prepared. 0.5g of the composite beads and were submerged in each solution in centrifuge tubes. Centrifuge tubes were shaken in the orbital shaker overnight. The adsorbent was sieved from the solution. The concentration of heavy metal ions remaining in the diluted solution was evaluated in a colorimeter. The procedure was repeated for each of the heavy metal ion solution, with 5 replicates carried out per metal ion. 5 setups containing pure calcium alginate beads without the tin (IV) oxide nanoparticles were made per metal ion.

Investigating the adsorption of dyes

200 cm³ of 25ppm dye solution was prepared. 5 replicates of 0.1g of the calcium alginate encapsulated SnO₂ NPs beads were submerged into 4 cm³ of 25ppm of dye solution and shaken in the orbital shaker overnight. 5 setups which only contained pure calcium alginate beads without the tin (IV) oxide nanoparticles were made and they underwent the previous procedure.

Investigating the inhibition on pathogenic bacteria

0.1g of the SnO₂ NPs and 0.5 cm³ of *E. Coli* was added to 9.5 cm³ of LB broth and shaken overnight. 3 replicates of this test were created. Serial dilution was performed 6 times for each sample, and 7 times for the control test. (Control did not contain any test sample) The bacteria were then distributed into agar plates and left at room temperature over 1 days. The number of bacterial colony counts was counted and compared to that of the control test without any samples used on it.

Investigating the reusability of calcium alginate encapsulated SnO₂ NPs

The test to investigate the effect of the heavy metal ion removal was conducted. Sodium dodecyl sulfate was added to leach out the heavy metal ions from the calcium alginate encapsulated SnO₂ NPs. Calcium alginate encapsulated SnO₂ NPs was placed back into a solution of heavy metal ions and the test to investigate the effect of the heavy metal ion removal was conducted again. This was repeated 5 times. The adsorption capability of the calcium alginate encapsulated SnO₂ NPs was measured after the 5th repetition. The percentage of final adsorption capability/initial adsorption capability was then found.

5. Results and Discussion

Scanning Electron Microscope Imaging of Nanoparticles

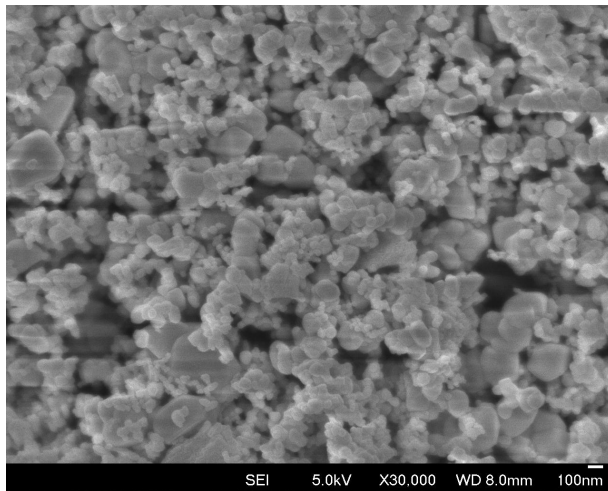


Figure 1: SEM imaging of SnO₂ NPs

To analyze the physical properties of the SnO₂ NPs synthesized with the assistance of tannic acid, the respective sample was dried, and their SEM images were taken at different resolutions. Figure 1 shows the SEM image of the SnO₂ NPs, which displays round and spherical morphology and are about 140nm in size. The SnO₂ NPs are characterized by a high surface area (Fig. 1), thereby enhancing its adsorption properties.

Results from Adsorption Tests

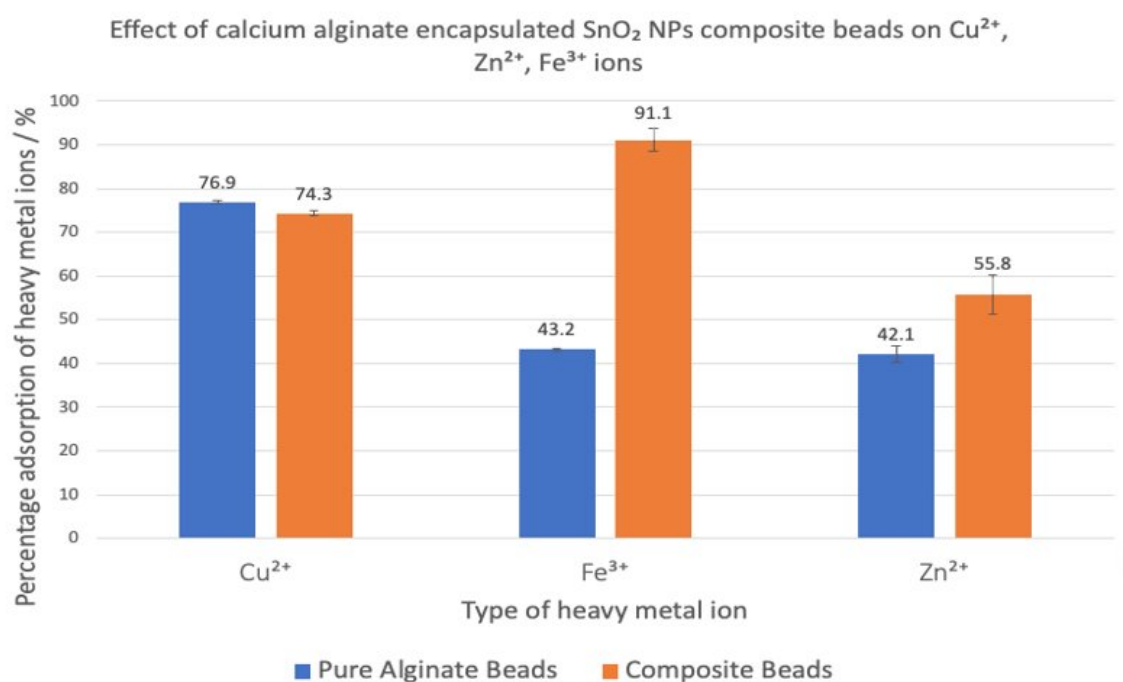


Fig. 2a: Adsorbents' effectiveness on Cu^{2+} , Fe^{3+} and Zn^{2+} ions

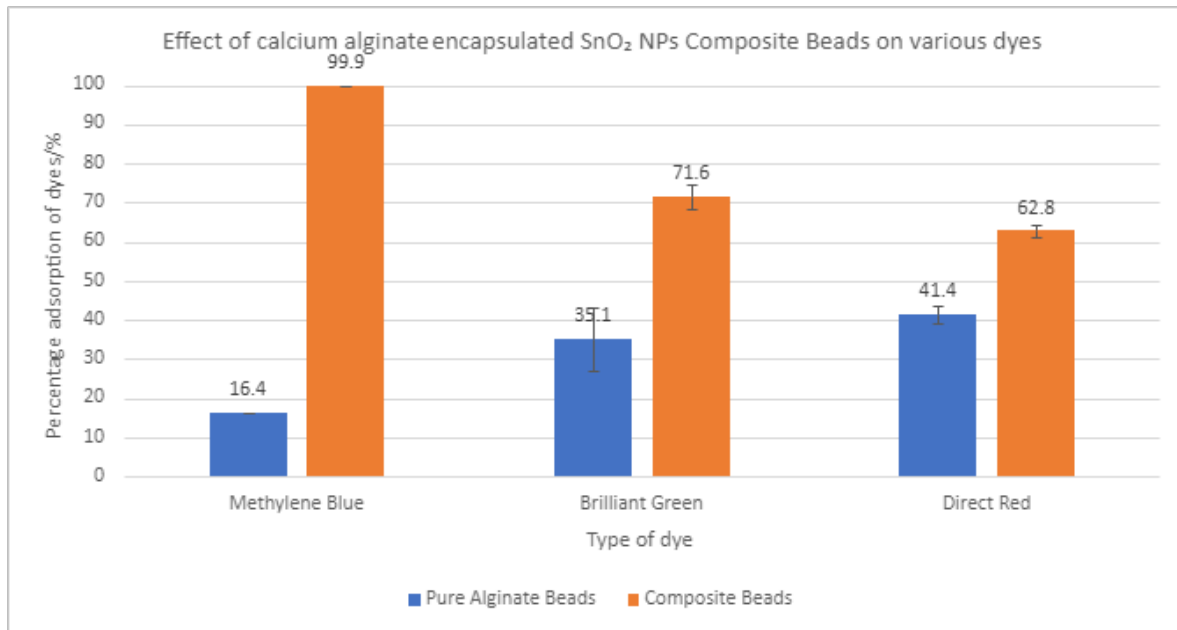


Fig. 2b: Adsorbents' effectiveness on methylene blue, brilliant green and direct red dyes

Discussion of Adsorption Test Results

The percentage of heavy metal ions and dyes adsorbed was calculated using the following formula:

$$\text{Percentage adsorbed} = \frac{\text{initial concentration} - \text{final concentration}}{\text{initial concentration}} \times 100\%$$

For the adsorption of copper (II) ions, iron (III) ions, methylene blue and brilliant green (cationic dyes), the calcium alginate encapsulated SnO_2 NPs composite beads showed ideal adsorption capabilities of 70% or more. For the adsorption of zinc ions and direct red (anionic dye), the composite showed unideal adsorption capabilities of less than 70%. Also, the addition of tin (IV) oxide NPs generally enhanced the adsorption capabilities of calcium alginate except for the adsorption of Cu^{2+} ions.

Difference in adsorption using composite beads compared to pure calcium alginate beads

The respective data sets were compared and analysed using the Mann-Whitney U statistical analysis test to conclude whether a significant difference exists between the adsorption capabilities of the composite beads and pure alginate beads.

Pollutant	p-value	Does a significant difference exist?
Cu ²⁺ ions	0.011	Yes
Fe ³⁺ ions	0.012	Yes
Zn ²⁺ ions	0.037	Yes
Methylene Blue	0.012	Yes
Brilliant Green	0.022	Yes
Direct Red	0.012	Yes

Fig 3. Results from Mann-Whitney U test of adsorption percentages using composite beads compared to pure calcium alginate beads

From Fig 3, there is a significant difference between the adsorption of the composite beads and the pure alginate beads for Cu²⁺ ions, Fe³⁺ ions and Zn²⁺ ions, methylene blue, brilliant green, direct red. Hence, the composite beads are significantly better at the adsorption of Cu²⁺ ions, Fe³⁺ ions and Zn²⁺ ions and methylene blue as compared to pure calcium alginate beads.

Overall, the composite beads have ideal adsorption capabilities ($\geq 70\%$) for Cu²⁺ ions, Fe³⁺ ions, methylene blue and brilliant green. Moreover, the addition of tin (IV) oxide NPs generally enhanced the adsorption capabilities of calcium alginate.

Colony Count Results

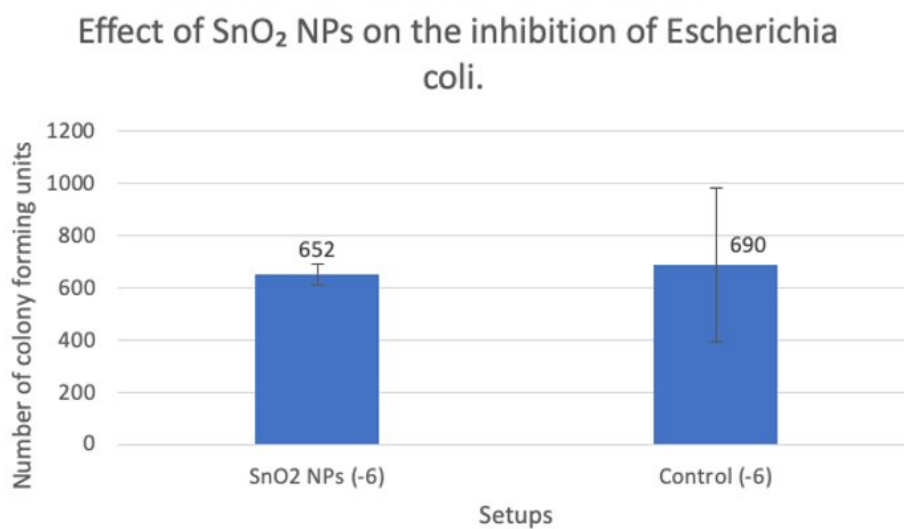


Fig 4: Graph showing effect of tin (IV) oxide nanoparticles on number of colony forming units

Discussion of Colony Count Results

From Figure 4, tin (IV) oxide nanoparticles exhibited poor inhibition capabilities of *Escherichia coli*, as there is a minimal difference of mean colony count between the control setup and the setup containing tin (IV) oxide nanoparticles. This could have been due to reasons such as, tin (IV) oxide nanoparticles being ineffective in inhibiting bacteria, the concentration of the tin (IV) oxide being insufficient to kill the bacteria or the contamination of the tin (IV) oxide NPs during the preparation process, accounting for the large error bar for the control setup as well. More antibacterial tests were unable to be conducted due to the long time spent because of the uncertainty of the ideal pH and amount of tin (IV) oxide to use in its preparation due to there being limited references online. Coupled with the coronavirus situation, this gave us little chance to conduct more antibacterial tests.

Discussion of Reusability Results

Due to the outbreak of the coronavirus, there were time constraints and we were unable to carry out the experiment to investigate the reusability of calcium alginate encapsulated SnO₂ NPs. Further work will be needed to investigate this property of the composite beads.

6. Conclusions and Recommendations for future work

Conclusions

This experiment involved the synthesis of a Tin (IV) oxide nanoparticle composite with the assistance of tannic acid and the encapsulation of these NPs in a calcium alginate matrix. The experiment also involved an analysis of the adsorption of the composite beads to its constituents for Cu²⁺ ions, Fe³⁺ ions, Zn²⁺ ions, methylene blue, brilliant green and direct red. The composite beads were hypothesised to have a higher adsorption capability and versatility compared to its constituent, calcium alginate. The alginate beads were found to have better adsorption capabilities for all pollutants except for Cu²⁺ ions when SnO₂ NPs was incorporated into it. Antibacterial properties of the composite were also investigated. Insufficient data was collected to have conclusive results for antibacterial properties of the tin (IV) oxide NPs.

All in all, an adsorbent with tin (IV) oxide and calcium alginate as its constituents has been produced.

Overall, the calcium alginate encapsulated SnO₂ NPs composite beads have higher versatility than solely calcium alginate beads. Although its adsorption of Cu²⁺, Zn²⁺ ions, brilliant green

and direct red dyes is only mildly effective, it is very effective in adsorbing Fe^{3+} ions and methylene blue dye.

Future Work

Our experiment has numerous possible aspects that can be further investigated. Future work can include investigating the reusability of the calcium alginate encapsulated SnO_2 NPs composite beads. Adsorption-desorption cycles could be carried out on the composites to find out the regeneration ability of the composite and how the ratio of its constituents affects the regeneration capability. Other dyes such as methyl orange and malachite green could be experimented on as well to see the effectiveness of the composite on a wider range of cationic and anionic dyes. The concentration of SnO_2 NPs in a pure calcium alginate bead could also be varied to find an ideal ratio that achieves maximum adsorption capability of heavy metal ions and dyes. The ratio of SnO_2 to tannic acid could also be varied to find an ideal ratio that achieves the maximum adsorption capability of the composite beads. Due to time constraints imposed by the outbreak of the coronavirus, we only managed to test our composite on a gram-negative bacteria species, *Escherichia Coli*. Moreover, the data gathered was insufficient and hence conclusive results are unable to be obtained. Thus, antibacterial tests on various gram-positive bacteria such as *Staphylococcus epidermidis* could be carried out as well.

7. References

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