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Biosynthesis Of Nanoparticles Using Milk Oligosaccharides

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Abstract

Milk oligosaccharides offer several benefits due to their unique properties. They have prebiotic actions that encourage the development of advantageous gut flora and support a balanced gut microbiome. Additionally, they exhibit antimicrobial activity against various microorganisms, helping to prevent infections. Milk oligosaccharides also possess anti-inflammatory properties and may have potential anti-allergic effects. These benefits make milk oligosaccharides valuable for applications in healthcare and food technology. Milk oligosaccharide nanoparticles offer the above benefits on nanoscale. They have prebiotic properties, promoting a healthy gut microbiome. They also exhibit antimicrobial activity against various microorganisms and possess anti-inflammatory and potentially anti-allergic effects. These nanoparticles have potential applications in healthcare and food technology, but further research is needed to fully understand their efficacy and safety.

Keywords: Bioactivity, prebiotic properties, nanoparticle.

I. Introduction

A variety of natural sources are there for metal nanoparticle synthesis including plants, fungi, yeast, actinomycetes, bacteria *etc*. The unicellular and multicultural organisms can produce intracellular and extra cellular inorganic nanoparticles. Oligosaccharides are a diverse group of molecules that are found in all living organisms. They can be used to synthesize a variety of nanoparticles, including gold, silver, zinc oxide, copper oxide and iron oxide nanoparticles. The biosynthesis of nanoparticles from oligosaccharide is a green and sustainable method that has several advantages over traditional methods of nanoparticle synthesis (Bhambulkar & Patil, 2020).

One advantage of biosynthesis is that it is a mild process that does not require harsh chemicals or high temperatures. This makes it a safer and more environmentally friendly method of nanoparticle synthesis. Another advantage of biosynthesis is that it can be used to produce nanoparticles with specific properties. For example, the size, shape, and surface chemistry of the nanoparticles can be controlled by the type of carbohydrate used and the reaction conditions (Sahoo, S., & Shahi, S. ,2021).

Oligosaccharides are natural constituents of all bacteria, fungi, plants and placental mammals' milk. The milk is a rich source of oligosaccharides it can provide number of novel oligosaccharides depends on the nature of their origin in to which mammals the milk belongs. Lactose, the main component of milk is full of glycoproteins with number of free oligosaccharides shows potential physiological benefits. The Pachyderms like elephant milk oligosaccharide is rich from sialyl oligosaccharides, which is important for brain development like gangliosides of the suckling calves while buffalo milk oligosaccharides have ability to stimulate non-specific immunological resistance of the host against parasitic infections. Donkey, resist non-specific and specific immunological stimulants. In the other hand dog's milk oligosaccharides is rich in N-acetylneuraminlactose sulphate, important for nutrition of the rat pups. Anti-inflammatory effects are present in Goat milk Oligosachharides due to presence of trinitrobenzenesulfonic (T) acid ability to cure inflammatory bowel disease and other intestinal infections. Most of the Indians use cow's milk as a supplement of mother milk, it reduces adhesion of enterotoxic Escherichia coli strains of the calf (Patil, R. N., & Bhambulkar, A. V.,2020).

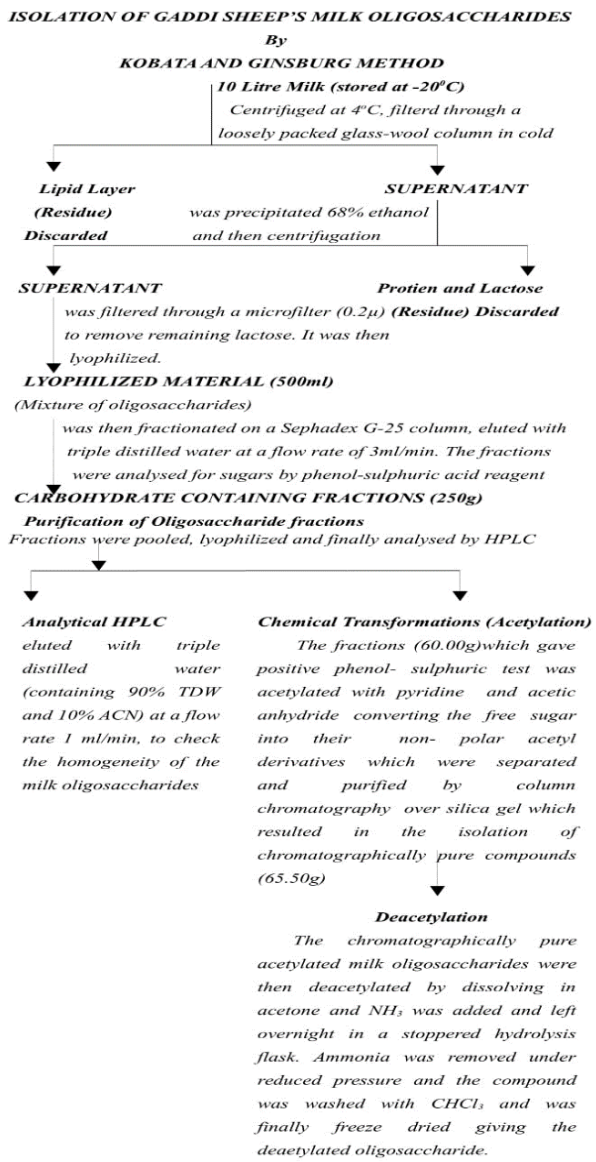
Due to presence of large number of free oligosaccharides HMOs are helpful of postnatal development of the immune system, the protective effect of human milk against viral and bacterial infections, and the enhancement of the bioavailability of minerals. Oligosaccharides with potential physiological benefit could be found in animal milks (Sanyogita Shahi, et al.,2022.

II. Methodology:

Various natural sources, such as plants, fungi, yeast, actinomycetes, bacteria, etc., are available for the creation of metal NPs. Intracellular and extracellular inorganic NPs can be produced by unicellular and multicellular organisms. All living things have a variety of molecules called oligosaccharides. They can be used to create a wide range of NPs, such as iron oxide, gold, silver, zinc, and copper oxide nanoparticles. In comparison to conventional techniques of NP synthesis, the biosynthesis of NPs from oligosaccharide is a green and sustainable process that has a number of benefits (Shah, S.,2022).

The fact that biosynthesis is a gentle process that doesn't require damaging substances or high temperatures is one benefit of it. Because of this, it is a safer and more environmentally friendly approach of producing NPs. The ability to create NPs with certain features is another benefit of biosynthesis. For instance, the kind of carbohydrate employed and the conditions of the reaction can determine the physical characteristics, shape, and surface chemistry of the NPs.

For the creation of NPs, a variety of techniques using different methodologies, such as chemical, physical, and biological protocols, are available. The chemical method of synthesis is useful since it can produce a large number of NPs in a short amount of time. However, this process requires capping chemicals in order to stabilize the size of the NPs. Additionally, the chemical reagents commonly utilized for the production and stabilization of NPs are hazardous and produce byproducts that are not good for the environment. Interest in biological systems that don't employ harmful chemicals as byproducts is growing in response to the demand for eco-friendly, non-toxic methods for synthesizing NPs. Various natural sources, such as plants, fungi, yeast, actinomycetes, bacteria, etc., are available for the creation of metal nanoparticles. The multicellular and unicellular creatures are capable of producing intracellular and extra cellular inorganic NPs. The NPs is created by milk oligosaccharide isolated from Gaddi sheep by modified Kobata and Ginsberg method (Shahi, S. 2019).



Usually, there are three processes involved in the production of nanoparticles from oligosaccharides:

1. **Stimulation.** The oligosaccharide molecule is activated first. This can be accomplished by decreasing the oligosaccharide using an enzyme like glucose oxidase or ascorbic acid. Free radicals are produced on the oligosaccharide molecule as a result of the activation phase.
2. **Nucleation.** Nucleation, or the production of the first few nanoparticles, is the second phase. The oligosaccharide molecule's free radicals start this process. The growth of the nanoparticles occurs after the nucleation stage.
3. **Flourish.** The mechanism by which the nanoparticles grow is known as the growth step. The free radicals on the molecule of the oligosaccharide reduce metal ions, which drives this action.

Preparation of Zn Nanoparticles:
0.1M concentration of Zn nanoparticle was made in distilled water using an oligosaccharide that was obtained from the milk of Gaddi sheep. Then, 0.1 M of zinc acetate solution was made in distilled water.

Making zinc oxide nanoparticles: Using a magnetic stirrer, the recently made zinc acetate solution was heated to 45–60 °C. Drops of the prepared sugar solutions were simultaneously added to the zinc acetate solution. The created nanoparticles are centrifuged for 90 minutes at 5,000 rpm. To assure purity, the washing procedure is repeated at least twice more. The cleaned particle is preserved for future research after a 15-minute sonication (Shahi, S., et al.,2020). .

Preparation of Cu Nanoparticles:
0.1M of an oligosaccharide extracted from the milk of Gaddi sheep was dissolved in distilled water. Then, in distilled water, copper sulphate solution (0.1 M) was also made.

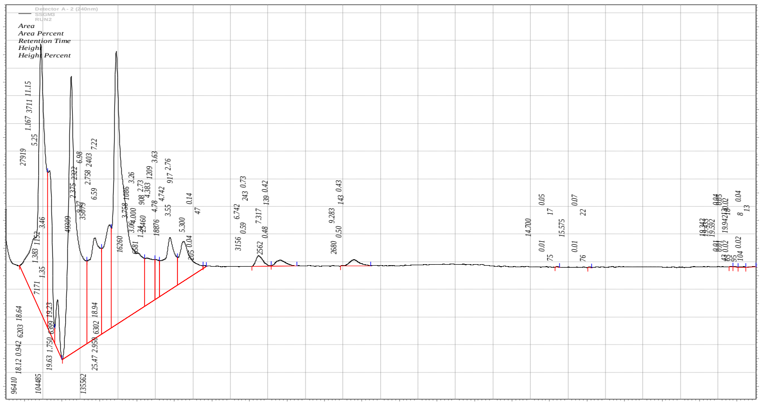
Copper nanoparticle preparation: Using a magnetic stirrer, the freshly made copper sulphate solution was heated to 45–60 °C. The produced oligosaccharide sample was added to the zinc acetate solution in drops at the same time. The created nanoparticles are centrifuged for 90 minutes at 5,000 rpm. To assure purity, the washing procedure is repeated at least twice more. The cleaned particle is preserved for future research after a 15-minute sonication.

Preparation of Ag Nanoparticles:
The 0.1M concentration of Ag nanoparticle was made in distilled water using an oligosaccharide that was obtained from the milk of Gaddi sheep. Then, in distilled water, silver nitrate solution (0.1 M) was also made.

Silver nanoparticle preparation: Using a magnetic stirrer, the freshly made silver nitrate solution was heated to 45–60 °C. The produced oligosaccharide sample was added to the zinc acetate solution in drops at the same time. The created nanoparticles are centrifuged for 90 minutes at 5,000 rpm. To assure purity, the washing procedure is repeated at least twice more. The cleaned particle is preserved for future research after a 15-minute sonication (Shahi, S., & Singh, S. K. ,2019). .

4. Result and Discussion:

Confirmation of Homogeneity of Gaddi's Milk Oligosaccharide by Reverse Phase HPLC- *90% TDW: 10% ACN in water 240 nm--*



Application of zinc nano-particles:
Antimicrobial activity:
Antimicrobial activity was determined by the agar well diffusion method with minor modifications. The ZnONPs (5, 10, 50 & 100 mg/ml) were treated with the grampositive S. epidermidis and gram-negative E. aerogenes. The synthesized ZnONPs showed dose-dependent increase in antimicrobial activity. Results implied the high antimicrobial activity of the synthesized ZnONPs. The growth inhibition was found higher in various strain of Saccharomyces and ZnONPs increase the growth of beneficial microbes by 90%. The observed particles size is 0.2 to 0.04nm. it also reduced the amount required for bioactivity of oligosaccharides. And it was also evident from the results that the antimicrobial activity of ZnONPs obtained from Avasose was a way higher than any other carbohydrate source.

Application of silver nano-particles:
Silver nanoparticles have been used extensively in anti-bacterial agents in the health industry, food storages, textile coating and number of environmental applications. But in contest of milk oligosaccharide NPs research is the part of future prospect.

Environmental Applications: The silver nanoparticles can be used for the treatment of water to avoid contamination of the environment. Environment friendly antimicrobial nano-paint can be developed. Silver nanoparticles can be used for water filtration. The silver nanoparticles used as an anti-bacterial agents and wide range of application from disinfecting devices and home appliances to water treatment.

Future Prospects: Nanoparticles are widely used to improve the various catalytic reactions due to their novel physico-chemical properties as compared to their bulk size components. Because of its their wide applications, there is need to produce nanoparticles in industries. As stated above the physical and chemical methods of nanoparticle synthesis are having many disadvantages. Biological synthesis would be a preferred method because of its environment friendly approach. However, very limited studies have been reported on the factors affecting or responsible for biosynthesis of metal nanoparticles. Synthesis of nanoparticles by using microorganisms has been developed over the last decades. It is known that the synthesis of nanoparticles using microorganisms is a quite slow process compared to physical and chemical approaches. Synthesis using microorganism is still on the laboratory scale. Efforts should be made to investigate the practical application of microorganism in nanoparticles production. The use of nanoparticles already established for specific medical applications.
Recent research has revealed exciting new biological properties of NS that could be translated into new therapeutic and pharmacological treatments. The full potential of this technology has yet to be discovered. The antibacterial, antifungal and antiviral properties of AgONPs have been extensively studied.
The present research showed that in compare to silver nanoparticles, Zinc nanoparticle are more effective in plant and microbes. Experimental trials are needed to understand the exact mode of function of both NPs on microbes, plants and animals.

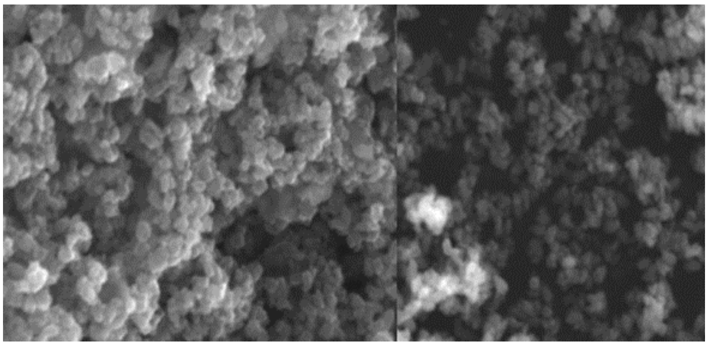


Fig: ZnNP of Oligosaccharide

The biosynthesis of nanoparticles from oligosaccharide is a promising method for the production of nanoparticles with specific properties. This method is green and sustainable, and it can be used to produce nanoparticles with a wide range of applications.
Here are some examples of the applications of oligosaccharides-based nanoparticles:
- **Biomedical imaging**. Oligosaccharide-based nanoparticles can be used to image cells and tissues. The nanoparticles can be labelled with fluorescent dyes or radioactive isotopes, which allows them to be tracked in vivo.
- **Drug delivery**. Oligosaccharide-based nanoparticles can be used to deliver drugs to specific cells or tissues. The nanoparticles can be coated with ligands that target specific receptors on cells.
- **Theragnostic**. Oligosaccharide-based nanoparticles can be used for both diagnosis and treatment. The nanoparticles can be used to image cells or tissues, and they can also be loaded with drugs.

The biosynthesis of nanoparticles from oligosaccharide is a new and amazing field. Because milk of several origin has various known bioactive properties. As the understanding of this process improves, new and innovative applications for oligosaccharide-based nanoparticles will be developed.

The study showed that, three fungal strains, namely Trichoderma (specifically T. polysporum, T. flavofuscum, and T. longibrachiatum), Saccharomyces cerevisiae ATCC 9763, and Penicillium communae ATCC 10248, were found to exhibit significant sensitivity to Gaddi sheep's milk oligosaccharides. After 24 hours of culture, these strains showed enhanced growth rates of approximately 58%, 52%, and 55%, respectively.
Trichoderma, known as bio-symbiotic fungi that promote plant growth, exhibited a growth rate of 58%. Saccharomyces cerevisiae ATCC 9763, a probiotic commonly used in the treatment of gut flora imbalances and certain forms of diarrhea in humans and animals, showed a growth rate of 52%. Penicillium communae ATCC 10248, recognized as a bio-pollutant controller for oil waste, demonstrated a growth rate of 55%.

Conclusion:

Based on the results obtained from this study, it can be inferred that Gaddi sheep's milk oligosaccharides have bioactivity that could be beneficial for the food and beverage industry, plant growth inhibition, and the promotion of a green and clean environment. These findings suggest that Gaddi sheep's milk oligosaccharides possess valuable natural remedies with novel functional properties for environmental purification and pharmaceutical applications.

In summary, the study indicates that the tested fungal strains, along with the bioactive Gaddi sheep's milk oligosaccharides, have the potential to contribute to the food and beverage industry, act as plant growth inhibitors, and play a role in maintaining a clean and sustainable environment. Furthermore, these findings highlight the potential applications of Gaddi sheep's milk oligosaccharides in the pharmaceutical industry, making them a valuable natural resource with unique functional properties. While ZnNPs of oligosaccharide is 90% more effective for growth of Trichoderma.

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