

Polymerase Chain Reaction (PCR) Enhancement Utilizing Magnesium Nanoparticles Fabricated from Agricultural Wastes via Green Methodology

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ABSTRACT

Polymerase chain reaction is among the major molecular biology techniques today, facilitating the precise amplification of DNA sequences for forensic investigations, agricultural biotechnology applications, and diagnostics within life sciences. Enzyme activity, heat cycle conditions, primer modification, and perhaps most notably, the availability of magnesium ions (Mg^{2+}), acting as a critical cofactor in DNA polymerases, are some of the major chemical and physical factors that determine the efficiency of the reaction. Optimization approaches traditionally centred on varying the magnesium salt concentrations and temperature conditions; however, this strategy suffers from constraints in terms of time, sensitivity, and specificity. Nanotechnology has emerged to prove that utilizing

magnesium nanoparticles (MgO NPs) produced by green synthesis techniques from agricultural wastes will optimize PCR reactions into an excellent nano-PCR technique.

INTRODUCTION

Polymerase Chain Reaction: Key Principles

There is a cyclic *in-vitro* technique known as Polymerase Chain Reaction (PCR) that employs a repeated sequence of denaturation, annealing, and extension reactions, which are facilitated by DNA polymerases to multiply specific DNA fragments in an exponential manner. Taq and similar polymerases need Mg^{2+} ions since they interact with the enzyme and nucleotides, helping maintain the integrity of the phosphate backbone and facilitating nucleotide addition. The choice and quantity of magnesium salts affect PCR outcomes such as specificity, output, and accuracy (Shen & Zhang 2013).

One of the disadvantages of PCR is its tendency for non-specific amplification due to primer mismatches. Sensitivity decreases at lower template quantities. Traditional reagents exhibit prolonged reaction periods and low yields. These problems have prompted researchers to explore nanotechnology-based reagents for optimizing reaction kinetics, enzymatic activity, and thermal transfer. (Gao *et al.*, 2019).

1. Nanoparticles Enhancing PCR

1.1 Nano-PCR Concept

Nanomaterials such as Fe_2O_3 , ZnO, CeO_2 , Fe_3O_4 , Al_2O_3 , CuO and TiO_2 and carbon nanotubes have all been utilized for stabilizing the polymerase complex, decreasing primer errors, and improving heat conductivity (Gao *et al.*, 2019). The inclusion of nanoparticles in order to enhance PCR performance by improving sensitivity and specificity is referred to as "nano-PCR." Some of the distinct physical and chemical properties of

nanoparticles which contribute to these improvements include their high surface-to-volume ratios and surface charge reactions with biological molecules (Shen & Zhang 2013).

1.2 Magnesium-Based Nanoparticle Effects on PCR

According to recent studies, the MgO nanoparticles can be used effectively as a source of magnesium ions, as well as nano-sized thermal conductors in the PCR reaction, whereas the noble metal nanoparticles like AuNPs are useful in enhancing the efficiency of PCR reactions via thermal conductivity. The use of MgO nanoparticles has been observed to significantly: Increase PCR sensitivity by multiple folds in comparison to traditional magnesium salt (Ali *et al.*, 2018; Narang *et al.*, 2016). Decrease the cycle number for visualization of amplicons. Possible role of co-factors for the polymerase enzymes because of controlled release of MgO NPs (Li *et al.*, 2005; Narang *et al.*, 2016; Gao *et al.*, 2019).

2. Green Synthesis of Magnesium Nanoparticles

Chemical and physical production methods of nanoparticles are known to require toxic precursors, energy, and by-products that are hazardous to health. In green synthesis, biological material including waste (plants, fruits, fungi) is used for nanoparticle formation, leading to the production of environment-friendly magnesium nanoparticles with very little toxicity (Arun *et al.*, 2025; Nazeer *et al.*, 2018).

2.1 Agricultural Waste

Agricultural waste like fruit skin, banana peels, and other green biomass sources include naturally occurring phytochemicals like polyphenols, flavonoids, and tannins which can be used for:

- Surface stabilization of nanoparticles, preventing their aggregation,
- Reduction of magnesium ions and conversion to nanoparticles, and
- Sustainable ways of nanoparticle synthesis at low cost.

For instance, MgO nanoparticles with controlled morphology were synthesized through a green chemistry approach involving walnut shell and sugarcane leafy trash extract (Zamani *et al.*, 2019).

2.2 Benefits of Green-Synthesized MgO NPs

The green synthesized MgO nanoparticles possess biocompatibility and nontoxic properties, making them suitable for biological applications, and exhibit higher stability compared to those prepared through chemical methods. Organic shells extracted from plant sources may also serve for functionalization purposes. Their unique features make them ideal choices for sensitive biological applications, including nano-PCR enhancers (Arun *et al.*, 2025; Narang *et al.*, 2016).

3. Mechanisms of PCR Enhancement by Mg Nanostructures

3.1 Controlled Mg²⁺ Ion Release

Release of Mg²⁺ ions from MgO nanoparticles may occur stepwise in the reaction mixture through surface hydration and subsequent dissolution, leading to (a) activation of Taq DNA polymerase with sufficient cofactor concentration, (b) Reduced clustering effect in contrast to magnesium salts in their bulk state

and (c) reduced interactions between the template and primers (Zamani *et al.*, 2019; Li *et al.*, 2005; Kuang *et al.*, 2013).

3.2 Improved Thermal Dynamics

The heat conductivity of nanoparticles is better than that of regular ionic solutions. This implies that the use of nanoparticles would improve heat distribution in PCR thermal cycling, and this would result in (a) improved rate of melting and annealing, and (b) improved heat distribution throughout the reaction mixtures (Li *et al.*, 2005; Narang *et al.*, 2016).

3.3 Surface Interactions and Molecular Crowding

Some of the mechanisms through which nanostructures can engage with DNA strands and polymerase include the following: (a) Electrostatic forces or surface functional groups (b) Local micro environment has high concentrations of Mg²⁺ ions are created, (c) Reduction of molecular artifacts due to secondary structure formation and (d) Such engagements could result in efficient primer annealing and DNA strand extension (Kuang *et al.*, 2013).

4. Practical Implementation and Optimization

The following considerations should be kept in mind while optimizing nano-PCR through the use of MgO NPs.

- Concentration of nanoparticles:** Too many could lead to the binding of DNA nonspecifically or hinder the enzyme.
- Size and surface characteristics of particles:** Improvements in nanoparticles tend to be more reliable when the particles are smaller and homogeneously dispersed.
- Parameters used in green synthesis:** Factors such as pH and the method used for

extracting plant extracts influence the biological compatibility of the synthesized nanoparticles (Kuang *et al.*, 2013; Iravani, 2011; Nazeer *et al.*, 2018).

5. Future Perspectives

Other potential areas of study can be involve:

- a) Studies to elucidate the mechanism of impact of particular MgO nanoparticles on the kinetics of the polymerase enzyme in PCR.
- b) Studies comparing traditional and eco-friendly sources of magnesium in different kinds of PCR reactions (for instance, multiplex PCR or quantitative PCR).
- c) The development of more eco-friendly MgO NP production methods for use in nano-PCR reagents via different morphologies and structural modifications.

CONCLUSION

One approach that can greatly enhance PCR efficiency involves the synthesis of magnesium nanoparticles from agricultural waste through green chemistry practices. This would result in an enhancement in PCR efficiency through controlled release of Mg²⁺, better thermal conductivity, and optimal surface kinetics. Another benefit is that the use of agricultural waste for nanoparticle synthesis brings next-generation diagnostics closer to green chemistry principles.

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