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Enhancement in Efficiency of Polymerase Chain Reaction by Silver Nano-Particles

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Abstract

This experiment evaluated the effect of silver nano-particles on the efficiency of Polymerase Chain Reactions. Silver nano-particles were prepared by chemical reduction method. The particles were analyzed by Particle Size Analyzer and UV-Vis Spectrophotometer. AFM image for the particles was taken. Different concentrations of the prepared nano-particles of median size 50.30 *nm were used with other components of PCR reaction mixture and the reactions were carried out along with the control. After Agarose Gel Electrophoresis, the DNA bands were observed. The experiment showed that the prepared Silver nano-particles enhance the PCR efficiency when added at a concentration of 0.02nM.*

Keywords: Silver nano-particles, UV-Vis Spectrophotometer, AFM, PCR.

Introduction

Polymerase chain reaction (PCR) is a widely used molecular technique to amplify a provided sample of DNA to generate millions of copies of the same. The technique is based on thermal cycling. The technique can also be modified to carry out genetic manipulation.

PCR is most indispensable technique for medical and biological research labs. But along with its advantages, it has lot of limitations. Peltier-effect restricts the thermoelectric heating of PCR. It also has some inherent limitations[1]. There may be bias in the template to product ratios of the given DNA sequences[2]. Nucleic acids extracted from various environmental samples sometimes contain PCR inhibitors and hence its effect on Q-PCR is well established[3]. Since the technique has extremely high sensitivity, presence of non-template DNA in lab environment (from bacteria, virus and your own DNA) may contaminate the whole process. Even the enzymes used in this experiment i.e., Polymerases has a higher error rate. Also, PCR of longer products are less efficient due to loss of enzyme activity.

Improvements have been done to overcome these limitations. Capillary and microchip PCR techniques were developed with high surface/volume ratio and less content volume. To reduce inherent limitations, '50 nuclease assay'[4-6], an adaptation of PCR method has been made. Quantitative estimations have been enabled by coupling Q-PCR with an initial R-T reaction. Use of aerosol barrier tip filters, laminar flow cabinet and a special preparation area for PCR, away from DNA isolation area are followed to minimize contamination. Conventionally used DNA Polymerases have been replaced by recombinant Polymerases and other Polymerases isolated (e.g., *Vent, Pfu, Pwo* etc.), which are more accurate, to prevent errors in the polymerizing activity.

Along with all these improvements, scientists are now more focused on the use of nano-particles in PCR to increase the yield. This is due to the changed properties exhibited by metals in nano meter size than in their bulk form. It has been reported that the nano-particle has higher thermal efficiency, super hardness, unexpected visible properties etc. In liquid, nano-particles can transport heat flux and cause thermal equilibrium with the environment within 10–200 ps.

High S/V ratio, molecular-level layering etc. is believed to be the reasons for this increase in thermal conductivities. Moreover nano-particles have been found to impart some extra properties such as self-cleaning effect, superior UV blocking properties, glass transition temperature to various daily products and are used in drug delivery systems, implantable materials, surgical aids and diagnostic tools, energy storage tools, nano-probes or sensors. Nano-encapsulation is one of the technique used in bioremediation as well as pollution indicator systems, where active ingredients are encapsulated leading to greater bio-availability, solubility and potency. Copper doping plays an important role in modifying the structural and optical properties of transition metal sulfide nanoparticle[7]. Influence of nano silica along with construction materials such as cement, concrete etc have been studied with respect to its properties like permeability, strength, durability etc[8].

Enhancement of PCR efficiency using gold nano-particles has been reported in 2005[9]. However, another report in 2008 says that Au nano-particles suppress the amplification of longer DNA molecules and favors the amplifications of shorter one, rather than enhancing the efficiency of PCR[10]. Due to this reason and its increasing cost, Au nano-particles possess limitations for its use in this technique.

In comparison to gold nano materials used so far, use of silver can be cost effective. It also has enhanced properties like higher electrical conductivity, chemical stability and is bacterio-static. Biologically synthesized silver nano-particles can be used as mosquito larvicidal agent[11]. Vermicidal activity and biophysico-chemical interaction of Ag nano-particle with bio-molecules has also been investigated[12].

Its chemical stability can also stabilize the DNA template. Report says that DNA forms stable complexes with Ag (I) because of its specific binding. This is due to the metal ions which get embedded inside the double helix[13].

Objective: The current research focuses on the effect of silver nano-particles on increase of PCR amplification when used at a specific concentration.

Material and Methods

Silver nitrate $AgNO_3$ (Sigma Aldrich, UK) and tri-sodium citrate $C_6H_5O_7Na_3$ (Sigma Aldrich, UK) of analytical grade purity, were used as starting materials. Qualified PCR reagents were used to amplify the DNA template by conventional PCR. The PCR kit was taken from Bangalore Genei.

Experimental protocol: Silver nano-particle synthesis: The silver colloid was prepared by using chemical reduction method[14]. Briefly, 50 ml of $1x10^{-3}$ M $AgNO_3$ was heated to boiling and 1 % tri-sodium citrate was added drop by drop with continuous stirring until color change is evident (pale yellow).

Mechanism of reaction could be expressed as follows:

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2(\uparrow)$$

The absorbance of silver colloidal solution was measured using UV-Visible Spectrophotometer. Further analyses were done to study particle size distribution of nano-particles using "MICROTRAC" Particle size and Zeta-Potential Analyzer which works on the principle of dynamic light scattering[15]. AFM image was also taken.

PCR reactions: the citrate of silver nano-particle solution was replaced with sterile distilled water since it inhibits Polymerase activity. Dilutions were made up to 1nM. PCR tubes were prepared containing 50ul reaction mix with added silver nano-particles by replacing sterile water to obtain final nano-particle concentration from 0.01nM-0.05nM.

Simultaneously, control PCR tube was prepared. PCR cycles were set as per the kit information, and the reactions were carried out using conventional PCR machine. Agarose gel electrophoresis of the amplified DNA was performed and the gel was visualized under UV trans-illuminator. The bands in each well were compared with the control and Ag nano-particle concentration that gives the highest amplification was determined.

Results and Discussion

The Ag nano-particles were prepared, which exhibited an absorption peak at 427nm when measured using 'UV-Visible Spectroscopy'.

Using "MICROTRAC" Particle size and Zeta-Potential Analyzer, the median diameter of the Ag nano-particles was found to be 50.30nm (figure1). The shape of the nano-particles was found to be spherical using AFM (figure2).

After performing the experiment in duplicates, the gel was visualized and band width of DNA from each well was compared with the control to observe the increase in PCR efficiency in DNA amplification (figure3). From the observation, the effective Ag nano-particle concentration which enhances DNA amplification in PCR was determined to be 0.02nM.

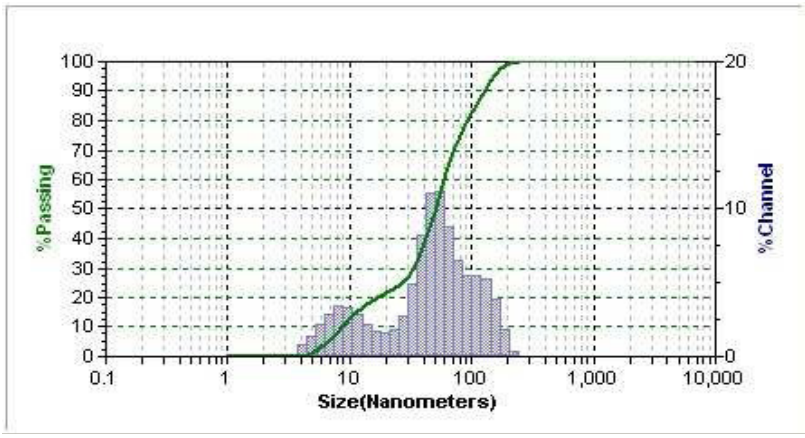

Figure-1
Particle Size Analysis

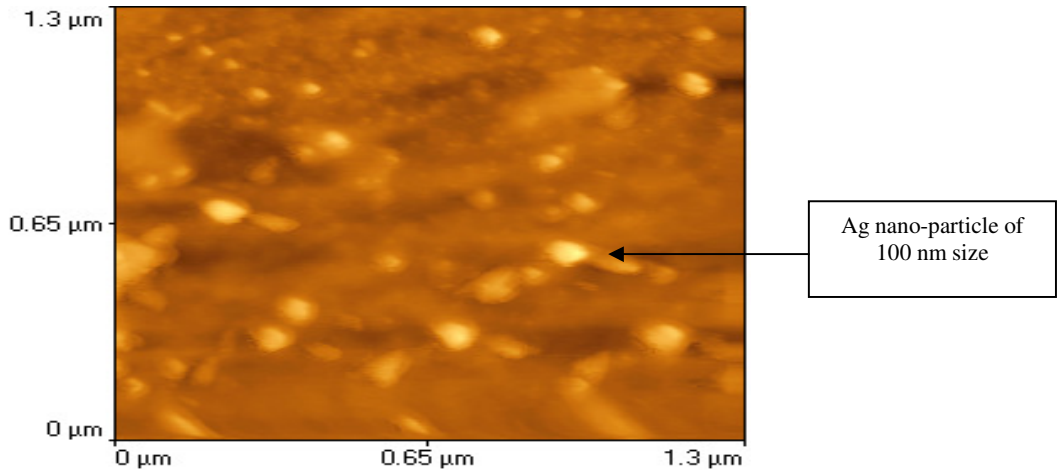

Figure-2
AMF image showing silver nano-particles

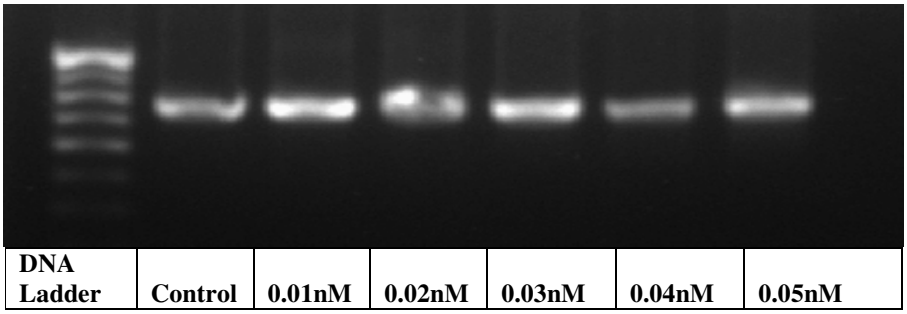

Figure-3
Gel under UV trans-illuminator showing amplified DNA after PCR

Conclusion

The silver nano-particles prepared were detected by measuring its absorption maxima. In this preparation, sodium citrate has been used as a protecting agent, reducing agent and a stabilizer for the particles. The solution can be stored for several months under sterile condition. Improvement in the PCR efficiency is contributed to the excellent uptake and dispersion of heat in the reaction, which has resulted in the quick amplification of the DNA strands. Ag nano-particles can also bind to phosphorus containing DNA[16].

This improved PCR technique has wide application in forensic investigations as well as fossil study where the available DNA is in trace amount. The technique will also have immense application in probe generation and diagnosis purpose.

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