SYNTHESIS OF IRON OXIDE NANOPARTICLES AND FUNCTIONALIZATION WITH SILICA FOR DNA PURIFICATION

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ABSTRACT

In our study, a simple and convenient process for efficient DNA purification was developed using iron oxide nanoparticles functionalized with silica. CTAB coated iron oxide nanoparticles were prepared by adopting an already established method. A change in the surface modification on CTAB coated iron oxide nanoparticles were introduced using tetraethyl orthosilicate (TEOS) as the silica source. Nanoparticles were then used for plasmid and genomic DNA purification. Binding capacity for plasmid DNA and genomic DNA was investigated. CTAB coated iron oxide nanoparticles functionalized with silica, possess high affinity for genomic DNA compared to plasmid DNA. Elution procedures were carried out at room temperature and elevated temperatures to compare the elution efficiency. The purification of DNA was monitored through agarose gel electrophoresis. More specifically, this preliminary study proves to be simple and convenient process when compared with the traditional DNA purification methods.

Keywords: Iron oxide nanoparticles, Silica coated, TEOS (tetraethyl orthosilicate), CTAB (cetyltrimethy ammonium bromide), DNA purification

1. INTRODUCTION

For majority of biomedical applications, DNA purification is generally needed. Some applications require extensively purified DNA because a variety of contaminants can inhibit amplification and diminish the success of some analytical instruments. There are number of existing methods through which DNA can be purified such as cesium chloride density centrifugation [1], hydroxyapatite chromatography [2] and anion exchange chromatography [3]. However, most of these methods require time consuming purification steps including centrifugation and organic extractions.

Magnetically driven separation techniques using silica, is an important approach in the development of biomedical applications [4]. Magnetic separation technology, using magnetic nanoparticles has several advantages [5]. This method is quick and easy to perform. Furthermore the analyte which we are interested, subjects to very little amount of chemicals compared to other methods. Thus for the purpose of purification of DNA, iron oxide nanoparticles play a vital role in the field of biotechnology [6]. Moreover magnetic bio separation is a very effective method in biological applications.

2.METHODOLOGY

2.1 Synthesis of CTAB Coated Iron Oxide Nanoparticles

The synthesis of iron oxide nanoparticles was carried out using an already established method [7]. Degassed solutions of Fe (III) (0.1 M) and Fe (II) (0.1M) were mixed with CTAB solution (0.1M) in a two necked round bottom flask at room temperature. A solution of NH_3 (5.0M) was added while stirring until pH of the media is equal to 12. Resulted black color solution was used for further modifications.

2.2 Synthesis of Silica Coated Iron Oxide Nanoparticles

This is a modified version of an already established method [8]. CTAB coated iron oxide particles were put into a round bottom flask and added aqueous ammonia, ethanol and double distilled water. The mixture was allowed to stir and added TEOS. Nanoparticles were collected through magnetic seperation. Finally they were washed twice with double distilled water and allowed to dry inside a desiccator.

2.3 Purification of DNA

Silica coated iron oxide nanoparticles were washed with binding buffer and DNA was added to it in the presence of binding buffer. Gentle mixing was

done for 10 minutes. Magnetic separation was done to separate DNA in the presence of elution buffer.

3. RESULTS

Prepared iron oxide nanoparticles showed magnetic properties and were attracted to an external magnet.

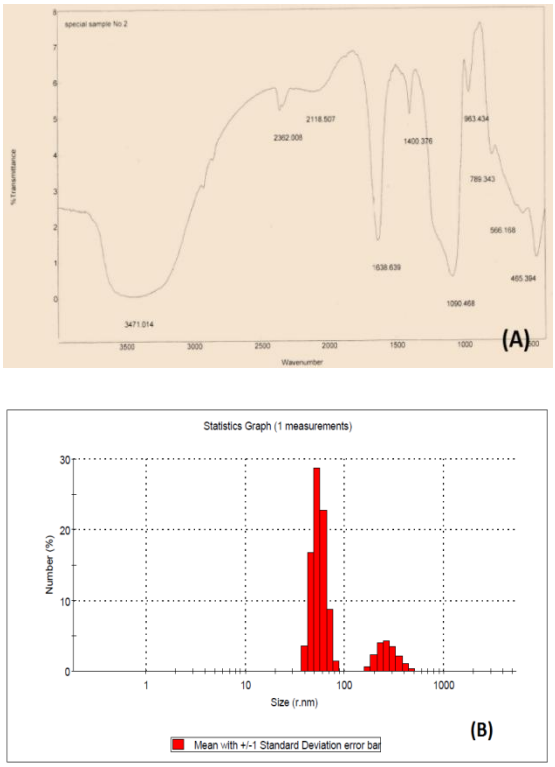

Figure 1: (A) FT-IR spectrum of silica coated iron oxide nanoparticles (B) Particle size distribution graph of silica coated iron oxide nanoparticles

In order to confirm the silica network was successfully covered onto the iron oxide nanoparticle surface, FT-IR spectrum was obtained. According to figure 1 (A), the absorption bands at 566 cm^{-1}, 1090 cm^{-1} , 963 cm^{-1} correspond to Fe-O-Si bond [9] , stretching vibration of Si-O-Si bond [10] and Si-OH bond vibrations [10], respectively. Accordingly, it can be concluded that silica has been coated on iron oxide nanoparticles. Moreover, large amount of functionalized particles have sizes less than 100 nm and only small amount of particles exceed nanometer range.

To determine the capability of binding silica coated iron oxide nanoparticles to DNA, binding capacity was determined. DNA adsorbed on silica surfaces was eluted and the quality and quantity of DNA was evaluated by agarose gel electrophoresis. Figure 2 illustrates the gel images of plasmid and genomic DNA at room temperature (RT) as well as high temperatures (High T). It is clear that the DNA purification at room temperature was efficient. Pictures also reflect that the quality of the DNA is intact.

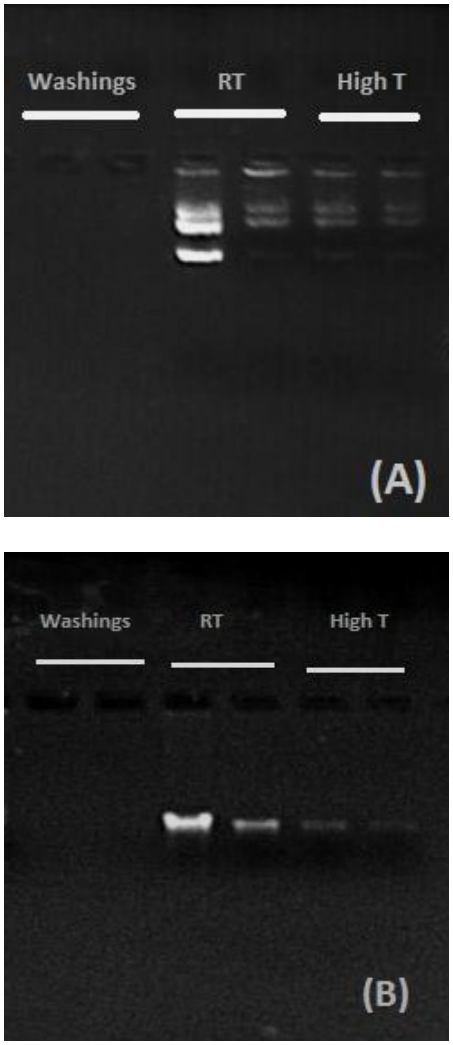

Figure 2 : Agarose gel electrophoresis image for (A) plasmid DNA elution (B) Genomic DNA elution

4. CONCLUSION

Iron oxide nanoparticles functionalized with silica can be prepared within very short period of time compared to reported methods. Functionalized nanoparticles possess high affinity for DNA. Moreover particles exhibited high binding capacity for genomic DNA compared to plasmid DNA. Elution of adsorbed DNA was successful at room temperature as well as elevated temperatures. Thus it is clear that the DNA can be purified easily and quickly with magnetic particles. This preliminary study could enable to design a simple and convenient method for direct DNA separation from biological fluids and also can be applied for clinical

diagnoses using appropriate surface modification techniques.

5. REFERENCES

[1] P. Stougaard, S. Molin, *Anal. Biochem*., vol. 118, pp. 191-193, 1981.

[2] A. Jangbauer, R. Hahn, K. Deinhofer, P. Luo, Biotechnol. Bioeng., vol. 87, pp. 364-365, 2004.

[3] M. M. Diogo, J. A. Queiroz, D. M. F. Prazeres, J. Chromatogr., vol 998, pp. 109-117, 2003.

[4] J. I. Taylor, C. D. Hurst, M. J. Davies, N. Sachsinger, I. J. Bruce, J. Chromatogr., vol. 890, pp. 159-166, 2000.

[5] S. Berensmeier, App. Microbiol. Biotechnol., vol. 73, pp. 495-504, 2006.

[6] P. Ashtari, X. He, K. Wang, P. Gong, Talanta, vol. 67, pp. 548-554, 2005.

[7] T. P. Ruberu, K. M. N. de Silva, C. S. S. R. Kumar, R. M. de Silva, SLASS 64th Annual Sessions, 2008.

[8] Y. H. Deng, C. C. Wang, J. H. Hu, W. L. Yang, S. K. Fu, J. Colloids Surf., A, vol. 262, pp. 87-93, 2005.

[9] M. Yamaura, R. L. Camilo, L. C. Sampaio, M. A. Macedo, N. Nakamura, H. E. Toma, J. Magn. Magn. Mater., vol. 279, pp. 210-217, 2004.

[10] A. L. Andrade, D. M. Souza, M. C. Pereira, J. D. Fabris, R. Z. Domingues, Ceramica, vol. 55, pp. 420-424, 2009.