



Entrapment of calf-thymus DNA on magnetic nanoparticles

Y. Sameena ^a, Israel V. M. V. Enoch ^{b,*}

^a Department of Chemistry, Sri Shakthi Institute of Engineering and Technology, Coimbatore - 641062, Tamil Nadu, India.

^b Department of Chemistry, Department of Nanoscience, Karunya Institute of Technology and Sciences, Coimbatore - 641114, Tamil Nadu, India.

* Corresponding Author: israelenoch@karunya.edu

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Abstract: Magnetic nanoparticles can be used to load bio-molecules for various biophysical applications. The direct attachment of bio-molecules such as protein and DNA to magnetic nanoparticles may lead to alter their structure. A method to immobilize and store biomolecules for example DNA on to a magnetic nanoparticles surface without much structural alteration is carried out in the present work. DNA is a target for many therapeutic small molecules as therapeutic drugs bind to DNA - interfering with protein factors involved in DNA mechanism, or cleave DNA cross-link - interfering with the cell division. This may find applications in drug interactions study with the stored DNA without much alteration in their structure.

Keywords: Magnetic nanoparticles, Glycol chitosan, DNA, Entapment

1.0 Introduction

Nanomaterials represent a bridge between bulk materials and molecules. Various methods of synthesis of nanomaterials for various bio-physical applications were adopted in the reports. An important feature of the nanomaterials synthesis deals with the specified size and shape, since the physico-chemical characteristics especially magnetism depends on the particle size [1]. The unique properties of nanoparticles are intrinsic in the particles with a core size of ~ 50 nm. Among various nanoparticles, Iron is a magnetically soft nanomaterial. Pure iron is not easily possible because they frequently contain oxides, carbides, and other impurities. Pure iron as nanoparticles can be obtained by evaporation of the metal in Argon atmosphere followed by deposition on a substrate. But they are not air stable, and are easily oxidized, resulting in the change or loss of their magnetization [2]. Various studies reported for the possibility of metal oxide nanoparticles not only as ferromagnetic fluids but also as nanoparticles covered by ligands or as particles included into rigid matrices like polymers, zeolites etc. Glycol chitosan (GC; Figure

1), a carbohydrate polymer can also be used to coat iron oxide magnetic nanoparticles [3]. Moreover, on account of biocompatibility, stability in tissue environment and resistance to enzymatic degradation, GC can be chosen to surface-immobilize on iron oxide magnetic nanoparticles for transporting drugs [4].

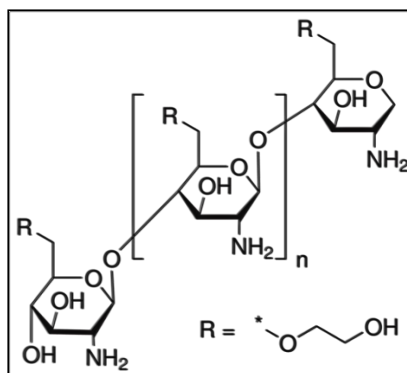
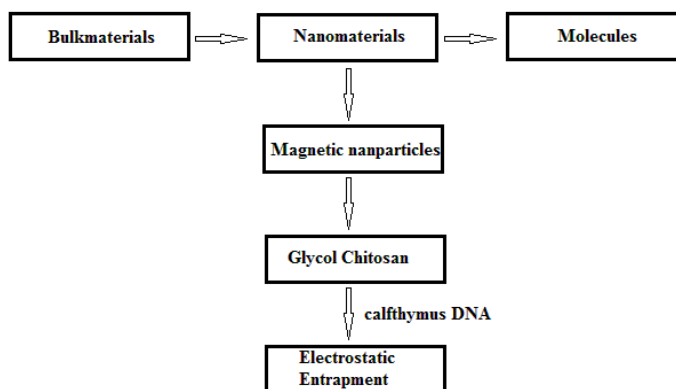


Figure 1. Molecular structure of Glycol chitosan

In addition, the nanoparticles can be used to load biomolecules for its future applications with small molecules such as drug molecules to find their small molecule-biomolecule interactions [5-6]. Because, the rational design of functionalized nanoparticles as tailor-made structures for biomedical applications requires exploration of the nanomaterials-to-DNA binding characteristics [7]. A way to obtain is binding to DNA is provided by the attachment of small molecules onto their surface and facilitate small molecule - DNA binding [8]. Another way is to load DNA inside magnetic nanomaterials to find its efficiency. The present work deals with the coating of GC to iron oxide magnetic nanoparticles and the electrostatic entrapment of DNA. The analysis of the binding by UV-Visible absorption and Scanning electron microscopic studies is carried out. The schematic representation of the work given in Scheme 1.



Scheme 1. The schematic representation of the work

2.0 Materials & Methods

2.1 Materials

Calf thymus DNA (ctDNA, DNA purified from Calf thymus), purchased from Genei (Merck), India was used without further purification. The presence of 1 mM EDTA with ctDNA is used to prevent nucleases from degrading the DNA. ctDNA is dissolved in NaCl solution of $50 \times 10^{-3} \text{ mol dm}^{-3}$ prior to use to obtain the required concentration per nucleotide phosphate which was calculated using the molar extinction coefficient of $6600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 260 nm. The sample purity of ctDNA was determined from the ratio of A_{260}/A_{280} . Glycol chitosan, GC of 1 mg/mL is prepared and refrigerated.

2.2 Characterisation

Absorption measurements were done with a UV-Vis spectrophotometer (V-630, Jasco, Japan) using a 1 cm path length cell. Ultra-sonicator PCI 9L 250H, India was used for sonication. The surface topology of the iron oxide magnetic nanoparticles, iron oxide magnetic nanoparticles-GC, iron oxide magnetic nanoparticles-GC-ctDNA was imaged by SEM (JEOL Model JSM 6360, Japan).

2.3 Preparation of Iron oxide magnetic nanoparticles

Equation 1 denotes the general method on preparing iron oxide magnetic nanoparticles which is followed by taking precursors such as Ferrous chloride tetrahydrate (2.6 g , 0.5 mol dm^{-3}) and 3.3 g of Ferric chloride hexahydrate (1 mol dm^{-3}) and dissolving in double distilled water. The yellow coloured solution was stirred for 1 hour at 50°C under nitrogen atmosphere. 40 ml of ammonia solution was added in very small portions to the above solution for a long period of time. The mixed solution was stirred vigorously. The addition of ammonia was continued till the yellow solution turned to dark brown coloured solution. The indication of the formation of iron oxide magnetic nanoparticles is further confirmed using UV-Visible absorption and Scanning electron microscopic (SEM) analysis. The reactions between ferrous and ferric salts in the presence of ammonia for the formation of iron oxide magnetic nanoparticles (Fe_3O_4 precipitate) are given in Equation (1) [5-6].



2.4 Entrapment of ctDNA onto iron oxide magnetic nanoparticles

Entrapment of ctDNA molecule onto iron oxide magnetic nanoparticles was carried out by addition of iron oxide nanoparticles (0.1 mg/mL) to the GC (1 mg/mL), sonicated at 25°C for 10 min. An external magnet was placed underneath the container to induce magnetic precipitation of the iron oxide nanoparticles/GC particles. The excess of supernatant GC was separated after the precipitation. Repeated application of external magnetic field to sediment the coated particles were carried out. The coating of glycol chitosan, GC on iron oxide magnetic nanoparticles is given in Figure 2. The same procedure repeated by the subsequent deposition

of the iron oxide magnetic nanoparticles/GC particles with 5mL of prepared DNA solution. The changes in morphology of nanoparticles can be seen before and after loading of ctDNA onto iron oxide magnetic nanoparticles-GC particles was analysed for alteration in their structure.

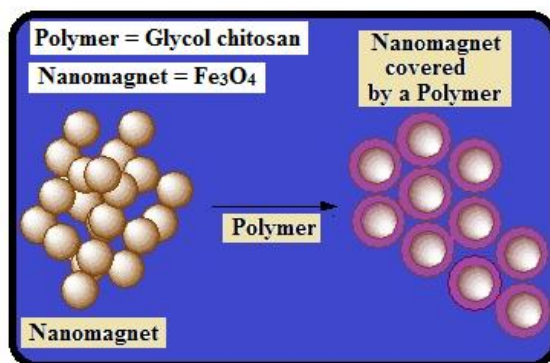


Figure 2. The schematic representation on the coating of glycol chitosan on iron oxide magnetic nanoparticles

3.0 Results and Discussion

3.1 UV-Visible absorption spectroscopic analysis

Figure 3 shows the UV-Visible absorption spectra of ctDNA at room temperature ($27 \pm 2^\circ \text{C}$). The sample purity of ctDNA was determined from the ratio of A_{260}/A_{280} which is commonly used to assess the amount of protein contamination that is left from the nucleic acid isolation process since proteins absorb at 280 nm. The purity of ctDNA sample used for electrostatic entrapment on magnetic nanoparticles was confirmed with the yield of A_{260}/A_{280} of approximately in the range of 1.8 - 1.9 (where A represents the absorbance).

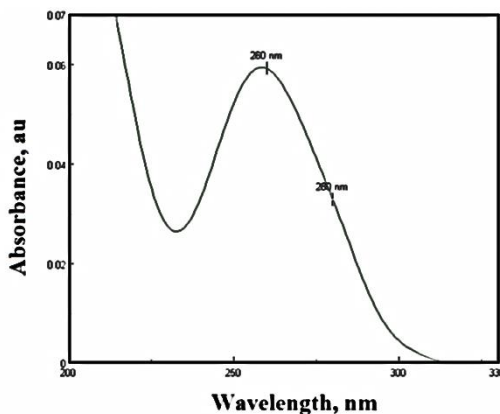


Figure 3. UV-Visible absorption spectra of calfthymus DNA.

The UV-Visible absorption spectra of iron oxide magnetic nanoparticles for the entrapment of the DNA was taken and given in Figure 4. It shows absorption maximum at

401nm. It shows the formation of iron oxide nanoparticles. The magnetic nanoparticles separated only analysed for its UV-Visible absorption spectroscopy.

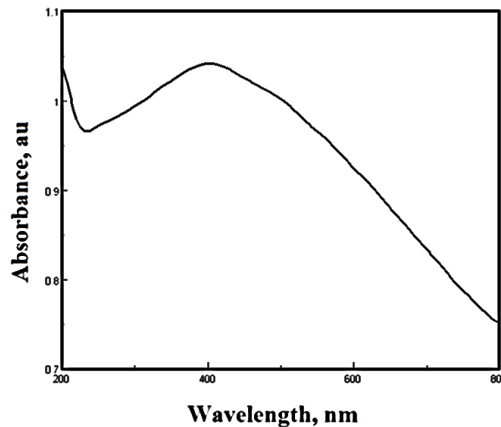


Figure 4. UV-Visible absorption spectra of iron oxide magnetic nanoparticles

Figure 5 shows the UV-Visible absorption spectra of iron oxide magnetic nanoparticles-Glycol chitosan-ctDNA. It shows the comparison on binding of ctDNA onto magnetic nanoparticles after modifying the magnetic nanoparticles with GC. Here Glycol chitosan shows the absorption maximum at 254 nm. A ~ 2 nm blue shift of absorption maximum of GC is obtained with the electrostatic entrapment of ctDNA. After the entrapment of ctDNA to GC coated magnetic nanoparticles, the supernatant solution was analysed for its excess amount of GC and ctDNA by UV-Visible absorption spectroscopic analysis.

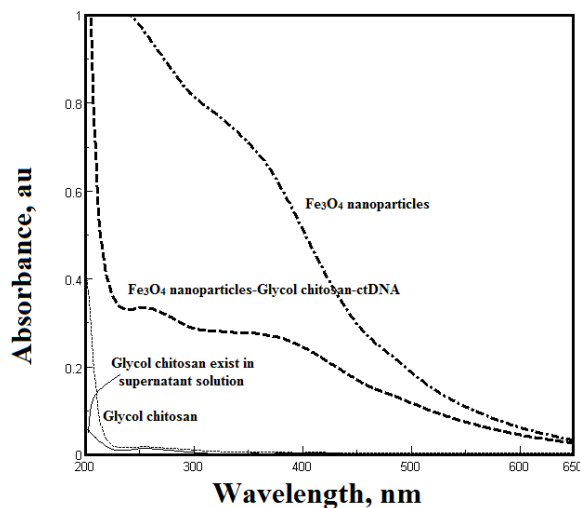


Figure 5. UV-Visible absorption spectra of iron oxide magnetic nanoparticles after entrapping with ctDNA

3. 2 Scanning electron microscopic analysis

If there is any alteration in the structure of magnetic nanoparticles by GC coating or after electrostatic entrapment of DNA, Scanning electron microscopic images were taken. The SEM images of iron oxide magnetic nanoparticles, iron oxide magnetic nanoparticles-glycol chitosan, iron oxide magnetic nanoparticles-glycol chitosan-ctDNA nanoparticles are given in Figure 6 to Figure 8. The nanoparticle size is increased with the coating of GC to the uniform distribution of iron oxide nanoparticles is possible with the coating of carbohydrate polymer, GC. The loading of DNA retained the nanoparticle size of iron oxide nanoparticles.

4.0 Conclusion

The electrostatic entrapment of calthymus DNA molecules through glycol chitosan coated iron oxide magnetic nanoparticles is carried out. UV-visible absorption spectroscopy in combination with Scanning electron microscopy suggest that the simple immobilization method can be adopted to entrap biomolecules inside magnetic nanoparticles for further small molecular interactions. Such immobilizations do not alter the structures of magnetic nanoparticles, revealed by Scanning electron microscopic studies. It may find applications in drug interactions study with the stored DNA without much alteration in magnetic nanoparticle structure.

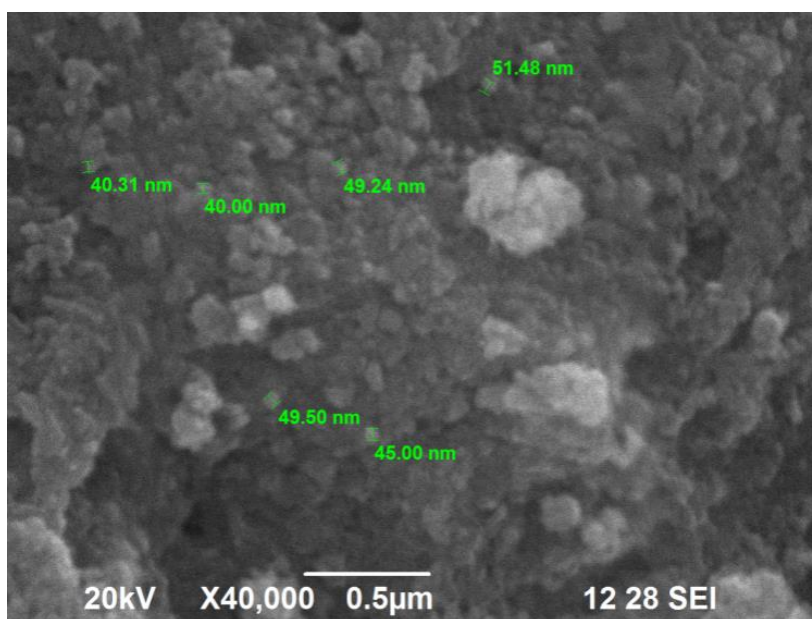


Figure 6. Scanning electron microscope spectra of iron oxide magnetic nanoparticles

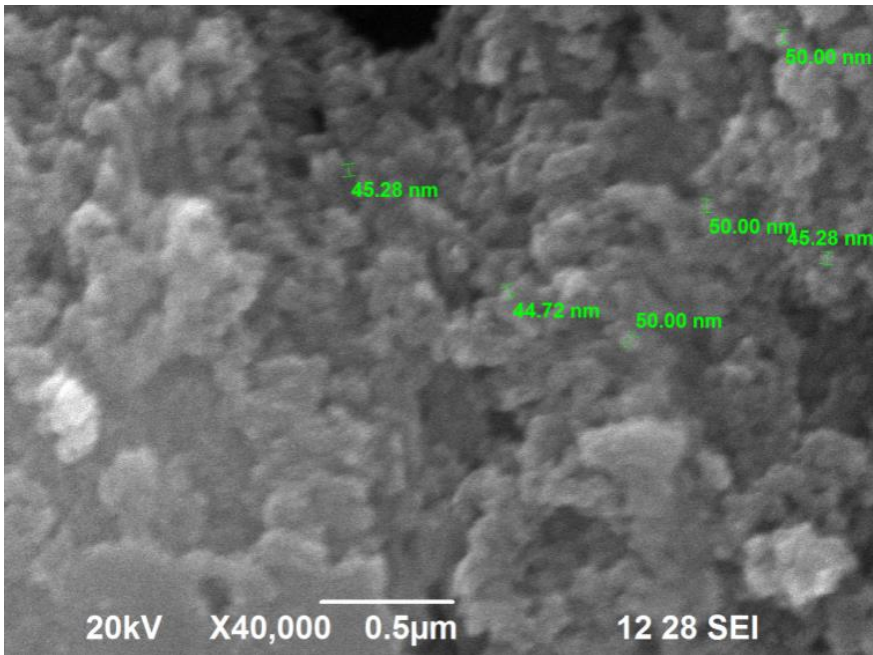


Figure 7. Scanning electron microscope spectra of iron oxide magnetic nanoparticles-GC

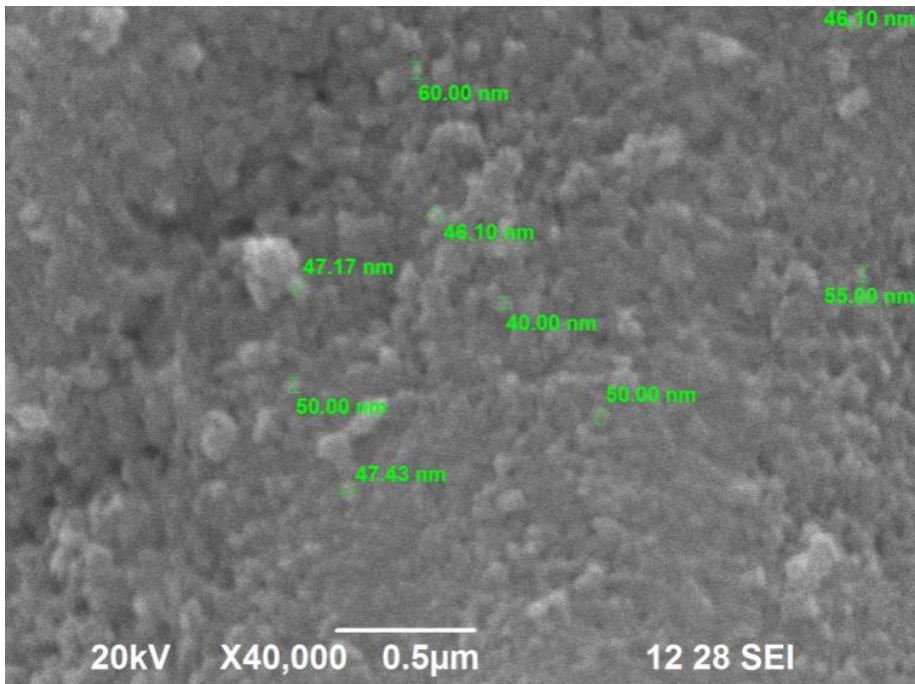


Figure 8. Scanning electron microscope spectra of iron oxide magnetic nanoparticles-GC-ctDNA

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Conflict of interest: NIL

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