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Research Article

# Dendrimer Coated SPIONs: Synthesis, Characterization and Potentials in Biomedical Applications

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## Abstract

The study focuses on the synthesis, characterization and potential applications of hybrid nanoparticles - dendrimer coated super paramagnetic iron oxide nanoparticles. Hybrid nanoparticles are usually superior in properties as compared to their individual components. Herein, we have made use of superparamagnetic inorganic component with a special class of polymeric organic component. Their synthesis and characterization techniques have been described in this study using methods like XRD, SEM, TEM, FTIR and VSM. Further, their properties have been evaluated using MTT assay and dye loading studies. In conclusion, dendrimer coated super paramagnetic iron oxide nanoparticles prove to be a better choice in order to enhance multifunctionality, biocompatibility while also retaining magnetic responsiveness and other advantages of inorganic materials. Overall, such kind of stable and readily customizable structures are promising tools in the future of biomedicine for applications in imaging, drug delivery, theranostics and cancer therapy.

**Keywords:** Dendrimers; SPIONs; Magnetic Nanoparticles; Hybrid Nanoparticles; Drug Delivery; Biomedicine; Theranostics

## Abbreviations

SPIONs: Super Paramagnetic Iron Oxide Nanoparticles; DC-SPIONs: Dendrimer Coated SPIONs; PAMAM: Polyamidoamide; EDA: Ethylene Diamine; MA: Methyl Acrylate; MTT: (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); RITC: Rhodamine B Isothiocyanate

## Introduction

Recent advances in the field of nanoscience and nanotechnology have helped in appreciating the advantages and limitations of different categories of nanoparticles. Inorganic nanoparticles like metallic nanoparticles possess excellent optical, mechanical, physiochemical properties, strength and stability [1-3]. But they may have shortfalls when they are evaluated for biological applications in terms of biocompatibility, bioavailability and overcoming biological barriers in live tissues. On the other hand, organic materials like polymeric nanoparticles possess great biostability and biocompatibility [4]. They are much more readily accepted by biological systems and show little to no adverse reactions. However, they do not possess the physiochemical properties and mechanical strength of an inorganic material. Thus, hybridizing these materials can be advantageous in order to overcome limitations and encompass unique combined properties [5-7].

In this study, we have made use of superparamagnetic iron oxide nanoparticles (SPIONs) as the inorganic component and PAMAM dendrimers as the organic counterpart. SPIONs have been one of the earliest and also the most popular choice of inorganic material for applications in biomedicine [8-10]. This is primarily

due to their magnetic properties which enable unique and highly sought-after applications which are not possible using other materials. Dendrimers on the other hand are unique structural moieties falling in the domain of supramolecular chemistry. Unlike other polymers, dendrimers have precise structural control and well-defined architecture. It also has several functional groups and high capacity loading structural voids making them capable of multifunctionality [11].

A combination of dendrimer coated SPIONs (DC-SPIONs) seems a promising combination for a variety of applications in the domain of biomedicine [12]. In this study, we have elaborated on the synthesis of DC-SPIONs, characterization and their potential applications in biomedicine.

## Materials and Methods

All the chemicals required were of analytical grade and used as received. Citric acid extra-pure was procured from S D Fine Chem Limited. Sodium hydroxide pellets (NaOH), Muller-Hinton agar, Tetracycline discs (30 mg) and NaCl of (99.5%) were procured from HiMedia. Potassium iodide (KI) from Merck (≥ 99.5%). RITC, Ferrous chloride anhydrous ($FeCl_3$) (97%), ferric chloride tetrahydrate ($FeCl_2$. $4H_2O$) (99%), DMSO reagent, Ethylene Diamine (EDA) and Methyl Acrylate (MA), Diphenyl-picryl hydrazyl (DPPH) were procured from Sigma- Aldrich.

### Synthesis

The synthesis of $Fe_3O_4$ nanoparticles was mainly carried out by the chemical co-precipitation method [13]. Briefly, an aqueous solution of 0.79M $FeCl_3$ and 0.26M of KI were mixed at room temperature under constant stirring and allowed to equilibrate for 1 hour. The iodide precipitate was filtered out and the obtained filtrate was collected for the synthesis of magnetite nanoparticles. To the filtrate, 1M NaOH was added drop wise under constant stirring until a pH of 9-11 was obtained. At the alkaline pH, magnetite nanoparticles precipitate out marking a change in solution from red to turbid brown to black. The precipitate was collected by magnetic separation (as shown in Figure 1) and washed thrice with double distillation. The precipitate was dried at 55°C for 3-4 hours. The same procedure was repeated for different combinations of time and temperature. Further, the surface of $Fe_3O_4$ nanoparticles was modified with citric acid to improve the particle size and dispersibility in water.

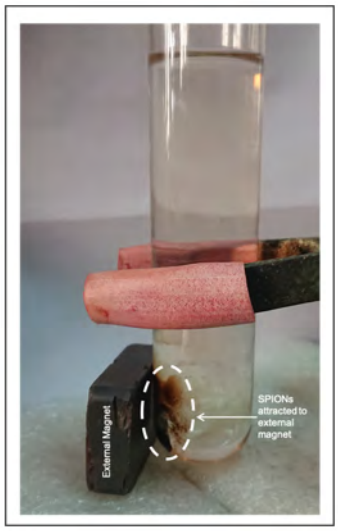


**Figure 1:** DC-SPIONs attracted to external magnet.

For surface activation, the SPIONs were functionalized with APTES. 5mL ethanolic solution of 0.5mg/mL of SPIONs was sonicated with 0.4 mL of APTES for about 30 minutes. Following this the solution was kept under constant stirring for 15 hours. Next, particles were magnetically collected, washed, dried and processed for coating with dendrimers. At this stage the particles are referred to as the "core" [14].

For coating with dendrimers, 40 mL of 50% v/v solution of MA is added to the core and sonicated for about 7 hours. After magnetic decantation and washing, 8 mL of 20% v/v EDA is added in a methanolic solution and sonicated for about 3 hours. This results in G1 PAMAM dendrimer coated SPIONs which are gently washed several times, dried and stored till further use [15,16].

### Characterization

Synthesis of SPIONs was characterized using XRD and TEM. FTIR was used to confirm dendrimer coating and VSM was used to evaluate the super paramagnetic potential of the bare and coated particles [17-19].

### MTT assay

MTT assay was used to evaluate cytotoxicity of the synthesized nanoparticles. Different concentrations of bare SPIONs and DC-SPIONs were incubated overnight with 70-80% 3T3 cells at 37°C

under 5% $CO_2$ in 96 well plates. Post incubation, the media was changed and MTT reagent was added to each plate and incubated once again for 4 hours in similar conditions. 10% DMSO was used to dissolve the formazan crystals after which the readings were immediately recorded using a Multiskan Sky High UV-Vis spectrophotometric reader between 550-600 nm.

### RITC loading

Functionalization and loading studies for SPIONs and DC-SPIONs were carried out using RITC dye. RITC dye is a ligand which can be easily conjugated and hence was chosen to perform these test studies. Briefly, low concentration of SPIONs and DC-SPIONs were incubated under shaking conditions with RITC dye overnight at 37°C. Post incubation, the contents of each vial were magnetically decanted using N52 neodymium magnets. Each sample was gently washed to remove excess or unloaded RITC and then resuspended in D.I. water. The samples were analyzed at λ= 550 nm and the data was recorded accordingly [20].

## Results and Discussion

### XRD

The crystal structure of SPIONs was analyzed by using XRD technique. The peaks corresponding to the crystal phase of $Fe_3O_4$ have been highlighted in *Figure 2*. (220) at 30.2, (311) at 35.6, 400) at 43.2, (422) at 53.6 and (440) at 62.8 [21].

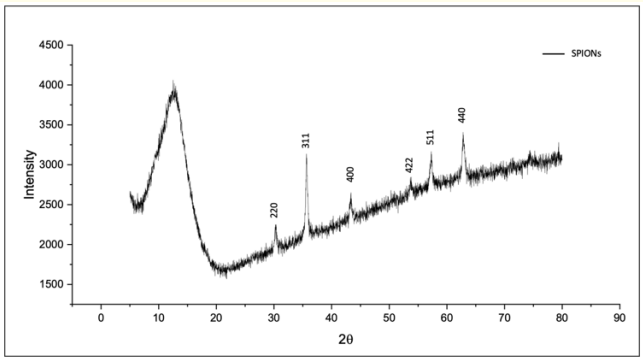


**Figure 2:** XRD of SPIONs.

### SEM, TEM

The morphological structure of SPIONs was observed through SEM and TEM as shown in figure 3. The morphology was observed to be more or less spherical in nature. The size of SPIONs was estimated using ImageJ software and was found to be in the range of 10-30 nm.

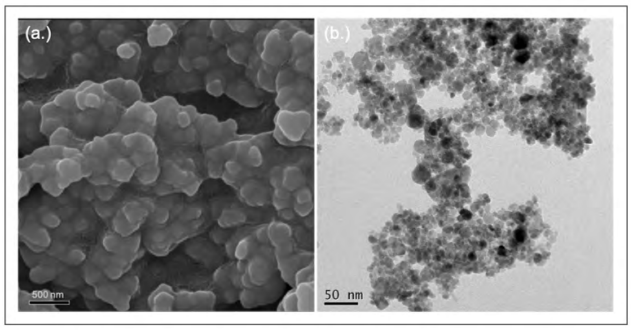


**Figure 3:** Morphology of SPIONs as seen in (a.) SEM, bar represented 500 nm; and (b.) TEM bar represents 50 nm.

### FTIR

The coating of dendrimer onto SPIONs was evaluated using FTIR spectroscopy as shown in figure 4. FeO bond of SPIONs is observed at 550nm which is well typical for these particles. The characteristic peaks observed at 1530 $cm^{-1}$, 1620 $cm^{-1}$ are attributed to dendrimers. Further the peak at 3250 $cm^{-1}$ is attributed to (-NH-) group which is also indicative of the presence of PAMAM dendrimers. Thus, it confirms the coating of dendrimers onto SPIONs.

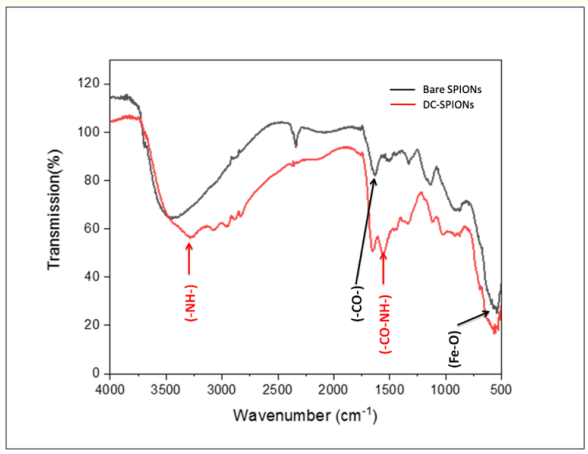


**Figure 4:** FTIR DC-SPIONs confirmation.

## VSM

Vibrating sample magnetometer was used to analyze the impact of coating on the superparamagnetic behavior of SPIONs. As it is seen in figure 5 that both the samples i.e. SPIONs and DC-SPIONs show no hysteresis indicating the superparamagnetic nature of both SPIONs and DC-SPIONs [22]. However, the saturation magnetization of DC-SPIONs is slightly reduced which can be attributed to the masking effect of the polymer coating on SPIONs [23,24].

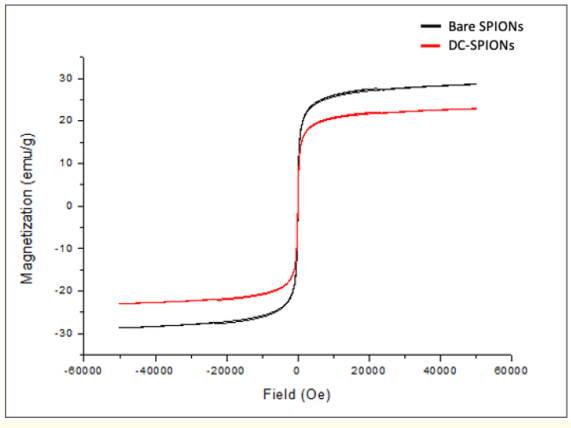


**Figure 5:** VSM DC-SPIONs.

## MTT assay

Cell viability and metabolic activity was evaluated using MTT assay. As represented in figure 6, cell viability is dose dependent and is slightly affected with increasing concentration of the test material. However, it can be clearly seen that, DC-SPIONs exhibit higher biocompatibility and tolerance by cells as compared to bare SPIONs. Thus, it reveals the biocompatibility advantage of DC-SPIONs over bare SPIONs [25,26].

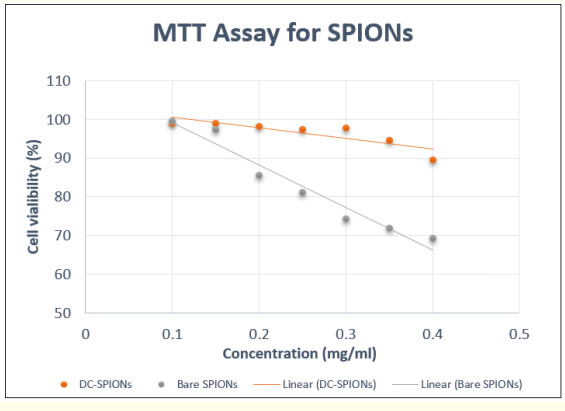


**Figure 6:** MTT assay results.

## RITC loading

RITC dye was chosen as a model ligand to evaluate the conjugation and loading capacity of SPIONs and DC-SPIONs using non-covalent bonding. As clearly seen in figure 7 DC-SPIONs exhibit a much superior loading capacity in comparison to bare SPIONs. Thus, DC-SPIONs would be a better choice of materials in case of both multifunctionality and loading capacity of nanoparticles [27-29].

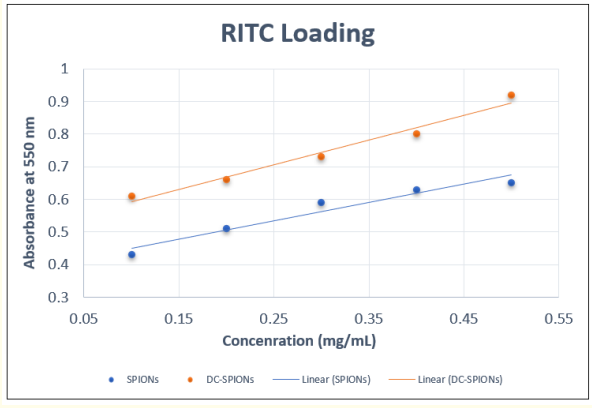


**Figure 7:** RITC Loading data.

## Conclusion

In this study, we have demonstrated the synthesis, characterization and potential applications of DC-SPIONs as a hybrid nano system. The XRD data confirms the crystalline nature of particles while the electron microscopy images give insights about the shape and size of the particles. FTIR confirms the dendrimer coating onto SPIONs which is also reflected in the VSM studies wherein the coated particles have reduced saturation magnetization but continue to remain superparamagnetic. Further, it is observed that DC-SPIONs are comparatively more biocompatible and also possess high loading capacity and enable multifunctionality.

With such a multifunctional and magnetically response material, the applications in the domain of biomedicine are abundant. Firstly, these particles can be used as MR contrast and imaging agents. They can be loaded with drugs, dyes, ligands and several other biomolecular entities to serve multiple functions using the same particle. Magnetic responsiveness can also be exploited to navigate and target the drugs apart from additionally functionalizing the particle with tissue targeted ligands. Magnetic nature has also been of great interest for treating solid tumor cancers using magnetic

hyperthermia therapy [30]. Additionally, this can also be coupled with photodynamic and photothermal therapy by attaching ligands of choice using surface groups of dendrimers.

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## Conflict of Interest

Authors declare no conflict of interest.

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