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Itraconazole Coated Super Paramagnetic Iron Oxide Nanoparticles for Antimicrobial Studies

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Abstract: In this present study, Superparamagnetic Iron Oxide Nanoparticles (SPIONs) were produced using $FeCl_3$ and $FeCl_2$ which were reduced to iron oxides using NaOH and ammonia solution (chemical co-precipitation). These naked SPIONs were further fabricated to form drug laden core-shell for controlled drug release and delivery. The fabrication was achieved by subjugating the naked SPIONs for oleic acid functionalization, drug tagging (Itraconazole) and finally encapsulated with a microbial derived polyester namely Polyhydroxybutyrate (PHB). Every stage of fabrication was characterized by scanning electron microscopy (SEM). The core-shell produced was checked for drug release kinetics, antibacterial and antifungal activities. These synthesized core-shells were carrying the drug and showed a slow drug release profile. The antimicrobial studies against bacteria - *Pseudomonas aeruginosa* & *Brevibacillus brevis* and fungi - *Candida albicans* by diffusion method proved that the core-shells inhibited bacterial and fungal activity. Furthermore, the naked SPIONs was found to be a good contrasting agent in X-ray imaging.

Keywords: SPIONs; Core-shell formation; antimicrobial activity.

1. Introduction

During these last decades, the growth of nanotechnology is humungous. Various types of nanoparticles are there, which are differentiated from each other by their nature and properties. Amongst all, metallic nanoparticles have tremendous scope and application in various fields including medicine, pharmaceutics, environmental remediation and others. Physical, chemical and biological properties of these nanoparticles aid them to be applied in various applications including theranostic, drug delivery, biosensing, imaging etc [1]. Metallic nanoparticles including gold, silver, Zn, iron oxides etc are most often used nanoparticles and proven to have various activities including anticancer activity, antibacterial, environmental bioremediation etc[2-10]. Amongst all these, iron oxides are cheaper to produce and versatile in action, moreover, they are biocompatible too. Iron oxides with magnetic properties are commonly called SPIONs (SuperParamagnetic Iron oxide nanoparticles), these nanoparticles are mostly exploited in imaging, drug delivery and so on. The major issue is its unstable in

nature, which can be overruled by functionalizing them with surfactant like SDS [11] or any other molecule like oleic acids [12]. Functionalization is usually performed to increase the colloidal stability as well as to prevent aggregation. The preparation of SPIONs is commonly done by chemical co-precipitation which is economically and sometimes they are also biologically derived [3]. On surface modification, the surface of these SPIONs becomes more functional to interact with further coating molecules. In this way, SPIONs are customized to form as effective drug nanocarriers with its characteristic feature of being able to be controlled by the applied magnetic field. In our earlier publications, we had prepared different types of SPIONs with varied precursors and reducing agents. The potential of each SPIONs was studied for different applications such as drug carriers [13], imaging [3,14], environmental bioremediation [6,15].

Here, it is an initial effort to produce SPIONs core-shells for drug tagging and drug delivery. Encapsulation with a biological source will help in increasing the biocompatibility and to confine the tagged drug onto the core-shells. Therefore, commercially available Polyhydroxybutyrate (PHB) was employed for encapsulating the core-shells. PHB is one of the most common polyhydroxyalkanoate (PHA) molecule produced by bacteria under stress conditions [16]. It is widely used as a natural biodegradable polymer for delivery drug / pesticide, tissue engineering and in medical devices [17 -19]. In this study, SPIONs were synthesized by co-precipitation method and coated with Itraconazole followed by PHB encapsulation. The drug release kinetics and efficiency of these core-shells were checked by antimicrobial studies. Also, chicken eggs were treated with the chemically prepared naked SPIONs to assess its competence as a contrasting agent in X-ray imaging.

2. Materials and Methods

2.1. Materials.

All the solvents were all analytical grade. The following chemicals were purchased with AR grade - Ferrous chloride (LOBACHEMIE), Ferric chloride (LOBACHEMIE), Ammonium solution (SRL), Sodium hydroxide (SRL), Hydrochloric Acid. PHB was obtained from Sigma Chemicals. The whole study was done using nitrogen purged MilliQ water.

2.2. Methods.

2.2.1. Synthesis of SPIONs.

Synthesis of SPIONs was done using the protocol followed by Samrot et al [15]. The precursor solution of 1 M of $FeCl_3$ and 3 M of $FeCl_2$ were prepared in nitrogen purged Millipore water. Equal volumes (10ml) of the two precursor solutions were mixed together and heated to 60 ^{0}C. To this, 15 ml of 8M NaOH and 5 ml of Ammonia solution were added in drops simultaneously and stirred vigorously. The temperature was maintained throughout the reaction. The black precipitate formed was settled down by applying a magnetic field. Thus separated black precipitate was washed with Nitrogen purged MilliQ water for multiple times to bring to neutral pH and later lyophilized to form dry powders.

2.2.2. Fabrication and characterization of drug loaded core-shells SPIONs.

Oleic acid functionalization, tagged with itraconazole and encapsulation with PHB was done in accordance with Sruthi et al [12]. Oleic acid solution in the ratio of 1:1 in ethanol was

prepared for 20 ml and 20 mg of SPIONs were added. The prepared mixture was placed in rotary shaker for 5h and then sonicated for 30 mins. Thus functionalized SPIONs were utilized for coating with Itraconazole. The drug solution was prepared at 1:2 ratio in ethanol for 30 ml and added with 20 mg of functionalized SPIONs. The prepared solution was left to interact with continuously stirring for 18 h. Further encapsulation of drug tagged SPIONs with PHB was successfully achieved by following Sruthi et al [12]. The powdered sample was characterized at every stage using Scanning Electron Microscopy (SEM).

2.2.3. Drug Release Kinetics.

After encapsulation with the biopolymer, the rate of drug release was determined by dialysis method [20-23], 10 mg of drug loaded SPIONs were dialysed against 50 ml PBS buffer [23]. At every time interval, 1 ml of sample dipped buffer was collected and OD value read at 267 nm wavelength (λ max of Itraconazole).

2.2.4. Antimicrobial Activity.

Agar well diffusion method was used to determine the anti-microbial activity against bacteria [24, 25] - *Pseudomonas aeruginosa* & *Brevibacillus brevis* and fungi - *Candida albicans*. The PHB encapsulated SPIONs were used in different concentrations such as 5 µg, 10 µg, 15 µg and 20 µg against a positive and negative controls. The plates were incubated at 37 ^{0}C for 24 h.

2.2.5. X-Ray imaging.

The X-ray imaging ability of SPIONs was tested on chicken's eggs to study its application as a contrasting agent. The experiment was carried out as described by Justin et al [14]. With the help of a syringe, 2ml of the glaire material was removed from the egg. It was replaced with 2ml of naked SPIONs solution (100mg/ml) and gently shaken to get the particles dispersed inside the egg thoroughly. After it is done, the egg was exposed to X-ray imaging

3. Results and Discussion

3.1. Scanning Electron Microscopy (SEM).

SEM results show that the SPIONs, Functionalized SPIONs, Drug coated SPIONs and PHB encapsulated SPIONs have a size range of 40 - 45 nm (Fig.1a), 40 - 65nm (Figure 1b), 50-70 nm (Figure 1c) and 100 -130nm (Figure 1d) respectively. As the result of coating, the nanoparticles seemed to increase in size gradually. Ultimately the PHB encapsulated core-shell reached a size range between 100 and 130nm. They were also seen to have spherical shape.

3.2. Drug Release Kinetics.

From the drug release kinetics study, it was clear that the drug is released in a sustainable manner overall, even with the presence of few troughs (Figure 2). It was seen to maintain a steady increase after 75th min up until 165th min. PHB nanoparticle is having the character of releasing drug slowly and steadily [26].

3.3. Anti-microbial study.

Anti-microbial study performed using agar well diffusion method showed that there was no activity seen against the bacteria species, *Pseudomonas aeruginosa* & *Brevibacillus brevis*. There was a significant zone of inhibition for Core-shell SPIONs at concentrations 15 & 20 mg i.e., 1.7 and 2 cm diameter (Table 1). A similar result was found by Sruthi et al [12].

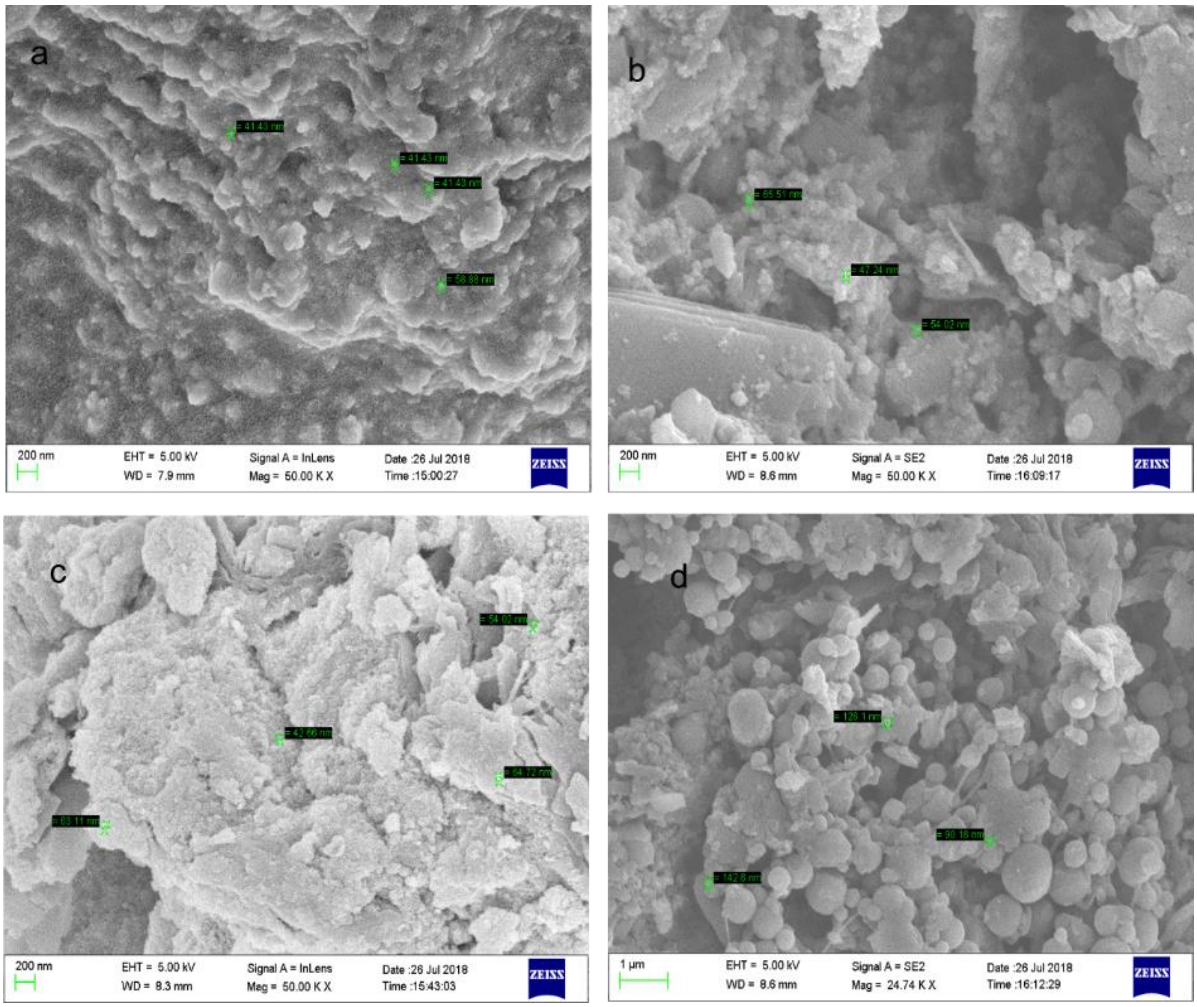

Figure 1. a) SPIONs b) Functionalized with oleic acids c) coated with itraconazole d) encapsulated with PHB.

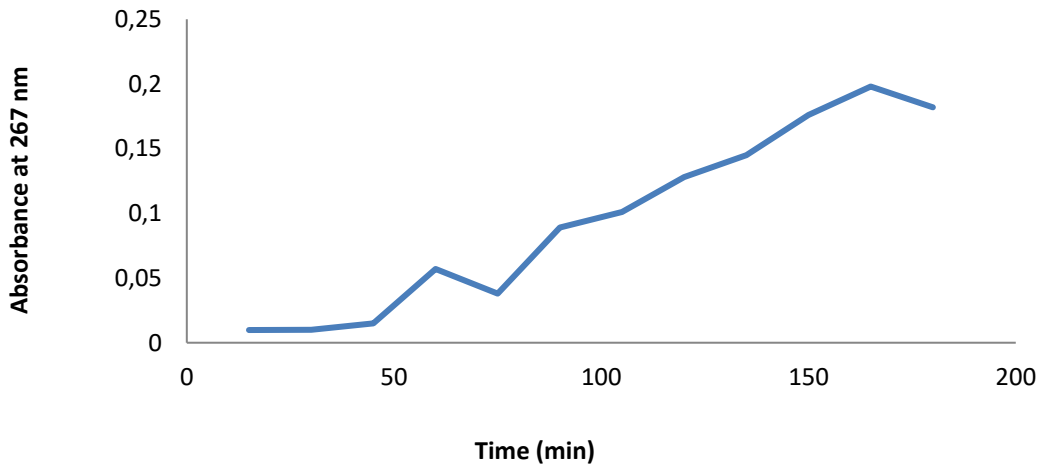

Figure 2. Drug release pattern of PHB encapsulated core-shell SPIONs.
c) *Candida albicans.*

Table 1. Antimicrobial activity of drug encapsulated Core shell SPIONs.

Concentration (µg/ml)	ZONE OF INHIBITION (cm)		
	Pseudomonas aeruginosa	*Brevibacillus brevis*	*Candida albicans*
POSITIVE CONTROL	4	3.5	2.8
NEGATIVE CONTROL	_	_	_
5	_	_	_
10	_	_	
15	_	_	1.7
20	3.2	0.3	2

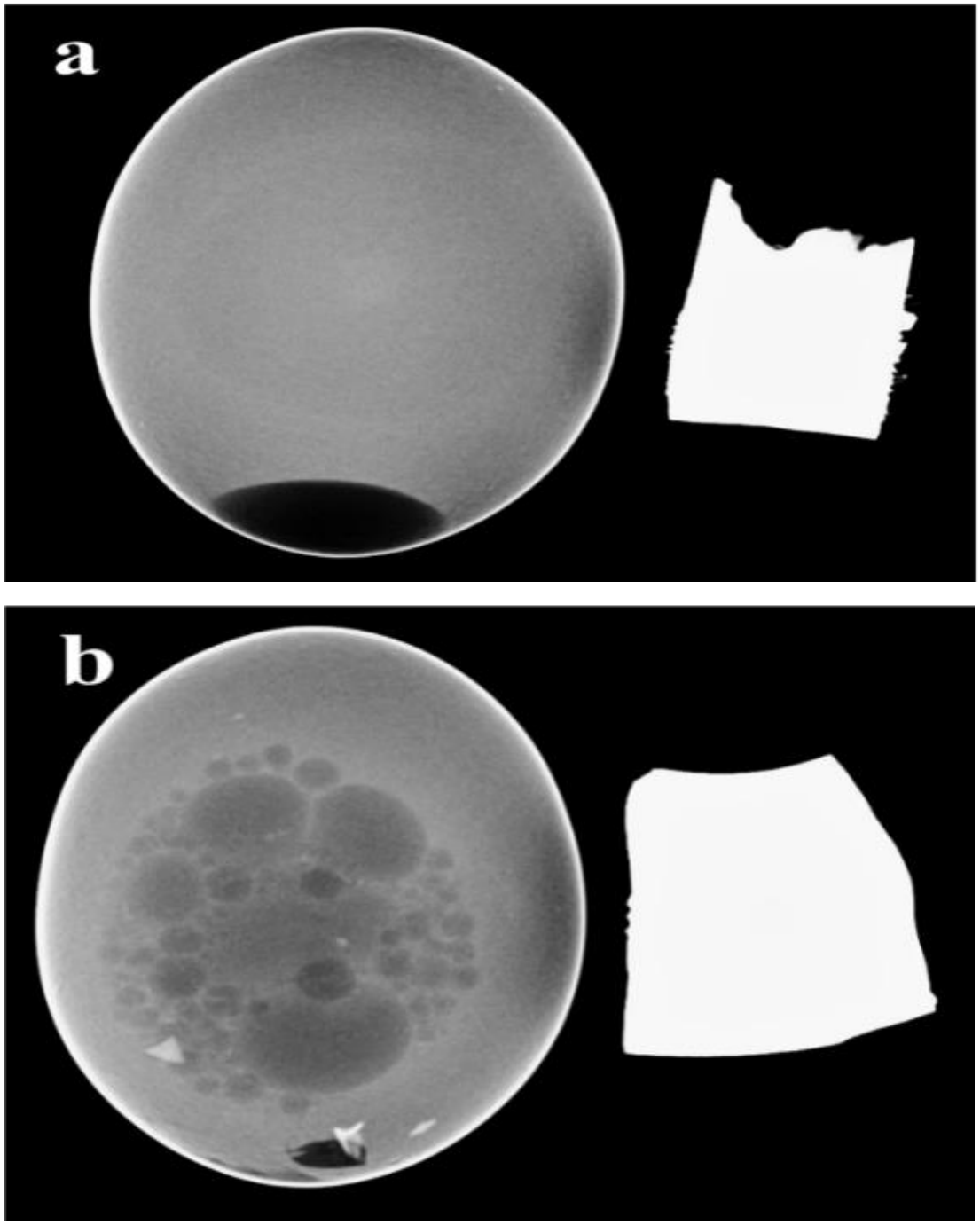

Figure 3. Imaging of egg under X-ray a) Control egg without SPIONs b) Egg injected with SPIONs.

3.4. X-ray Imaging.

Internal components of the egg could be visualized more clearly when injected with SPIONs (Figure 3). Hence SPIONs has the tendency to enhance the x-ray imaging. In our earlier report with quail's egg, SPIONs treated egg showed similar contrasting effect with increased quality of imaging [12,14, 27]. And with the help of an externally applied magnetic field, it is even possible to retrieve the SPIONs from the treated egg. There are more reports where these iron oxides were used in various pharmaceutical industries [28].

4. Conclusions

The synthesized core-shell SPIONs were successfully loaded with the drug, itraconazole and further encapsulated with PHB. The drug-encapsulated SPIONs reached to a size of 100-130nm. These drug laden core-shells expressed steady drug kinetics and released out the maximum drug at 160th min. The core-shell SPIONs exhibited antibacterial activity only against *P.aeruginosa* and antifungal activity against *C.albicans* at the highest concentration of 20 µg/ml. The chemically synthesized naked SPIONs were found to improve the X-ray visualization by acting as a good contrasting agent.

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Conflicts of Interest

The authors declare no conflict of interest.

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