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Tunable Self-Assembled Peptide Structure: A Novel Approach to Design Dual-Use Biological Agents

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Abstract

Micro/nanostructures based naturally occurring building blocks have attracted much attention as potential materials in the field of bio-nanotechnology. In this context, peptides are ideal naturally occurring materials for tissue regeneration, scaffolding, and drug delivery. Herein we report an initial study of fabrication of spherical and tubular structures by self-assembly process using butoxy carbonyl (Boc)-diphenylalanine as a naturally occurring peptides under different solvent conditions in the presence and absence of superparamagnetic iron oxide (SPIONs) core. The novel nanocomposites have been characterized using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Dynamic Light Scattering (DLS) Thermogravimetric Analysis (TGA) and Magnetic Hyperthermia under Alternating Magnetic Field (AMF).

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Keywords: Self-assembling peptides; Superparamagnetic iron oxide; core-shell nanoparticles.

1. Introduction

The molecular self-assembly is an attractive approach to engineer the nanostructures in various applications of nanoscience such as in the bioelectronics as sensor devices, drug delivery system, scaffolds, micro/nano fluidics and

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tissue and organ regeneration. The common strategy of nanoscale materials is based on “top-down” approach using photolithography to build two-dimensional structures. The nanostructures with the size of 50 nm are successfully achieved using common lithographic techniques. On the other hand, the molecular self-assembly is a unique “bottom-up” strategy to manufacture the functional materials. Superparamagnetic nanoparticles with the diameter range from 20-100 nm have already been studied in gene delivery, as MRI contrast agents, or for typical separation techniques [1–5].

Iron oxide core-shell nanomaterials have received great attention in the field of biomedical sciences [6]. The superparamagnetic feature and tunable physico-chemical properties such as, size, shape and surface charge make them an ideal candidate for biomedical applications. However, they are not yet reached to regulatory approvals due to their safety and efficacy concerns. Initial pre-clinical trials showed some of the limitation in the magnetic nanoparticles based drug delivery systems. These limitations include the poor drug availability, targeted drug release and aggregation *in vivo* system. To overcome these limitations, variety of techniques have been studied such as size optimizing and proper coatings with biocompatible molecules such as proteins, lipids, dextran-PEG and other polymers [7–9]. These studies suggest that coated SPION uptake by macrophages cells is lower than the bare SPIONs [10]. Drug loaded SPIONs (drug incorporated in the coatings) can be systematically targeted by using an external magnetic field. Additionally, the fate of loaded drug composite can be tracked via imaging.

The coating material self-assemble as spontaneous diffusion or aggregation of individual molecules governed by the ionic interaction, Van der Waals interactions and hydrophobic interactions. Various biomolecules such as proteins [11], peptides [12], lipids [13] and aromatic compounds [14] have a tendency to self-assemble and stabilise the other molecules after coating. The self-assembling peptides are either the short sequences of certain proteins or the sequences of de novo design. These peptides derived from naturally occurring proteins, such as amyloid peptides have shown greater tendency to form aggregates in aqueous solutions [15]. Along with these naturally occurring peptides from proteins, peptides can also be designed for the self-association. The synthetic amphiphilic peptides are well-studied for the formation of micro/nanostructures in order to produce the supermolecules of various functions [16]. Due to their unique properties, these self-assembling peptides are classified into four groups according to their chemical compositions; peptide amphiphiles, Bolaamphiphiles, cyclic peptides and ionic-complementary peptides.

The aim of this research is to study the ability of a self-assembling peptide (Boc-diphenylalanine) to coat the SPIONs to fulfil the requirements of systematic administration of nanoparticle along with magnetite properties of nanoparticles. These particle were characterized using a variety of techniques for size (light scattering), composition (FTIR, EDAX) shape (SEM) and stability (TGA). Furthermore, the heating ability of these novel biocompatible nanocomposite has been studied and also their possible use in cancer theranostics is discussed.

2. Materials and Methods

2.1. Materials

The studied peptide, diphenylalanine N-terminal modified analogues; Boc-Phe-Phe-OH were purchased from Bachem (Bubendorf, Switzerland) with a purity > 95 %. Ethanol and Iron (II) chloride tetra hydrate were purchased from (Sigma-Aldrich, UK). Iron (III) chloride hexahydrate and Hexa-fluoro-isopropanol (HFIP) were purchased from (Alfa Aesar, UK) and ammonia was purchased from (VWR Scientific, UK). Aluminium stub for SEM analysis was from (Agar Scientific, UK). All solutions were prepared in Milli-Q water with 18 MΩ·cm resistances otherwise discussed.

2.2. Synthesis of peptide self-assemblies

Lyophilised peptide powder was initially dissolved in HFIP at a concentration of 100 mg/ml as a stock solution. For each set of experiments, fresh stock solutions were prepared in order to avoid pre-aggregation of the peptide. The stock solutions were subsequently diluted to 10 mg/ml solution in ethanol/water (1:1) in order to obtain spherical structures.

2.3. Synthesis of Superparamagnetic Iron Oxide Nanoparticles (SPIONs)

SPIONs were prepared following a method outlined by [17,18]. Briefly, 50 ml of 0.085 M solution of $FeCl_2$ .4H2O and 0.16 M solution of $FeCl_3.6H_2O$ were mixed at 80°C under nitrogen atmosphere. The pH of the mixture was checked and found to be in the range of 1.6 to 1.8. 25 ml of 28% Ammonia was added to the above solution drop wise for approximately 30 minutes in the presence of nitrogen gas at 80°C. The solution exhibited a colour change, turning black indicating that nanoparticles were formed as magnetite (black colour appearance). The reaction was allowed to proceed for a further 1 hour, at this stage, the pH value of suspension was 10. The black nanoparticles obtained were washed with deionised water until a pH of 7.0 was reached via magnetic sedimentation and decantation using a slab magnet.

2.4. Synthesis of core-shell nanocomposites

1 ml of (1 mg/ml) of SPIONs were dispersed in 1ml of (5 mg/ml of peptide Boc–diphenylalanine in ethanol/water 50%) dropwise in bath ultra-sonication for 30 minutes. Solutions of peptide-functionalised SPIONs have characterized accordingly.

2.5. Morphological characterization

Peptide-coated SPIONS were dried on 12 mm aluminum sample stubs using carbon tabs. Samples were deposited with a thin layer of gold by low-vacuum sputter coating to facilitate imaging (argon gas with an ion current of 30 mA). SEM images were recorded using FEI Quanta 200 (JEOL, Tokyo, Japan) operating at 20 kV, WD 10 mm with spot size 2.5 -5.0.

2.6. Particle size and zeta potential measurement

The size of diphenylalanine structures was measured using dynamic light scattering technique which is based on laser diffraction. The data was obtained from the Dispersion Technology Software (DTS) provided by (Malvern Mastersizer 2000 Instruments, Worcestershire, U.K.). Zeta potential of diphenylalanine was measured with Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). 1 ml of (10 mg/ml) each of the peptide solutions (water and water/ethanol mixtures) were used for each measurement. Peptide solutions were transferred in zeta potential folded capillary cuvette (Malvern, Worcestershire, UK). Zeta potential was measured by electrophoretic mobility of the charged particles using Smoluchowski formula [69] by applying the constant voltage supplied from the electrodes of the instrument sample chamber. The results are shown in mV of the average of three independent samples. For each sample, three measurements were performed with 20 scans at 25°C. The acquisition time was set 120 seconds. The temperature was set at 25°C and acquisition time was 120 seconds. Ten runs were performed with an average of three measurements.

2.7. Hyperthermic determination of magnetic heating

The Specific Power Absorption (SPA) of peptide coated SPIONs was determined from experiments performed in a commercial A/C field applicator (DM100, nB nanoscale Biomagnetics, Spain, The parameters were set as f = 406 kHz. All measurements were performed within a thermally-insulated working space of about 1 cm^3, using a closed capped vials of 1.0 ml volume.

2.8. Measurement of thermal stability of materials

Thermal stability of Boc-diphenylalanine, SPIONs and composites were characterised with Mettler-Toledo equipment (TGA/SDTA 851, Mettler-Toledo Ltd., Leicester, UK). The instrument was calibrated for both the

parameters i.e. weight and temperature. Approximately 5 mg of the sample was taken in an aluminium pan. The pan was kept on the balance arm in a closed chamber of the furnace. The chamber was filled with nitrogen gas to initiate the combustion of the sample. The heating temperature was set at a rate of 10°C /minute, starting from 25°C to 500°C. The weight of the sample was recorded using the software connected balance. The exact output of analysis was plotted with a loss in weight over the range of temperature analysed.

3. Results and Discussion

Boc-diphenylalanine, when dissolved in 1:1 ethanol/water, it shows the spherical structure of size less than 1 μm (Fig. 1a). Boc-diphenylalanine, when dissolved in water showed tubular structure (Fog. 1b), changes in the solvent system have also changed the shape of the nanostructure. The configurations may be related to the molecular interactions between the various groups present and their stoichiometric arrangements.

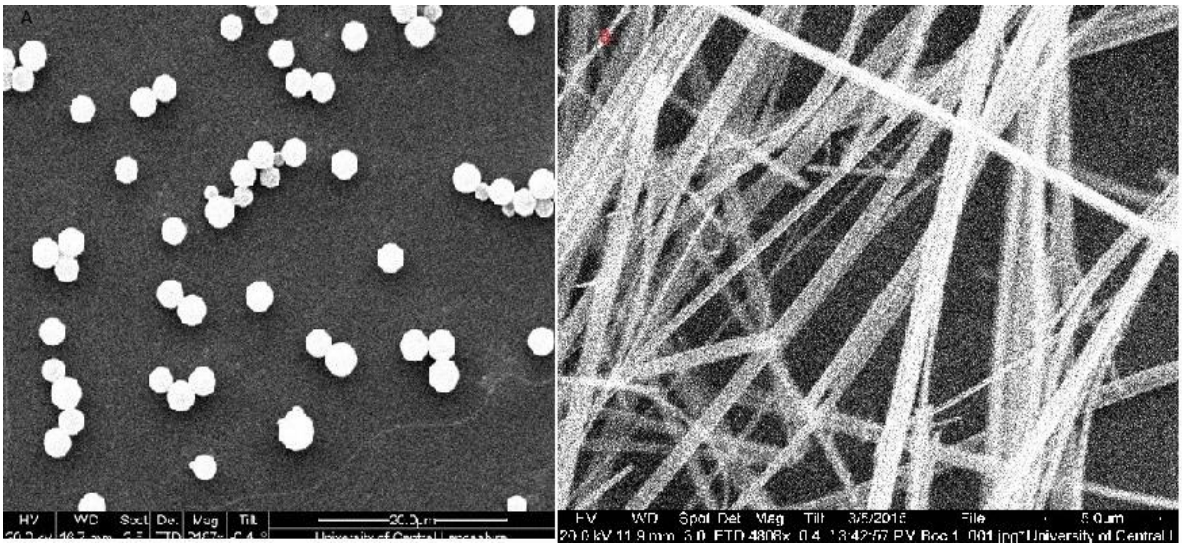


Fig. 1. SEM image of Boc – diphenylalanine (dissolved in HFIP/50% ethanol/water) (a) and in water (b)

Fig. 2, shows the SEM micrograph of SPIONs synthesized following the published method [18] by coprecipitation of ferrous and ferric ions with a ratio of 1:2 in an aqueous medium in the presence of ammonium hydroxide. These particles have a spherical shape and the diameter (size) ranging from 90 to 100 nm (see Fig. 2a). Figure 2b exhibited SPIONs embedded peptide nanoparticles as core-shell nanoparticles. The core-shell nanoparticles were separated with the influence of an external magnetic field due to the presence of superparamagnetic core.

FT-IR spectrum (see Fig. 3) of bare SPIONs exhibited Fe-O stretching at 548 cm^{-1}, which reveals the presence of Fe_3O_4 [19] whereas core-shell (SPIONs core + peptide shells) nanoparticles exhibited peaks at around 1672 cm^{-1} and 1278 cm^{-1} correspond to C=C stretching vibration and at 1416 cm^{-1} due to C=O stretching along with asymmetric stretching of C-H at 2970 cm^{-1} [20]. The broad peak at 3200 cm^{-1} to 3400 cm^{-1} correspond to O-H stretching, superimposed with N-H stretching of amino groups presence in peptides. In addition, a characteristic weak absorption at 548 cm^{-1} corresponds to Fe-O vibration (19, 21) indicating the presence of SPIONs as a core in the core-shell structure.

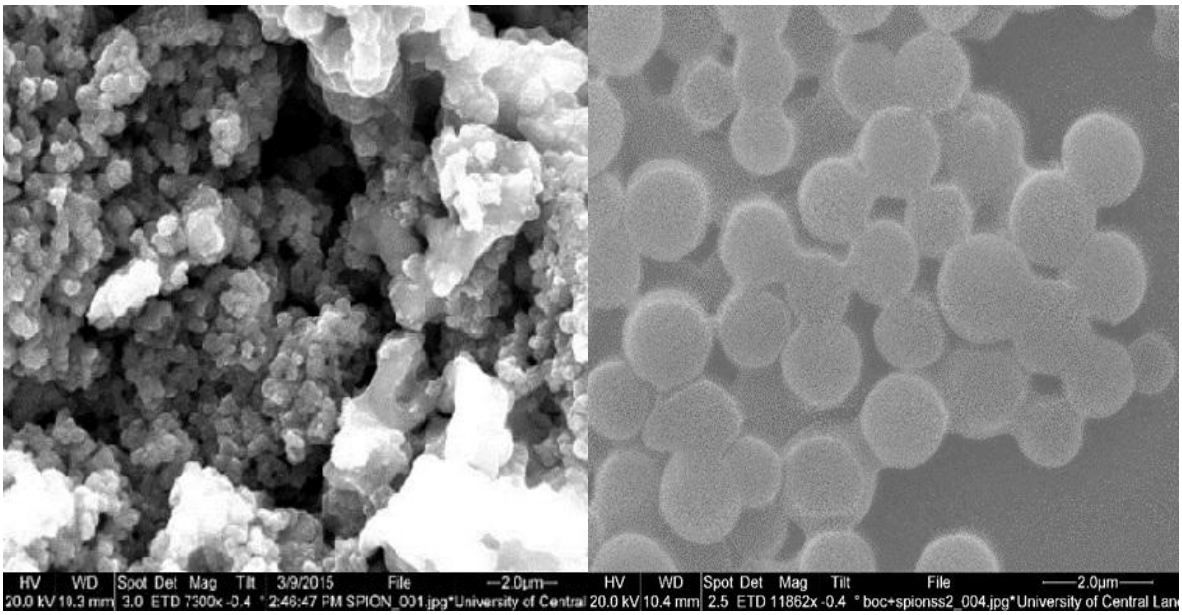
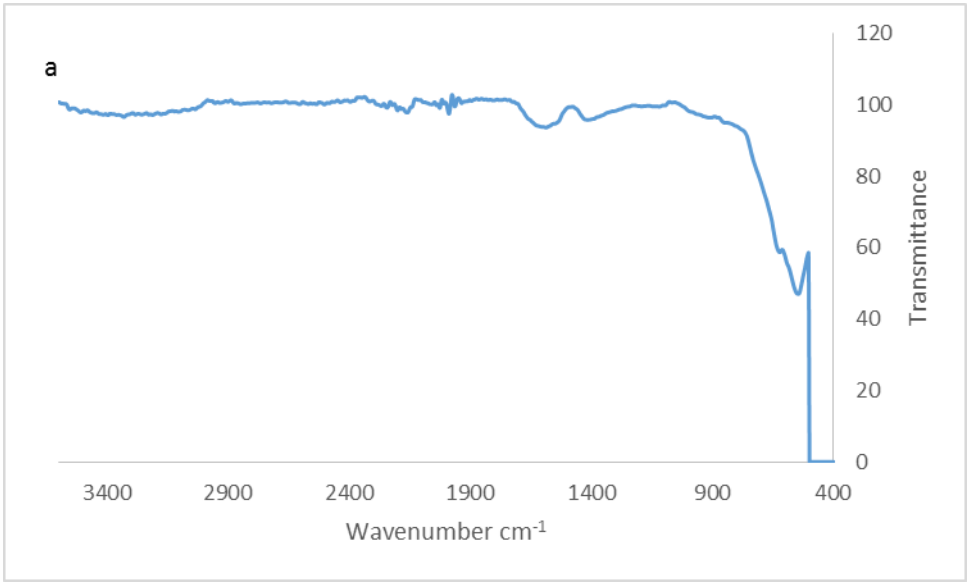


Fig. 2. SEM image of SPIONs (left) and peptide coated SPIONs (right)



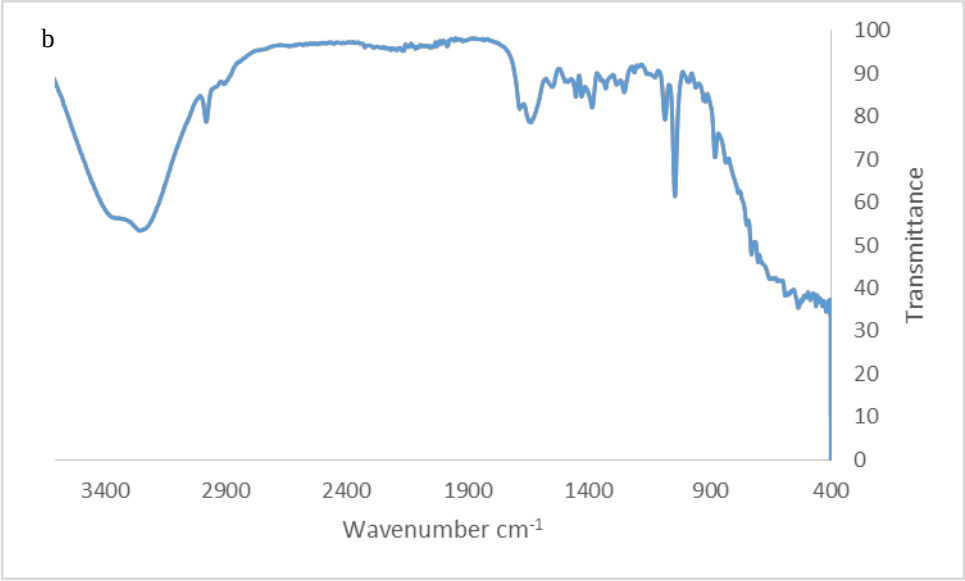


Fig. 3 FT-IR spectra of bare SPIONs (a) and Boc- diphenylalanine coated SPIONs (b)

The heating ability of SPIONs and Boc-coated SPIONs was tested for hyperthermia under AMF which indicated good heating response (see Fig. 4). The SPIONs showed heating ability within the range 30 °C to 39 °C within less than 100 seconds whereas for core-shell nanoparticles took much longer (about 600 seconds) to reach 39 °C.

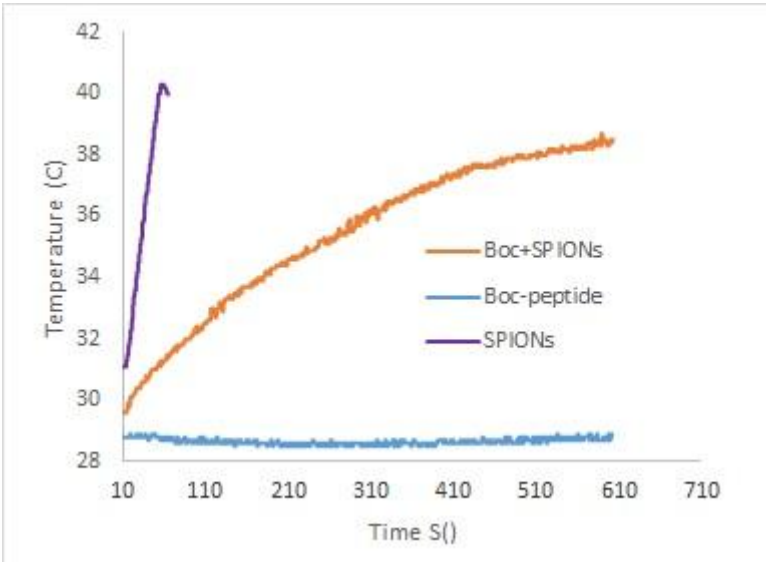


Fig 4. Heating profile of various nanoparticles

Table 1 present the EDAX analysis data indicating the presence of iron along with carbon and oxygen due to the SPIONs. An additional peak due to Nitrogen in core-shell nanoparticles could be due to the BOC-peptide as shell structure.

Table 1. EDAX analysis of nanocomposites

Sample	Elemental Composition	Atomic %
Boc-diphenylalanine	C	66.15
	O	27.54
Boc-diphenylalanine and SPIONs	C	74.17
	O	2.9
	Fe	0.38

Multiple peaks ranging from 80-200 µm (see Figure 5, DLS data) indicating that core-shell nanoparticles were highly aggregated.

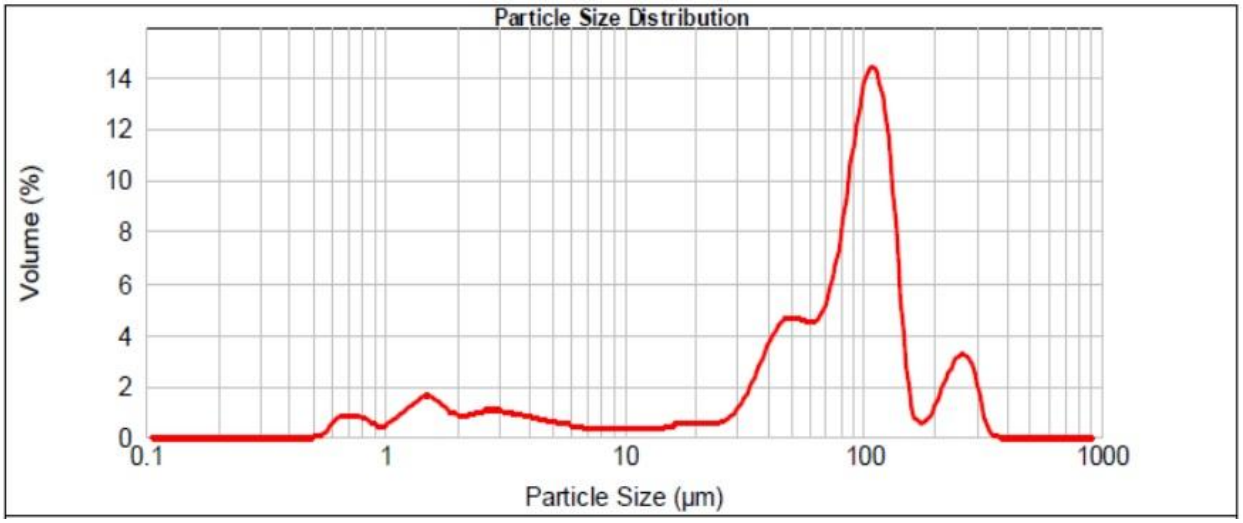


Fig. 5 Size distribution of core-shell nanoparticles (Boc- diphenylalanine shell + SPIONs core)

The zeta potential results of pure Boc-diphenylalanine in the presence of water and ethanol / water (50 %) are shown in Fig. 6. Zeta potential of Boc-diphenylalanine was observed to be – 31.5 ± 1.2 mV in water and-and 1.3 ± 0.3 mV in ethanol/water mixture (50 %). The high value of zeta potential is reported to be due to the lower level of aggregation.

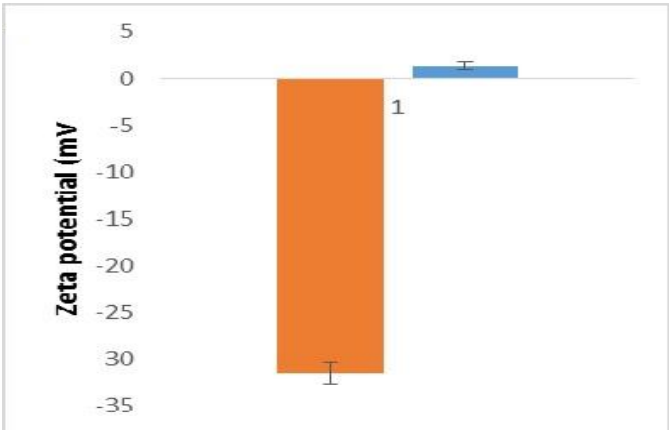


Fig. 6. Zeta potential of Boc Diphenylalanine in different solvents: water (orange) and 50 % ethanol/water (blue).

The stability profile of single material or in the form of composites was characterised using thermogravimetric analysis. Thermal degradation curves of pure Boc-diphenylalanine, bare SPIONs, and core-shell nanoparticles are presented in figure 7. A two-step degradation of pure Boc-diphenylalanine has been observed. The onset degradation starts at 158°C for pure Boc-diphenylalanine and SPIONs coated Boc-diphenylalanine. The complete weight loss is observed at 340°C for Boc-diphenylalanine is in good agreement with earlier published results [22]. Core-shell nanoparticles exhibited around 60% weight loss due to the decomposition of Boc-diphenylalanine indicating the presence of around 40 % inorganic SPIONs in the composites. Bare SPIONs exhibited only around 10 % weight loss indicating no loss of inorganic component except adsorbed water.

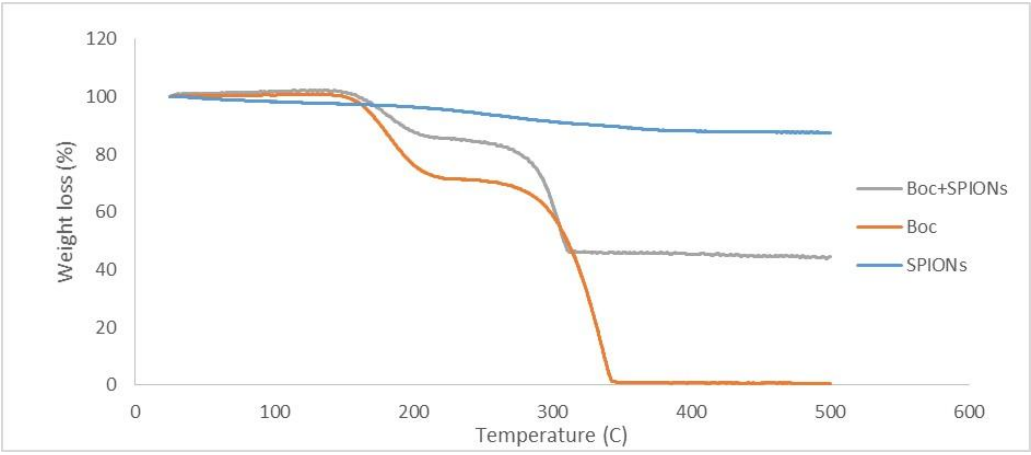


Fig. 7. TGA of Boc-Diphenylalanine (brownish-orange) showing stability up to 158 °C, SPIONs (green) and Boc+SPIONs (grey)

4. Conclusions

In this paper, the aromatic amino-terminal-modified with butoxy carbonyl (Boc-Phe-Phe-OH) peptide and their self-assembly pattern have been studied. Boc modified diphenylalanine peptide in different solvent conditions have shown tunable structures of two different morphologies such as tubular or spherical. Magnetic nanoparticles (SPIONs) were embedded inside the peptides nanostructures which have been confirmed by FT-IR, EDAX, and Thermal gravimetric experiments. However, the core-shell nanoparticles were largely aggregated into micrometer

sizes in suspension which may limit their applications in drug delivery. Both bare SPIONs and core-shell nanoparticles were efficient in generating heat under AMF which could be useful for hyperthermia-induced cancer therapy.

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