Easy-Synthesis of BDA/ABDA Functionalized Hydrophilic Superparamagnetic Iron Oxide Nanoparticles for Magnetic-Hyperthermia Application

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Herein, we have reported an easy process for the synthesis of superparamagnetic iron oxide nanoparticles (SPIOs, with a size of ~ 10 nm), where these SPIOs are surface-functionalized with novel pi-electron rich surfactants such as 1,4-benzene dicarboxylic acid (BDA) and 2-amino-1,4-benzene dicarboxylic acid (ABDA). The BDA/ABDA capped SPIOs have demonstrated well crystalline character, excellent colloidal stability, and high saturation magnetization. Moreover, these capped SPIOs have shown good heating ability in magnetic-hyperthermia studies under an alternating magnetic field at a medically suitable frequency, as compared to the previously reported SPIOs based heating-agents. Thus, the as-prepared BDA/ABDA-SPIOs can be used as promising heating agents for magnetic-hyperthermia based biomedical applications.

Introduction

Superparamagnetic iron oxide nanoparticles (SPIOs - especially magnetite – Fe_3O_4) have been extensively deployed in numerous biomedical applications such as heating-agents in magnetic-hyperthermia therapy (MHPT) [**1–5**], contrast-agents in magnetic resonance imaging (MRI) [**6**], magnetically-targeted delivery agents for different drugs and also in magnetic separation [**7–10**]. The potential benefit of these applications comes from the low toxicity and the high chemical stability of the SPIOs, which is a direct function of the engineered surface coating functionalities. But for effective biomedical applications, especially as heating agents, it is essential to achieve stable water-based colloidal suspensions (ferrofluid), which should consist of ultrafine SPIOs. It is, however, a challenge to stabilize the ultrafine synthetic SPIOs in an aqueous media because of the induction of the agglomeration due to magnetic dipoles for compensating their large surface free energy. Moreover, the physical effect that leads to agglomeration is mainly based on the emergence of larger magnetic interactions among the SPIOs under an externally applied alternating magnetic field. Hence, it is very difficult to evenly disperse the SPIOs in the aqueous media for effective usage as heating agents in magnetic hyperthermia therapy applications for cancer treatments.

Several chemical methods including co-precipitation, microemulsion, hydrothermal and thermal decomposition have been commonly used to synthesize water-stable SPIOs. But, the direct synthesis of water-soluble SPIOs is challenging and yet necessary, to exploit their full potential as a nanomedicine. Many different synthetic routes have been explored by several groups to set up protocols for direct synthesis of hydrophilic SPIOs, however done with limited successes [**11**]. Therefore, in this work, we have reported an easy, inexpensive and straightforward approach to synthesize the SPIOs, where their surfaces have been in-situ modified using novel surfactants – (i) BDA (1,4-benzene dicarboxylic acid) and (ii) ABDA (2-amino-1,4-benzene dicarboxylic acid) molecules to make BDA/ABDA coated SPIOs. The engineered hydrophilic BDA/ABDA surface coatings might bring good dispersibility to the SPIOs in aqueous media and/or improve their heating properties under the alternating magnetic field. It is anticipated that the reduced spacer dimension of the BDA/ABDA canopy, combined with their pi (π)-electron rich and rigid aromatic rings, might enhance the spin-transfers between the metallic SPIOs cores, and the shells (BDA/ABDA). In this way, a large amount of spin-density will be directly exposed to a bulk scenario, hence differing from previously explored sigma-conjugated long aliphatic chains. This effect may enhance the relaxation mechanisms (Neel and/or Brownian) of the SPIOs, which in turn can improve their heating properties in comparison to the reported surface-functionalized SPIOs. Besides, the surface attached functional –COOH/NH_2 groups from BDA/ABDA molecules allow the assembly (post-synthesis) of various bio-conjugations, by interacting with biomolecules like monoclonal antibodies and/or folate receptors for site-specific targeting of the SPIOs as heating agents for use in *in-vitro/-vivo* applications.

Experimental

Materials

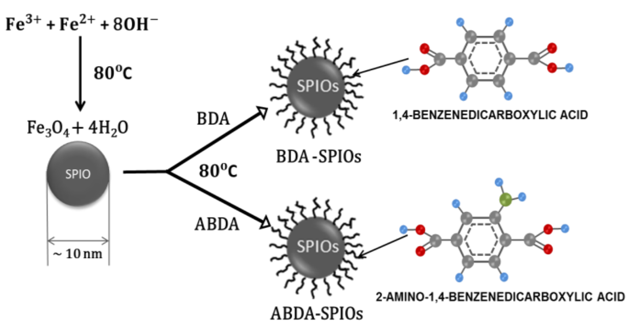
BDA (C_6H_4 $(CO_2H)_2$), ABDA ($H_2NC_6H_3$-1,4-$(CO_2H)_2$), and Iron (II)/ferrous chloride tetrahydrate ($FeCl_2.4H_2O$) are obtained from Sigma Aldrich. Ammonium hydroxide (NH_4OH with 25% H_2O) and iron (III)/ferric chloride hexahydrate ($FeCl_3.6H_2O$) are purchased from Fisher Scientific and Loba chemicals, respectively

Methods

(i) Synthesis and Characterization of SPIOs

SPIOs are prepared via a simple chemical co-precipitation process (refer **Scheme 1**) as reported elsewhere [**12**].

Briefly, 2.34 g of $FeCl_3.6H_2O$, 0.86 g of $FeCl_2.4H_2O$, and 1.51/1.65 g of BDA/ABDA are mixed with a specific amount of distilled water (DW). The resulting mixture is heated to a temperature of 80 ºC for 60 minutes (min) under magnetic stirring and nitrogen gas flow. Then, NH_4OH is poured to the above solution while maintaining the temperature at 80ºC for 1 hour. Finally, the precipitated nanoparticles are magnetically-isolated and washed with DW/ethanol mixture. Moreover, the BDA/ABDA functionalized SPIOs are obtained and labeled as BDA-SPIOs and ABDA-SPIOs, respectively. Subsequently, the as-prepared BDA/ABDA-SPIOs are either suspended in water to prepare aqueous ferrofluids or dried in an oven overnight at 40ºC to prepare powder samples for further characterizations to investigate their structures and properties. Particle sizes (using transmission electron microscopy - TEM), crystalline phase (using X-ray diffraction), surface-modifications (using Fourier transform infrared spectroscopy/thermogravimetric analyzer) and magnetic saturation (using superconducting quantum interference device) are determined.



Scheme 1. Schematic representation of the synthesis of BDA-SPIOs and ABDA-SPIOs.

(ii) Calorimetric Magnetic-Hyperthermia (CMH)

CMH studies are performed via hyperthermia equipment, where 1 ml of the BDA-SPIOs and ABDA-SPIOs based ferrofluids at concentrations of 0.5-8 mg/ml are initially prepared, and then subjected to an alternating magnetic field (AMF of 13.96 kA/m) at a fixed medically suited frequency. Subsequently, the temperature rise in these ferrofluids is monitored via an optical fiber-based temperature probe for a specific time. Then, the calorimetric heating efficacies of the BDA/ABDA SPIOs are determined by using a specific absorption rate (SAR) as per the following equation.

$$SAR = C*(\Delta T/\Delta t)*(1/m_{Fe}) \quad (1)$$

where C is the specific heat of water (4.18 Jg^{-1} °C), $\Delta T/\Delta t$ is the initial slope in the time versus (vs) temperature graphs and m_{Fe} is the mass fraction of the iron (in grams) in BDA/ABDA SPIOs. Moreover, intrinsic loss power (ILP in nHm^2/kg) is determined based on the normalization of SAR value as per equation (2). ILP is an equipment-independent parameter specifying the direct efficiency in the conversion between the electromagnetic and thermal energies of the ferrofluids.

$$ILP = SAR/(H^2 f) \quad (2)$$

Results and discussions

The crystalline phase (magnetite/Fe_3O_4), surface-modifications (BDA/ABDA), and magnetic saturation (Ms ~73-74 emu/g) are reported in our previous studies [**12,13**].

Herein, **Fig. 1(a)** and **Fig. 1(b)** respectively show the TEM micrographs of the BDA-SPIOs and ABDA-SPIOs, where the average particle sizes are measured to be 10 ± 4.0 nm and 10 ± 3.0 nm, respectively. Besides, the TEM sizes are in correspondence with the calculated XRD crystallite sizes – i.e., 8.5 and 7.7 nm of BDA-SPIOs and ABDA-SPIOs [**12,13**].

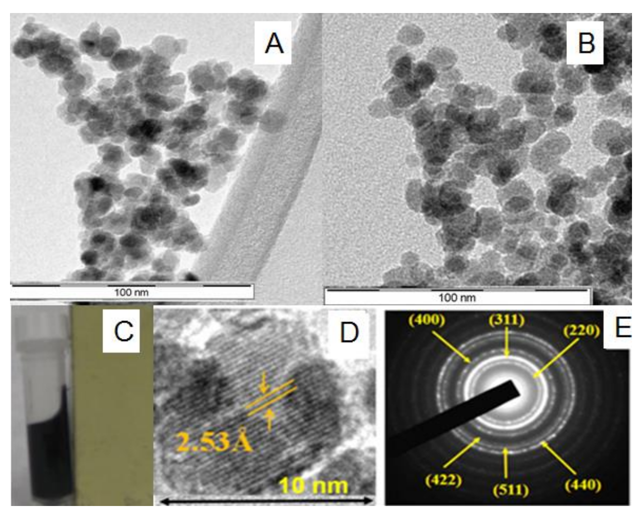


Fig. 1. TEM micrographs of (A) BDA-SPIOs & (B) ABDA-SPIOs respectively. Panel (C) shows the aqueous ferrofluid suspension of BDA-SPIOs under a static magnetic field. Panel (D) displays the TEM micrograph of single BDA–SPIOs (crystalline planes are highlighted). Panel (E) illustrates the SAED pattern of the BDA-SPIOs.

Moreover, **Fig. 1(c)** shows the flow of the BDA–SPIOs in DW under a static magnetic field indicating the stability of the aqueous ferrofluid suspension. This colloidal stability is confirmed by their zeta potential result i.e., +17.5 mV for BDA–SPIOs [**12,13**]. Besides, **Fig. 1(d)** shows a single BDA–SPIO (via TEM micrograph) with the lattice spacing of 2.53 Å, which confirms the Fe_3O_4 phase of the as-prepared SPIOs and the corresponding lattice fringes indicate their high crystalline nature. Furthermore, the selected area electron diffraction (SAED) pattern of the BDA–SPIOs has been displayed in **Fig. 1 (e)**, which exhibits the diffraction rings matching with their crystalline planes including (511), (422), (220), (440), (311) and (400). Similar single-particle TEM image/SAED pattern are observed for the ABDA-SPIOs.

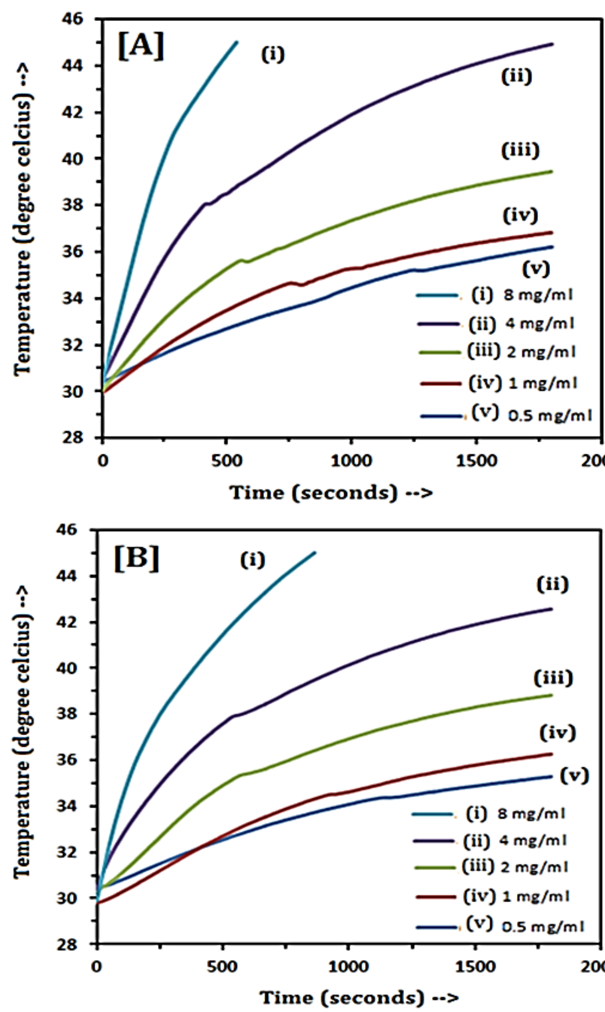


Fig. 2. (A) & 2 (B) Time vs temperature curves of BDA-SPIOs and ABDA-SPIOs respectively at concentrations of 0.5-8 mg/ml under an alternating magnetic field at a fixed frequency of 175.3 kHz.

Fig. 2(A) and **Fig. 2(B)** show the time-dependent temperature graphs for the BDA-SPIOs and ABDA-SPIOs, respectively when they are exposed to the AMF at a medically suited frequency of 175.3 kHz in CMH studies. It can be seen that the rate of temperature rise is amplified with the increase in concentrations from 0.5 to 8 mg/ml of the BDA-/ABDA-SPIOs. The temperature has reached at 45°C for both the BDA-SPIOs and ABDA-SPIOs in a period of 538.9 and 863.3 seconds at their 8 mg/ml concentration, respectively. It can be noted that ABDA-SPIOs have taken slightly longer time to reach 45 °C as compared to the BDA-SPIOs, which could be due to the hindrance to the Neel and Brownian relaxation mechanisms as induced by the extra $–NH_2$ groups from the surface attached ABDA molecules.

Besides, the calculated SAR and ILP values (as shown in **Table 1**) for the BDA-SPIOs and ABDA-SPIOs are relatively higher than the already reported SPIOs based heating agents [**14–16**]. This could be due to their (i) higher Ms/smaller sizes and (ii) π- electrons rich conjugation paths which enhanced the spin-transfers between the metallic SPIOs cores and the shells (BDA/ABDA). Thus, the as-prepared BDA-SPIOs and ABDA-SPIOs are found to be very promising heating-agents for MHPT applications.

Table 1. SAR and ILP values of BDA/ABDA ferrofluids at 175.3 kHz of an applied alternating magnetic field.

	BDA		ABDA	
Concentration	SAR (W/g_{Fe})	ILP (nHm^2/kg)	SAR (W/g_{Fe})	ILP (nHm^2/kg)
0.5	45.22	1.32	16.75	0.49
1	31.82	0.93	14.24	0.42
2	28.68	0.84	18.63	0.55
4	22.82	0.67	36.53	1.07
8	22.98	0.67	25.80	0.76

Conclusions

In summary, hydrophilic SPIOs are prepared via a simple chemical co-precipitation process. The as-prepared SPIOs consist of approximately 10 nm Fe_3O^4 core with π-electron rich organic shells (such as BDA/ABDA) that provide hydrophilicity to disperse the SPIOs into a stable aqueous ferrofluid suspension and also to avail surface functional groups ($-COOH/-NH_2$) for their further bio-conjugations. BDA-SPIOs/ABDA-SPIOs have demonstrated strong magnetic response and improved AMF based heating with high SAR/ILP values. Thus, it can be concluded that the as-synthesized BDA SPIOs/ABDA-SPIOs could be used as potential heating-agents for MHPT applications. In near future, the as-synthesized SPIOs might be tested as potential thermal agents in *in-vitro* and *in-vivo* MHPT applications. Moreover, the as-synthesized SPIOs might be utilized (i) as dual-mode contrast agents for use in magnetic resonance imaging by synthesizing these SPIOs in association with gadolinium-based nanoparticles, and also (ii) in combinatorial therapies (i.e., hyperthermia therapy + chemotherapy) by integrating these SPIOs with chemotherapeutic agents inside carrier vehicles such as niosomes.

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Keywords

Ferrofluids, biomedical applications, magnetic hyperthermia, superparamagnetic nanoparticles.

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References

1. Bakoglidis, K.D.; Simeonidis, K.; Sakellari, D.; Stefanou, G.; Angelakeris, M.; *IEEE Trans. Magn.*, **2012**, *48*, 1320.
2. Kandasamy, G.; Sudame, A.; Bhati, P.; Chakrabarty, A.; Maity, D.; *J. Mol. Liq.*, **2018**, *256*, 224.
3. Kandasamy, G.; Sudame, A.; Luthra, T.; Saini, K.; Maity, D.; *ACS Omega*, **2018**, *3*, 3991.
4. Kandasamy, G.; Soni, S.; Sushmita, K.; Veerapu, N.S.; Bose, S.; Maity, D.; *J. Mol. Liq.*, **2019**, *274*, 653.
5. Kandasamy, G.; Sudame, A.; Maity, D.; *Adv. Mater. Lett.*, **2017**, *8*, 873
6. Maity, D.; Chandrasekharan, P.; Pradhan, P.; Chuang, K.-H.; Xue, J.-M.; Feng, S.-S.; Ding, J.; *J. Mater. Chem.*, **2011**, *21*, 14717.
7. Krishnan, K.M.; *IEEE Trans. Magn.*, **2010**, *46*, 2523.
8. Kandasamy, G.; Sudame, A.; Maity, D.; *Adv. Mater. Lett.*, **2019**, *10*, 185.
9. Masud, M.K.; Na, J.; Younus, M.; Hossain, M.S.A.; Bando, Y.; Shiddiky, M.J.A.; Yamauchi, Y.; *Chem. Soc. Rev.*, **2019**, *48*, 5717.
10. Tanaka, S.; Kaneti, Y.V.; Septiani, N.L.W.; Dou, S.X.; Bando, Y.; Hossain, M.S.A.; Kim, J.; Yamauchi, Y.; *Small Methods*, **2019**, *3*, 1800512.
11. Du, Y.; Li, L.; Leung, C.W.; Lai, P.T.; Pong, P.W.T.; *IEEE Trans. Magn.*, **2014**, *50*, 1.
12. Kandasamy, G.; Surendran, S.; Chakrabarty, A.; Kale, S.N.; Maity, D.; *RSC Adv.*, **2016**, *6*, 99948.
13. Maity, D.; Zoppellaro, G.; Sedenkova, V.; Tucek, J.; Safarova, K.; Polakova, K.; Tomankova, K.; Diwoky, C.; Stollberger, R.; Machala, L.; Zboril, R.; *Chem. Commun.*, **2012**, *48*, 11398.
14. Maity, D.; Kandasamy, G.; Sudame, A.; Superparamagnetic Iron Oxide Nanoparticles (SPIONs) Based Magnetic Hyperthermia: A Promising Therapy in Cancer Treatment, in: Leon V. Berhardt (Ed.), Adv. Med. Biol., 117th Ed., Nova Science Publishers, **2017**, pp. 99–160.
15. Kandasamy, G.; Maity, D.; *Int. J. Pharm.*, **2015**, *496*, 191.
16. Subramanian, M.; Pearce, G.; Guldu, O.K.; Tekin, V.; Miaskowski, A.; Aras, O.; Unak, P.; *IEEE Trans. Nanobioscience*, **2016**, *15*, 517.