ATA and TA coated superparamagnetic iron oxide nanoparticles: Promising candidates for magnetic hyperthermia therapy

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Received: 24 March 2017, Revised: 29 March 2017 and Accepted: 09 April 2017

DOI: 10.5185/amlett.2017.1730
www.vbripress.com/aml

Abstract

In recent times, superparamagnetic iron oxide nanoparticles (SPIONs) are widely used as heating agents in magnetic hyperthermia therapy (MHT) to kill malignant cells in cancer treatments, which is mainly due to their excellent magnetic properties and biocompatibility. However, it is still a challenge to coat SPIONs with suitable surfactants and to apply an appropriate alternative magnetic field (AMF) at specific frequency to achieve enhanced heating effects in MHT. In this work, the as-synthesized novel short-chain surfactants (i.e., amino-terephthalic acid (ATA) and terephthalic acid (TA)) coated hydrophilic SPIONs are synthesized and subsequently involved in calorimetric hyperthermia studies to investigate their intrinsic heating capability by varying (i) their concentrations from 1 - 8 mg_{Fe}/ml and (ii) AMFs at different frequencies (263.2 – 752.39 kHz) while achieving the temperature above 42 °C – therapeutic hyperthermia temperature. It is found that the heating rate of TA-SPIONs is faster as compared to ATA-SPIONs on exposure to the AMF. However, the highest specific absorption rate (SAR) value of 129.80 W/g_{Fe} is attained for ATA-SPIONs with 2 mg_{Fe}/ml concentration on exposure to AMF at 752.39 kHz. Thus, ATA/TA coated SPIONs are very promising agents for magnetic hyperthermia and could be further investigated in *in vitro*/*in vivo* cancer treatments. Copyright © 2017 VBRI Press.

Keywords: Terephthalic acid, amino-terephthalic acid, hydrophilic SPIONs, magnetic hyperthermia, specific absorption rate.

Introduction

Recently, superparamagnetic iron oxide nanoparticles (SPIONs - Fe_3O_4 and Fe_2O_3 nanoparticles) are significantly involved in various biomedical applications such as magnetic targeting, magnetic resonance imaging (MRI) and especially magnetic hyperthermia therapy (MHT) [**1–5**]. This is mainly because of their exceptional size/shape dependent superparamagnetism and biocompatibility. However, the magnetic properties of the SPIONs, particularly saturation magnetization values, can be improved by modifying their synthesis conditions and surface coatings, which may enhance their employment in the above-mentioned applications [**6–10**].

In many research investigations, SPIONs are commonly synthesized using the conventional aqueous chemical co-precipitation approach by utilizing the surfactants such as citric acid, maleic acid, oleic acid, mannose, lactose, maltose, galactose, silica, poly(ethylene glycol), poly(vinyl pyrrolidone) and so on [**11**, **12**]. But, there are the risks of reduction in the magnetization values and colloidal stability of the SPIONs due to the usage of these long-chained nonmagnetic surface coatings. Consequently, the heating effect in MHT - which is measured in terms of specific absorption rate (SAR) value – is also decreased. Therefore, there is still a need for preserving the magnetic properties and water stability of the hydrophilic SPIONs to enhance their heating effects/SAR values for subsequent MHT applications by choosing/applying suitable surface coatings and alternative magnetic field (AMF) at specific frequencies.

Very recently, our group has reported one-pot facile synthesis of stable hydrophilic SPIONs via co-precipitation process by using short-chained molecules such as amino-terephthalic acid (ATA) terephthalic acid (TA) as surface coatings [**13**]. This resulted in ATA- and TA- coated SPIONs with high saturation magnetization values (73.6 and 74.3 emu/g) and MRI relaxivity values (450.8 and 735.3 1/(mM.s)) respectively. In the present work, the interest arises from the possibility of investigating the calorimetric heating capabilities of ATA- and TA- coated SPIONs by varying (i) their concentrations in the range of 1 - 8 mg_{Fe}/ml and (ii) AMFs at frequencies in the range of 263 – 752 kHz. Moreover, the respective specific absorption rate (SAR) values are calculated by considering the initial slope of the heating graphs (temperature vs time) to determine an effective concentration of those SPIONs to efficiently involve them in further *in vitro/in vivo* MHT studies.

Experimental

Materials

Iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$), TA ($C_6H_4(CO2H)_2$), and ATA ($H_2NC_6H_3$-1,4-$(CO2H)_2$) are purchased from Sigma Aldrich. Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) and ammonium hydroxide (NH4OH – 25% inH_2O) are obtained from Loba chemicals and Fisher Scientific respectively.

Material synthesis

Hydrophilic SPIONs are synthesized by chemical co-precipitation as reported elsewhere [**13**]. For ATA-SPIONs, 1.17 g of iron (III) chloride hexahydrate, 0.43 g iron (II) chloride tetrahydrate, 0.82 g of ATA and 22.5 ml of distilled water are added sequentially to a round bottom flask. Then, the above mixture is magnetically stirred and heated to 80 °C for 1h under the flow of nitrogen gas. Further, 2.5 ml of NH_4OH is rapidly added to the reaction mixture, and vigorously stirred for next 1h by maintaining the same temperature. Then, the resultant black SPIONs solution is cooled down to room temperature, magnetically separated and washed 3 times with ethanol/distilled water. Finally, one half of the washed particles is re-dispersed in distilled water to get ferro-fluid samples and the other half is dried overnight in a hot air oven at 40 °C to obtain dry powder of ATA-coated SPIONs for subsequent characterizations. Similarly, TA-SPIONs are synthesized and characterized.

Characterizations

ATA- and TA- SPIONs are characterized for morphology (size/shape) by using TEM, for phase purity by using XRD, for surface coatings by using FTIR and thermogravimetric analysis (TGA) and for saturation magnetization (M_s) by using superconducting quantum interference device (SQUID).

Calorimetric hyperthermia study

The time-dependent calorimetric heating efficacy of the as-synthesized ATA- and TA- SPIONs is determined by using a magnetic hyperthermia machine (magneTherm – nanoTherics, United Kingdom). 1 ml of the water dispersed SPIONs (with the concentrations 1-8 mg_{Fe}/ml) is subjected to the alternating magnetic fields (AMFs) at four frequencies (such as 263.2, 330.28, 634 and 752.39 kHz). Consequently, the time-dependent temperature rise is monitored by using an optical probe. Moreover, the SAR values are calculated using the following equation (1) [**14**].

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

where, C is the specific heat of solvent (here, C_{water} = 4.18 J g^{-1} °C), $\Delta T/\Delta t$ is the initial slope of the time-dependent temperature curve and m_{Fe} is the weight fraction of the magnetic element (i.e. Fe) in the samples.

Results and discussion

Structure and morphology

The phase purity and surface coatings of ATA- and TA-SPIONs are characterized by using XRD and FTIR/TGA as reported elsewhere [**13**]. It has been found out that the as-synthesized SPIONs demonstrated Fe_3O_4 phase as per the XRD results. Moreover, the FTIR spectra and TGA curves have confirmed that the surfaces of the SPIONs are adsorbed with the corresponding surface coating (ATA/TA) molecules based on (i) the cluster of absorption peaks in the range of 1400–1600 cm^{-1} corresponding to the symmetric/asymmetric vibrations of amino and/or carboxylic groups associated to the ATA/TA molecules and (ii) secondary weight losses in the temperature range of 200–800 °C due to the decomposition of the respective coatings of the SPIONs. **Fig. 1(a)** and **Fig. 1(b)** show the TEM images of the ATA-SPIONs and TA-SPIONs respectively. The average particle sizes are determined to be 10 ± 2.0 and 10 ± 3.0 nm for ATA- and TA-SPIONs correspondingly. It can also be noted that both of the SPIONs are spherical in shape.

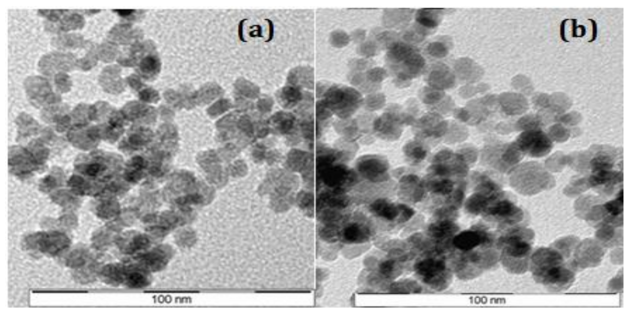

Fig. 1. TEM images of (a) ATA-SPIONs and (b) TA-SPIONs.

Magnetic properties

Fig. 2 displays the room temperature magnetization-magnetic field (M-H) curves of the (A) ATA-SPIONs and (B) TA-SPIONs respectively. It can be noted that the saturation magnetization (M_s) values of the ATA- and TA-coated SPIONs are measured as 73.4 and 74.3 emu/g respectively [**13**].

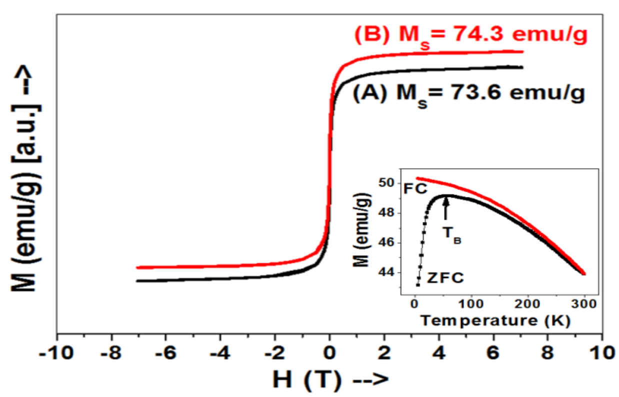

Fig. 2. Room temperature (T=300 K) magnetization-applied magnetic field (M-H) curves of (A) ATA-SPIONs and (B) TA-SPIONs. Inset show zero-field cooled and field cooled (ZFC and FC) magnetization curve of TA-SPIONs recorded in an applied magnetic field (H) of 1 kOe.

Here, the M_s values of both of the SPIONs are lower than that of bulk magnetite (i.e. 92 emu/g), which could be due to (i) their smaller size and (ii) the surface attached non-magnetic coatings (ATA/TA) [**15**].

Also, the zero or negligible coercivity and residual magnetization of the M-H curves indicate that the SPIONs are superparamagnetic in nature at room temperature. This has been further confirmed by the temperature-dependence magnetization of TA-SPIONs (refer inset in **Fig. 2**) as measured by SQUID under an applied field of 1 kOe. Similar results can be obtained for ATA-SPIONs also.

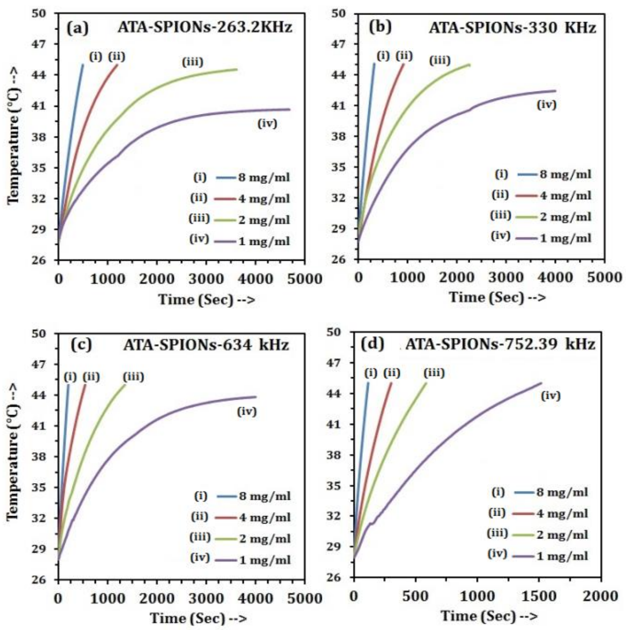

Fig. 3. (a-d). Time-dependent temperature rise of 1 ml aqueous suspension of ATA-SPIONs with different iron concentrations ((i) 8, (ii) 4, (iii) 2 and (iv) 1 mg_{Fe}/ml) on exposure to AMF at frequency of (a) 263.2 kHz, (b) 330 kHz, (c) 634 kHz and (d) 752.39 kHz respectively.

Calorimetric hyperthermia study

Fig. 3 (a-d) and **Fig. 4 (a-d)** show the time-dependent temperature rise of 1 ml aqueous suspensions of ATA-SPIONs and TA-SPIONs, respectively on exposure to AMFs at four different frequencies in the range of 263 – 752 kHz. It can be seen that the temperature is increased with the increase in concentrations (1-8 mg_{Fe}/ml) of the ATA-/TA- coated SPIONs. This heating effect could be due to Neel and Brownian losses, which arise from the rotation of magnetization vector and the physical rotation of the ATA-/TA- coated SPIONs, respectively [**16–18**].

Herein, the time taken by the ATA- and TA- coated SPIONs to reach 42 °C is considered since it is the temperature that can lead to lethal hyperthermia in *in vitro/in vivo* scenarios to cause cell death by apoptosis. For instance, the ATA-coated SPIONs (at 8 mg $_{Fe}$/ml concentration) have taken approximately 6.3, 4.2, 2.6 and 1.49 mins to reach the hyperthermia temperature at the respective frequencies of 263.2 kHz, 330 kHz, 634 kHz and 752.39 kHz. However, the TA-coated SPIONs (at 8 mg_{Fe}/ml concentration) have taken only 2.6, 2.4, 2.2 and 1.06 mins at corresponding frequencies.

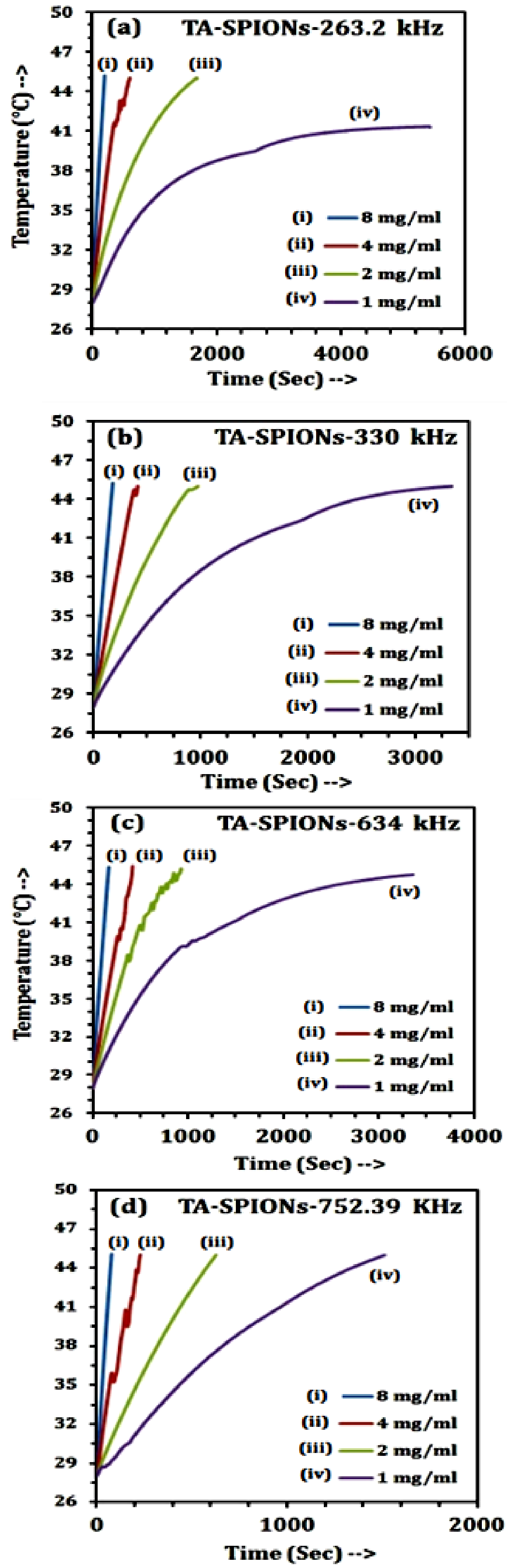

Fig. 4. (a-d). Time-dependent temperature rise of 1 ml aqueous suspension of TA-SPIONs with different iron concentrations ((i) 8, (ii) 4, (iii) 2 and (iv) 1 mg $_{Fe}$/ml) on exposure to AMF at frequencies of (a) 263.2 kHz, (b) 330 kHz, (c) 634 kHz and (d) 752.39 kHz respectively.

Similarly, the TA-SPIONs have taken less time to reach 42 °C in other concentrations as well (e.g., 4 and 2

mg_{Fe}/ml) when compared to ATA-SPIONs (data not shown). The reason for reaching the hyperthermia temperature at a faster rate could be attributed to higher magnetization and smaller size of the TA-SPIONs than the ATA-SPIONs as confirmed by SQUID/TEM analyses.

Table 1. SAR values of ATA- and TA-SPIONs.

SAR Values (W/g_{Fe}) at 2 mg_{Fe}/ml Concentration		
Frequency (kHz)	ATA SPIONs	TA SPIONs
263.2	46.06	62.81
330	64.90	77.46
634	90.02	81.65
752.39	129.80	94.21

Besides, the SAR values of ATA- and TA-SPIONs are also calculated at 2 mg_{Fe}/ml concentration (i.e., the least concentration to reach 42 °C at all the frequencies) by considering the initial slope from the respective time-temperature graphs as per equation (1). The SAR values of ATA- and TA- SPIONs are in the range of 46.06-129.80 W/g_{Fe} and 62.81-94.21 W/g_{Fe} respectively for the frequency range of 263.2 - 752.39 kHz **(**refer **Table 1)**. Moreover, the SAR value of the ATA- and TA- SPIONs increases linearly with the increase in the frequency (as per **Fig. 5 (a)** and **Fig. 5(b))**. It can be observed that the SAR value of ATA and TA SPIONs are comparable to each other at different frequencies. Initially, the ATA-SPIONs have lower SAR values for AMF at 263.2 and 330 kHz frequencies as compared to TA-SPIONs. However, their SAR values have dramatically increased for the ATA-SPIONs at higher frequencies (i.e. 634 and 752.39 kHz). This could be due to the additional amino group of ATA molecules which hinder the physical rotation and thus eventually leads to the higher Brownian losses for the ATA-coated SPIONs as compared to the TA-SPIONs.

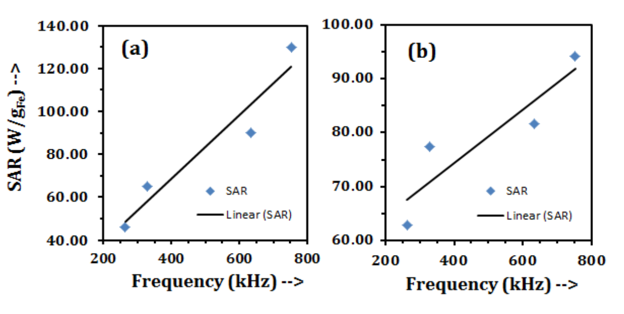

Fig. 5. Frequency dependent SAR values of 1 ml sample with 2 mg_{Fe}/ml iron concentration of (a) ATA SPIONs and (b) TA-SPIONs.

Nevertheless, the SAR values of ATA- and TA- SPIONs are higher as compared to the reported values in the literatures [**19–21**]. Therefore, the as-synthesized magnetite nanoparticles (i.e., ATA- and TA- SPIONs) are potential candidates to be used in *in vitro*/*in vivo* cancer hyperthermia treatments.

Conclusion

ATA- and TA- SPIONs are successfully synthesized via chemical co-precipitation method and studied for their calorimetric heating efficacy through magnetic hyperthermia studies. The heating rate of TA-SPIONs is faster as compared to ATA-SPIONs on exposure to AMF at different frequencies. However, the highest SAR value of 129.80 W/g_{Fe} is attained by ATA-SPIONs at 752.39 kHz for 2 mg_{Fe}/ml concentration, regardless of their lower SAR values at lower frequencies. Nevertheless, the SAR values of both ATA- and TA- SPIONs increased with the increase in AMF frequency. Thus, the optimal frequencies for ATA-/TA- coated SPIONs can potentially be chosen for further involving them in *in vitro*/*in vivo* cancer hyperthermia treatments.

Acknowledgements

The financial support from the Department of Science and Technology (DST) under Nanomission, New Delhi, India (Grant No. SR/NM/NS-1141/2015 (G)) and Shiv Nadar University is gratefully acknowledged.

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