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Systematic investigations on heating effects of carboxyl-amine functionalized superparamagnetic iron oxide nanoparticles (SPIONs) based ferrofluids for in vitro cancer hyperthermia therapy

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ABSTRACT

Here, we have studied the colloidal properties of carboxyl-amine functionalized superparamagnetic iron oxide nanoparticles (SPIONs with high saturation magnetization) based ferrofluids and their heating efficacies in magnetic fluid hyperthermia (MFH) via specific absorption rate (SAR)/intrinsic loss power (ILP). Moreover, we have systematically investigated the impact of the following heat influencing factors in MFH: (i) concentrations (0.5–8 mg/ml), (ii) surface coatings (trimesic acid (TMA), pyromellitic acid (PMA), terephthalic acid (TA) and aminoterephthalic acid (ATA)), (iii) applied alternating magnetic fields (AMFs - with amplitudes (H)/frequencies (f)) - chosen near to Hergt's biological safety limit, and (iv) dispersion media (biological/non-biological) for using SPIONs in in vitro cancer hyperthermia therapy. SPIONs (particularly decorated with dual-surfactants, i.e., TA-ATA) based aqueous ferrofluids have displayed excellent time-dependent temperature rise even at lower concentrations for the applied AMFs, which resulted in enhanced SAR values ranging from 12.5–200.1 W/g_{Fe} because of their high colloidal stability and enhanced π-π conjugations from the close structural orientations of TA/ATA molecules due to high electrostatic attractions of the respective functional groups (-$COOH/NH_2$). Moreover, high ILP values of up-to 3.9 nHm^2/kg (higher than the best commercial ferrofluids) are attained on exposure to magnetic fields below the safety limit. Besides, TA-ATA coated SPIONs dispersed in biological/non-biological media have exhibited better thermal responses as compared to their aqueous counterpart and reached therapeutic temperatures at faster rates due to prominent Neel relaxation mechanisms. The highest SAR value of 276.3 W/g_{Fe} is recorded for TA-ATA coated SPIONs dispersed in triethylene glycol (TEG - with high viscosity), ascribed to the lesser inter-particle interactions from electrostatic repulsions of negative charges among carboxyl/oxygen molecules from SPIONs/TEG respectively. Moreover, TA-ATA coated SPIONs have induced almost 90% cell death in MCF-7 cancer cells in in vitro MFH studies. Thus, the TA-ATA coated SPIONs based ferrofluids have great potential for in vivo/clinical MFH cancer therapies.

1. Introduction

Ferrofluids are stable colloidal suspensions of ultrafine magnetic nanoparticles - superparamagnetic iron oxide nanoparticles (SPIONs – especially magnetite (Fe_3O_4)/meghemite (Fe_2O_3)) - in a carrier liquid, where SPIONs are single domain structures without any domain walls. Ferrofluids are widely employed in bio-medical applications including magnetofection [1–3], drug delivery [4,5], magnetic targeting [6,7], and magnetic resonance imaging (MRI) [8–10] because of their size dependent superparamagnetism, better chemical stability and/or lesser cellular-toxicity. In recent times, SPIONs based ferrofluids are extensively utilized for treating cancers via magnetic fluid hyperthermia (MFH) therapy since they can produce heat (~42–45 °C) locally inside the tumors on exposure to the externally applied alternating magnetic fields (AMFs – with specific amplitudes, marked as H) at definite frequencies (marked as f) [11–16]. The generated heat might be useful in inducing death in cancer cells by bringing alterations in their membranes/nucleic-acids/proteins [17,18]. Moreover, MFH based clinical therapeutics are already being performed for treating prostate cancers/glioblastoma-multiforme in humans [19].

The heating capacities of the ferrofluids are usually quantified via specific absorption rate (SAR - W/g_{Fe} - i.e., heat/power dissipation per unit mass of the SPIONs) [20]. In addition, Kallumadil et al. introduced another parameter known as intrinsic loss power (ILP - nHm^2/kg - a normalized SAR value) which is an equipment independent parameter that directly specifies the efficiency of the ferrofluids in converting the applied electromagnetic energy to thermal energy [21]. Generally,

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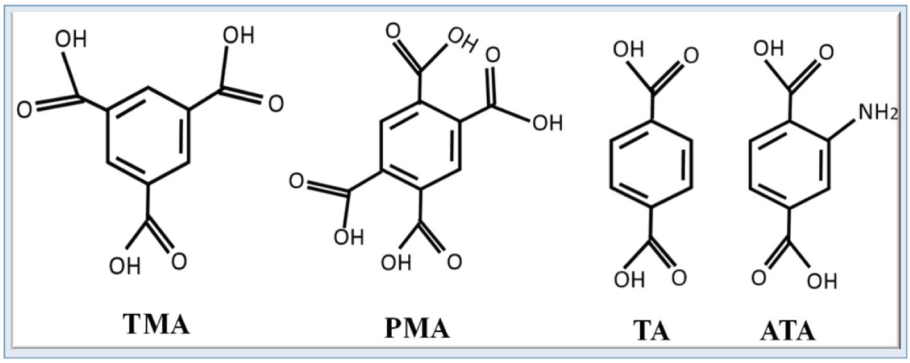

Scheme 1. Molecular structures of trimesic acid (TMA), pyromellitic acid (PMA), terephthalic acid (TA) and 2-aminoterephthalic acid (ATA).

these heating capacities depend upon the (i) physicochemical properties such as size, shape, and phase composition, and (ii) magnetic properties, i.e., saturation magnetization and magnetic anisotropy of the SPIONs, which are broadly investigated in many research works [22–29]. Conversely, very less works are focused on the impact of the other significant factors including (i) concentrations, (ii) surface coatings, (iii) applied magnetic fields (H/f), and (iv) dispersion media of the SPIONs, on the heat generating efficiency of the ferrofluids [30–35]. However, these reported works also lack in complete investigation of the effects of all the above-mentioned influencing parameters in the heat induction process in ferrofluids (lab-made and commercial), which make them unsuitable for further biological MFH applications. Therefore, more crucial investigations are needed to understand the impact of these important factors on the thermal responses of SPIONs based ferrofluids to attain enhanced heating efficacies in suitable medium and magnetic fields, while utilizing lower concentrations for clinical circumstances.

Recently, our group has reported one-pot facile synthesis of the hydrophilic SPIONs with high saturation magnetization (Ms) and better cytocompatibility in MCF-7 cancer cells, where the SPIONs are in situ surface functionalized using the carboxyl-amine based novel short-chained surface coatings such as trimesic acid (TMA), pyromellitic acid (PMA), 2-aminoterephthalic acid (ATA) and terephthalic acid (TA) [36,37]. In this paper, we have systematically investigated the impact of the key factors such as concentrations, surface coatings, AMF (H/f), and dispersion media on the intrinsic heating effects of the SPIONs (based ferrofluids) that are synthesized with single/combination of the surface coating molecules (TMA/PMA/TA/ATA) via chemical co-precipitation method, whereas the H/f combination (i.e., known as C = =H*f) is selected near to Hergt's biological safety limit (5×10^9 kAm^{-1} s^{-1}). Later, based on the calorimetric MFH results, ferrofluids with high heating efficacy have been chosen and investigated for their killing efficiency in MCF-7 cancer cells at therapeutic temperature of 42 °C in a concentration-dependent manner (0.5 and 1 mg/ml) so that they could be further employed as effective nanomedicines in in vivo and clinical MFH applications.

Table 1
Comparison of TEM size, saturation magnetization (Ms), hydrodynamic diameter and zeta potential of the SPIONs dispersed in the aqueous media (F1, F2, F3, F4, F5 and F6).

Code	Surface coatings	TEM size (nm)[a]	Ms (emu/g)[a]	Mean hydrodynamic diameter (nm)[b]	Mean zeta potential (mV)[b]
F1	TMA	7 ± 3	58.2	186.2	−31.5
F2	TMA-ATA	9 ± 2	61.8	180.0	−14.5
F3	PMA	8 ± 3	62.2	303.2	−50.2
F4	PMA-ATA	8 ± 2	63.2	199.8	−32.5
F5	ATA	10 ± 3	73.6	137.0	−47.3
F6	TA-ATA	9 ± 3	66.3	145.6	−34.5

[a] Reported in our previous work.
[b] Measured in this work.

2. Materials and methods

2.1. Materials

Iron (III) chloride, iron (II) chloride, 2-aminoterephthalic acid (ATA), and terephthalic acid (TA) are purchased from Sigma Aldrich. Trimesic acid (TMA) and pyromellitic acid (PMA) are purchased from Alfa Aeser. 0.1 M potassium thiocyanate (KSCN), ethylene glycol (EG), diethylene glycol (DEG), triethylene glycol (TEG), ammonium persulfate (APS), NH_4OH and ethanol are obtained from Fisher Scientific. Phosphate buffer saline (PBS), Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) are obtained from Gibco Life Technologies.

2.2. Synthesis and characterization of SPIONs

SPIONs with different surface coatings (single/combination - TMA/PMA/TA/ATA) are synthesized via chemical co-precipitation method as reported elsewhere [36,37]. Briefly, for TMA coated SPIONs, 2:1 ratio of iron (III) chloride and iron (II) chloride are mixed in distilled water (DW, Millipore) along with appropriate amount of TMA and heated to 80 °C under continuous N_2 gas flow with magnetic stirring. Then NH_4OH is added to the above mixture and vigorously stirred for 60 minutes (min). At last, the resultant black solution is cooled down to room temperature, magnetically-separated and washed with DW/ethanol for 2–3 times. The washed TMA-coated SPIONs (marked as S1) are re-dispersed in DW to obtain an aqueous ferrofluid (marked as F1). In similar fashion, TMA-ATA, PMA, PMA-ATA, ATA and TA-ATA coated SPIONs (marked as S2, S3, S4, S5, and S6 respectively) are synthesized, washed and re-dispersed in DW to get respective aqueous ferrofluids of F2, F3, F4, F5, and F6, where ATA is used in equal molar ratios with TMA/PMA/TA to synthesize SPIONs with combined surface coatings. Then, hydrodynamic sizes/zeta potentials of the aqueous ferrofluids are characterized using dynamic light scattering (DLS, Horiba nanoPartica SZ-100-Z) to determine their colloidal stability.

2.3. Determination of iron concentration using spectrophotometry

Ferrofluids are analysed for the concentration of the magnetic element (iron - Fe) by using KSCN via UV–vis spectrophotometry [38]. Briefly, 20 μl of as-prepared ferrofluids are added to 30 μl of HCl solution and incubated at 70–80 °C for 2 h. Then, the mixture is cooled down to room temperature and added with 100 μl of 1 weight-percent (wt%) of APS. Then, 0.1 M KSCN is added to above mixture and UV–vis spectroscopy is taken by measuring the absorbance at 474 nm.