Chapter 4

Studies On Surface Functionalization And Colloidal Stability Of PVP Coated LSMO Nanoparticles

The part of this chapter has published as a research articles:

**Studies on colloidal stability of PVP-coated LSMO nanoparticles for magnetic fluid hyperthermia**

4.1 Introduction

The revolution in nanotechnology has brought us new tools not only in analytical systems, but also for human applications. One of these new tools of great interest for researchers is MNPs [1]. Because of their ultrafine size, biocompatibility and SPM properties, these MNPs are already approved for various biomedical applications such as MFH [2], MRI [3], drug delivery [4-5], fluorescent biological labels [6-8] and all these applications of MNPs arise from the combination of their magnetic properties with biological phenomenon. For the application of MNPs in biomedicine, they should form stable suspension in physiological media like water or phosphate buffer saline (PBS). In order to enhance the colloidal stability, biocompatibility and reduce toxicity, these NPs are often encapsulated with suitable biocompatible polymers.

Among various MNPs, until last decades magnetite Fe$_3$O$_4$ and maghemite γ-Fe$_2$O$_3$ have been mostly preferred for the MFH application because of their inherent biocompatibility, their easy synthesis in the form of stable aqueous magnetic fluids and their parallel development as contrast agents in MRI. Thus, the majority of in vitro and in vivo tests on animals, and until very recently on humans, have occurred with heat mediators based on magnetite or maghemite cores. However, these nanomaterials do not exhibit self-control of the in vivo temperature rise after induction heating (as their $T_C$ are very high above 500°C), with their ramp-up time being typically $\sim$10–15 minutes. This is not only because of the heat conduction and energy adsorption in vivo are insufficiently known but also local overheating may damage healthy tissue [9], as these particles retained their magnetic properties even at high temperature. Even though substituted iron oxides have their $T_C$ above 100°C, simultaneously their magnetization value decreases significantly because of the high amount of substitution [10]. In order to overcome this difficulty, ferromagnetic perovskite doped transition metal
oxide LSMO has attracted great attention in biomedicine. This is mainly due to controllable Tc (by Sr\textsuperscript{2+} doping) in between 283-380 K and large magnetic moment at room temperature.

Researchers have used several coating materials on LSMO MNPs for improving the colloidal stability and biocompatibility of LSMO MNPs, including SiO\textsubscript{2} [11], dextran [12], citrate ligands [13], and fatty amine [14]. However, very few reports are available in literature on LSMO NPs coated with amphiphilic polymer PVP for biomedical applications. Excellent features of PVP required for biomedical applications are explained in chapter 2. In present chapter, LSMO MNPs have been synthesized by combustion method and functionalized with PVP polymer. The main objective of this work is to study the functionalization of the NPs to enhance the colloidal stability for hyperthermia therapy along with the structural, morphological and magnetic characteristics. Uncoated NPs are abbreviated as S1 and coated NPs are abbreviated as S2 throughout the chapter.

4.2 Synthesis

4.2.1 Synthesis of LSMO NPs

The strontium doped perovskite LSMO NPs were synthesized by solution combustion method by using PVA as a fuel. Detailed synthesis method of LSMO is described in chapter 3.

4.2.2 Coating of LSMO NPs with PVP

Core LSMO NPs were encapsulated with PVP as a capping agent. Briefly, 150 mg of LSMO NPs were suspended in DDW under ultrasonication treatment for 20 min to obtain a good dispersion. 5% (w/v) PVP solution was prepared in DDW under constant stirring for 15 min. To tailor the surface of NPs, the PVP solution was slowly poured into a suspension of LSMO NPs under vigorous mechanical stirring at 100 ℃ for 2h to enable sufficient
interaction between LSMO and PVP. This step has been included especially to prevent clustering of the MNPs by ferromagnetic contact interaction as well as to enhance the dispersion stability of NPs in liquid media. After that, the mixture was centrifuged and washed several times with DDW, ethanol and acetone, respectively, to remove excess unreacted chemicals. After purification, NPs were dried naturally and are subjected to characterizations such as XRD, FE-SEM, FTIR spectroscopy etc.

4.2.3. Characterizations of MNPs

The phase composition, lattice parameter and the mean size of the crystallites were determined by XRD (RIGAKU Miniflex 600) equipped with a crystal monochromator employing Cu-K$_{\alpha}$ radiation of wavelength 1.54 Å and applied scanning rate of 3° min$^{-1}$, ranged from 20 to 80°. The patterns were analysed by X'Pert High score software and compared with standard JCPDS (reference code: 00-051-0409). The average crystallite size was calculated from the broadening of the XRD peaks using the Scherrer’s equation (3.5). Elemental analyses were performed by ICP/OES (720ES Varian). The powder (5 mg) was dissolved in concentrated hydrochloric acid (3 mL) and DDW was added to get 50 mL of solution. The oxidation state of manganese was determined by standard Mohr’s salt titration. Surface analyses were performed with XPS. XPS measurements were carried out with X-ray photoelectron spectrometer model (VG Multilab 2000-Thermo Scientific, USA, K-Alpha) with a multi-channel detector having high photonic energies from 0.1 to 3 keV. Surface morphology was recorded by FE-SEM (Model: Carl Zeiss Ultra 55) for coated and uncoated samples. FT-IR spectroscopy (Perkin Elmer spectrometer model no. 783, USA) analysis was used to estimate chemical bonding and the interaction of PVP with LSMO NPs. The shape, size and uniformity of the NPs were measured by TEM and high resolution TEM (HR-TEM) with TECNAI F20 Philips operated at 200 KV.
The average hydrodynamic diameter was measured (DLS) and Zeta potential were measured by using NICOMP™ 380 ZLS (Particle sizing system, Santa Barbara, CA) in acidic, neutral and alkaline solution at a scattering angle of 90° at 25 °C with He-Ne laser of wavelength 632.8 nm. Magnetic measurements were done by using a VSM of Lakeshore, model -7410.

4.3 Results and Discussion

4.3.1 XRD Study

![Fig. 4.1 XRD patterns of uncoated (S1) and PVP coated (S2) LSMO NPs.](image)

Fig. 4.1 shows XRD patterns of uncoated (S1) and PVP coated (S2) LSMO NPs. It shows formation of phase and purity without any noticeable trace of impurities. The XRD patterns showed pseudo-cubic perovskite structure. All peaks are fully indexed in R-3c space group. The lattice parameters $a =$
5.5307 Å and c = 13.394 Å are determined. The Gaussian fit of the most intense peak (110) was used to calculate the FWHM for determination of crystallite size ($D$) by Scherrer’s formula. The average crystallite size calculated by the equation (3.5 from chapter 3) is found to be 21 and 20 nm for uncoated and coated NPs, respectively, suggesting the formation of crystallites of nanosize. There is no pronounced change in the lattice constant however crystallite size varies slightly after surface coating because the coating does not influence the crystal structure of LSMO [15]. In addition, the peaks appeared broad with slight decrease in intensity after coating with PVP (Fig. 4.1(S2)). The decrease in signal to noise ratio is due to effect of PVP which affects the surface of MNPs and induces internal strain on MNPs which readily decreases the intensity of the peaks after coating.

4.3.2 Elemental analysis

4.3.2.1 ICP-OES

ICP-OES study was performed to investigate the internalization of the as synthesized sample. The LSMO particles obtained after calcinations set for ICP-OES analysis confirmed unchanged cationic ratios. The real composition of LSMO (La/Sr/Mn) was (0.700: 0.300: 1). The result of the chemical analysis obtained by ICP-OES for the as synthesized samples shows the La/Sr/Mn concentration in (mmol/L) as (0.0357: 0.0151: 0.0502). The result evidenced the same stoichiometry for La, Sr, and Mn in the sample as expected. In earlier research work, Epherre et al. [16] studied evidence of non-stoichiometric effects in nanometric LSMO perovskites. Mohr’s salt titration was performed on uncoated particles and the deduced oxidation state of manganese was found to be Mn$^{3.37+}$ (37% Mn$^{4+}$). It was then clear that the oxidation state of manganese deviated from the theoretical one (Mn$^{3.18+}$ considering that the material is perfectly stoichiometric both on the cationic and anionic sites) [9].
4.3.2.2 XPS

Fig. 4.2 The survey XPS spectra of slow scan of each metal ion of LSMO NPs

Fig. 4.2 shows XPS spectrum of LSMO in the binding energy range of 0-1100 eV and slow scan of separate metal ions. Only La, Sr, Mn and O elements are observed on the sample surface and no other impurity element was detected in the spectrum calibrated by the binding of C 1s (284.60 eV) (Fig. 4.2 (d)). According to the Gauss fitting-curves, the binding energies of La 3d_{5/2} are between 835.14 and 837.29 eV, and the binding energies of La 3d_{3/2} are between 852.39 and 854.83 eV, confirming the existence of La^{3+} ions (Fig. 4.2 (a)). Spectrum contains the Sr 3d a doublet, whose binding energies are 132.8 and 134.6 eV, which can be assigned as Sr 3d_{5/2} and Sr 3d_{3/2} lines, respectively (Fig. 4.2 (b)). Their binding energies are very close to the similar compounds [17] which can be contributed to the Sr^{2+} ions in LSMO.
Fig. 4.2 (c) illustrates the fine peaks of Mn 2p core levels. The interpretation of the Mn 2p spectrum is complicated due to the multiple splitting of the Mn 2p spectra of Mn$^{4+}$, Mn$^{3+}$, and Mn$^{2+}$ ions [17]. But according to the observed binding energies, 643.3 and 652.23 eV for Mn 2p$_{3/2}$ and Mn 2p$_{1/2}$ respectively, which indicate the oxidation state of Mn ions is 4+ in present LSMO sample. One main emission line was detected of the O 1s core level having binding energy 531.02 eV (Fig. 4.2 (e)).

4.3.3 FT-IR analysis

![FT-IR spectra of uncoated (S1), PVP coated (S2) LSMO NPs.](image)

The presence of PVP can be illustrated by means of FT-IR analysis. Fig. 4.3 shows FT-IR spectra for PVP coated LSMO (S2) and uncoated LSMO (S1) NPs. Uncoated NPs show characteristics absorption peaks at 600...
cm\(^{-1}\). This suggests that uncoated LSMO strongly contain the metal oxygen bonds which involve the internal motion of a change in Mn-O-Mn bond length. The stretching mode associated with internal motion of change in Mn-O-Mn bond length and the bending mode associated with the change of Mn-O-Mn bond angle [18]. The peaks at 1630 cm\(^{-1}\) and 3440 cm\(^{-1}\) are because of the deformation mode of adsorbed molecular water and O-H stretching of the same respectively [19].

Fig. 4.3 (S2) shows characteristic peaks at 1290 cm\(^{-1}\) and 1666 cm\(^{-1}\) attributed to C-N linkage in which bands couple with the stretching of adjacent bands and C=O due to the interaction of carbonyl group and LSMO NPs [20-21]. Also, the spectrum of coated sample consists of the bands at 2964 cm\(^{-1}\) and 1455 cm\(^{-1}\), bands corresponding to -CH\(_2\) stretching and asymmetric stretching vibrations of -CH\(_2\) groups, respectively. The PVP coated sample had only a little difference compared with pure PVP, which revealed that the interaction between PVP and MNPs were intermolecular interactions [22-24]. All these results confirm that surface of LSMO NPs was successfully attached with PVP.

4.3.4 FE-SEM study

Fig. 4.4 shows FE-SEM images of uncoated (S1) and PVP coated (S2) LSMO NPs. There are large aggregates and porous foam like structures which is a characteristic of solution combustion synthesis observed in uncoated LSMO NPs while PVP coated NPs show few aggregates and somewhat enhanced dispersed characteristics. Due to dipole-dipole interaction uncoated particles are attracted strongly, resulting in cluster formation. After coating, the nonmagnetic PVP layer may separate the particles, due to decreased interparticle interaction (dipole-dipole interaction), since the extent of dipolar coupling is related to the distance between particles. The use of
PVP polymer as a surfactant may prevent the large aggregation of particles by enhancing the distance between the particles as shown in Fig. 4.4 (S2).

**Fig. 4.4** FE-SEM images of uncoated (S1) and PVP coated (S2) LSMO NPs.

### 4.3.5 TEM and HR-TEM Study

Fig. 4.5 (a) represents TEM images for both uncoated (S1) and PVP coated (S2) LSMO NPs. From Fig. 4.5 (a) one can see most of the coated particles are separated from each other while uncoated particles are strongly aggregated with average size of 23 nm. After surface modification with PVP, improvement of the dispersibility was observed compared to uncoated particles. (see Fig. 4.5 (a) S2). The SAED pattern simultaneously obtained from the TEM measurements (Fig. 4.5 (b) (I)) suggests the single crystalline structure of uncoated LSMO NPs. The corresponding HR-TEM image of uncoated LSMO (Fig.4.5 (b) (II)) confirms the crystallinity of the NPs in their structure. The interplanar distance of the fringes is measured to be about 0.27 nm, corresponding to the distance between (110) planes of the LSMO crystal lattice of the NPs in their structure.
Fig. 4.5 (a) TEM images of uncoated (S1) and PVP coated (S2) LSMO NPs, (b) SAED pattern (I) and HR TEM images (II) of uncoated LSMO NPs.

4.3.6 DLS Study

Measurement of the hydrodynamic diameter in physiological media is one of the significant issues for in vitro or in vivo MFH applications. In order to study this parameter and elucidate the binding of groups to the magnetic core of LSMO NPs, the NPs were dispersed in DDW as well as in PBS. 7.5 µl of 1 mg/ml solution was taken in 3 mL DDW and PBS for uncoated as well as PVP functionalized NPs to carry out the experiment. Each solution was sonicated in a bath type ultrasonicator for 5 min to get well dispersion.
Fig. 4.6 (a) shows the hydrodynamic diameter at physiological pH 7.4 for PVP coated NPs dispersed in water (S2\textsubscript{w}) and dispersed in PBS (S2\textsubscript{p}). The main peaks for coated particles dispersed in water were centred at 55 to 60 nm and for coated particle dispersed in PBS were measured 40 to 45 nm, as shown in Fig. 4.6 (a). As indicated by Qiao et al. [25], PBS is often used to mimic the pH and ionic strength of physiological conditions as the osmolarity and ion concentrations of the PBS buffer match those of the human body.

Fig. 4.6 (b) shows mean hydrodynamic diameter as a function of pH for uncoated NPs dispersed in water (S1\textsubscript{w}), coated NPs dispersed in water (S2\textsubscript{w}) and PBS (S2\textsubscript{p}). It was observed that the mean diameter varies from 110 to 50 nm for uncoated particles and 105 to 38 nm for coated particles in DDW as shown in Fig. 4.6 (b) (S1\textsubscript{w} and S2\textsubscript{w}). However, in PBS the size of PVP coated NPs varies from 90 to 32 nm with varying pH which can be useful for \textit{in vitro} or \textit{in vivo} MFH applications [26-27]. All these measurements were carried out with varying pH. These results indicate that significantly larger NPs formed in the acidic solution (pH 2-5) as compared to neutral and
alkaline solutions which highlights that the acidic solution contains a higher degree of aggregation indicating strong hydrophobic-hydrophobic interaction resulting in increased particle size. The hydrodynamic sizes for coated MNPs dispersed in PBS remained significantly smaller than uncoated and coated MNPs dispersed in DDW. The possible reason behind it is that the reduction in Debye length with phosphate buffer ions appears to reduce the electrostatic attraction, which leads to decreased hydrodynamic diameter. In all cases, the polydispersity index (PDI) was ≤ 0.2 indicating monodisperse nature.

Fig. 4.6 (b) Mean hydrodynamic diameter as a function of pH for uncoated LSMO NPs dispersed in water (S1_w), PVP coated LSMO NPs dispersed in water (S2_w) and PVP coated LSMO NPs dispersed in PBS (S2_p).

An increase in average particle size for uncoated NPs may be due to the large magnetic moment which results in larger aggregates compared to PVP coated NPs. This means that the PVP coating attenuates the cluster
Chapter 4

behaviour in aqueous media by reducing interparticle interactions, as shown in Fig. 4.6 (b). These PVP coated MNPs could be easily dispersed in physiological media (PBS) to form stable suspensions. This indicates that the ionic strength of the PBS enhances the dispersibility and stability. All these DLS measurements were repeated 5 to 7 times and the average values were finally noted as a mean. DLS cannot discriminate between inorganic and organic material, also we cannot exclude the presence of small aggregates of two-to-three nanocrystals capped together within the same polymer shell [28], which are thus measured as having larger overall particle size. Therefore, this particle size or hydrodynamic size can differ significantly from the true physical size (i.e. measured by the XRD or TEM). In any case, it is remarkable that with the polymer coating procedure, it is possible for these particles to stay well dispersed/stable in water and for stability to significantly increase in PBS.

Long term stability is very important for biomedical applications, and is estimated in our experiment at physiological pH 7.4 for uncoated NPs dispersed in water (S1w), and for coated NPs dispersed in water (S2w) and PBS (S2p) as being about 15 days. Remarkably, for uncoated particles dispersed in water, significantly larger variation in hydrodynamic diameter was observed (65 to 79 nm) with a lack of stability over 15 days. In the second case, for coated particles dispersed in water, a slight change is observed in size (hydrodynamic diameter 50 to 60 nm). Interestingly, enhanced colloidal stability was observed when coated particles were dispersed in PBS. Particles remained stable with no appreciable change in size (hydrodynamic diameter 40 to 45 nm; (see Fig. 4.6 (c)) over 15 days. This means that at these points, attractive and repulsive interactions between approaching particles have reached equilibrium. This may be due to the effect of the ionic strength of the phosphate group on the colloidal stability of coated
MNPs which is one of the primary issues for their successful MFH application that is fulfilled in our study.

Fig. 4.6 (c) Long term Stability for uncoated LSMO NPs dispersed in water (S1w), PVP coated LSMO NPs dispersed in water (S2w) and PVP coated LSMO NPs dispersed in PBS (S2p) as a function of time at physiological pH 7.4.

One of the important issues for in vivo study is renal clearance, which depends on the size as the filters in the kidneys have very stringent restrictions on the size of the molecule that can pass through to the bladder. Without such clearance or their biodegradation into biologically benign components, toxicity is potentially amplified. In some previous studies on small NPs with specific sizes in the range of ~5 to 10 nm, these particles could be rapidly removed through extravasation and renal clearance [29]. On
the other hand, the particles with size >200 nm could be sequestered by the spleen and eventually removed by the phagocytes or, in the worst case, could pose a detrimental risk of pulmonary embolism. However, in both these cases, the blood circulation times of NPs, and hence their effectiveness, is affected [11]. However, the size of the particles in the range of 10 to 100 nm appears to be ideal for biomedical use because they are small enough to evade the reticulo-endothelial system of the body and are able to penetrate very small capillaries within body tissue for more effective distribution, ensuring adequate blood circulation times [11,30]. On the contrary, in some cases, the mechanism of NPs having larger size being excreted through the renal system is still unclear. For these excretion processes, NPs might produce glomerular damage and then increase the glomerular permeability or can lead to the glomerular liquefaction degeneration in which NPs might permeate through glomerular filtration and excrete through urine [31].

4.3.7 Zeta potential

For NPs to be useful in biomedical applications they must be stable in biological media. This stability can be studied by electrostatic interaction and can be controlled by variation in their surface charges, which is determined by measuring the zeta potential of these particles. The electrostatic potential on the ion surface of NPs is called the zeta potential. DC voltage is applied to the solution in which particles are dispersed. Particles within the dispersion with a zeta potential migrate towards the electrode of opposite charge with a velocity (electrophoretic mobility) proportional to the magnitude of the zeta potential. Smoluchowski equation was used to convert electrophoretic mobility ($\mu_{\text{ep}}$) into zeta potential ($\zeta$) as,

$$\zeta = \frac{\mu_{\text{ep}}}{\eta}$$

(4.1)
Where, \( \eta \) and \( \varepsilon \) are coefficient of viscosity and the dielectric constant of dispersion medium, respectively.

**Fig. 4.7** Zeta potential as a function of pH for uncoated LSMO NPs dispersed in water\((S1_w)\), PVP coated LSMO NPs dispersed in water \((S2_w)\), and PVP coated LSMO NPs dispersed in PBS \((S2_p)\).

Zeta potential measurements were carried out for NPs dispersed in DDW as well as in PBS. Fig. 4.7 illustrates the zeta potential as a function of pH for uncoated as well as coated NPs. By using an autotitrator, the pH was varied between 2-12. Each measurement was the average of at least 3 runs at 25 °C. From Fig. 4.7 it can be seen that at lower pH, the value of the zeta potential is more negative for coated NPs while that of uncoated NPs was more negative at higher pH. The IEP is the point at a particular pH where particles carry no net electric charge on their surfaces. The IEP in DDW for uncoated NPs is found to be \(-7\) as represented in Fig. 4.7.
(S1w) while after coating with PVP it decreases from pH 7 to 4 as shown Fig. 4.7 (S2w). It was found that the attachment of PVP on the surface of LSMO particles can greatly shift the IEP point of LSMO particles away from neutral pH [23].

In PBS, the IEP value for coated particles was observed at pH 5. The zeta potential value in PBS (Fig. 4.7 (S2p)) at physiological pH for coated particles was measured to be -22.51 mV which is higher than that measured in DDW (-14.24 mV for coated particles). This result suggests that enhancing the ionic strength of the aqueous media (phosphate salts from the PBS) will increase the electrical double layer around the particle which eventually reduces the steric repulsion and enhances stability. It is quite obvious that NPs with highly charged surfaces form stable solutions more readily. Also, the difference in zeta potential for uncoated NPs may be due to hydrophobicity and bundles of uncoated NPs affect the stability in the dispersion medium (DDW) resulting in lower negative zeta potential at lower pH compared to coated particles. Coating of PVP on the surface of the NPs allows electrostatic repulsion between the particles and steric stabilization is thus achieved. Hence, higher negative zeta potential was observed which could prevent attraction and collision between particles caused by Brownian motion.

4.3.8 Magnetic properties

The effect of surface coating on magnetic properties was explored by VSM at room temperature (300 K). The magnetization values were measured to be 42.90 and 36.44 emu/g for uncoated and coated particles, respectively. The value of magnetization correlated with the reported literature for many polymer-stabilized LSMO formulations [11, 14, 32].
Fig. 4.8 M-H curves of uncoated (S1) and PVP coated (S2) LSMO measured at 300 K.

In order to confirm the magnetic degradation due to surface modification of NPs caused by the PVP coating, the M-H behaviours of uncoated and coated particles are compared. Fig. 4.8 shows M-H curves for uncoated (S1) and PVP coated (S2) NPs. The PVP coated NPs have smaller magnetization values compared to uncoated particles. Reduction in magnetization may be explained in terms of reduction of the effective mass and the degradation of the particle dipole–dipole interaction caused by the non-magnetic PVP coating layer which might lead to decreased aggregation between the NPs. Rajagopal et al. reported that the lower magnetization value for a coated system may a priori reflect the extra weight of surfactant per particle [14]. Further magnetization measurements showed no coercivity and no remanence for any sample in the absence of the external field which
indicated that they are SPM at room temperature (300 K) and can be successfully considered for hyperthermia agents.

4.4 Conclusions

LSMO NPs with size ranging from 20-25 nm were synthesized by a simple and cost effective combustion route and were successfully coated with PVP as a stabilizing agent. FTIR spectroscopy confirmed the covalent attachment of PVP to the surface of the NPs. Magnetic measurements showed the SPM behaviour of LSMO MNPs with almost zero coercivity before and after coating with PVP. HR-TEM image of uncoated particles confirmed the crystallinity of the NPs. From DLS measurements, it was found that, the hydrodynamic diameter of the coated sample in PBS decreased more than in DDW. The most important part of this work is to demonstrate the long term stability of the coated NPs dispersed in PBS for biomedical application. Similarly, from zeta potential measurements, it was found that at physiological pH, the colloidal stability of coated sample dispersed in PBS was much higher than in DDW, which ensures their suitability for MFH. Hence, it is revealed that PVP encapsulated LSMO NPs form stable suspension in PBS. This seems to be the best suited candidate for magnetic fluid hyperthermia, since the dispersibility of the nanocrystals in physiological saline is a very important prerequisite for magnetic fluid hyperthermia is fulfilled in our study.
References:


Chapter 4


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