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Short Communication

Synthesis and Characterization of Magnetic Nanostructured Lipid Carriers (mNLCs) for Drug Delivery

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Magnetic nanoparticles (MNPs) are a class of nanoparticles, which can be manipulated using magnetic fields. Currently, MNPs are recognized as one of the most important mode as a drug carrier while they can also be potentially used as carriers for gene delivery. In this article, magnetic nanostructured lipid carriers (mNLCs) are prepared through co-precipitation method. The particle size and zeta potential, structure and thermal properties of the MNPS have been studied. The produced MNPs were also hydrophobically modified with a long chain of fatty acid namely lauric acid. These modified MNPs were mixed with nanostructured lipid carriers (NLCs) to form mNLCs. The heat capacity and flow of mNLCs was profligate in the presence of a magnetic field which is a worthy attribute for targeted drug delivery applications.

Keywords: Magnetic nanoparticles (MNPs), magnetic nanostructured lipid carriers (mNLCs), drug delivery.

1. INTRODUCTION

Magnetic nanoparticles (MNPs) are nanocrystals that can be manipulated using magnetic forces and are promising for the application in medicine due to their size, surface area, and drug carrying ability and biodegradable nature [1-4]. There are different types of magnetic nanoparticles such as magnetite (Fe_3O_4) [5], maghemite (Fe_2O_3) [6], wustite (FeO)[7] and Bermalite ($Fe(OH)_3$) [8] etc. Among which the most important ones are the magnetite nanoparticles, which are non-poisonous, non-immunogenic and possess good stability in suspensions [9]. For pharmaceutical and medicinal

purposes, the size, shape and high magnetism of MNPs are very important. After Physio-chemical characterization, these MNPs are coated with a desired coating material to form various products.

In human bodies, MNPs have several applications because of their low toxicity besides the biocompatibility. Owing to superparamagnetic nature, MNPs are very good for MRI, where they can be used as a contrast agent for cancer diagnosis, gene expression, angiogenesis, atherosclerosis, stem cell marking and inflammation identification [10-12]. MNPs coated with polymers can deliver drugs to the targeted area. In physio-chemical conditions, MNPs are very effective at cellular as well as molecular level [7, 13]. Some common methods for the synthesis of MNPs are wet chemical method, thermal decomposition, co-precipitation, hydrothermal, micro emulsion, sol-gel and sonolysis [14-17]. In this article, to prepare magnetic nanostructured lipid carriers (mNLCs), magnetic nanoparticles (MNPs) were synthesized through co-precipitation method. The aim of the work was to synthesize and investigate the MNPs for intended applications in targeted drug delivery.

2. MATERIALS AND METHODS

Materials: The chemicals such as $FeCl_3(H_2O)_6$ (Hexahydrated Iron (III) chloride), $FeCl_2(H_2O)_4$ (Tetrahyderated Iron (II) chloride), NH_4OH (Ammonium hydroxide), Tween 40, Poloxamer 188 and 2-nitrobenzaldehyde were purchased from Sigma Aldrich while $C_{12}H_{22}O_2$ (Lauric acid) and CH_3OH (methanol) were obtained from Friedman Schmidt Chemicals UK. Deionized Water was obtained from Q-POD Elix technology and hydrochloric acid HCl was purchased from Gain land Chemicals UK.

Synthesis of magnetic nanoparticles: The stock solutions of $FeCl_2(H_2O)_4$ and $FeCl_3(H_2O)_4$ were prepared by dissolving 2 grams of analytical grade iron (II) chloride, 5ml of 2 molar HCl and 4 grams analytical grade of Fe(III) chloride in 20ml of 2molar HCl. Magnetic nanoparticles were synthesized through a well-known "co-precipitation" technique. All the glassware was purged with nitrogen gas. All the required solutions were freshly prepared. The Deionized water was treated with nitrogen gas to remove oxygen. The ratio of hydrated ferrous chloride and hydrated ferric chloride was 1:2. 2g of iron (II) chloride and 4g of iron (III) chloride were dissolved in 5 ml and 20 ml of 2 M HCl, respectively, in nitrogen gas. These solutions were combined in three necks round bottom flask which is tightly closed with rubber septum and flushed through nitrogen gas for fifteen minutes to remove all the oxygen and create an inert atmosphere. 5% of Tween 40 solution was added to prevent agglomeration of magnetic nanoparticles. 50 ml of 0.7 M ammonium hydroxide was added drop wise into the mixture. The solution was stirred continuously under the nitrogen atmosphere for fifteen minutes. The product of the reaction was appeared as black precipitate according to the following reaction.

$$2FeCl_3 + FeCl_2 + 8NH_3 + 4H_2O \rightarrow Fe_3O_{4\,(magnetite)} + 8NH_4Cl \quad (1)$$

Characterization of prepared MNPs: The hydrodynamic particle size and zeta potential were measured by using a DLS, Malvern zeta Sizer NANO ZS (Malvern Instruments, UK), which is also called photon correlation spectroscopy (PCS). An X- ray diffractometer (Rigaku D/Max, Japan) were used for structural analysis. Fourier Transform Infrared (FTIR) spectra were recorded using Perkin Elmer 400 spectrometer. Thermal properties were investigated using dynamic scanning calorimetry (DSC) 823, Mettler Toledo, Switzerland.

Then the magnetic nanoparticles were subjected to magnet for separation and found precipitated MNPs were settled at the bottom of the flask. The magnetic nanoparticles were re-dispersed in 5% surfactant solution to prevent agglomerations. Then the solution was centrifuged for two minutes at 1200 RPM. The supernatant was decanted while the sediment was washed with deionized water four or five times to remove excess of ammonium hydroxide (NH_3OH). The washed magnetic nanoparticles were filtered through filter paper and the precipitates were collected. The precipitates were dried in a vacuum oven for 12 hours at 50 °C [18]. Magnetic nanoparticles were core coated with lauric acid. Magnetic nanoparticles and lauric acid were mixed at a ratio of 1:1.5. 2.5g of the prepared magnetic nanoparticles were suspended in 40ml of deionized water. 3.75 g of lauric acid was dissolved in 40 ml of deionized water at 75 ºC. The prepared magnetic nanoparticle suspensions were added into lauric acid solution at the same temperature (75 ºC). Immediately, the reactants change into two layers core coated magnetic nanoparticles was formed. Through a strong magnet, all the core coated magnetic nanoparticles were precipitated at the bottom. The solvent was decanted, and the synthesized nanoparticles were washed with Deionized water to remove excess of lauric acid. The synthesized precipitate formed in the form of the cluster, which was then dried in oven at 50 ºC for 14 hours.

Magnetic nanostructured lipid carriers are prepared through a well-known melt-emulsification method. In this method, 10.5 g of solid lipids and 4.5 g liquid lipids were mixed which was melted at75 ºC with the drug. 5 g of poloxamer 188 and magnetic nanoparticles were dispersed in water and heated to a temperature high enough to melt. This melted mixture of lipid and drug were added into surfactant solution to form emulsion. The prepared mixture was sonicated for two minutes using an ultrasonic processor to produce a black emulsion. The emulsion formed was mixed with chilled water in the ratio of 1:1 and kept in an ice bath. The rapid crystallization process occurred to form magnetic nanostructured lipid carrier.

3. RESULTS AND DISCUSSION

Dynamic light scattering (DLS) was used to measure particle size and zeta potential of pure MNPs and coated MNPs with long fatty acid chain lauric acid. For size estimation, pure MNPs were diluted in deionized water while coated MNPs were diluted in methanol. The size vs concentration of pure MNPs and coated MNPs are plotted in Figure 1 (a) and Figure 1 (b), respectively. The DLS results showed that the average diameter of the MNPs was 160.44 nm while the average size of the cMNPs was 187.46 nm. The size of coated MNPs increased which shows that MNPs are coated with lauric acid. Figure1 (c) shows the comparison between the size of uncoated MNPs and coated MNPs. DLS showed that the both uncoated MNPs and coated MNPs are poly-dispersed due to size distribution in suspension. For ideal physiological use, the magnetic nanoparticles are in the range of 200 nm. Here it is important to note that the size of magnetic nanoparticles is a very important parameter which affects the drug delivery physiologically. Absorption of smaller particles is easy compared to larger particles. Table 1 shows the concentration and size of the bare and coated MNPs at different dilutions. The average Zeta potential of the particles was found to be 25.28 mV as shown in Table 2.

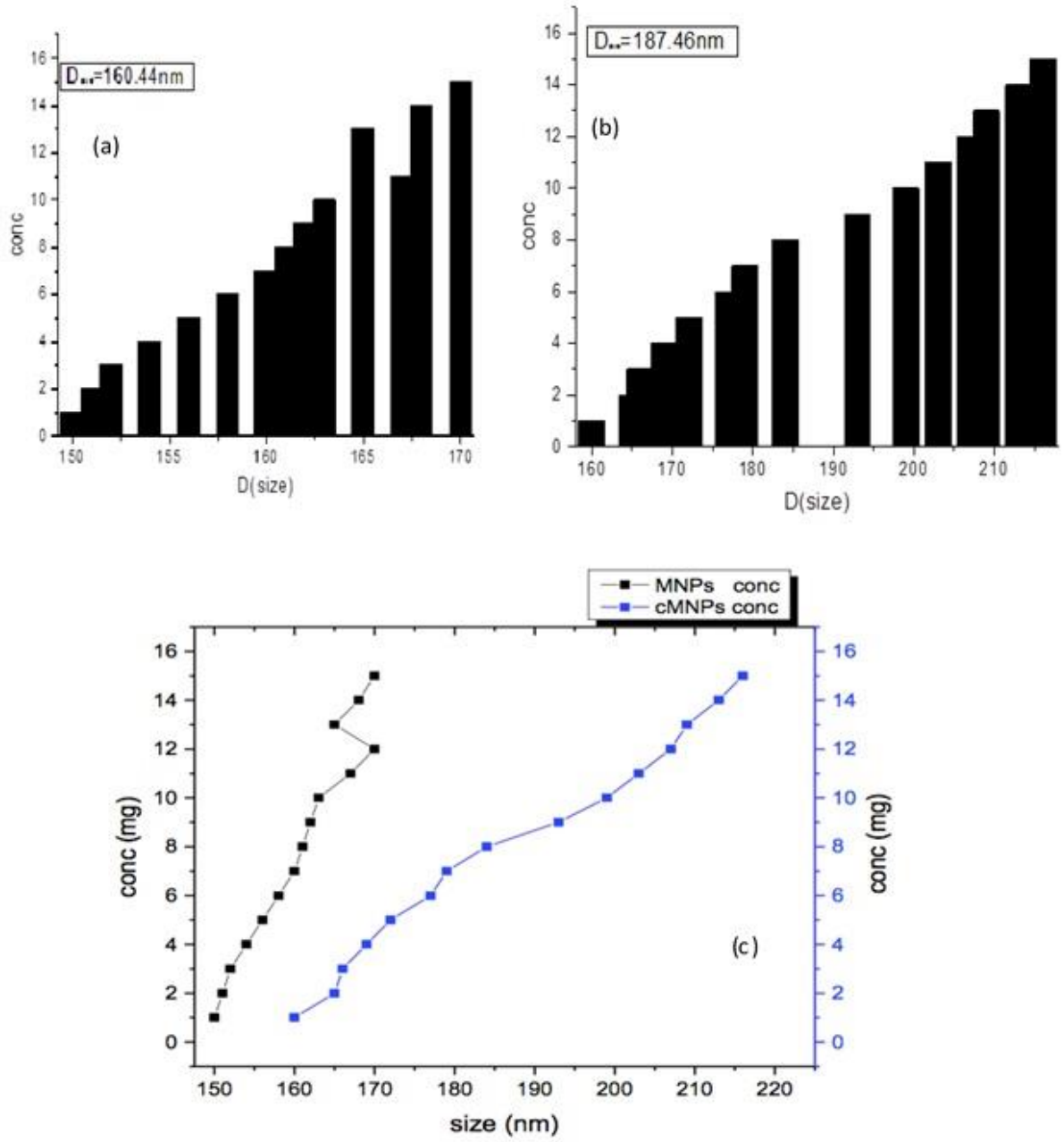

Figure 1. (a) Particle size of pure MNPs, (b) Particle size of coated MNPs and (c) comparison (particle size) of bare MNPs and coated MNPs

Table 1. Concentration of MNPs at different dilutions.

Concentration of pure MNPs(g) in10g DI water	Size of Pure MNPs (nm)	Concentration of coated MNPs (mg) in 10g of D.I water	Size of coated MNPs (nm)
0.001	149.87	0.001	160.12
0.002	150.90	0.002	163.96
0.003	151.93	0.003	165.88
0.004	154.00	0.004	168.68
0.005	155.90	0.005	172.05
0.006	158.01	0.006	176.63

0.007	159.94	0.007	178.69
0.008	160.80	0.008	183.06
0.009	161.97	0.009	199.09
0.0010	162.79	0.0010	203.18
0.0011	165.07	0.0011	206.57
0.0012	166.88	0.0012	2008.98
0.0013	168.00	0.0013	213.08
0.0014	170.16	0.0014	216.21

Table 2. Zeta Potential of prepared MNPs

Concentration of pure MNPs(g) in10g DI water	T (°C)	ZP (mV)
0.001	25	25.5
0.002	25	23.2
0.003	25	28.8
0.004	25	29
0.005	25	25
0.006	25	25
0.007	25	23
0.008	25	24
0.009	25	25.6
0.0010	25	23.7
Average Zeta potential = 25.28 mV		

XRD was also performed for uncoated MNPs, and coated MNPs. Figure 2 (a) shows that the XRD peaks for the pure MNPs were found at 9.09, 21.63, 23.92, 30.23, 35.78, 43.39, 56.96 and 62.91 values of 2θ. These peaks were well matched with a reference card. Figure 2 (b) shows the peaks of core coated MNPs with lauric acid. The peaks of the coated MNPs existed at 2θ 6.5,9. 54, 20.87, 21.68, 21.86, 24.14, 30.23, 35.78, 43.49, 56.96 and 62.91. These peaks were compared with both, pure lauric acid and pure MNPs. The XRD graph of coated MNPs consisted of all the peaks of both lauric acid and MNPs, which confirms that the MNPs were core coated with lauric acid.

FTIR analysis was performed for the detection of functional groups in reactants as well as in products. All the samples were crushed to obtain powder form. The instrument crystal was cleaned thoroughly using methanol to prevent contamination. Figure 2 (c) shows the graph of uncoated MNPs. Uncoated MNPs peaks in the range of 3000-2850 cm^{-1}, which belongs to (C-C). This absorption shows that the bonding is a stretch (C-C). According to correlation table these peaks are for C-H and C=C. This means that pure MNPs contain the above functional groups, i.e. alkyl, aromatic hydrocarbons and functional groups. Figure 2 (d) shows FTIR graph for coated MNPs. Peak at 3000-2850 cm^{-1} shows alkanes (C-C) group, whereas peaks at 1680-1600 cm^{-1}, 1400-1000cm^{-1} and 800-600 cm^{1} represent alkenes (C=C) group, aldehyde (C=O) group and sulfone group, respectively.

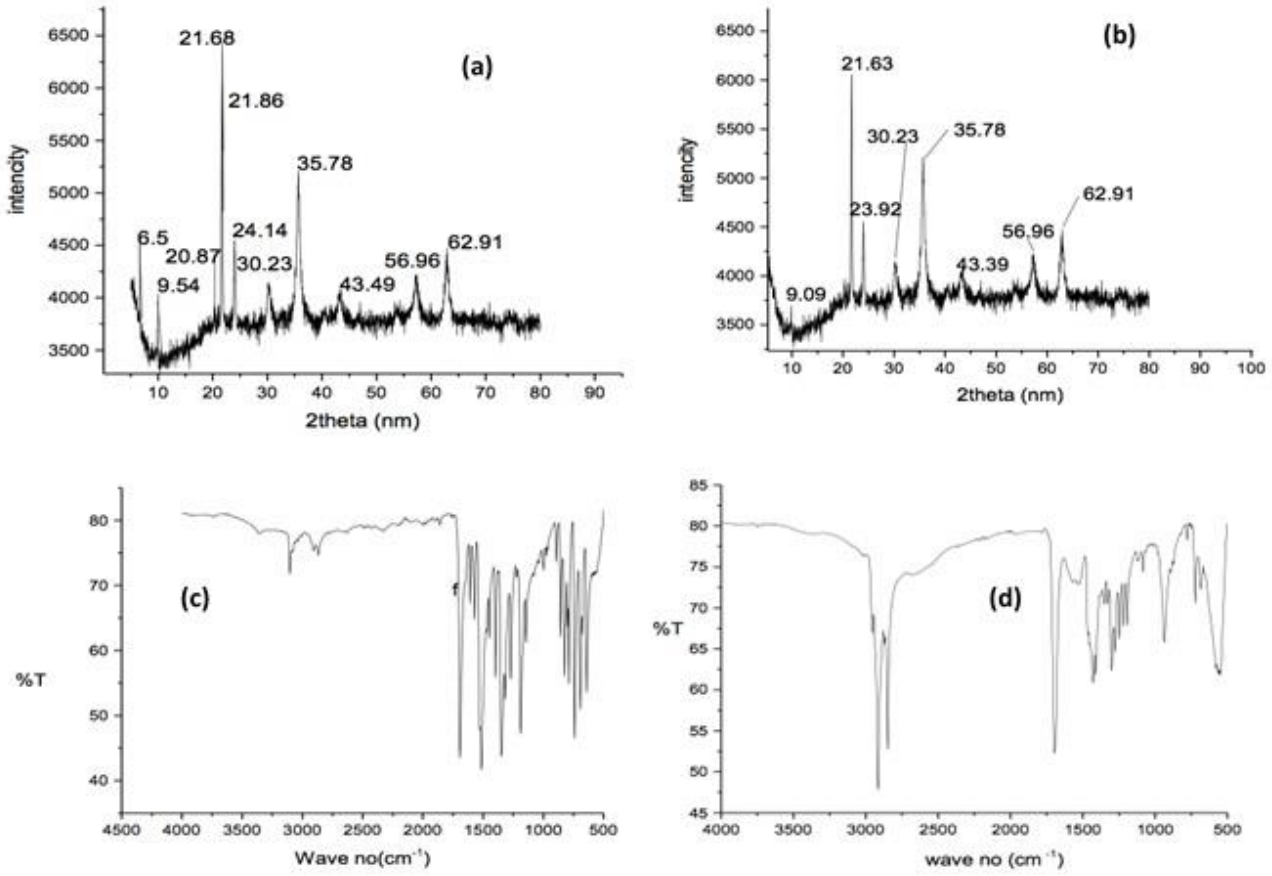

Figure 2. (a) XRD of pure MNPs, (b) XRD of core coated MNPs with lauric acid, (c) FTIR analysis of pure MNPs, (d) FTIR analysis of cMNPs

Thermal properties (phase transformation) or energetics changes in the samples were studied using DSC. The thermos-tropic behavior was measured for nano lipid, drug and mixture of nano lipid and drug. The samples were dried prior to the analysis of DSC and packed in aluminum covets. However, before measurements, the sample's temperature was reduced to 10 °C by keeping the samples in ice bath. The results indicate that 2-nitrobenzyldehyde and Nano lipids are stable at body temperature as their melting points are above that of body temperature (as seen in Figure 3). Thus, it can, easily deliver drugs to the body without melting. The in *vitro* study for mNLCs was performed to find the drug release at the targeted area and movement in body fluids. For this purpose, mNLCs were taken in permeating dialysis bags. The solubility of mNLCs was checked through bulk equilibrium dialysis method. These dialysis bags were filled with mNLCs and put in a buffer solution. A magnet was used along the walls of the container to drag the loaded mNLCs along the movement of the magnet. Initially, the pH of water was 7 at room temperature and it was start changing with the passage of time, which indicates that the drug was released from mNLCs.

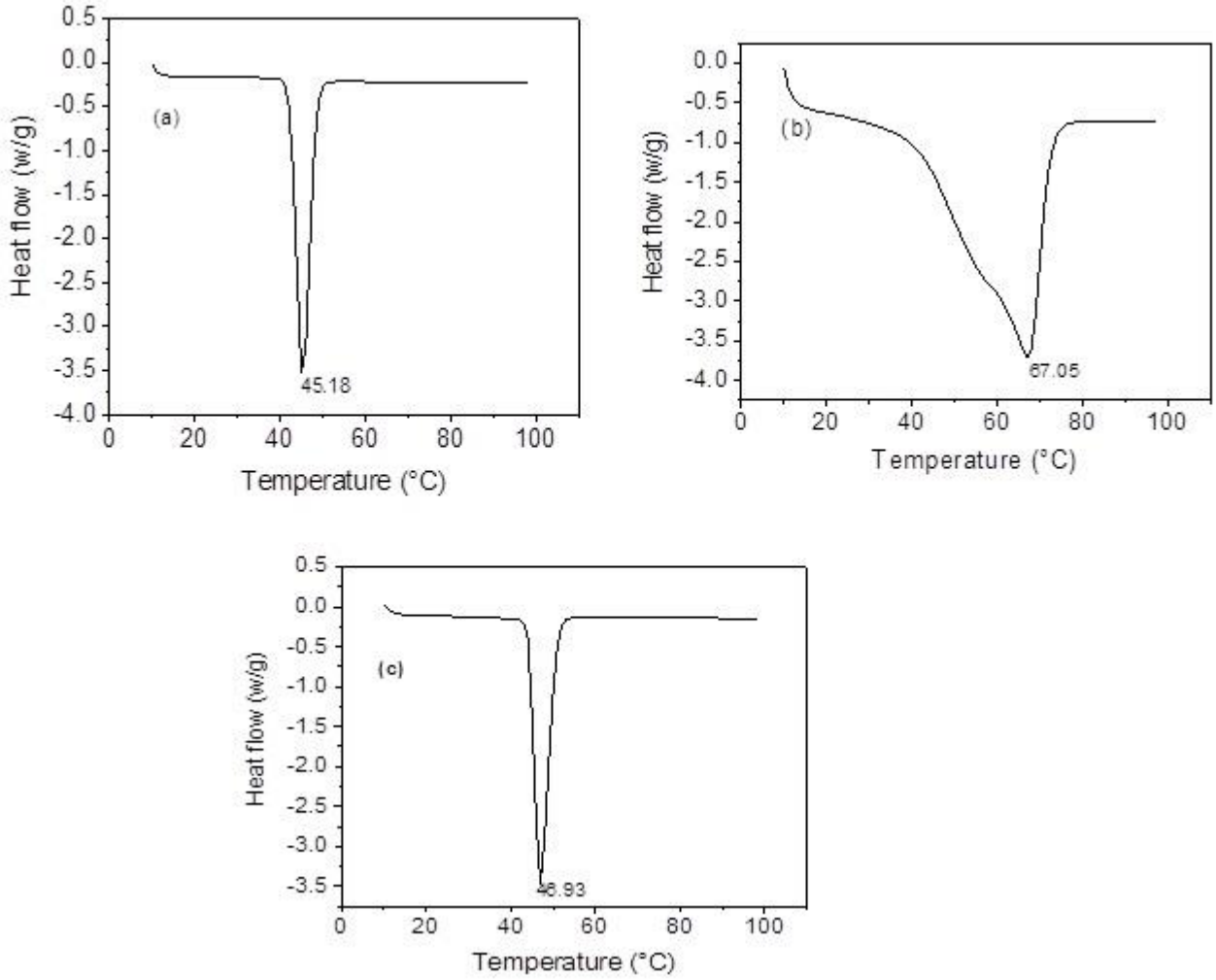

Figure 3. DSC analysis of (a) Lauric acid, (b) Beeswax and (c) 2-Nitrobenzyldehyde

This behavior demonstrated that the bare and coated MNPs can be concentrated in the affected areas through external magnetic field. This behavior also proved that drug loaded MNPs, can be a strategic technology for in vitro drug carrier, which can very easily target the affected areas in the body. The zeta potential average for both the bare MNPs and coated MNPs are 25 to 30 mv. The result of zeta potential shows that these magnetic nanoparticles are stable and can easily move in the body.

Darwish et. al. [19] prepared magnetic nanoparticles through co-precipitation method. The prepared magnetic nanoparticles were coated with oleic acid and polyvinyl benzyl chloride. Due to the oleic acid coating, magnetic nanoparticles were stabilized. Uprit et. al. [20] have prepared Nanostructured Lipid Carriers (NLCs) encapsulated with minoxidil and apply *in vitro*. Their drug release was very abrupt. Pardhan et. al. [21] prepared magnetic nano particles coated with different materials, including dextran and lauric acid. These coated magnetic nanoparticles were studied *in vivo* and *in-vitro* (human and mouse cells). The analysis showed that the dextran-coated magnetic nanoparticles have higher cytocompatibility but lower cellular uptake. While the MNPs coated with lauric acid have higher cellular uptake and lower cytocopetablity. Mamani et. al. [22] prepared MNPs through co-precipitation method. These MNPs were core coated with long chain fatty acid, lauric acid.

These MNPS were biodegradable and biocompatible. Thus, MNPs coated with lauric acid have an effective role in vitro and in vivo study.

4. CONCLUSION

In this work, the MNPs have been successfully prepared through a wet method known as 'co-precipitation' technique for application in magnetically controlled drug delivery. The observed flow of mNLCs was very fast in the presence of a magnetic field which is crucial for targeted drug delivery. The prepared mNLCs had higher melting points than body temperature, which is vital for a drug carrier. Drug release can be easily controlled from external magnetic field. The results show that the synthesized MNPs possess interesting properties which can make them suitable for magnetic nanostructured lipid carriers for targeted drug delivery.

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ABBREVIATIONS
Magnetic nanoparticles (MNPs)
Magnetic nanostructured lipid carriers (mNLCs)

AUTHORS' CONTRIBUTIONS
NA, YT and ZB conducted the experiments and collected the data. NA, MN and ZA analyzed the data and NA and MN wrote this manuscript. All the authors read and approved the final manuscript.

COMPETING INTERESTS
The authors declare that they have no competing interests.

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