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Original article

Synthesis and in vitro anticancer evaluation of 2-isopropyl-5-methylphenol Loaded PLGA based iron oxide nanoparticles

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ABSTRACT

For precise delivery of 2-isopropyl-5-methylphenol (IPMP), Poly (lactic-co-glycolic acid) (PLGA) based superparamagnetic nanoparticles loaded with IPMP (IMNPs) were synthesized and characterized. IMNPs with a particle size of 223 ± 20.7 nm were synthesized by solvent evaporation method. Fe_3O_4 nanoparticles of 33 ± 3.63 nm encapsulated in the PLGA polymer provides the superparamagnetic property to the IMNPs. The entrapment efficacy of Fe_3O_4 nanoparticles, 2-isopropyl-5-methylphenol and average cumulative percentage release profile of IPMP from the IMNPs were investigated. The magnetic nanocarrier enhanced the stability and activity of IPMP. Release of IPMP from IMNPs occurred in a controlled manner. The cytotoxicity of IMNPs against human hepatoma Hep3B cell line was investigated and the IC_{50} value was found to be 20 μg/mL for IMNPs compared to 25 μg/mL for free IPMP. The result showed that IMNPs had significant antitumor activity. Therefore, IMNPs may be considered as an effective anticancer drug delivery system for cancer chemotherapy.

1. Introduction

Little did the world knew of nanotechnology's potential, when in 1959 Richard P. Feynman, a physicist at California Institute of Technology, said, in one of his class "There is plenty of room at the bottom", starting from the bottom and scaling down to nanolevel holds key to future technology and advancement [1]. From there nanotechnology has now evolved into spearhead of many scientific innovation in all areas of medicine, magnetic separation, new therapeutic, diagnostic concepts, drug delivery, tissue engineering and vehicles for gene and targeted drug delivery [2–5]. Magnetic nanoparticles (MNPs) are promising candidates for applications in drug delivery due to their ultra fine sizes, biocompatibility and superparamagnetic behavior (i.e. responsiveness to an applied magnetic field without retaining any magnetism after removal of the magnetic field), reducing particle aggregation risk in capillary [6]. The surface of the MNPs are loaded with the pharmaceutical drugs and are made to release at the target site with externally localized magnetic field gradient, thereby administering lower but more accurate dose [7]. To prevent the aggregation of magnetic nanoparticles, they are coated with polymers or surfactants containing long-chain hydrocarbons for more effective stabilization [8]. Various research groups mostly use long-chain polymer oleic acid (OA) and its salts for the stabilization of iron oxide nanoparticles [9]. PLGA, a polymer approved by food and drug administration (FDA) is considered in our study for achieving stability of the magnetic nanoparticles [10].

Liver cancer is the fifth most common cancer in men and eighth in women. Among primary liver cancers occurring worldwide, essentially hepatocellular carcinoma (HCC) likely accounts for 70%–85% of cases. More than 80% of these cases occur in developing countries. In contrast, incidence rates are lower in the developed countries, with the exception of Japan [11–13]. IPMP, a phenolic compound present in essential oils of *Carum copticum sp (Apiaceae)*, *Thymus vulgaris (Lamiaceae)* has been reported for its antioxidant [14], antispasmodic [15], antibacterial and anti-inflammatory effects [16].

The main objective of this study was to synthesize 2-isopropyl-5-methylphenol-loaded PLGA based magnetic nanoparticles, comprised of magnetite nanoparticles coated with PLGA and IPMP for active drug delivery to human hepatoma Hep3B cell line.

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2. Materials and methods

2.1. Materials

Iron(III)acetylacetonate,1,2-Hexadecanediol, Benzyl ether, Oleic acid, Oleylamine, Dichloromethane (DCM), Polyvinyl alcohol (PVA) (Molecular weight 30,000-70,000), Poly (lactic-co-glycolic acid) (polylactide/glycolide = 50:50, Molecular weight = 1,30,000 Da), and 2-isopropyl-5-methylphenol were purchased from Sigma Chemicals Pvt Ltd. Human hepatoma Hep3B cell line were obtained from the National Centre for Cell Science (NCCS), India. All other chemicals used were of analytical grade. Milli Q water was used throughout the experiment.

2.2. Preparation of IPMP-loaded PLGA based magnetic nanoparticles

Oleic acid-stabilized 30 nm Fe_3O_4 magnetic nanoparticles (OA-MNPs) were synthesized by a high temperature reaction according to a reported method [17]. 100 mg of PLGA, 20 mg of 30 nm Fe_3O_4 and 7 mg of IPMP were dissolved in 8 mL dichloromethane and vortexed for 10 min to attain organic phase. The obtained organic phase was then poured into 50 mL of stirred aqueous solution containing 1% PVA as emulsifier. The mixture was then sonicated for 120 seconds. The formed emulsion was then stirred at room temperature overnight on a mechanical stirrer to evaporate the organic solvent. Then, it was washed three times with Milli Q water at 20,000 rpm for 20 min at 10 °C (Sigma centrifuge, Germany). The acquired pellets were lyophilized to get powdered IPMP-loaded PLGA based magnetic nanoparticles.

2.3. Characterization of magnetic nanoparticles

2.3.1. Structure of crystal, particle size, surface and magnetic properties

Powder X-ray diffraction patterns were used to determine the crystal structure of the samples in the range of 20–70 degrees on X-ray diffractometer (GE Inspection Technologies, TT3003-Germany) using Cu Kα radiation (λ = 1.540 A°) operated at 40 kV and 100 mA. The morphology of the Fe_3O_4 nanoparticles was imaged using high resolution scanning electron microscopy (FEI Quanta FEG 200) operating at 30 kV on carbon coated powder sample. The size distribution and zeta potential of the nanoparticles (1 mg mL^{-1}) suspended in Milli Q water (after sonication) were performed in triplicate using the Zeta Plus analyzer (Malvern Instruments Ltd). Fourier transform infrared spectra were recorded on a Bruker spectrometer over the range of 400–4000 cm^{-1}. The sample powders were ground with KBr and compressed into a pellet whose spectra were recorded. Measurement of magnetization of the nanoparticles was carried out with a Vibrating Sample Magnetometer (Lakeshore, VSM 7410).

2.3.2. Fe_3O_4 loading and IPMP entrapment efficiency

The loading efficiency of Fe_3O_4 nanoparticles incorporated in the magnetic carrier was determined by thermogravimetric analysis (TGA), on Thermogravimetric Analyzer (Exstar SII DT/TGA-6300). Thermogravimetric analysis was carried out on powder samples (~5 mg) with a heating rate of 10 °C/min in Nitrogen atmosphere up to 800 °C. The amount of IPMP entrapment on to the nanoparticles was determined by quantifying the amount of IPMP present in the supernatant after IMNPs synthesis. The supernatant obtained was subjected to UV-spectrophotometer analysis at 276 nm. The entrapment efficiency was calculated from the following formula,

% of unentrapped Drug = (drug present in the supernanant/drug added in formulation) * 100% of entrapment efficiency = 100 - % of unentrapped Drug

2.4. In vitro drug release

Five milligrams of IMNPs was suspended in 100 mL of phosphate-buffered saline solution (PBS, pH 7.4) and placed in a shaker at 37 °C at 120 rpm for 48 hours. At a predetermined time intervals, 2 mL of aliquots was withdrawn and replaced by 2 mL fresh PBS to maintain the sink conditions. The amount of released drug was determined by UV-spectrophotometer at 276 nm. The cumulative amount of IPMP released was integrated from each measurement, with concentrations determined from a calibration curve.

2.5. In vitro cytotoxicity assay of IMNPs against human hepatoma Hep3B cell line

Human hepatoma Hep3B cell line was obtained from the National Centre for Cell Science (NCCS), India and the culture was maintained in Modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum and incubated at 37 °C in humidified 5% CO_2 atmosphere. The cells were seeded on to a 96-well plate at a density of 1×10^5 cells per well in 100 μL culture medium and allowed to grow for 24 h. A stock solution of IMNPs and IPMP was prepared in dimethyl sulfoxide (DMSO), and subsequently diluted in medium to obtain the desired concentration of 5–30 μg/mL at a final volume of 200 μL in each well and incubated at 37 °C for 48 h. Twenty micrograms of MTT solution (5 mg/mL in PBS) was then added to each well. Only functionally active mitochondrial dehydrogenase enzymes from viable cells can reduce MTT to formazan. The media were removed and the formazan crystals were solubilized with 100 μL DMSO. The amount of formazan was then determined from the optical density at 570 nm by a microplate reader.

2.6. Statistical analysis

All experiments were repeated at least three times. Statistical analyses were performed using a Student's *t* test.

3. Results and Discussion

3.1. Characterization of the magnetic nanoparticles

3.1.1. X-ray diffraction (XRD)

The X-ray diffraction patterns of iron oxide nanoparticles coated with Oleic acid is shown on Fig. 1. Five characteristic peaks were observed at 2θ = 30.0°, 35.4°, 43.0°, 56.9°, and 62.5°, which can be assigned as (220), (311), (400), (511), and (440), respectively [17]. XRD patterns of the synthesized nanoparticles are well crystalline. The position and relative intensity of the diffraction peaks of the sample match well with the standard Fe_3O_4 powder diffraction data (JCPDS file No: 19-629). No peaks of any other phases were observed in particles, indicating the high purity of the synthesized nanoparticle. The black color of the OA-MNPs (Fig. 2) further proves that it contains mainly magnetite phase [18].

3.1.2. High Resolution Scanning electron microscopy (HR SEM) and Dynamic light scattering (DLS)

High Resolution Scanning electron microscopy was used to observe the surface morphology of the magnetic iron oxide nanoparticles coated with Oleic acid. Fig. 3 shows fairly uniform and spherical morphology of OA-MNPs with sizes around 30 nm. Table 1 shows the Particle size of OA-MNPs and IMNPs calculated by DLS to be 33 ± 3.63 and 223 ± 20.7. Increase in the particle size