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Dendrimer-magnetic nanoparticles as multiple stimuli responsive and enzymatic drug delivery vehicle

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ABSTRACT

Two different chain lengths of (poly)ethylene glycol-PAMAM dendrimers namely, L6-PEG-PAMAM and S6-PEG-PAMAM with six end-grafted ethylene glycol ether-tentacles of type $CH_2CH_2C(O)O(CH_2CH_2O)_9CH_3$ and $CH_2CH_2C(O)O(CH_2CH_2O)_2C_2H_5$, respectively, were synthesized. These dendrimers have multiple σ-donor capabilities and therefore, were used for stabilizing the magnetite (Fe_3O_4) nanoparticles. Both the dendrimer-magnetic nanoparticles (L6-PEG-PAMAM-MNPs and S6-PEG-PAMAM-MNPs) were characterized by different spectroscopic and microstructural techniques. The nanoparticles were mesoporous and superparamagnetic and therefore, explored for their possible use in delivery of cancer drug, doxorubicin (DOX). In the developed drug delivery system, achieving high drug-loading efficiency with controllable release were the main challenges. The change in zeta potential and quenching of fluorescence intensity suggests chemical interaction between DOX and the nanoparticles. The loading efficiency was calculated to be over 95% with a sustained pH and temperature sensitive release. Further, enzyme cathepsin B has also been used to degrade the dendritic shell to trigger sustained drug release in the vicinity of tumor cells.

1. Introduction

Dendrimers are nano-sized highly ordered structure with numerous functional groups and internal cavities. These features make the dendrimers suitable for many biomedical applications like drug and gene delivery, biochemistry and nanomedicine [1]. Further, dendrimers are also considered as nonviral synthetic vectors due to its biocompatibility, simplicity of use, and easy synthesis as compared to viral vectors which has inherent risk for clinical use [2].

Magnetic nanoparticles (MNPs), on the other hand, have proven applications in hyperthermia, magnetic resonance imaging contrast agent, targeted drug and gene delivery, tissue engineering, cell tracking, biosensing and bioseparation [3]. When functionalized with macromolecules, MNPs form distinct particulate systems that can pass through cellular barriers and offer organ-specific therapeutic and diagnostic tools [4]. Surface chemistry plays an important role in regulating the physiochemical characteristics of MNPs, *viz.*, size, solubility, state of dispersion and magnetization and also influences the fate of the MNPs in the biological system. MNPs coated with dendrimers can have better prospective in terms of surface charge, functionality, and reactivity as well as enhanced stability and dispersibility in solution [5]. Nanotechnology researchers have combined these two very effective materials to produce a nanoscale construct that can be effectively used for various biological applications.

Targeted drug-delivery systems can effectively convey drugs to the desired site of action, increase patient compliance, extend the product life cycle, and reduce healthcare costs [6]. However, in the current scenario, targeted drug delivery is a bottleneck since most of the drugs have low solubility, rapid excretion and high toxicity. They are also limited by untargeted biodistribution and non-specific delivery, *in vivo* degradation and short circulating half-lives [7]. All these drawbacks can be addressed by introducing polyethylene glycol groups (PEGylation) in the nanosystems. After PEGylation, many drugs have been found to attain increased solubility, improved pharmacokinetics and targeting [8].

The motivation for the study is to combine the functions of longevity (PEGylation in dendrimer), targetability (use of MNP to assist in magnetic guidance to tumor site) and stimuli sensitivity (pH, temperature) in addition to leveraging the tumor microenvironment (acidic pH, over-expression of enzymes promoting degradation) to design an efficient, minimally toxic drug delivery

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system. It was also aimed to fabricate the system in such a way that it would release maximum payload at the tumor site and minimum during circulation.

The present study aims to synthesize dendrimer based MNPs wherein the dendrimer stabilizes the nanoparticles and provides functional groups for attachment of drug molecules. Thorough characterization of the functionalized MNPs has been performed to gage particle size distribution, surface area, porosity and magnetic properties. Loading of doxorubicin and their subsequent release in acidic, hyperthermic and enzymatic (cathepsin B) environment has also been investigated.

2. Experimental

2.1. Materials used

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), sodium hydroxide and doxorubicin hydrochloride were received from Sigma Aldrich, USA. All other chemicals were of analytical grade and used as received.

The poly(ethyleneglycol)–poly(amidoamine) dendrimers were synthesized [9,10] and used to stabilize and functionalize the magnetic nanoparticles. One of the dendrimers had 6 long dendritic arms (L6-PEG-PAMAM) and the other dendrimer had 6 comparatively short arms (S6-PEG-PAMAM) (Fig. 1).

The MNPs were prepared by the conventional co-precipitation technique with 2:1 M ratio of Fe^{3+}/Fe^{2+}. Typically, 6 g $FeCl_3 \cdot 6H_2O$ and 2.1 g $FeCl_2 \cdot 4H_2O$ in 80 mL deionised water was stirred in a five-necked flask under inert atmosphere for 30–45 min until a temperature of 80 °C was reached. To this solution, 20 mL of 5 M NaOH was added drop by drop, following which the solution turned from orange to black. The reaction mixture was then vigorously stirred at 1000 rpm for 1 h. 10 mL dendrimer solution (1 mg/mL concentration) was added and the refluxing was continued for another 30 min, after which the system was cooled to room temperature. The solution was washed alternatively with deionised water and ethanol for 3–4 times. A permanent magnet was then used to separate the dendrimer stabilized magnetic nanoparticles. The magnetically separated nanoparticles are named as L6-PEG-PAMAM-MNPs and S6-PEG-PAMAM-MNPs.

2.2. Characterization techniques

The phase purity and identification of the MNPs were done by X-ray diffraction (XRD) with PanAnalytical X-Pert diffractometer using a monochromatised X-ray beam with nickel-filtered Cu-Kα radiation at 4°/min scan rate. Fourier transform infrared (FT-IR) spectra were obtained using a Jasco, FT-IR 300E spectrometer with a resolution of 4 cm^{-1}. The TEM micrographs were observed by JEOL JEM 2100 for particle size determination. The specific surface area, pore volume and pore size distribution of the nanoparticles were measured by ASAP 2020 Micromeritics instrument. Magnetic properties of MNPs were studied using Vibrating Sample Magnetometer Model: 7410, Lake Shore Cryotonics Inc., U.S.A.

2.3. Drug loading and release

The anticancer agent, doxorubicin hydrochloride (DOX) was used to study the drug loading and release efficiency of the dendrimer-MNPs. Fluorescence spectroscopy was used to investigate the interaction of DOX with L6- and S6-PEG-PAMAM-MNPs.

A typical drug loading method is as follows: The aqueous dispersion of varying amounts of L6- and S6-PEG-PAMAM-MNPs (1, 2, 4, 6 and 8 mg/mL from a stock suspension) were added to a fixed amount of DOX solution (50 μg) and incubated by shaking at ambient temperature for 24 h. The loading percentages for different concentrations of nanoparticles were calculated by comparing the fluorescence peak intensities of the supernatant of the DOX loaded nanoparticles against the fluorescence spectrum of pure DOX solution. The standard curve of DOX solution was prepared by recording the individual fluorescence intensities under similar conditions using Cary Eclipse fluorescence spectrophotometer ($R^2 = 0.998$) [10,11]. The loading efficiency (w/w%) was calculated using the following relation:

$$\text{Loading efficiency } (\%) = \frac{I_{DOX} - I_S}{I_{DOX}} \times 100$$

where, I_{DOX} is the fluorescence intensity of pure DOX solution and I_S the fluorescence intensity of the supernatant solution. The loading interactions were evaluated at $\lambda_{ex} = 490$ nm and $\lambda_{em} = 590$ nm for DOX.

For studying the drug release profile, various external stimuli like change in pH, temperature and enzymatic degradation was used. The drug release under the influence of pH was carried out in a reservoir-sink condition. Typically, 4 mg of drug loaded

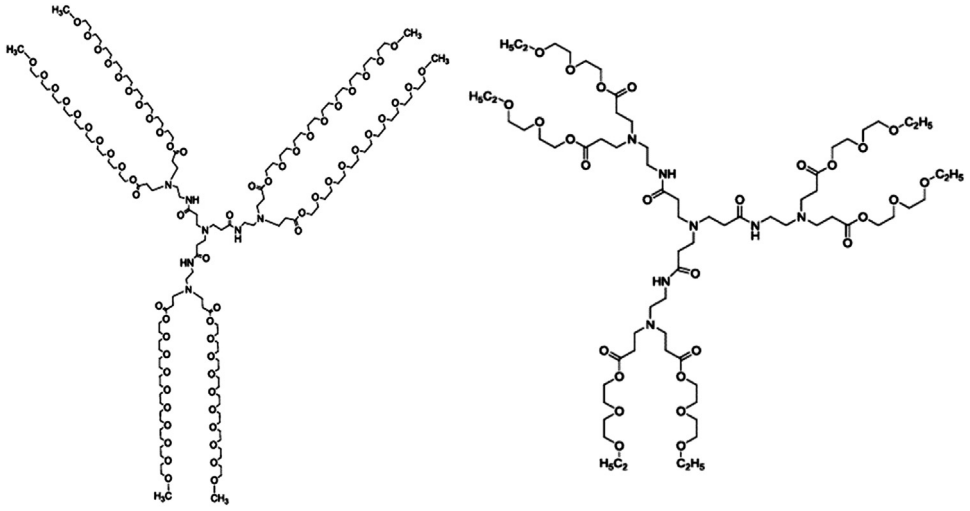

Fig. 1. Structure of dendrimers: (a) long branch or L6-PEG-PAMAM and (b) short branch or S6-PEG-PAMAM.