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Dendritic multi-walled carbon nanotube with thermoresponsive shells: A good carrier for anticancer drugs

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ABSTRACT

In this research, multi-walled carbon nanotubes (MWCNTs) were modified by dendritic macromonomer. Herein, polyamidoamine with acrylamide end groups was incorporated on MWCNTs. Afterwards, poly(N-isopropylacrylamide), (PNIPAM), was grafted on polyamidoamine in a facile synthesis. Then, doxorubicin as anticancer drug was loaded on this nanocarrier. The drug release was studied at below and above the lower critical solution temperature of PNIPAM, (LCST 32 °C), 27 °C and 37 °C, respectively. At 37 °C (body temperature) the polymer shell dehydrated and the drug release increased. The profile of drug release was expressed by Higuchi's equation which indicated that the drug release mechanism was diffusion controlled.

Introduction

A suitable carrier for intelligent drug delivery systems should be able to host, protect and transport the embedded drug to the target site under the action of a defined physical stimulus, such as the cellular pH, the local temperature, or the tumor reducing environment [1]. Among the nanoparticles which have been used in drug delivery systems, carbon nanotubes, CNTs, represent a novel class of carriers for the delivery of drugs in a specific site [2–11]. CNTs posses extraordinary physical, chemical and mechanical properties, which make them as a potent biological carrier to deliver anticancer drugs. The functionalization of CNT with biodegradable polymers provides biocompatibility. Also, functionalized CNTs possess high propensity to traverse cell membrane either via, endocytosis dependent or independent pathways [12]. CNTs without surface modification are cytotoxic to certain mammalian cells. For example, pure MWCNTs can injure plasma membrane of human macrophages [13]. Covalent functionalization of CNTs with the therapeutically active molecule or the biocompatible surfactants is governed by the oxidation of CNTs using strong acids (conc. H_2SO_4 or conc. HNO_3) which generates substitutable hydrophilic functional groups such as COOH, OH on the CNTs which then further undergo into the chemical reactions. It is worth mentioning that the oxidation treatment shortens the nanotubes, in addition to the generation of oxygenated species.

Dendrimer modified CNTs can reduce the cytotoxicity of CNTs and enhance the cellular uptake of the CNTs [14,15]. Dendrimers are highly branched and monodisperse macromolecules that are three-dimensional and generally spherical in shape, have a large number of reactive terminal groups, and have space available within their interior. Among the various dendrimers, polyamidoamine (PAMAM) dendrimer is the most prevalent. It contains many primary amine, amide, and tertiary amine groups in the interior and/or at the terminals [16]. On the other hand, pure commercially PAMAM, solely, do not show controllable release, very well [17]. Hence, some modifications would be very interesting to overcome this drawback. The integration of dendrimers with CNTs provides multivalent amine-rich periphery for the combination of drug molecules with CNT surfaces, greatly improving the effective therapeutic payload by incorporating drug molecules into dendrimer cavity [14,18,19]. Additionally, using intelligent polymers which respond to the local temperature could be more effective in smart drug delivery systems. A number of polymers are known to exhibit thermosensitive properties such as poly(N-isopropylacrylamide), (PNIPAM) [20–23]. This polymer is highly soluble in water at low temperature, and becomes water-insoluble at temperature higher than 31–32 °C. In this work, to achieve thermosensitivity and controlled releasing, PNIPAM was

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grafted on the PAMAM. Herein, MWCNT@PAMAM-*g*-PNIPAM with peripheral acrylamide end groups was prepared in a facile synthesis. The oxidized MWCNT was amine functionalized by 3-aminopropyltriethoxysilane (APTS) for growing up PAMAM. After grafting of the PAMAM, the PNIPAM was grafted on the MWCNT@PAMAM by free radical polymerization method. This nanocarrier would be good candidate to deliver chemotherapeutics to tumor cells. This nanocarrier was characterized by Fourier transform infrared (FT-IR), thermogravimetric analysis (TGA), dynamic light scattering (DLS), elemental analysis (CHN analysis), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Also the drug release profile (in vitro) was studied by UV–vis spectrophotometer.

Materials and methods

Materials

MWCNT (95–98% purity, 30–50 nm in diameter, 20 μm in length, synthesized by catalytic chemical vapor deposition) was purchased from Neutrino Company (Tehran, Iran). 3-aminopropyltriethoxysilane (APTS, Merck), potassium persulfate (KPS, Sigma), ethylenediamine (Merck), methyl acrylate (Merck) and N-isopropylacrylamide (NIPAM, Alfa Aesar) were used without further purification. Acryloyl chloride was purchased from Alfa Aesar. Doxorubicin hydrochloride (DOX) was obtained from India. Phosphate buffer saline (PBS) and all other materials were chemical grade and used as received.

Characterization

Fourier transform infrared (FT-IR) analysis was carried out using KBr discs in the region of 4000–400 cm^{-1} by an ABB Bomem MB-100 FT-IR spectrophotometer. The drug release was studied by UV–vis spectra which were obtained using a Perkin–Elmer Lambda 25 spectrophotometer. The hydrodynamic diameter of the nanoparticles were measured by dynamic light scattering (DLS) using an autosizer 4700 (Malvern) in aqueous solution with pH 7.4 at 25 °C, 37 °C and 42 °C. Thermogravimetric analysis was performed with a Perkin Elmer-Pyris Diamond TG/DTA from room temperature to 600 °C, at heating rate of 10 °C/min under N_2 atmosphere. The elemental analysis (C, H, and N) was done by CHNS-O Elemental Analyzer, Costech ECS 4010. The morphology of MWCNT was examined using a scanning electron microscope (SEM; Philips, Natick, MA, XL30) operated at 15 kV after coating the samples with gold film. Transmission electron microscopy (TEM) images were obtained on a JEOL's JEM-1200 EXII Transmission Electron Microscope. For this purpose one drop of sample was placed on copper grid covered with carbon and dried at room temperature.

Preparation of MWCNT@PAMAM (G3)

In order to prepare MWCNT@PAMAM, first, pristine MWCNTs were oxidized in a mixture of concentrated sulfuric and nitric acids (3:1, 98% and 69%, respectively) at 50 °C for 6 h. Then the mixture was neutralized with sodium hydroxide solution and the solution of oxidized MWCNTs was vacuum-filtered. The filtered solid washed with deionized water and finally, the oxidized MWCNTs were dried in oven for 24 h at 100 °C [24]. The second step was the functionalization of the oxidized MWCNTs with amine groups for growing up dendrimer. For this purpose, 0.5 g oxidized MWCNTs were dispersed in 30 mL of ethanol/water mixture (v/v: 4/1) and 1.0 mL (0.5 mmol) 3-aminopropyltriethoxysilane was added. This mixture was stirred at reflux condition for 24 h. The preparation of MWCNT@PAMAM up to the third generation was performed according to procedure reported by Tomalia [25,26]. 0.5 g aminopropyl modified MWCNTs and methyl acrylate (4.0 mmol, 0.35 g) were stirred at 50 °C under nitrogen for 3 days in methanol (20 mL). The suspension was cooled and vacuum-filtered, washed first with 20 mL methanol (three times) and then with 20 mL ether (three times). The residual solvent was removed in vacuum. The product of this stage was methyl propylaminopropionate MWCNT. The latter was then added to ethylenediamine (3 mL) in methanol (20 mL) and stirred at room temperature under nitrogen for 5 days. The resulting first generation MWCNT@PAMAM was isolated by filtration, and then washed with methanol (three times 20 mL) and dichloromethane (three times 20 mL). The residual solvent was removed in vacuum. The second-generation MWCNT@PAMAM can be prepared by following the above procedure starting with the first-generation MWCNT@PAMAM and methyl acrylate (10 mmol, 0.87 g), and by changing the reaction time to 5 days. After isolation, the ester was added to ethylenediamine (6 mL) in methanol (20 mL) and stirred at room temperature under nitrogen for 7 days. The third generation was prepared in the same manner.

Preparation of MWCNT@PAMAM with acrylamide end groups (MWCNT@PAMAM macromer)

For this purpose, 0.5 g MWCNT@PAMAM prepared from previous step was dispersed in 20 mL dried dichloromethane containing 30 mmol triethylamine. Then 30 mmol acryloylchloride was added dropwise at 0 °C, the resulting mixture was stirred under argon overnight at room temperature. Finally, it was filtered and washed with dichloromethane two times.

Preparation of MWCNT@PAMAM-g-PNIPAM

The procedure for the preparation of MWCNT@PAMAM-*g*-PNIPAM was according to free radical polymerization method. 2.0 g (28 mmol) NIPAM was dissolved in 20 mL distilled water and 0.5 g MWCNT@PAMAM macromer was dispersed in this solution. Then 0.05 g KPS as initiator was added and stirred at 70–80 °C for 12 h. The resulting product was washed with hot water several times and dried at 70 °C.

Drug loading

In this study, water soluble doxorubicin hydrochloride was used as anticancer drug. 1.0 mL (2 mg mL^{-1}) DOX was added to 1.0 mL PBS buffer and 5.0 mg MWCNT@PAMAM-*g*-PNIPAM was dispersed in this solution and stirred for 24 h at room temperature. When these particles were swollen, the uptake of the drug molecules occurred. After this, the drug loaded nanoparticles were filtered and washed with water. The supernatant was kept for calculating the drug loading content.

In vitro drug release

5.0 mg MWCNT@PAMAM-*g*-PNIPAM was dispersed in 1.0 mL PBS buffer (pH 7.4). The dispersion was then transferred into a dialysis bag (cut off molecular weight 12,000 g mol^{-1}) and the bag was placed in a 5.0 mL PBS solution. At timed intervals, 3.0 mL solution was withdrawn from the solution and the amount of released drug was estimated by UV–vis spectrophotometer at λ = 480 nm. After each measurement the sample was returned in to release medium to keep the medium constant. The release measurements were carried out at 27 °C and 37 °C below and above LCST, respectively.

Results and discussion

Preparation of MWCNT@PAMAM-g-PNIPAM

In this work, MWCNT@PAMAM-*g*-PNIPAM was prepared in a facile synthesis. At first, the MWCNT was oxidized and functionalized by APTS for growing up PAMAM on its surface. The standard