



Shiraz University

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Research proposal

Temperature-responsive poly (*N*-vinylcaprolactam) grafted magnetic nanoparticles modified with carbon quantum dots (CQDs)

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1. Introduction

In recent years great progress in the field of medicine and pharmacy has been observed, especially in the case of controlled drug delivery and release systems as well as diagnostics. Rapid development of nanotechnology provides many valuable solutions for doctors and their patients suffering from various diseases such as cancer or diabetes due to the extraordinary features of materials in the nanoscale. One of the most emerging nanomaterials are carbon quantum dots (CQDs) which are defined as particles of the size below 10 nm with a carbon core. Although pure carbon is poorly soluble in water and has weak fluorescence abilities their unique characteristic include interesting properties such as tunable luminescence, high stability in water, and resistance to photobleaching and photoblinking. Contrary to many organic dyes or semiconductor quantum dots (inorganic nanomaterials), they are also quite soluble in aquatic solutions and are susceptible to various modifications. At the same time, they maintain their inertness to chemicals. Finally, they are biocompatible. Therefore, carbon quantum dots are prior to most currently used photoluminescent substances and materials. CQDs due to their low cytotoxicity maybe applied in biomolecules/drugs delivery, biosensors manufacturing, and bioimaging. This class of nanomaterials can be also used in catalysis, optoelectronics, or optronics [1]. Among the electronic and physicochemical characteristics of CQDs, their optical properties and their fluorescence emissions in particular have attracted increasing interest in recent years. For many years, semiconductor quantum dots have been extensively investigated for their strong and tunable fluorescence emission properties, which enable their applications in biosensing and bioimaging. However, semiconductor quantum dots possess certain limitations such as high toxicity due to the use of heavy metals in their production. It is known that heavy metals are highly toxic even at relatively low levels, which may prove prohibitive to any clinical studies. This prompted the creation of CQDs to replace semiconductor quantum dots due to their low toxicity, biocompatibility, low cost and chemical inertness in addition to having similar fluorescence properties [2].

Compared to atomic or bulky counterparts, nano-sized materials owe superior physical and chemical properties due to their mesoscopic effect, small object effect, quantum size effect and surface effect. Recently, Fe_3O_4 magnetic nanoparticles (MNPs) have been intensively investigated because of their superparamagnety, high coercivity and low Curie temperature. In addition to these characters, Fe_3O_4 MNPs are also non-toxic and biocompatible. Therefore, Fe_3O_4 MNPs have brought out some new kinds of biomedical applications such as dynamic sealing, biosensors, contrasting agent in magnetic resonance (MR) imaging, localizer in

therapeutic hyperthermia and magnetic targeted-drug delivery system, *etc.* It is well known that it is very important to ensure the narrow size distribution, good dispersion and high magnetic response of Fe_3O_4 MNPs in tissue fluid for applications. However, magnetic attractive forces combined with inherently large surface energies (>100 dyn/cm) make them easy for the aggregation Fe_3O_4 MNPs in fluids. Therefore, lots of synthesized polymers (*e.g.*, poly (vinyl alcohol) phosphate, polyethylene glycol, polyamides, polyglycidyl methacrylate, poly(acrylic acid), chitosan (CS) and o-carboxymethylchitosan) were employed as coating agent in order to modify the surface of iron oxide particles. Although the polymeric coatings can reduce the aggregation of MNPs, they also increase the overall size of the particles and thus limit the expression of magnetic property, tissue distribution, metabolic clearance and penetration ability into interstitial spaces. So it is very important to develop an efficient surface-modification method for preparing Fe_3O_4 MNPs with narrow size distribution and excellent dispersion in aqueous or non-aqueous solution using small molecular compounds. [3]

Poly(*N*-vinyl caprolactam) (PVCL) is a stimuli-responsive polymer with many significant characteristics, such as solubility in water and organic solvents, non-toxicity, biocompatibility, nonionic nature, temperature sensitivity, and the presence of hydrophilic and hydrophobic groups in its structure. PNVCL has hydrophilic amide groups directly connected to the hydrophobic carbon-carbon backbone chain and does not produce undesirable amide compound by-products after hydrolysis and thus has better biodegradability and compatibility than other polymers. Recently, the number of studies of its applications has increased considerably. From an application point of view, PVCL is an interesting polymer because it is biocompatible and shows a temperature-induced phase transition near physiological temperatures (LCST ~ 303 – 323 K depending on the molecular weight). Unlike most polyacrylamides, PVCL has a remarkable advantage because its hydrolysis does not yield toxic low-molecular weight amines, which have been proven by evaluations of the cytotoxicity. Furthermore, PVCL is used widely in controlled drug delivery and release, stabilization of proteases, hair-care and cosmetics, immobilization of enzymes and cells, fluorescent thermometers, and biomedical materials. Therefore, the incorporation of PVCL onto CQDs might be promising because it leads to the formation of biocompatible nanomaterials with unique properties for a wide range of applications. To the best of our knowledge, few attempt has been made to functionalize CQDs using PVCL for drug-delivery applications [4, 5].

Bare surface of CDs is rather defenseless due to the existence of many defect sites, such as dangling bonds, non-radiative states and radicals, which usually cause the low PL efficiency (QY) and impair of the optoelectronic properties of CDs. Surface passivation is the most effective technique to enhance the PL intensity and common polymer passivation agents include polyethylene glycol (PEG), polyethyleneimine (PEI), poly(ethylenimine)-co-poly(ethylene glycol)-co-poly(ethyl-enimide) (PPEI) and 4,7,10-trioxa-1,13-tridecanediamine (TTDDA). The mechanism of surface passivation can be explained by cutting off other non-emitting ways to amplify the emission intensity caused by irradiation. After passivation with polymers or other organic molecules, the QY of CDs can increase dramatically.

Besides, functionalization usually occurs along with the passivation process and they are the same process when polymer passivation agents are doped. Functionalization is the process of adding new features and properties to a material by changing the surface chemistry of the material and is a useful technique to develop more tailoring work for CDs. After passivation, it can also modify the surface of CDs by bringing in or generating various functional groups such as carboxyl, carbonyl, and hydroxyl groups in the defect sites of their surface. The process is usually assisted by doping polymers or organic compounds. In terms of the category of doping, there are currently N-doping, S-doping, P-doping, and Si-doping, among which N-doping has proved to be the most effective and popular doping method.

2. Literature review

Since the discovery of CQDs, a large variety of techniques for the preparation of CQDs have been developed. Generally, synthetic methods of CQDs can be clarified into two groups: top-down and bottom-up methods (Figure 1). In top-down process, the macromolecule is destroyed or dispersed into small-sized CQDs by physical or chemical methods; while the bottom-up approach mainly refers to the polymerization and carbonization of a series of small molecules into CQDs through chemical reaction.

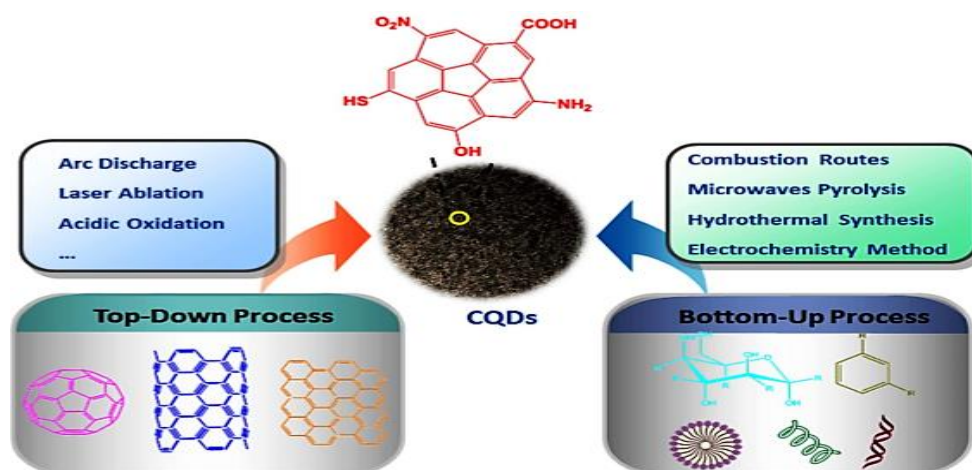


Figure 1. The typical approaches for the synthesis of CQDs

Among the bottom-up approaches, the microwave pyrolysis method has been well-established due to the rapid synthesis and commercialization. Zhu *et al.* reported a facile microwave pyrolysis approach to synthesize CQDs by combining poly(ethylene glycol) (PEG200) and a saccharide (glucose, fructose, *etc.*) in water to form a transparent solution, followed by heating in a microwave oven. The obtained CQDs exhibited an excitation-dependent photoluminescence (PL) properties. This is a simple, fast and environment-friendly preparation method for CQDs rich in oxygen-containing groups, which would become the coordination sites of metal ions for the design of carbon-based electrocatalysts [6].

Strong oxidizing acids carbonize small organic molecules to carbonaceous materials, which can be further cut into small sheets by controlled oxidation. This method may suffer from harsh conditions and drastic processes. Peng and Trivas-Sejdic reported a simple route to prepare luminescent CQDs in an aqueous solution by dehydrating carbohydrates with concentrated H_2SO_4 , followed by breaking the carbonaceous materials into individual CQDs with HNO_3 , and finally passivating with amine-terminated compounds (4,7,10-trioxa-1,13-tridecanediamine). The surface passivation was essential for the PL of these CQDs. The emission wavelength of these CQDs can be tuned by differing the starting material and the duration of the nitric acid treatment. The multicolor emission capabilities and nontoxic nature of these CQDs enable them to be applied in life science research [7].

Bhunia *et al.* synthesized two kinds of the CQDs, hydrophobic and hydrophilic with diameters less than 10 nm from the carbonization of carbohydrates. The hydrophobic ones were produced by mixing different amounts of carbohydrate with octadecylamine and

octadecene before being heated up to 70–300 °C for 10–30 minutes. The hydrophilic ones can be synthesized by heating an aqueous solution of carbohydrate within wide pH ranges. The hydrophilic CQDs with yellow and red emissions can also be synthesized by mixing an aqueous solution of carbohydrate with concentrated phosphoric acid followed with heating at 80–90 °C for 60 min [6].

In 2012, an interesting method was reported for the preparation of Fe₃O₄ magnetic nanoparticles (MNPs) by a *co*-precipitation method using sodium citrate and oleic acid as modifiers. Phase composition and microstructure analysis indicate that the sodium citrate and oleic acid have been successfully grafted onto the surface of Fe₃O₄ MNPs. The magnetic behaviors reveal that the modification can decrease the saturation magnetization of Fe₃O₄ MNPs due to the surface effect. Fe₃O₄ MNPs modified by sodium citrate and oleic acid show excellent dispersion capability, which should be ascribed to the great reduction of high surface energy and dipolar attraction of the nanoparticles [8].

Sugimot and Matijevic succeeded in synthesizing spherical Fe₃O₄ nanoparticles of narrow size distribution with average diameter between 30 to 100 nm by partially oxidizing Fe (II) salt with a base and a mild oxidant.[9]

Wulandari et al. used *ex situ* *co*-precipitation to produce and coat biomaterial (chitosan) Fe₃O₄ nanoparticles. A tripolyphosphate/sulfate combination was used to cross-link chitosan and stirring time to adjust the size of the nanoparticles. [10]

AG Magdalena et al. prepared EDTA-functionalized Fe₃O₄ nanoparticles at different temperatures and in an aerobic and oxygen-free environment. As a result, the temperature did not seem to significantly affect the stability of the phase. [11]

Ensafi, et al. reported an optical sensor based on carbon quantum dots (CQDs) coated with molecularly imprinted polymers (MIPs) for selective and sensitive determination of promethazine hydrochloride (PrHy). Water-soluble and fluorescent CQDs were synthesized by a simple, low cost and green approach using orange juice as a carbon source. The special advantage of the synthesized CQDs with this method is low toxicity. The surface of the CQDs was covered with MIPs matrix (CQDs-MIPs) *via* sol-gel polymerization. This sensor was used for determination of PrHy in real samples and showed satisfactory results [12].

Xiaona Li, et al. reported smart mesoporous silica nanoparticles (MSNs) coated with carbon dots (CDs) and poly(*N*-vinylcaprolactam) (PNVCL) as a mixed shell (CDs/PNVCL) polymer

grafted MSNs) for pH triggered anticancer drug release and real-time monitoring. The amino-terminated PNVCL and amino-rich CDs were grafted onto the surface of aldehyde group functionalized MSNs through Schiff base reaction. Doxorubicin (DOX) was loaded into the prepared nanoparticles. DOX could be quickly released in the tumor environment, leading to cell apoptosis. The linear fit between the percentage of released DOX and the fluorescence intensity of CDs indicated that the change in fluorescence intensity could be used to monitor drug release in real time. The as-prepared CDs/PNVCL polymer grafted MSNs are promising candidates for integrating controllable release and real-time monitoring in cancer treatment [13]

Guoping Li, *et al.* designed PEGylated CQD-DOX conjugate prodrug (DOXHy-CQD-SS-PEG) by conjugating DOX *via* acid-labile hydrazone linkage onto the few PEG brushes modified CQDs *via* bio reducible disulfide bond. Owing to the hydrophobic DOX conjugated CQDs and the few hydrophilic PEG brushes, comet-shaped conjugate prodrug was obtained with DOX content of 28.5%, and could self-assemble into unique micelles with mean diameter of 127 nm. The DOX-Hy-CQD-SS-PEG micelles possessed excellent pH/reduction dual responsive drug release with low drug leakage of 9% in 150 h. After DOX release and de-PEGylation in the simulated tumor intracellular media, high fluorescence intensity of the CQDs was recovered, demonstrating their potential application for real-time response of chemotherapy [14].

3. Experimental

3.1 Synthesis of carbon quantum dots

The C-dots were synthesized using a simple one-step hydrothermal reaction process, with polyacrylamide (PAM) as the primary carbon source. In a typical experiment, 0.1 g of PAM was vortexed in 40 mL of deionized water until homogenisation was achieved. The solution was then transferred to a Teflon-lined autoclave followed by hydrothermal treatment of 24 h at 270 °C. The reactor was then allowed to cool down to room temperature and the yellow solution was filtered to obtain clear dispersion of C-dots (Figure 3) [11].

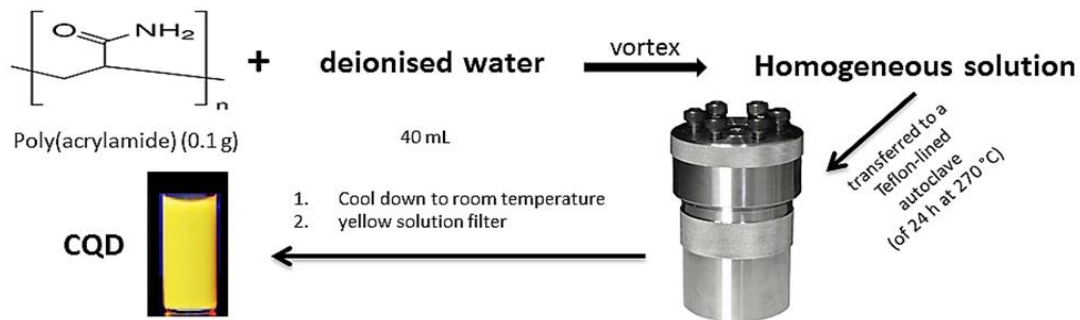


Figure 3. Preparation of CQDs from polyacrylamide (PAM) source

3.2. Synthesis of Fe_3O_4 nanoparticles

The Fe_3O_4 NPs were prepared using a simple chemical *co*-precipitation method. Briefly, 1.26 g of FeCl_2 and 3.24 g of FeCl_3 were solubilized together in the presence of glutaric acid (0.1 g). Chemical precipitation was then achieved by drop-wise addition of NH_4OH (1.0 M) until the pH of the solution reached 10. The formation of small black precipitates indicated the completion of reaction. The solution was then maintained at 80 °C for 5 h followed by high speed centrifugation to obtain nano-sized iron oxide. The as-synthesized Fe_3O_4 NPs were thoroughly washed with deionized water and ethanol/acetone to ensure removal of any unattached functionalizing agent. In this method, the glutaric acid was used to ensure size control of the NPs and to induce carbonyl functionality over the surface of Fe_3O_4 NPs, which would then facilitate their coupling with amine containing C-dots (Figure 4) [11].

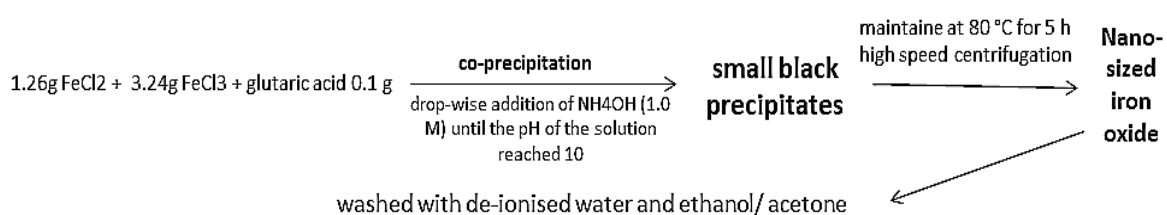


Figure 4. Preparation of Fe_3O_4 MNPs

3.3. Coupling of carbon quantum dots with Fe_3O_4 nanoparticles

To obtain a hybrid material of C-dots with Fe_3O_4 NPs, the glutaric acid functionalised Fe_3O_4 NPs (0.1 mg) were dispersed within 2 mL of pure C-dots. The mixture was allowed to shake overnight followed by centrifugation to collect the hybrid composites. Since the constituents of hybrid material were charged with favorable functionalities, the coupling of the Fe_3O_4 NPs

and C-dots is expected to be facilitated by electro-static interactions. The hybrid composites are hereon referred as C-dot/Fe₃O₄ for better readability [11].

3.4. Grafting of poly(*N*-vinylcaprolactam) onto CQD-Fe₃O₄ nanoparticles

CQD-Fe₃O₄@ poly(*N*-vinylcaprolactam) prepare by using a microfluidic system composed of a microfluidic chip and two automated pumps. Briefly, the organic phase (*i.e.* CQDs-Fe₃O₄ dispersed poly(*N*-vinylcaprolactam) solution) and the aqueous phase (*i.e.* HEPES buffer (10 mM, pH 7.4)) pump into the inner channel and the outer channels with the flow rates of 1mL/min and 5mL/min, respectively. The resulted nanoparticles dispersion collect in a beaker with magnetic stirring for 2-3 h to evaporate the organic solvent. To quantify the encapsulation efficiency (EE%) and loading capacity (LC%) of the CQDs in the nanoparticles, the resulted CQD-Fe₃O₄@ poly(*N*-vinylcaprolactam) dispersion was filtered by using 0.02 μm membrane filter.

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