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Sustained release profile of quatro stimuli nanocontainers as a multi sensitive vehicle exploiting cancer characteristics

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ABSTRACT

A versatile drug delivery carrier that responds to external stimuli was synthesized *via* the emulsion polymerization process. This simple two-step process was carried out by using Poly (Methyl Methacrylate) as a soft template and a series of monomers, with desired properties, as coating monomers. It is noteworthy that during shell fabrication (2nd step) an inner cavity is created inside the nanocontainers that can be used as a host for small drug molecules. The thermo-, pH- and redox sensitive monomers used in the coating procedure were Dimethyl Amino Ethyl Methacrylate (DMAEMA), Acrylic Acid (AA) and *N,N′*-(disulfanediylbis(ethane-2,1-diyl))bis(2-methylacrylamide) (Disulfide or DS), respectively. It has to be noted that DMAEMA is also pH- sensitive and acts synergistically with AA. The surface of the multi-stimuli nanocontainers was functionalized with magnetite nanoparticles in order to induce an alternating magnetic field (AMF) sensitivity. By using AMF in various strenghts and frequencies, the temperature of the final multi-stimuli nanocontainers (Q-NCs) can be increased in a controlled manner resulting in the Hyperthermia phenomenon. Loading and release studies were carried out using the anthracycline drug, Doxorubicin, aiming at the confirmation of the release mechanism.

1. Introduction

Everyday new smart Drug Delivery Systems (DDS) are fabricated seeking to improve cancer therapy as well as the patients' life. The development of these systems is substantial in order to achieve more effective treatment and fewer side effects [1–12]. Conventional treatments, like chemotherapy and radiotherapy, have the disadvantage of killing normal cells and destroying neighboring tissues, causing toxicity. The role of DDS is to avoid these side effects by treating only cancer cells. Some of the characteristics that can be used for fabricating an intelligent DDS are: 1) temperature difference between cancer cells and their surrounding area, 2) vulnerability of cancer cells at 42–43 °C in contrast to 45–46 °C for normal cells 3) difference between intra- and extra-cellular pH with values for cancer cells around 6.0–4.5 and 6.8, respectively and 6.5 and 7.4, respectively for normal cells, and finally 4) enzyme copiousness that creates reductive and oxidizing (redox) conditions. By combining all these factors [25], a smart nanocarrier can be fabricated aiming at specific targeting and drug release in a controlled manner. The idea of the aforementioned nanocarrier has been studied for the past few years and lots of ideas came to forefront. The thermo-, pH-, and redox sensitivities were used as a single property, or by combining two of the properties, for the fabrication of polymer nanocarriers [13–25]. These nanocarriers can be in the form of nanospheres, micelles, nanorods, nanoparticles, nanocontainers, etc. and are synthesized by monomers that have specific properties taking advantage of some unique characteristics of cancer cells. One of the many properties that are currently investigated and used for the fabrication of nanocarriers is thermo-sensitivity [26–29]. Hydroxy Propyl Methacrylamide (HPMA) and Dimethyl Amino Ethyl Methacrylate (DMAEMA) are monomers that exhibit thermo-sensitivity. Similar to thermo-sensitive monomers are, pH-sensitive monomers [30–32] like Acrylic Acid (AA) and redox-sensitive monomers [33–35] like *N,N′*-(disulfanediylbis(ethane-2,1-diyl))bis(2-methylacrylamide). An ideal combination of monomers can result to a co-polymer that integrates thermo- and pH- sensitivity, or thermo- and redox-sensitivity, or any other combination of the above properties. A

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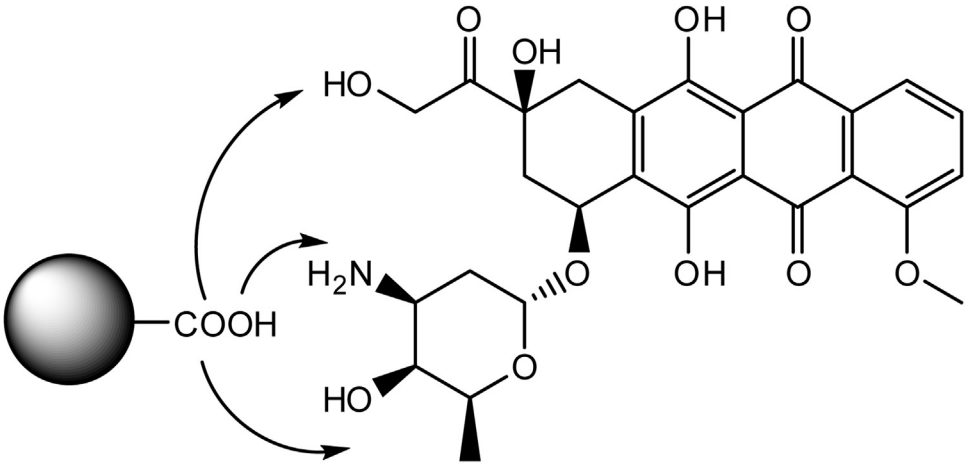
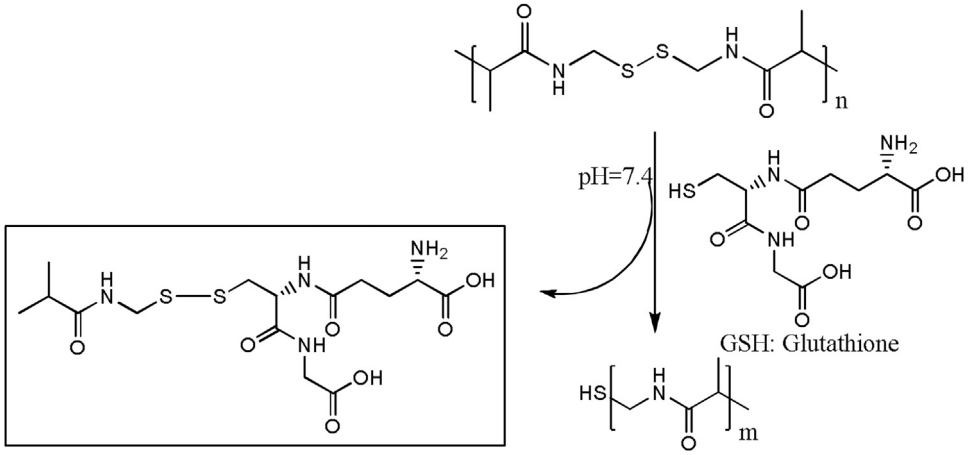


Scheme 1. The possible interacted groups between DOX and Q-NCs.



Scheme 2. The Disulfide segment degradation.

way to enhance the efficacy of the nanocarriers is to use magnetic nanoparticles either on the surface or in the interior of the nanocarriers. Magnetic nanoparticles, like Fe_3O_4 (magnetite), have been used extensively in medicine for cancer therapy [36–40], as Magnetic Resonance Imaging (MRI) contrast agents or for local Hyperthermia. According to Habash et al.[41], hyperthermia is the state where the temperature of the body (general) or of the tissue (local or regional) is abnormally increased above 37 °C for a certain period of time, aiming at the destruction of cancer cells. The influence of hyperthermia for human cancer cells has been stimulated by the consistent evidence that low pH exerts a major effect in sensitizing cultured cells to heat [24]. By using an alternating magnetic field (AMF) the temperature of the nanocarriers with the magnetic nanoparticles can be increased in a control manner to a desired temperature, adding this way one more capability to the smart DDS.

Great progress has been also achieved in the design of nanocarriers, that are able to selectively carry radionuclides, in order to improve the outcome of cancer diagnosis and/or treatment [42,43].

In this report we quote the synthesis and characterization of a versatile DDS with pH-, thermo- and redox sensitivity that can be used in hyperthermia treatment. We loaded the drug Doxorubicin, as model drug, in the hollow Q-NCs and studied the release behavior under external stimuli, such as pH, redox, temperature and hyperthermia (Schemes 1 and 2).

2. Materials and methods

2.1. Materials

Acrylic Acid (AA) and Dimethyl Amino Ethyl Methacrylate (DMAEMA) were purchased from Sigma Aldrich and distilled before their use. Divinyl Benzene (DVB) and Poly(ethylene glycol) methacrylate (average Mn = 360) (PEG-360) were also purchased from Aldrich and used as received. Methyl Methacrylate (MMA) which was purchased from Merck was freshly distilled before its use and Potassium persulfate (KPS) was purchased from Panreac and used as received. Ethylene Glycol (EG) provided by Merck, Iron (II) Chloride tetrahydrate ($FeCl_2 \times 4H_2O$) provided by Riedel-de Haën, Potassium Nitrate (KNO_3) provided by Acros and Hexamethylenetetramine (HETM) provided by Alfa Aesar were used as received. 95° ethanol was used as received. *N*,*N*′-(disulfanediylbis(ethane-2,1-diyl))bis(2-methylacrylamide) (Disulfide) was synthesized in our lab (See the synthetixc approach in Supl. material file). Doxorubicin·HCl was provided by Pharmacia & Upjohn and used as received. Phosphate