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Synthesis and characterization of modified starch hydrogels for photodynamic treatment of cancer

Mohammad Reza Saboktakin[a,b,*], Roya M. Tabatabaie[a], Parvaneh Ostovarazar[a], Abel Maharramov[b], Mohammad Ali Ramazanov[b]

[a] Nanostructured Materials Synthesis Lab., International Research Institute of Arian Chemie Gostar, Tabriz, Iran
[b] Nanotechnology Research Center, Baku State University, Baku, Azerbaijan

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ABSTRACT

The objective of the present study was to develop carboxymethyl starch (CMS) and dextran sulfate (DS) hydrogels that are able to efficiently encapsulate 5-,10-,15-,20-tetrakis(*meso*-hydroxyphenyl)porphyrin (mTHPP), a porphyrin-based PS agent. The study showed that the lifetime of the triplet state for porphyrin PS is significantly increase when encapsulate into hydrogel. In addition to the possible enhancement of $^1O_2$ generation, other advantages to incorporating porphyrin-based PS agents into hydrogel include the ability to solubilize these generally hydrophobic agents, the small and uniform size of hydrogels, and potential for passive targeting of solid tumors via the enhanced permeation and retention effect decreasing systemic photosensitization. This novel type of carboxymethyl starch (CMS) hydrogel using dextran sulfate (DS) as a polyanionic polymer was developed to achieve complex coacervation for the incorporation and controlled release of an anti-angiogenesis hexapeptide, this was the first report describing the use of DS to formulate CMS based hydrogels.

1. Introduction

Photodynamic therapy (PDT) is a light-activated treatment for malignant or premalignant cancer tumors and other diseases. It has emerged as important research area in biophotonics [1–3]. PDT is based on the fact that some photosensitizers (PSs) can be accumulated to a higher concentration in tumor cells than in healthy cells upon systemic administration. By matching the wavelength of the therapeutic light to the absorption peak of the sensitizers, most of the light is absorbed by the PSs, and the excited PS molecules can then transfer their energy to surrounding oxygen molecules, which are normally in their triplet ground state [4]. These results are in the formation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) or free radicals. ROSs are responsible for oxidizing various cellular compartments, resulting in interversible damage to tumor cells. PSs, oxygen, and light are three crucial components for this photon-induced toxicity effect [5–7]. Most exiting PSs are hydrophobic and may aggregate easily in a biological environment. Even for hydrophilic PSs, the selective accumulation in tumor tissues is not high enough for clinical use. To overcome these limitations, colloidal carriers for PSs, such as liposomes and polymeric micelles, have been investigated [8]. This study described in this paper, carboxymethylstarch (CMS)–dextran sulfate (DS) hydrogels with encapsulated mTHPP, which has been widely investigated and officially approved for use in clinical treatments were synthesized and utilized as drug carriers. The PS-encapsulated CMS–DS hydrogels can be easily prepared with desired size, shape, and porosity, and are very stable [9]. They are more biocompatible than other nanomaterials, and they can effectively protect the PSs against denaturation induced by the extreme bioenvironment [10]. The CMS–DS hydrogel encapsulation is relatively transparent for both the activated light and signal light. To accumulate PSs selectively in tumor tissues and reduce the dosage of PSs, their surfaces can be functionalized with various groups and further conjugated with antibodies for specific uptake in an in vivo experiment [11–15]. In this paper, we reported an investigation that used CMS–DS hydrogels with encapsulated mTHPP for PDT treatment. The CMS–DS hydrogels were synthesized, characterized, and utilized for in vitro imaging of tumor cells. The effect of photon-induced toxicity of this hydrogels was also demonstrated after comparison with some control experiments [16]. The mTHPP-encapsulated CMS–DS hydrogels showed good potential applications to, e.g., photodynamic therapy for cancers. One potential challenge of PDT therapy is that many PS agents are lipophilic, making parenteral administration problematic [17–19]. In addition, systemic administration of a PS leads to generalized photosensitivity and the temporary need to avoid light exposure. Various strategies to overcome these

* Corresponding author at: International Research Institute of Arian Chemie Gostar, Tabriz, Iran. Tel.: +98 411 6694803; fax: +98 411 6694803.
E-mail address: saboktakin123@yahoo.com (M.R. Saboktakin).

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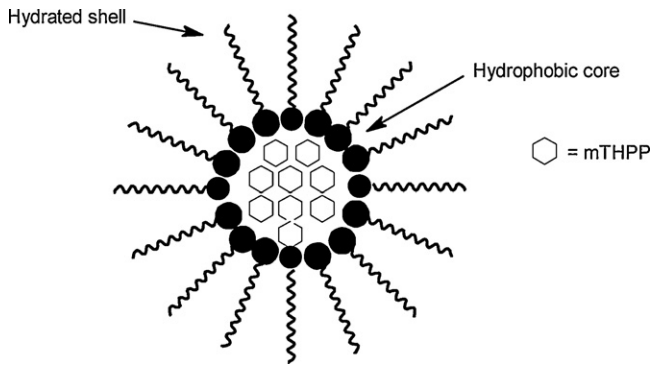

Scheme 1. Chemical structure of CMS–DS hydrogels and mTHPP.

limitations have been investigated, including conjugation of PS agent to water soluble polymers and colloidal administration, as well as encapsulation in hydrogel carriers such as micelles. Recent progress has been made in the design of polymeric hydrogels for nanoscale therapeutic and diagnostic applications [20–23]. Polymeric hydrogels are composed of amphiphilic block copolymers that contain distinguished hydrophobic and hydrophilic segments. The distinct chemical nature of the two blocks results in thermodynamic phase separation in aqueous solution and formation of nanoscale supramolecular structures [24–28]. A new type of carboxymethyl starch (CMS) hydrogel using dextran sulfate (DS) as a polyanionic polymer was developed to achieve complex coacervation for the incorporation and controlled release of an anti-angiogenesis hexapeptide, this was the first report describing the use of DS to formulate CMS based hydrogels. Although there have been investigations of how the properties of CMS and formulation variables such as CMS molecular weight (M_W), concentrations of CMS and mTHPP, and formulation pH affect the formation and encapsulation capability of hydrogels, to our knowledge, no attempts have been made to study how the ratio of CMS to the oppositely charged polymer influences the formulation and properties of hydrogel. This system is able to target different organs and control the release of the PS molecules by the incorporation of site-specific moieties. The system is based on a CMS–DS/mTHPP conjugate that uses for PDT [29,30] (Scheme 1).

Many functional properties of CMS–DS such as paste and gel storage stability are significantly improved. The studies of effects of degree of substitution starch on the physicochemical and properties of CMS–DS hydrogel have been revealed that when degree of substitution of hydrogel increased from 0.10 to 0.20 both viscosity and freeze–thaw stability of hydrogel decreased. The amylase content of starch influences many physicochemical and functional properties of starch products. It is also worthwhile to investigate whether amylase content plays role on the properties of hydrogel with similar degree of substitution level [31].

2. Materials and methods

2.1. Materials

The cornstarch was purchased from Merck Co., Germany. Sodium salt of dextran sulfate (M_W 12,750 Da), and mTHPP were purchased from Sigma Chemical Co. All other solvents and materials were of analytical grade. Deionized water (Milli-Q water) was used in the preparation of buffers and standard solutions. All other chemicals and reagents used in this study were of analytical grade. Sodium hydroxide and chloroacetic acid were used as received.

2.2. Instruments

Melting points were obtained on a Mel-Temp melting point apparatus. Analytical TLCs were run on commercial Merck plates coated with silica gel GF250 (0.25 mm thick). The amount of released drug was determined on a Philips PU 8620 UV spectrophotometer at the absorption maximum of the free drug in aqueous alkali, using a 1 cm quartz cell. The nanoparticle samples were obtained by Freeze dryer Model FD-10 (Pishtaz Engineering Company). The samples were examined to determine the mean diameter and size distribution. The powder morphology hydrogel in the form of pellets (to measure grain size) was investigated using Philips XL-30 E scanning electron microscope (SEM) at 30 kV (max). The samples were prepared by physical vapor disposition method. The gold layer thickness were about 100 Å at these samples.

2.3. Preparation of carboxymethylstarch (CMS)

Firstly, the 0.5 g corn starch and 120 mL 2-propanol were placed in a 500 mL vessel and stirred fro 2 h. The 5 g sodium hydroxide was added and reacted for 1 h at 78–80 °C. After that, the 10 g chloroacetic acid was added to the vessel and stirred for another 2 h at 50 °C. The product was filtered and washed several times with ethanol, then dried under vacuum. The resulting CMS was crushed in a mortar [degree of substitution (DS) = 0.49].

2.4. Preparation of CMS–DS hydrogel with mTHPP

Copolymer (50 mg) and mTHPP (10 mg) were dispersed with stirring in 25 mL deionized water. After approximately 180 min, the sample was sprayed into a liquid nitrogen bath cooled down to 77 K, resulting in frozen droplets. These frozen droplets were then put into the chamber of the freeze-dryer. In the freeze drying process, the products are dried by a sublimation of the water component in an iced solution.

2.5. Measurement of stability of mTHPP-loading in hydrogel

Free mTHPP was removed from the hydrogel solution by three cycles of centrifugal filtration at 4 °C (Amicon Ultra, [Millipore Corporation, Billerica, MA], M_W cut off = 10 kDa). Total concentration of free mTHPP in the combined filtrate was then determined by obtaining its UV–vis absorbance (λ_{max} = 418 nm, ε = 546.2 mL/(cm mg)). Hydrogel solutions were then freeze-dried, weighed, dissolved in THF, and analyzed via UV–vis spectrophotometry to determine the total amount of encapsulated mTHPP. Hydrogel yield, mTHPP loading efficiency and loading density were determined utilizing the following equations:

$$\%\,\text{net mTHPP loading effieciency} = \frac{\text{mTHPP weight}}{\text{theoretical hydrogel weight}} \times 100$$

$$\%\,\text{hydrogel yield} = \frac{\text{total hydrogel weight} - \text{free mTHPP weight}}{\text{total hydrogel weight}} \times 100$$

$$\%\,\text{mTHPP loading density} = \frac{\text{mTHPP weight}}{\text{theoretical hydrogel weight} - \text{free mTHPP weight}} \times 100$$