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Starch-based microspheres for sustained-release of curcumin: Preparation and cytotoxic effect on tumor cells

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ABSTRACT

Curcumin (CUR) has been proved to be highly cytotoxic against different tumor cell lines. However, its poor solubility in aqueous medium and fast degradation in physiological pH are the common drawbacks preventing its efficient practical use. Herein, we report the development of original microspheres based on the biopolymer starch crosslinked with N,N-methylenebisacrylamide (MBA) to be applied as an efficient delivering system for CUR. The starch-based microspheres showed high loading efficiency even in loading solution with different CUR concentrations. In vitro release assays data showed that the CUR release is governed by anomalous transport ($n = 0.73$) and it is pH-dependent. Cytotoxicity assays showed that starch microspheres could improve the cytotoxicity of CUR toward Caco-2 and HCT-116 tumor cell lines up to 40 times than that found for pure CUR. This behavior was attributed to the slowly and sustained release of CUR from the microspheres.

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1. Introduction

Curcumin, *1,7-bis-(4-hydroxy-3-methoxy-phenyl)-hepta-1,6-diene-3,5-dione*, is a natural pigment derived from an active component of *curcuma longa* (Mandeville, Froehlich, & Tajmir-Riahi, 2009; Tang et al., 2010). It has been demonstrated by several studies that curcumin (CUR) has a wide range of biological and pharmacological activities, especially against cancer propagation and prevention (Cabrespine-Faugeras, Bayet-Robert, Bay, Chollet, & Barthomeuf, 2010; Radhakrishna Pillai, Srivastava, Hassanein, Chauhan, & Carrier, 2004). Although, CUR has great efficiency in the treatment of some human tumors its water insolubility and instability limits its application (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). The hydrophobic nature disables, for instance, its vascular and oral administration (Sharma, Gescher, & Steward, 2005). One alternative to overcome such limitations is the application of an adequate releasing system (hydrogels, dendrimers, liposome, micro, and nanoparticles) for CUR delivering. The employment of such systems in controlled drug delivery has been widely explored for long time (Sun, Shoji, Lu, Liotta, & Snyder, 2006; Vemula, Cruikshank, Karp, & John, 2009). A variety of polymeric materials (synthetic and natural) have been used to synthesize/prepare efficient carriers (Jain, Gupta, & Jain, 2007; Saito et al., 2005). That was possible because polymeric materials include a huge variety of molecules, which can enable adhesion at the delivered site and allow the sustained drug release. Additionally, functional components can be combined with the polymeric carrier to target the drug or to control the releasing rate, for instance.

A promising natural polymer widely applied in several fields that shows interesting physical, chemical and biological features is starch. Starch is a complex polysaccharide composed by amylose and amylopectin, two macromolecules that have different characteristics. Amylose is a linear macromolecule consisting of α-D-glucopyranose units linked through (1 → 4) linkages and has a molecular weight of 10^5–10^6 g/mol. Amylopectin is a branched macromolecule formed by short chains linking linear chains via (1 → 6) linkages and has molecular weight of 10^6–10^7 g/mol (Namazi & Dadkhah, 2010) that possesses hydrophilic nature. It is important to emphasize that starch has natural abundance, low cost of production, renewability, biocompatibility and biodegradability, features that support its application in the development of drug delivery systems. In order to improve and/or create its end-use properties, starch can be chemically modified (Angellier, Molina-Boisseau, Dole, & Dufresne, 2006; Xu et al., 2010). One starch modification example is the introduction of chemical groups on the

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starch chains to allow its crosslinking with other polymer (Kim, Cho, & Park, 2001). The crosslinked network improves considerably the mechanical properties and the water resistance of starch-based materials. Furthermore, some reports show that crosslinked starch chains acquires resistance against enzymatic hydrolyzes enabling it to be used, for instance, as carrier in oral drug delivery systems (Chen et al., 2007; Reis et al., 2008). Taking into account these considerations, we present the development and characterization of original microspheres based on chemically modified starch crosslinked with *N,N′*-methylenebisacrylamide (MBA) to act as an efficient deliverer of CUR.

2. Materials and methods

2.1. Materials

Cassava starch was kindly supplied by INPAL S.A. (São Tomé, Brazil) M_w 5.4×10^3 kDa determined by GPC/SEC as described elsewhere (Pereira, Gouveia, de Carvalho, Rubira, & Muniz, 2009). Glycidyl methacrylate (GMA, 97% CAS: 106-91-2), curcumin from *curcuma longa* (Turmeric) (CUR, CAS: 458-37-7), sodium dodecyl sulfate (SDS, CAS: 151-21-3), and sodium persulfate (SPS, CAS: 7775-27-1), were purchased from Sigma–Aldrich (St. Louis, USA). Sunflower oil (CAS 8001-21-6) was purchased from Cocamar (Maringá, Brazil). *N,N*-methylenebisacrylamide (MBA, CAS: 110-26-9) was purchased from Biorad (Hercules, USA); and sodium hydroxide (NaOH, 95%, CAS: 1310-73-2), acetone (99.5%, CAS: 67-64-1), absolute ethanol (99.5%, CAS: 64-17-5), hexane (99%, CAS 110-54-3), dimethyl sulfoxide (DMSO, 99%, CAS 67-68-5), and tetrahydrofuran (THF, CAS 109-99-9) were purchased from Nuclear (São Paulo, Brazil). All materials and reagents were used as received.

2.2. Synthesis of chemically modified starch (starch-mod)

Starch was chemically modified adapting the methodology developed by Reis et al. (2008). Briefly, starch (4 g) was solubilized in distilled water/DMSO mixture (ratio 1:1) at 80 °C under vigorous stirring. After complete solubilization, the temperature was reduced to 60 °C and the pH was adjusted to 10.5 with NaOH solution (0.2 M). GMA (4 ml) was added to the reaction system, which was kept under mechanic stirring at 60 °C for 24 h. The chemically modified starch (starch-mod) was recovered by precipitation in ethanol. The precipitated material was separated by vacuum filtration. At last, the starch-mod was washed several times with ethanol and water portions to complete eliminate unreacted materials and then it was lyophilized (at −55 °C and 0.1 mBar for 12 h).

2.3. Starch-mod characterization

2.3.1. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of raw starch, GMA, and starch-mod was recorded using a Shimadzu 8300 FTIR spectrophotometer operating in the region from 4000 cm^{-1} to 500 cm^{-1} with resolution of 4 cm^{-1} and 64 scan acquisitions. The dried samples were mixed with KBr powder and pressed into pellets for spectrum acquisition.

2.3.2. Nuclear magnetic resonance (NMR)

^1H NMR spectra of raw starch, GMA, and starch-mod were recorded on Varian 300 MHz unity (Oxford, model 300). Approximately, 10% sample solutions in DMSO-d_6 (raw starch and starch-mod samples) and $CDCl_3$ (GMA sample) were prepared in 5 mm tubes with samples referred to tetramethysilane (the tetramethysilane signal was suppressed from the ^1H NMR spectra). Chemical shifts in ppm were obtained by first-order analysis of spin patterns (here it was considered that the proton is only coupled to other protons that are far away in chemical shift) for ^1H NMR experiments with the following conditions: number of data points 12 K; relaxation delay 30 s; angle pulse 90°; acquisition time 5 s; temperature 298 K; number of scans 32.

2.4. Microspheres preparation

Microspheres based on modified starch (starch-mod) crosslinked with MBA were prepared in water/oil emulsion system. 2 g of starch-mod were dissolved in distilled water (90 ml) at 80 °C to form the aqueous phase. The solution was cooled to room temperature and MBA (40 mg) and SPS (0.25 mg) were added. The oleaginous phase, composed by sunflower oil (90 ml), was deposited in a round bottom flask coupled to a mechanical stirrer apparatus. Sodium dodecyl sulfate (SDS – 3.5 g) was added as emulsifier and the system was stirred for 15 min (1000 rpm) before the temperature was increased to 90 °C. Both phases were deoxygenated through N_2 bubbling for 15 min. The aqueous phase was poured slowly to the oil phase, and then the stirring was increased to 2000 rpm and kept for 30 min to crosslink. Finally, the system was cooled to room temperature and the microspheres were recovered by filtering under vacuum. The recovered microspheres were washed several times successively with portions of hexane (2× 20 ml) to eliminate some oil residue, acetone (2× 20 ml), ethanol (2× 20 ml) and water (2× 20 ml) to eliminate the non-reacted chemicals (MBA, sodium persulfate, SDS, starch-mod). Then, microspheres were lyophilized before analyses.

2.5. Microspheres characterization

2.5.1. Scanning electron microscopy (SEM)

The morphology was investigated by SEM images (Shimadzu, model SS 550, Japan). The microspheres were sputter-coated with a thin layer of gold and image acquisition was taken by applying an electron accelerating voltage of 12 kV. The microspheres average diameter was calculated by the Size Meter© software with differentiation threshold set according to the image scale.

2.5.2. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy and analytical procedures were performed according to parameters described in the previous section.

2.5.3. Thermogravimetry (TGA)

TGA was performed on freeze-dried samples from 25 °C to 600 °C at a heating rate of 10 °C/min under 30 ml/min of flowing N_2 in a thermogravimetric analyzer (Netzch, model STA 409 PG/4/G Luxx, USA).

2.6. Curcumin loaded microspheres

CUR (1.25 mg) was solubilized in water/THF (ratio 1:1, 100 ml) to prepare the stock solution. After, 1.0 g of microspheres was deposited in that solution. The system microspheres/CUR stock solution was kept under magnetic stirring overnight at room temperature. So, the loaded microspheres were removed by high-speed centrifugation and oven-dried at 40 °C for 24 h. The stock solution was analyzed through UV spectroscopy technique to evaluate the loading efficiency. The supernatant was analyzed at 430 nm using a UV-Vis Femto (model 800Xi, Brazil) apparatus. Using a previously built analytical curve, the concentration of CUR remaining in the supernatant was calculated. The analytical curve was designed from standard CUR solutions varying from 0.17 to 7.68 mg/l using water/THF (ratio 1:1) as solvent. The linear correlation coefficient

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