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Synthesis of colloidal delivery vehicles based on modified polysaccharides for biomedical applications

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ABSTRACT

Recent years research in the field of medicinal chemistry, led to the development of drug delivery systems to improve the efficacy and effectiveness of various therapeutic agents for a variety of diseases. Such systems are used for the transport of diagnostic and therapeutic factors, either experimentally or clinically. Particularly, the use of colloidal carriers made of polysaccharides, has emerged as a promising alternative, for improving the transport of pharmaceutical agents on pathogenic areas. In the present work, bio-compatible microspheres-with a synthetic polymeric core and three layers of polysaccharides [PMMA@HPC@CS@CH microspheres (Poly(methylmethacrylate)@hydroxypropyl cellulose@cellulose succinate@chitosan)] were synthesized by combining two techniques. PMMA nanospheres were used as a core in order to coat the surface with polysaccharides *via* layer by layer deposition. This composition is advantageous in comparison with other synthetic routes because at all stages of the synthesis, it is used only water as a solvent and not organic solvents and includes all of the unique properties of two polysaccharides (cellulose and chitosan) in one vehicle. Fabricated vehicles were characterized structurally by FT-IR spectroscopy and morphologically by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Finally, daunorubicin an anticancer agent was encapsulated as a drug model into the vehicles in order to evaluate their loading and releasing behavior.

Polysaccharides have several advantages to be used as raw materials for synthesis of advanced delivery systems [1]. These systems exist lack of toxicity, presenting pretty good biocompatibility and low production cost [2]. The combination of polysaccharides with synthetic co-polymers provide the advanced materials with adequate biochemical and mechanical properties [3]. Regarding the polysaccharides' benefits, these materials can be used to improve drugs and macromolecules' lifetime in the body, increasing the absorption of entrapped molecules. Combining these materials in smart carriers, it can be fabricated a multi-functional delivery system. For all these qualities, natural polysaccharides are ideal raw materials for synthesis of promising biomaterials in the field of delivery systems [4,22,23].

Hydroxypropyl cellulose (HPC) is a biocompatible, thermosensitive derivative of cellulose [5,6]. It is soluble in water and organic solvents. Also, it presents sensitivity in temperature variations changing their hydrophilic/hydrophobic behavior, with a minimum critical solubility temperature (LCST) at 41 °C in water [6]. Hydroxypropyl cellulose has applied in ophthalmic formulations [7], mainly as artificial tears and presents great antimicrobial activity.

Although chitosan was discovered by the early 19th century, only the last two decades it is used in biology and medicine, emphasizing in drug delivery systems. Microspheres with chitosan have been synthesized by various techniques and have been used to entrap drugs [8,9] and biomolecules such DNA [10], releasing them in a controlled manner. Additional, it improves the solubility of water-insoluble drugs and their modifications can bring specific ligands for peptides or antibodies [11], magnetic nanoparticles [12], *etc*. enhancing their targeting ability [13]. Chitosan is a pharmacological active natural polymer, enhancing wound healing [14] and presents antimicrobial activity also [15]. In conclusion, biological, chemical and mechanical properties of chitosan showing this as a promising material for the synthesis of drug delivery systems.

In this paper biocompatible microspheres PMMA@HPC@CS@CH were synthesized by combining the below mentioned techniques. Initially, PMMA nanospheres synthesized by emulsion polymerization of methyl methacrylate. Continuously, PMMA nanospheres were used as core for the polysaccharides' coating by sequential deposition technique of cortices (layer by layer deposition) [16].

The first shell formed by deposition of hydroxypropyl cellulose, while the second shell was developed by succinic acid modified cellulose. The last coating was fabricated by chitosan deposition aiming at improving the microsphere-proteins interactions. The intermediate layer was selected to be formed by use of modified cellulose which

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functions as cross-linker for stabilization purposes between the other two layers. This stabilization has performed by crosslinking between the modified cellulose and hydroxyl propyl cellulose *via* ester bonds and between chitosan through amides bonds formation. This composition is advantageous in comparison to the literature, due to the fact that in all synthetic stepes, water is used as solvent, avoiding the use of organic solvents. The fabricated vehicles were characterized structurally by FT-IR spectroscopy and morphologically by scanning and transmittance electron microscopy. Finally, the encapsulation of the anticancer drug daunorubicin has been carried out and the accomplished loading and releasing mechanisms were investigated.

In a round bottom flask were added 28 ml H_2O and allowed to warm to 70 °C for 30 min with vigorous stirring in a nitrogen flow. After the solution conditions were stabilized methyl methacrylate (MMA) (3 ml) was added and the mixture was left for additional 30 min. Continuously, the KPS initiator ($K_2S_2O_8$, 0,015 g) ($t_{1/2}$ = 8 h at 70 °C) was added. The reaction was left over night for completion and the milky emulsion was centrifuged for the nanospheres' isolation. The emulsion was centrifuged by various cycles of re-suspension in water (3 × 8000 rpm for 5 min) [17].

0.4 g of hydroxypropyl cellulose (HPC) dissolved in 50 ml acidic solution (aqueous solution of HCl, pH 3). 0,4 g of spheres of PMMA were dispersed in 50 ml aqueous solution (aqueous solution of NaOH, pH 10). The mixture was left under stirring at room temperature for 24 h at 50 °C. The resulting emulsion was centrifuged and purified *via* various re-suspension cycles in water (4 × 10,000 rpm for 5 min).

0.1 g of cellulose succinate [18] dissolved in 50 ml of basic solution (aqueous solution of NaOH, pH 10). In a 250 ml beaker 0,4 g of HPC coated spheres were added and dispersed in 50 ml of acidic aqueous solution (aqueous solution of HCl, pH 3). The suspended microspheres were added to the aqueous solution of cellulose. The mixture was left under stirring at room temperature for 24 h. The resulting emulsion was centrifuged and re-suspended in water for purification (3 × 10,000 rpm for 5 min).

0.05 g of chitosan was dissolved in 25 ml aqueous acidic solution (aqueous solution of HCl, pH 3). 0.4 g of modified spheres dispersed in 50 ml basic aqueous solution (aqueous NaOH, pH 10) and the dispersion added to the aqueous solution of chitosan. The mixture was left under continuous stirring for 24 h at room temperature. The resulting emulsion was centrifuged and re-suspended in water for further purification (3 × 10,000 rpm). Aiming at crosslinking the three layers which are deposited on vehicle's surface, 0.4 g of coated PMMA@HPC@CS@CH spheres dispersed in 2 ml water. In the resulting emulsion were added aqua solution of EDC (10 mg, 0.06 mmol) and NHS (5 mg, 0.04 mmol).

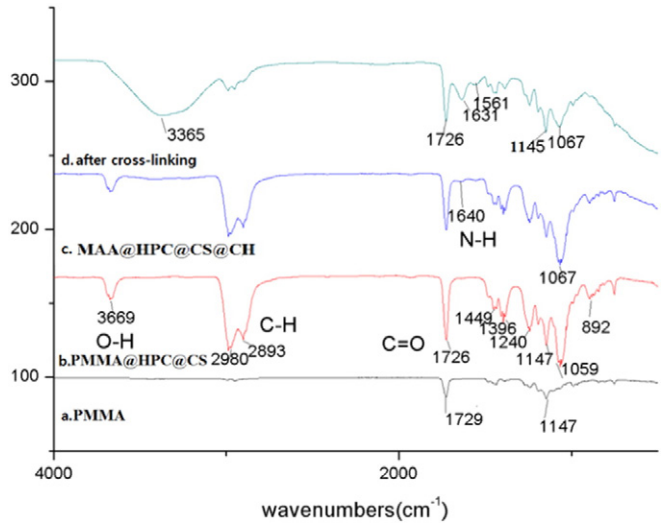

Fig. 1. FT-IR spectra for each step of synthesis.

The mixture was left for 30 min under vigorous stirring. Then the final mixture was centrifuged (3 × 10,000 rpm for 5 min) and purified after suspended in water.

FT-IR spectra at various stages of the synthesis are presented in Fig. 1. The new observed peak appears at 3669 cm^{-1} assigned to the vibration of the C—H bond of hydroxypropyl cellulose's propyl group , and the peaks at 2980 and 2893 cm^{-1} can be assigned to the vibrations of the aliphatic bonds C—H. The sharp peak at 1726 cm^{-1} corresponding to the carbonyl absorption of methyl methacrylate and succinic acid segment of modified cellulose. In addition, the peak at 1059 cm^{-1} can be attributed to the vibrations of C—O—C pyranose ring (structural units of cellulose). Finally, the characteristic peak at 892 cm^{-1} is assigned to beta-glycosidic bonds between the structural units of the cellulose. The successful deposition of chitosan is confirmed by the appearance of the peak at 1640 cm^{-1} in spectrum 1c due to the vibration of N—H bond of chitosan. 1d. spectrum obtained the material after crosslinking of the layers. Successfull crosslinking was confirmed by the appearance of the peak at 1631 cm^{-1} due to the absorption of the carbonyl amide bond and the absence of the peak at 3669 cm^{-1} due to vibration of O—H group of HPC. The absence of peak confirms that atributed to hydroxyl groups of hydroxypropyl cellulose and the carboxylic acids of the CS confirms the ester bond formation.

Scanning (SEM) and transmission electron microscopy (TEM) images were obtained on an FEI Inspect microscope with W (Tungsten) filament operating at 25 kV and a FEI CM20 microscope operating at 200 kV, respectively. The spectra were scanned over the range 4000–500 cm^{-1}. UV–vis absorption spectra in the wavelength range of 200–800 nm were obtained on a Jusco V-650 spectrometer, UV–vis at 480 nm spectrometer. An ultrasonic bath was used for sonication (Elma Sonic, S. 30H).

In Fig. 2, it is observed the configuration of polymethyl methacrylate microspheres. The microspheres diameter ranges at 200 ± 15 nm with low polydispersity. In the next step, coated spheres with hydroxypropyl cellulose are presented in Figure B. It is observed an increasing in initial diameter confirming the successful deposition. After coating with modified cellulose, their diameter of vehicles is increased to 300 ± 30 nm, while is further increased to 350–370 nm after coating with chitosan (Fig. 2D).

Fig. 3, presents TEM images of spheres PMMA@HPC@CS@CH. Inside, with a dark color is distinguished the core of Polymethyl methacrylate and with a light gray layer are distinguished the deposited layers of polysaccharides. Typical configurations of spheres are due to the intermolecular hydrogen bonds that grow between the spheres. It is also observed that the coating is relatively uneven and varies forming core-shell morphology.

The drug encapsulation is a process of integration of the pharmaceutical compound in a polymeric network or in a capsule. The entrapment of the drug can be done by two methods: either during the synthesis of the carrier or through by carriers' incubation in the appropriate buffer drug solution. The method chosen determines the mechanism and rate of drug release. The drug releases in a controlled manner *via* inverse process by which drug molecules existing from the solid phase and the polymeric network can now act as a smart carrier.

Entrapment and drug release is closely related processes which depends on the physicochemical properties of the carriers (size, molecular weight, porosity, and reactive groups), physicochemical properties of the entrapped drug compound (hydrophobicity/hydrophilicity, functional groups) and finally the influence of environment (pH, temperature, ionic strength, redox environment). The drug may be encapsulated in carriers through intermolecular interactions due to the polymer network, such hydrogen bonds, ionic and intermolecular dipole-dipole interactions. It can also be trapped by adsorption on the surface. In most drug delivery systems the loading and release mechanisms, can follow different mechanisms simultaneously [19–21].

In this study, we investigated the ability of synthesized microspheres to entrap a pharmaceutical compound, particularly the anticancer drug, daunorubicin (Daunorubicin Hydrochloride DNR).