Materials Science and Engineering C 70 (2017) 169–177

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Cationic cyclodextrin/alginate chitosan ***nanoflowers*** as 5-fluorouracil drug delivery system

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ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form 3 August 2016
Accepted 27 August 2016
Available online 29 August 2016

Keywords:
Cationic-β-cyclodextrin
5-Fluorouracil
Nanoflowers
Alginate
Chitosan
Inclusion complex and oral delivery system

ABSTRACT

Cyclodextrins (CDs) have widely been used as component of drug delivery systems. However unmodified cyclodextrins are associated with cytotoxicity and poor water solubility thus limiting their use in pharmaceutical industry. The cationic-β-cyclodextrin (Cat-β-CD) polymer cores were synthesized using β-CD, epichlorohydrin and choline chloride *via* a one-step polycondensation process. The main aim of this study was to synthesize hierarchical ***nanoflowers*** composed of cationic-β-CD as polymeric core along with alginate and chitosan "petals" (Cat-β-CD/Alg-Chi ***nanoflowers***) as carriers for oral delivery of 5-Fluorouracil (5-FU) *via* an ionic-gelation technique. The drug loading capacity, particle size, zeta potential and surface morphology of the synthesized ***nanoflowers*** were determined. The prepared ***nanoflowers*** were formed with an average size of 300 nm and a zeta potential of +9.90 mV with good encapsulation efficiency of up to 77.3%. *In vitro* release of 5-FU from the loaded ***nanoflowers*** showed controlled and sustained release compared to the inclusion complex alone. Cat-β-CD/Alg-Chi ***nanoflowers*** were assessed against L929 cells and found to be effectively inhibiting the growth of L929 cells in a concentration dependent manner.

1. Introduction

An antimetabolite, 5-Fluorouracil (5-FU), has been used as an anticancer agent against various cancers like those of the colon or rectum for many years [1–3]. The main challenge of using 5-FU as with many other chemotherapy agents is its short biological half-life, toxic side effects and death of normal healthy cells causing side effects such as hair loss, fatigue, ulcers, and liver disorders [4]. Out of all delivery system, the oral route still continues to be most viable pathway for administration of drugs, especially where numerous doses are obligatory because it is non-invasive, avoids injections and risks associated with intravenous or subcutaneous delivery, such as acute infection. It has been suggested that chitosan nanoparticles can be used an as effective delivery system to overcome the side effects caused by the 5-FU [5]. In a recent study, pH responsive, (Tripolyphosphate) TPP-crosslinked 5-FU encapsulated chitosan nanoparticles were evaluated and computational experiments were conducted to judge whether it could be used as drug delivery agents for anticancer therapy [6]. Dubey & Parikh, (2004) prepared chitosan microspheres of 12 μm size loaded with 5-FU [7] whereas, B. Arica et al. developed alginate bead nanoparticles loaded with 5-FU for breast cancer [8]. A poly-(ε-caprolactone) delivery system for 5-FU has also been developed for anticancer therapy where binding of 5-FU onto poly-(butylcyanoacrylate) nanoparticles showed enhanced efficacy towards anticancer activity [9]. All the systems mentioned above seem to face disadvantages associated with low encapsulation of the drug or low stabilization of the nanoparticles. It seemed to us that there would be an advantage in exploiting the best aspects of these current systems in order to overcome some of the limitations.

To achieve oral drug delivery for 5-FU, a nano drug delivery system is required to have certain important properties including: a) substantial biocompatibility b) protection from enzymatic degradation c) reduced leakage of drug during transportation d) controlled release of drug using biodegradable and biocompatible polymers e) reduced toxicity and f) increased therapeutic efficiency of the drug with reduced dosage frequency [10–12]. Ideally, the development of a nanocarrier system with controlled release could enhance the therapeutic drug effect at the specific target site and reduced dosage frequency leading to a better patient compliance and shorter duration of treatment [13,48].

Cyclodextrins (CDs) have attracted a great deal of attention in the pharmaceutical industry not only for their structure but also for their unique characteristics. CDs are not easily hydrolyzed or absorbed in the stomach or intestine but can be fermented to small saccaharides

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http://dx.doi.org/10.1016/j.msec.2016.08.073
0928-4931/

by colonic microflora [14]. These characteristics are attractive for an ideal specific carrier delivery system to the colon. CDs when conjugated with the drug can reach the colon in an intact form and will release the drug after enzymatic degradation by micro-organisms in the lower intestinal tract without getting absorbed in the stomach. Udo et al. (2010) synthesized 5-FU/β-CD conjugates through ester or amide bond linkages and evaluated the drug release behavior for colon specific delivery [15].

β-cyclodextrin (β-CD) is made up of 7 glucopyranose units with hydrophobic cavity interior and hydrophilic exterior. Cyclodextrins are known to form inclusion complexes with organic compounds thus changing the physicochemical properties such as solubility, stability and bioavailability by non-covalent interactions [16]. CDs can break down to smaller derivatives and can get easily absorbed in the large intestine making them an ideal carrier for oral administration of drugs [17]. However, unmodified cyclodextrins are associated with reduced solubility and some hepatotoxicity, which reduces their application in the pharmaceutical industry [18]. To overcome this problem various cyclodextrin derivatives like hydropropyl-β-cyclodextrin (2HPβ-CD) and sulfobutylether-β-cyclodextrin (SBEβ-CD) have been developed. Cationic cyclodextrins have gained more attention due to their high solubility and stability. One of the most prominent groups of cationic cyclodextrins used in this study is the polycationic derivative that can be synthesized using a one-step poly-condensation method [19]. This cyclodextrin derivative has greater solubility in water as compared to parent β-CD. The inimitable trait of this cationic cyclodextrin makes it an ideal host to perform dual function during synthesis. Firstly, it acts as a host to form an inclusion complex with 5-FU and secondly its polycationic nature promoted the formation of ***nanoflowers*** (by ionic gelation technique). Cationic cyclodextrin polymers have been reported to play a safe role of drug delivery for gene delivery [9]. This system has also been shown to be an ideal, controlled released system for protein delivery such as insulin, by forming a complex with cationic cyclodextrin and encapsulation of the complex within the polymers [20].

A polyelectrolyte complex formed by alginate and chitosan is very well known [21]. Alginate and chitosan have received great attention in the pharmaceutical field because of their biological properties such as biodegradability, biocompatibility and non-toxicity. Alginate is a hydrophilic polymer made up of D-mannuronic (M) and L-glucoronic acid (G) residues joined linearly by 1, 4-glycosidic linkages. It is extensively associated with gel-forming capacity [22]. Chitosan, a mucoadhesive polymer is randomly composed of β (1–4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D- glucosamine (acetylated unit). Alginate, when crosslinked with divalent cations and chitosan has been widely studied for biomedical applications [23,24]. A nanocarrier system using combination of chitosan and alginate has been used as a carrier of 5-FU in ophthalmic delivery [25–27]. A mucoadhesive characteristic of alginate and chitosan helps to maintain contact with the epithelium for a longer period of time thus giving scope for the drug to pass through intestinal membrane. For effective delivery of insulin and small molecule drugs, alginate/chitosan nanoparticles have been studied for oral absorption and oral bioactivity [28,49].

In this work, we propose the combined use of these two systems for the delivery of 5-FU thereby taking advantage of the properties of both cationic cyclodextrin and biodegradable polymers. This was achieved by firstly forming an inclusion complex of the drug with cationic cyclodextrin acting as a carrier for the drug and secondly by linking the cationic cyclodextrin with polyanionic polymer, alginate. We report for the first time, an oral formulation of Cat-β-CD/5-FU loaded alginate/chitosan ***nanoflowers***. Polyelectrolyte complexes and Cat-β-CD play a vital role by retaining the drug within the core of alginate/chitosan ***nanoflowers***. This may certainly lead to a decrease in the degradation of the drug in the gastric environment avoiding the toxicity caused by the drug along with the enhancement of permeability of drug.

2. Material and materials

2.1. Materials

β-cyclodextrin (β-CD) was obtained from Walker Chemie (Munich, Germany). Epichlorohydrin (EP), Choline chloride (CC), 5-Fluorouracil (5-FU), deuterium oxide (99%, purity), low viscosity sodium alginate and low molecular weight chitosan (molecular weight 7464 g/mol) were purchased from Sigma-Aldrich (South Africa). Hydrochloric acid, acetic acid and sodium hydroxide were purchased from Merck chemical company. Deionized water was used for all the experiments. All other reagents were of analytical grade and were used without further purification.

2.2. Preparation of cationic-β-cyclodextrin polymer (Cat-β-CD)

Cationic cyclodextrin (Cat-β-CD) was synthesized following a procedure reported earlier [19] with slight modifications. In this study, the molar ratio of β-CD/EP/CC was selected to be 1:15:1, respectively. A typical polymerization reaction of β-CD for molar ratio β-CD/EP/CC is as follows: Sodium hydroxide, (NaOH) (1 g) was dissolved in water (20 mL), and then β-CD (5.675 g) was dissolved in the sodium hydroxide solution. The resulting solution was magnetically stirred at room temperature for 24 h. Thereafter, choline chloride (0.698 g) was fed into the solution rapidly and epichlorohydrin (6.940 g) was added dropwise at a flow rate of 0.1 mL/min. After completion of EP addition, the mixture was heated at 60 °C under stirring at 600 rpm for 2 h. The polymerization was terminated by neutralizing with aqueous hydrochloric acid solution (3 N). The solution obtained was dialyzed for 24 h with a dialysis membrane (Nominal Molecular weight-cut off 2000) to remove unreacted EP and CC. The solution obtained was evaporated and the solid pulverized to powder (Fig. 1a).

2.3. Preparation of Cat-β-CD/5-FU inclusion complex

The inclusion complex of Cat-β-CD with 5-FU was prepared using lyophilisation method as follows. An accurately weighed amount of Cat-β-CD (1.135 g) was placed into 100 mL round bottom flask and 50 mL of deionized water was added. The mixture was then stirred until dissolved. 5-FU (0.1308 g) was dissolved in deionized water (50 mL), slowly added (dropwise) into the Cat-β-CD solution and stirred for about 24 h at room temperature. After stirring, a nearly clear solution formed that was lyophilized using a freeze dryer (VirTis BenchTop K) yielding cyclodextrin inclusion complex as white powder. This material was assayed spectrophotometrically to determine the drug content included. The inclusion complex was characterized by FTIR, DSC, ^{1}HNMR, 2D-NMR and SEM.

2.4. Preparation of Cat-β-CD/5-FU loaded Alg/Chi nanoflowers

The Alg/Chi ***nanoflowers*** were spontaneously obtained using method modified described earlier [29]. Briefly, sodium alginate was dissolved in deionized water under continuous stirring overnight. Upon ensuring that all alginate powder was completely dissolved, the resulting solution (0.063 w/w %) (19.5 mL) was then mixed with the inclusion complex (3 mL of 3.5 mg/mL) under magnetic stirring for 30 min. $CaCl_2$ (1.3 mL of 18 mM) was then slowly added (dropwise) under constant magnetic stirring to the alginate solution containing the inclusion complex to provide pre-gel. Subsequently different concentrations of aqueous chitosan solutions (0.05–0.08% w/w) (4.2 mL) were added to the calcium alginate pre gel and stirred for 30 min. Chitosan was previously dissolved in 1% acetic acid under magnetic stirring for 24 h. The resulting ***nanoflowers*** were collected by centrifugation (10,000*g*, 4 °C) for 30 min using a Merck Eppendorf 5403R centrifuge. It is being represented schematically in Fig. 1b.