European Journal of Pharmaceutics and Biopharmaceutics 117 (2017) 29–38

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

pH responsive controlled release of anti-cancer hydrophobic drugs from sodium alginate and hydroxyapatite bi-coated iron oxide nanoparticles

Danushika C. Manatunga [a], Rohini M. de Silva [a,*], K.M. Nalin de Silva [a,b], Nuwan de Silva [b], Shiva Bhandari [c], Yoke Khin Yap [c], N. Pabakara Costha [d]

[a] Department of Chemistry, University of Colombo, Colombo 03, Sri Lanka
[b] Sri Lanka Institute of Nanotechnology (SLINTEC), Nanotechnology & Science Park, Mahenwatte, Pitipana, Homagama, Sri Lanka
[c] Department of Physics, Michigan Technological University, 118 Fisher Hall, 1400 Townsend Drive, Houghton, USA
[d] Ratnapura General Hospital, Colombo Road, Ratnapura, Sri Lanka

ARTICLE INFO

Article history:
Received 11 January 2017
Revised 4 March 2017
Accepted in revised form 17 March 2017
Available online 19 March 2017

Chemical compounds studied in this article:
Curcumin (PubChem CID: 969516)
[6]-Gingerol (PubChem CID: 442793)
Calcium nitrate tetra hydrate (PubChem CID: 16211656)
Diammonium hydrogen phosphate (PubChem CID: 24540)
Ammonium iron (II) sulfate hexahydrate (PubChem CID: 16211143)
Ammonium iron (III) sulfate dodecahydrate (PubChem CID: 16211144)
Alginic acid sodium salt (PubChem CID: 5102882)

Keywords:
Magnetic Fe_3O_4
Sodium alginate
Hydroxyapatite
Curcumin
6-Gingerol
Controlled release

ABSTRACT

Developing a drug carrier system which could perform targeted and controlled release over a period of time is utmost concern in the pharmaceutical industry. This is more relevant when designing drug carriers for poorly water soluble drug molecules such as curcumin and 6-gingerol. Development of a drug carrier system which could overcome these limitations and perform controlled and targeted drug delivery is beneficial. This study describes a promising approach for the design of novel pH sensitive sodium alginate, hydroxyapatite bilayer coated iron oxide nanoparticle composite (IONP/HAp-NaAlg) via the co-precipitation approach. This system consists of a magnetic core for targeting and a NaAlg/HAp coating on the surface to accommodate the drug molecules. The nanocomposite was characterized using FT-IR spectroscopy, X-ray diffraction, scanning electron microscopy, transmission electron microscopy and thermogravimetric analysis. The loading efficiency and loading capacity of curcumin and 6-gingerol were examined. *In vitro* drug releasing behavior of curcumin and 6-gingerol was studied at pH 7.4 and pH 5.3 over a period of seven days at 37 °C. The mechanism of drug release from the nanocomposite of each situation was studied using kinetic models and the results implied that, the release is typically via diffusion and a higher release was observed at pH 5.3. This bilayer coated system can be recognized as a potential drug delivery system for the purpose of curcumin and 6-gingerol release in targeted and controlled manner to treat diseases such as cancer.

1. Introduction

The development of an ideal drug delivery system consisting of increased therapeutic index, increased therapeutic efficacy, biocompatibility, controlled release, reduced side effects and improved patient compliance is a much needed area in pharmaceutics and medicine. The main concern out of above has been devoted to increase the therapeutic index of a drug delivery system [1]. An increased therapeutic index has been obtained by directing the delivery of the drug to a predetermined target [2–4] and maintaining a sufficient amount of a specific drug over a period of time avoiding the necessity for frequent dosages [2]. The concept of targeted delivery depends on the interaction between the targeted site, carrier molecule and the therapeutic drug [1,4].

In many of the drug designing strategies, controlled release of the drug at the target site has been achieved by the change of the environmental factors such as temperature or pH [5–8]. The significance of pH factor has been used for the delivery of drugs particularly at the tumor cell sites [1,5,9]. As a result, the use of

* Corresponding author.
E-mail address: rohini@chem.cmb.ac.lk (R.M. de Silva).

http://dx.doi.org/10.1016/j.ejpb.2017.03.014
0939-6411/

pH sensitive drug carrier systems has gained significant consideration over the years [10–12].

The process of controlled release of drug molecules has been performed using polymer blends [13,14], hydrogels [15], hydrogel incorporated nanoparticles [9,16], microparticles and nanoparticles made out of polymers [17,18] and liposomes [19]. However, the broad size distribution has triggered in increased initial burst of drug release resulting in unmanageable releasing pattern which has restricted the application of these systems in drug delivery applications [17]. This has led to the development of nanomaterials which could be functionalized to eliminate the problem of aggregation, broad size distribution, toxicity which could result in the clearance by reticular endothelium system (RES) [1,20].

Much work on targeted delivery has been carried out using external magnetic field by incorporating magnetic nanoparticles as delivery vehicles [13,14,21]. On this regard iron oxide nanoparticles (IONPs) have gained much consideration as a biocompatible material over the years [22–24]. However due to agglomeration, the direct use of these nanoparticles has limited its applications [25] and this has been overcome by using surface functionalizing compounds such as dextran [25], polyethylene glycol (PEG) [1,26], polyvinylpyrrolidone (PVP) [25,27], and sodium alginate (NaAlg) [28]. However, biocompatible inorganic coatings on the IONPs have identified as the most suitable candidate to eliminate instability arising from functionalizing compounds [25]. On this regard IONPs incorporated with nano hydroxyapatite (HAp) have been utilized as a novel approach for targeted delivery of drugs and growth factors [21,23]. In addition, the ability of proliferation and differentiation of osteoblasts in the presence of HAp/IONPs has been utilized in bone tissue engineering. [22,25,29,30].

Incorporation of natural anti-cancer agents into a stable drug carrier [31–33] is becoming an interesting area in cancer research as it could enhance the stability and activity. On this regard extensive studies have been performed with liposomes [4,34], polymer nanoparticles [5,34], solid lipid nanoparticles [35,36], complexation with phospholipids [37], cyclodextrins [13,37,38], microspheres [39], dendrosomes [4] and hydrogels [38]. It has also been attempted for the development of a carrier molecule to eliminate common issues encountered in liposomes and solid lipid nanoparticles [40,41].

Curcumin, which is a natural polyphenolic compound extracted from the rhizome of *Curcumin Longa* is identified as having both anti-cancer and cancer preventive properties [14,38,42]. Curcumin is also known to be a multifunctional drug molecule which is safe to be used even at high concentrations with fewer side effects [4,14]. However, the poor aqueous solubility [42], instability in the presence of light and high pH [42], limited absorption, poor bioavailability, rapid metabolism, excretion and short half-life has limited its practical applications as an oral or intravenous drug [34,37,38]. Similar description can be given to the 6-gingerol, extracted from the rhizome of *Zingiber officinale,* which is one of the main pharmacological compounds in the ginger extract [43–46]. It has known to inhibit both melanogenesis in melanoma cells [44] and synthesis of both leukotrienes and prostaglandins showing ability to reduce the side effects arising from commonly used cancer treatments [44]. As a result, both curcumin and 6-gingerol have been incorporated into suitable drug carrier systems to enhance the pharmacokinetic profile, stability and solubility. [1,43].

Most of the delivery systems for curcumin and 6-gingerol are based on the use of polymer nanoparticles [34,37,43], polymer incorporated systems [43,46,47], cellulose fibers [45] magnetic IONPs [5,14,48] and multilayer coated magnetic nanoparticles [13,20,48]. However, the delivery of hydrophobic drug molecules like curcumin and 6-gingerol using magnetic-HAp (m-HAp) is not heavily investigated, other than recently reported work on delivery of curcumin to breast cancer cells using HAp coated IONPs together with polyethylene imine and cyclodextrin [20].

Therefore, this study focused on the development of pH sensitive NaAlg HAp bi-coated IONP nanocomposite (IONP/HAp-NaAlg) system for the loading, targeted and controlled release of curcumin and 6-gingerol. This delivery system has not been reported for pH sensitive and targeted delivery of these drug molecules. In addition, this study has also attempted to perform the extended release of 6-gingerol for four days using the above system.

In this study, a simple co-precipitation technique was employed to synthesize IONP/HAp-NaAlg system to load curcumin and 6-gingerol to assess its ability to encapsulate anti-cancer drugs and to perform pH sensitive extended release.

2. Materials and methods

2.1. Materials

Curcumin (≥98.0%, HPLC), [6]-gingerol (≥98.0%, HPLC), calcium nitrate tetra hydrate ($Ca(NO_3)_2{\cdot}4H_2O$, 99%, ACS), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, ≥99.0%,), ammonium iron (II) sulfate hexahydrate ($(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$, 99.0%, ACS), ammonium iron (III) sulfate dodecahydrate ($NH_4Fe(SO_4)_2$. 12 H_2O, 99.0%, ACS), ethanol (EtOH, ≥99.8%, HPLC), methanol anhydrous (MeOH, 99.8%), dichloromethane (CH_2Cl_2 anhydrous, ≥99.8%), alginic acid sodium salt (NaAlg, low viscosity), Cetyltrimethyl ammonium bromide (CTAB, ≥98%) and TWEEN® 80 (Viscous liquid) were purchased from Sigma Aldrich, Bangalore, India. Polyethylene glycol 200 (PEG 200) was purchased from Merck Millipore Corporation, Darmstadt, Germany. Snakeskin dialysis tubing (MWCO 3.5 kDa) was purchased Thermo Fisher, Bangalore, India.

2.2. Synthesis of polyethylene glycol (PEG) coated IONPs

IONPs were synthesized following a simple co-precipitation technique. In a typical procedure, 0.1 M Fe^{3+} and Fe^{2+} ion solutions were mixed in 2:1 ratio in degassed ultra -pure water in the presence of PEG 200 at 45 °C. Degassing was done for further 20 min under nitrogen atmosphere. Later 10 mL of 25% ammonium hydroxide was added in dropwise with vigorous stirring. Reaction was continued till a black colored suspension is obtained. This was subjected to magnetic separation and the black pellet resulted from the separation was washed thoroughly with degassed ultrapure water to remove excess ammonia and unreacted salts in the medium. The product obtained was directly used for the HAp coating.

2.3. Synthesis of NaAlg HAp bi-coated IONPs nanocomposite (IONP/HAp-NaAlg)

IONPs (0.500 g) were dispersed in ultra-pure water and mixed with 40% (w/w) NaAlg powder. Vigorous stirring was carried out at 50 °C until a homogeneous suspension is obtained. Then it was mixed with 0.25 M $Ca(NO_3)_2$ where the pH was then adjusted to 8. While these contents were mixed vigorously under stirring at 50 °C, $(NH_4)_2HPO_4$ kept at pH 9 was added dropwise to the nanosuspension and the stirring was continued for another couple of hours. Throughout the reaction the pH of the medium was maintained at 8.5–9.0. The HAp: IONPs molar ratio was maintained at 1:6 during the preparation. At the end of the reaction, the black brown color suspension was magnetically separated and washed thoroughly to remove excess ions and ammonia.