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Magnetically responsive release of 5-FU from superparamagnetic egg albumin coated iron oxide core-shell nanoparticles

Neha Sonker, Jaya Bajpai, A.K. Bajpai*

Bose Memorial Research Laboratory, Department of Chemistry, Government Autonomous Science College, Jabalpur, MP, India

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ABSTRACT

The present study aimed at preparing egg albumin coated iron oxide (EAIO) core shell nanoparticles and evaluating their ability to release 5-FU drug under applied external magnetic field. EAIO nanoparticles were prepared by simultaneous co-precipitation and emulsion crosslinking technique and characterized by FTIR, DSC, TEM, XRD, particle size analysis and surface potential measurements. The influence of chemical composition of EAIO nanoparticles, pH and temperature of release media, presence of simulated biological fluids as the release media, and applied magnetic field was investigated on the release profiles of 5-FU drug. The drug release was analyzed kinetically using Fickan power law, zero order, first order and Korsmeyer-Peppas models and the amount of released drug was correlated and the relation was drawn between the quantity of released drug and water holding capacity of nanoparticles. The nanoparticles were judged for *in vitro* cytotoxicity and the chemical integrity of the drug was also accessed.

1. Introduction

It is always exciting to see how the technology of nanostructures has advanced to find applications in biomedical fields like tissue engineering, controlled drug delivery, gene delivery, imaging of tumor and cancerous sites, and probing DNA structures [1,2]. For complex diseases like cancer and tumors, diabetes, allergy, infection and inflammation therapeutic treatments, use of nanoparticles has been enormously successful [3]. One of the valid reasons for using nanoparticles in therapeutic applications is due to their small size that is comparable to many of the proteins and other macro-drugs [4]. These nanoparticles possess a large number of functional groups on their surfaces that enable them to function like a ligand to hold a variety of other bioactive and pharmaceutically active moieties. Furthermore, they also offer enormous potential to show rapid fluid absorption and drug release characteristics due to their abilities to undergo rapid diffusion and volume transitions. Another specialty of these nanostructures is that they can be easily tailored to seek desired functions and properties with controlled performance [5,6]. The submicron size of nanoparticles enables them to actively take part in biological processes and occlude through terminal blood vessels [7].

There are certain unusual physico-chemical properties of nanoparticles such as greater stability, low toxicity, appropriate hydrophilic-hydrophobic balance, large surface to volume ratio and ease of transformation of their surface functionalities [8]. All these properties have made the nanoparticles as one of the versatile drug delivery carriers for vaccines, anticancer drugs and oligonucleotides [9]. It is known that most of the tumors have fenestrated vasculature and poor lymphatic drainage which produce an enhanced permeability and retention (EPR) effect which is known to be responsible for accumulation of drug carrying nanoparticles in the immediate vicinity of tumor site [10]. Besides causing an EPR effect the nanoparticles also play a crucial role in protecting encapsulated drug from rapid metabolism and clearance, non specific recognition and distribution, and the stealth shielding [11] which helps to avoid the uptake of reticulo endothelial system [12] and mononuclear phagocytes [13].

Cancer is one of the most fatal diseases that still involve chemotherapy as the major therapeutic route. Some of the major chemical compounds investigated as chemotherapeutics are water insoluble and, therefore, cannot be used directly due to their solubility issues. It is, therefore, mandatory to design a drug delivery system that ensures good dispersion of drug in aqueous solution, proper clearance *in vivo* and precisely target the cancer sites [14]. Nanoparticles of iron oxide are widely used nanostructures which have large surface to volume ratio and possess high surface energies. Some of the unusual features of iron oxide are that they possess hyper chemical reactivity, prone to oxidation by atmospheric air and routinely undergo loss of magnetism and ease of dispersibility. It is, therefore, mandatory to protect iron

* Corresponding author.
E-mail address: akbmrl@yahoo.co.in (A.K. Bajpai).

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oxide nanoparticles to retain their intrinsic physico-chemical properties which enable them to be employed in drug delivery applications. Among existing possible options to address the above mentioned issues, coating of nanoparticles with suitable polymer materials has great potential to achieve the desired properties. Thus, being motivated by the designing of polymer coated nanoparticles, we have attempted to prepare core-shell nanoparticles wherein egg albumin is present as shell while iron oxide as core. The rational for selecting albumin as a biopolymer for shell lies in the fact that albumin is well known as nanocarriers for the transport of hydrophobic chemotherapeutic agents. It is known to carry hydrophobic molecules through reversible noncovelant binding. Moreover, it also has ability to bind to the glycoprotein (gp60) receptor and mediate the transcytosis of albumin bound molecules [15,16]. The proposed studies further include characterization of as prepared core-shell nanoparticles, loading of 5-Fluorouracil drug into nanoparticles, and their release study under applied magnetic field. The hydrophilic nature of the nanoparticles may enable them to be used as magnetically responsive and swelling controlled drug delivery system for 5-FU.

5- Fluorouracil (5-FU) drug, chosen for the present study, is a vital drug for numerous tumors such as breast, pancreas, gastrointestinal, liver and gastrointestinal tract related cancers. This drug is easily metabolized in the body and for improving its therapeutic activity the drug is needed to maintain its high serum concentration which demands continuous administration of the drug. In spite of these specialties, this drug is not free from severe toxic effects that demand the use of low concentration of this drug [17]. The release of 5-FU from egg albumin iron oxide (EAIO) nanoparticles may open up new possibilities for achieving optimum efficacy of the drug, desirable drug release rate, site specific targeting ability, prolonged pharmacological activity, and minimum side effects that eventually take care of patients comfort [18].

2. Experimental

2.1. Materials

Egg albumin and iron salts (ferrous and ferric chloride salts) were obtained from Merck, India. For crosslinking of egg albumin, glutaraldehyde was used as a crosslinker (Loba Chemie, India). The oil phase was prepared by paraffin oil (Merck, India). The drug 5-FU was procured from Dabur Research Foundation (India). Other required chemicals like toluene, acetone, and sodium carbonate were used of analytical grade and all solutions were prepared in distilled water.

2.2. Methods

2.2.1. Preparation of iron oxide nanoparticles

In order to prepare iron oxide nanoparticles, known molar ratio of ferrous and ferric salts was dissolved in 250 mL water and stirred for 1 h under nitrogen atmosphere. Now to this mixture of iron salts solutions, 2 M solution of NaOH was added dropwise with continuous stirring that resulted in precipitation of iron oxide. The as precipitated nanoparticles of iron oxide were left in mother liquor for 6 h at room temperature so that iron oxide nanoparticles could be completely precipitated. The precipitated nanoparticles were filtered, purified by repeatedly washing with distilled water, dried at 70 °C, and stored in air-tight poly (ethylene) bags for further studies.

2.2.2. Preparation of iron oxide –egg albumin (IOEA) core-shell nanoparticles

In order to perform coating of egg albumin onto the surfaces of iron oxide nanoparticles a co-precipitation method was followed as published in literature [19,20]. In a typical experiment, 8% (w/v) egg albumin solution was prepared in 1:1 (w/v) mixture of N/50 NaOH and paraffin oil and the mixture was constantly stirred for 1 h to yield a stable emulsion. Prior to crosslink egg albumin, a known weight of iron oxide was added to the emulsion and, thereafter, 31.7 mM of glutaraldehyde was added as a crosslinker. The crosslinking reaction was allowed to continue for 6 h with continuous stirring which eventually resulted in formation of glutaraldehyde crosslinked and egg albumin coated iron oxide nanoparticles. After complete crosslinking of egg albumin the excess of oil was removed by washing the nanoparticles excessively with toluene and acetone. The dried nanoparticles were stored in air-tight containers.

2.2.3. Detoxification of nanoparticles

Glutaraldehyde is one of the known crosslinking agents of proteins which crosslinks lysine residues of protein by reacting with amine groups of the proteins. In spite of its ability to form covalent bonds with proteins, it has been established that its unreactive residues cause severe physiological disorders and, therefore, must be removed from the end product. This task was accomplished by extracting aldehyde from nanoparticles with L-glutamic acid at low pH.

3. Characterization techniques

3.1. FTIR spectral analysis

Fourier transform infrared spectroscopy was used to characterize the functional groups of native and 5-FU loaded EAIO nanoparticles. FTIR studies of the powdered specimens were recorded on a FTIR-8400 S, Shimadzu spectrophotometer over the region of 4000–400 cm^{-1}. For the spectral analysis the required pellets of KBr and as prepared nanoparticles were mixed in the ratio of 1:10 (wt/wt) followed by uniaxial pressing the powders under vacuum.

3.2. Particle size analysis

The size and size distribution of the native EAIO and 5-FU loaded EAIO nanoparticles were determined by particle size analyzer using Zetasizer Nano ZS 90, Malvern Instruments, UK.

3.3. Zeta potential measurements

To characterize the surface charge properties of nanoparticles zeta potential of the prepared 5-FU loaded EAIO nanoparticles was determined using Zetasizer (ZS 90 'Malvern Instruments, UK). This technique can also be used to ascertain encapsulation of charged active materials within the center or onto the surfaces of nanoparticles [21].

3.4. XRD analysis

The crystalline features of the as prepared nanoparticles were explored using XRD technique on Bruker D8 Advance X-ray diffractometer with Cu-K_α radiation operated at 40 kV, 40 mA and over the 2θ range of 10°–80° with a step size of 0.02° and counting time of 2 s·$step^{-1}$. Debye-Scherer equation was used to determine crystallite size of EAIO and 5-FU loaded EAIO nanoparticles in accordance with Equation-(1) where d is mean grain size, k is the shape factor (0.9), β is diffraction angle broadening, and λ is the diffraction wavelength (1.54 A^0).

$$d = K\lambda / \beta Cos\theta \quad (1)$$

The degree of crystallinity was also calculated using Equation- (2), where Ac = Area of the crystalline phase, and Aa = Area of the amorphous phase, respectively [22,23].

$$Xc\ \% = \frac{Ac}{Aa + Ac} \times 100 \quad (2)$$

3.5. DSC

Differential scanning calorimetry (DSC) enables one to study the