Colloids and Surfaces B: Biointerfaces 162 (2018) 163–171

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

# pH sensitive surfactant-stabilized $Fe_3O_4$ magnetic nanocarriers for dual drug delivery

Bijaideep Dutta[a], Neena G. Shetake[b,c], B.K. Barick[d], K.C. Barick[a,b,*], B.N. Pandey[b,c], K.I. Priyadarsini[a,b], P.A. Hassan[a,b,*]

[a] Chemistry Division, Bhabha Atomic Research Centre, Mumbai 400085, India
[b] Homi Bhabha National Institute, Anushaktinagar, Mumbai 400094, India
[c] Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Mumbai 400085, India
[d] Department of Physics, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

ARTICLE INFO

*Article history:*
Received 10 August 2017
Received in revised form 14 October 2017
Accepted 21 November 2017
Available online 22 November 2017

*Keywords:*
$Fe_3O_4$
Nanoparticles
Drug delivery
Hyperthermia
Magnetic
Doxorubicin
Curcumin

ABSTRACT

Highly water-dispersible surfactant-stabilized $Fe_3O_4$ magnetic nanocarriers (SMNCs) were prepared by self-assembly of anionic surfactant, sodium dodecyl sulphate (SDS) on hydrophobic (oleic acid coated) nanoparticles and their biomedical applications were investigated. These nanocarriers have an average size of about 10 nm and possess tunable surface charge properties. The formation of an organic coating of SDS was evident from infrared spectroscopy, dynamic light scattering, zeta-potential and thermogravimetric measurements. These nanocarriers were used for loading of both hydrophilic and hydrophobic anticancer agents such as doxorubicin hydrochloride (DOX) and curcumin (CUR), respectively. DOX was conjugated onto the surface of nanocarriers through electrostatic interaction, whereas CUR was encapsulated into the hydrophobic interlayer between oleic acid and SDS. The toxicity and cellular internalization of drug loaded nanocarriers were investigated against WEHI-164 cancer cell line. Specifically, the drug loading, pH sensitive drug release and cellular internalization studies suggested that these nanocarriers are suitable for dual drug delivery. Furthermore, they show good heating ability under AC magnetic field, thus can be used as effective heating source for hyperthermia treatment of cancer.



## 1. Introduction

Recently, combination chemotherapy involving two or more drugs is emerging as a promising approach for the treatment of cancer. Such combination therapy approaches could significantly reduce multidrug resistance and side effects, leading to enhanced therapeutic efficacy [1–5]. It has been reported that combination of drugs efficiently disrupts cell repairing as well as their reproduction cycle, which in turn enhanced the apoptotic cell death [6]. It is feasible to alleviate multi drug resistance (MDR) of some cancer cells by combining broad spectrum of anticancer drug, DOX and natural herbal product, CUR. The majority of failures in anticancer drugs administration have been attributed to their poor water-solubility [7]. The problems related with poor water-solubility of drugs can lead to low bioavailability resulting in suboptimal drug delivery. The carrier with poor payload requires multiple injections to achieve desired therapeutic effect, which may result in systemic toxicity and serious inflammatory response [8]. Therefore, it is highly desirable to develop novel carrier having capability to accommodate high payload of multiple drugs for combination chemotherapy. The advancements in the area of nanotechnology help in creating nanoparticles with specific functional properties to address these shortcomings.

Among the others, magnetic nanoparticles (MNPs) have gained significant attention in drug delivery due to their unique physicochemical properties and low toxicity [9,10]. The biocompatibility, water-dispersibility and narrow size distribution are some of the primary requirements of MNPs to be used as drug carrier [11–13]. A critical step in developing such carriers is to engineer the surface of nanoparticles with suitable bioactive molecules. Recently, organic and inorganic functionalities were being introduced on the surface of MNPs, namely carboxylate, phosphate, phosphonates, amines, organosilane, silica and gold which have been proven of having better efficacy without affecting the properties of MNPs [14–18]. The presence of these organic/inorganic layers on surface not only stabilizes the nanoparticles, but also provides desired

* Corresponding authors at: Chemistry Division, Bhabha Atomic Research Centre, Mumbai 400085, India.
*E-mail addresses:* kcbarick@barc.gov.in, kcbarick@iitb.ac.in (K.C. Barick), hassan@barc.gov.in (P.A. Hassan).

https://doi.org/10.1016/j.colsurfb.2017.11.054
0927-7765/

properties required for conjugation/encapsulation of drugs. Further, the selective binding of specific functional groups on MNPs surface provides stimuli responsive shell that is susceptible to external exogenous/endogenous environments such as pH, temperature, ionic strength, ultrasound intensity, enzyme, light or electric pulses etc [19–22]. The stimuli responsive shell ensures that the loaded drug molecules will not freely extravasate during blood circulation (i.e., the drug should not released before reaching target tissues/organs or with extremely slow rate), but only release at the target sites, where the nanocarriers accumulate by active or passive targeting strategy. In an interesting review article, Mura et al. [22] discussed the recent advances in the design of nanoscale stimuli-responsive systems that are capable of controlling biodistribution of drug in response to different environments. Owing to physiological differences between cancerous and normal cells/tissues, among the others pH-sensitive nanocarriers represent smart vehicles for transport and delivery of anticancer agents. At the cellular level, pH sensitivity can trigger the release of drugs into endosomes or lysosomes, as well as promote the escape of nanocarriers from the lysosomes to the cell cytoplasm. For instance, Vivek et al. [23] developed pH-responsive chitosan nanoparticles for delivery of tamoxifen in breast cancer cells, and observed that drug loaded nanoparticles increase intracellular concentration of tamoxifen and enhance its anticancer efficiency by inducing apoptosis. Manatunga et al. [24] reported pH responsive controlled release of anti-cancer hydrophobic drugs (CUR and 6-gingerol) from sodium alginate and hydroxyapatite bilayer coated iron oxide nanoparticles. Rana et al. [19] demonstrated the pH sensitive release of DOX into KB cells from DOX loaded folate conjugated $Fe_3O_4$ MNPs. Further, the targeted delivery of drug molecules to a diseased area in the body can be magnetically guided by using MNPs [25]. Being MNPs as carrier, it is also possible to combine magnetic hyperthermia (heat activated killing of cancer cells at 5–6 °C above body temperature) to chemotherapy [26,27]. Such combination therapies are proven to be superior for successful alleviation of cancer [28]. Magnetic delivery system not only increases the accumulation of drug carrier at tumor site but also enhances the drug toxicity in certain cancer cells that are otherwise drug resistant [29].

Herein, we report the development of novel nanocarriers by self-assembly of anionic surfactant, SDS onto the surface of hydrophobic $Fe_3O_4$ nanoparticles. These nanocarriers have good aqueous colloidal stability, magnetic responsivity and have capability of loading both hydrophilic (DOX) and hydrophobic anticancer (CUR) drugs. The high payload for both the drugs with their sustained release behaviour and good cellular internalization capability makes these nanocarriers suitable for dual drug delivery. Furthermore, these nanocarriers can be used as effective heating source in hyperthermia treatment of cancer.

## 2. Material and methods

### 2.1. Materials

Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, ≥99%), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, ACS reagent, 97%), DOX (98%), bovine serum albumin (BSA), oleic acid (OA) were purchased from Sigma Aldrich, USA. CUR (>99%) was received as a gift from Win Herbal Care, India. Hexane (99%), SDS (99%) and ammonia solution (25%) were bought from SRL Pvt. Ltd., India. Dulbecco's Modified Eagle Medium (DMEM), fetal calf serum (FCS), MTT reagent (thiazolyl blue tetrazolium bromide) and dialysis membrane-60 were procured from Himedia Laboratories Pvt. Ltd., India. Tween-80 and 1, 10-phenanthroline monohydrate were obtained from Alfa Aesar, Canada and Merck, India, respectively. WEHI-164 cells were purchased from National Centre for Cell Science (NCCS), Pune, India. The acetate buffer (AB, pH 5) and phosphate buffered saline (PBS, pH 7.4) were prepared using standard protocols. All chemicals used were of AR grade unless otherwise specified. All the aqueous solutions were prepared using deionised water from a MilliQ system, Millipore Corporation, USA (resistivity ~18 MΩ cm).

### 2.2. Synthesis of oleic acid coated $Fe_3O_4$ hydrophobic magnetic nanoparticles (HMNPs)

HMNPs were prepared by co-precipitation of Fe-chloride precursors in basic medium followed by in-situ coating of oleic acid. In a typical synthesis, 5.406 g of $FeCl_3{\cdot}6H_2O$ and 1.988 g of $FeCl_2{\cdot}4H_2O$ in 80 ml water in a round bottom flask and temperature was slowly increased to 70 °C under $N_2$ atmosphere with constant stirring. The temperature was maintained at 70 °C for 30 min, after which 30 ml of 25% $NH_3$ solution was added to the reaction mixture and kept for another 30 min at 70 °C. Then, 5 ml of oleic acid was added to the reaction mixture and the temperature was maintained at 90 °C for 1 h. The obtained precipitate was thoroughly rinsed with ethanol and separated from supernatant using permanent magnet (field strength ~0.25 kOe).

### 2.3. Synthesis of surfactant stabilized $Fe_3O_4$ magnetic nanocarriers (SMNCs)

HMNPs are hydrophobic in nature due to the coating of oleic acid on their surface. In order to make them hydrophilic anionic surfactant, SDS was introduced onto their surface through self-assembly process. In a typically synthesis of SMNCs, 0.2 wt.% aqueous solution of SDS (5 ml) was added into the hexane dispersion of HMNPs (20 mg particles dissolved in 5 ml hexane). The resulting solution was stirred under shaking for 24 h, and then particles were separated and thoroughly washed with nanopure water.

### 2.4. Characterizations

#### 2.4.1. Structural, colloidal stability and magnetic studies

X-ray diffraction (XRD) analysis was performed on Phillips PW1729 diffractometer with Cu Kα radiation (λ = 1.5405 Å). The crystallite size is estimated from X-ray line broadening using Scherrer formula. The transmission electron micrographs and selected area electron diffraction (SAED) were taken by Philips CM 200 transmission electron microscopy (TEM). The infrared spectra were recorded on a Fourier-transform infrared spectrometer (FTIR, Bomen MB series). The thermogravimetric analysis (TGA) of samples was carried using Mettler Toledo TG/DSC system at a scan rate of 10 °C/min under $N_2$ atmosphere. Dynamic light scattering (DLS) measurement was performed using a Malvern 4800 Autosizer employing a 7132 digital correlator. The zeta-potential measurements were obtained by Zetasizer nanoseries, Malvern Instruments. The colloidal stability assay was investigated by measuring the absorbance of SMNCs suspensions (0.1 mg/ml) in water and cell culture medium (DMEM supplemented with FCS and antibiotics) at a wavelength of 350 nm using JASCO V-650, UV–vis spectrophotometer. The field-dependence magnetization was measured by physical properties measurement system (PPMS), Quantum Design.

#### 2.4.2. Drug loading and release studies

The anticancer agents, DOX and CUR were used as model drugs to estimate the drug loading and release behaviour of SMNCs. The binding isotherm of both drugs were investigated by shaking (under vortex) 1 ml solution of drug and nanocarrier having 10 μg drug (10 μl from a stock of 1 mg/ml, stock solutions were prepared in water and methanol for DOX and CUR, respectively) and different concentrations (0, 20, 40, 60, 80, 100, 120, 140 and