Colloids and Surfaces B: Biointerfaces 161 (2018) 18–26

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Full Length Article

Studies on the adsorption and desorption of mitoxantrone to lauric acid/albumin coated iron oxide nanoparticles

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ARTICLE INFO

Article history:
Received 13 March 2017
Received in revised form
21 September 2017
Accepted 27 September 2017
Available online 28 September 2017

Keywords:
Magnetic drug targeting
Iron oxide nanoparticles
Nanomedicine
Small-angle scattering of neutrons and X-rays

ABSTRACT

A rational use of superparamagnetic iron oxide nanoparticles (SPIONs) in drug delivery, diagnostics, and other biomedical applications requires deep understanding of the molecular drug adsorption/desorption mechanisms for proper design of new pharmaceutical formulations. The adsorption and desorption of the cytostatic Mitoxantrone (MTO) to lauric acid-albumin hybrid coated particles SPIONs ($SEON^{LA-HSA}$) was studied by Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), surface titration, release experiments and small-angle neutron and X-ray scattering. Such MTO-loaded nanoparticles have shown very promising results in *in vivo* animal models before, while the exact binding mechanism of the drug was unknown. $SEON^{LA-HSA}$ formulations have shown better stability under drug loading in comparison with uncoated nanoparticle and sustainable drug release to compare with protein solution. Adsorption of MTO to $SEON^{LA-HSA}$ leads to decreasing of absolute value of zeta potential and repulsive interaction among particles, which points to the location of separate molecules of MTO on the outer surface of LA-HSA shell.

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1. Introduction

Superparamagnetic iron oxide nanoparticles (SPIONs) are a very promising vehicle for drug delivery, imaging and several other biomedical applications [1–5]. In Magnetic Drug Targeting (MDT), particles are accumulated in a certain body region using magnetic field gradients [6]. Aggregation behaviour of SPIONs is crucial for proper application and is mostly determined by interface properties. The different approaches of interface modification are used to get particles of proper size in determined medium i.e., the most promising approaches for water based solutions are electrostatic [7] or steric [8] approaches. Furthermore, these coating molecules are also used as binding moieties for pharmacologically active substances. Drugs can thus be concentrated accordingly if they are bound to the SPIONs with sufficient stability. We have demonstrated earlier that the bioavailability of drugs can thus be enhanced over 56-fold, which greatly increases efficacy of therapy and reduces side effects [3]. A pharmaceutically active moiety can be attached to the particles by either covalent binding or adsorption. While both binding strategies offer advantages and disadvantages [9] it is important to understand the binding location of drugs in order to understand the drug release *in vivo* as well as the influence of drug binding on important particle characteristics such as colloidal stability, hydrodynamic size or surface charge. For potential use in complex biosystems, i.e. *in vivo*, it is thus preferable to understand the mechanism of drug binding to the particles and its influence on their ordering and stability [10]. Recently we have reported about a hybrid lauric acid/human serum albumin coated formulation called $SEON^{LA-HSA}$. These particles showed very promising properties for MDT after the cytotoxic drug mitoxantrone (MTO) was added to the particle suspension to yield

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https://doi.org/10.1016/j.colsurfb.2017.09.057
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$SEON^{LA-HSA*MTO}$ [11]. The stabilisation mechanism of $SEON^{LA-HSA}$ seemingly is a mixture between electrostatic and steric stabilisation. While MTO showed a slow, pseudo-zero order release kinetics from $SEON^{LA-HSA*MTO}$ the exact binding location remains unclear. Due to its chemical nature MTO possesses charged, polar groups as well as a conjugated π system. This gives rise to its amphiphilic nature, as the logarithm of its partition coefficient in *n*-octanol/water (log*P* value) lies at −3.1 [12]. MTO shows significant and specific binding to albumin [13] as well as being a strong complexing agent for iron [14]. Classical methods such as nuclear magnetic resonance (NMR) based spectroscopy are not able to investigate the binding mechanism of MTO to the particles due to the magnetic properties of the particles. Fourier transform infrared spectroscopy (FTIR) or fluorescence spectroscopy did not produce conclusive results. This, together with the proposed binding modality of MTO to $SEON^{LA-HSA}$, leaves speculation whether the drug is located at the iron oxide core, in the albumin hull around the particles or even just in the surrounding Helmholtz layers around the individual colloid particles.

In the present study we investigated adsorption and desorption of MTO to different compounds including human serum albumin (HSA), uncoated iron oxide particles and lauric acid/HSA coated particles. We investigated adsorption efficiency and also the desorption kinetics of MTO from the respective compounds using high performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). The effect of surface adsorption on colloidal properties of the system was investigated using dynamic light scattering (DLS), surface titration techniques and FTIR. We furthermore studied structure and interactions of particle clusters using small-angle scattering (SAS) methods [41]. Application of X-rays [37] and neutrons [39,40] as probes gives us the possibility to visualize different parts of clusters (iron and LA/HSA/MTO) and find out the possible location of MTO by comparison of the alternation of structure and interaction among clusters after drug addition. With our findings we show the effects of surface adsorption and desorption of small molecules on the cluster interaction as well as on the colloidal properties of such materials.

2. Experimental section

2.1. Chemicals and reagents

Iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) EMSURE quality and deuterium oxide (D_2O) were purchased from Merck (Darmstadt, Germany). Iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) Ph. Eur. quality, heavy metal-free dialysis tubes (Spectrapor 7, MWCO 8 kDa), ammonium chloride Ph.Eur quality, hydrochloric acid 25%, sodium chloride, sodium hydroxide, sodium hydrogencarbonate, magnesium sulphate, methanol, formic acid, nitric acid 65% and ammonia solution 25% Ph.Eur quality were supplied by Roth (Karlsruhe, Germany). Lauric acid, Bovine serum albumin (BSA), potassium dihydrogenphosphate, D-glucose and acetone were purchased from Sigma-Aldrich (St Louis, MO, USA). Iron reference standards 1 g/L were bought from Bernd Kraft GmbH (Duisburg, Germany). Recombinant human serum albumin without octanoate stabilization (Albix®) was purchased from Novozymes Biopharma (Bagsvaerd, Denmark). Strata Phenyl columns (500 mg/3 ml) were purchased from Phenomenex (Torrance, CA, USA). Mitoxantrone solution 2 mg/ml was bought from Hexal AG (Holzkirchen, Germany). 500 µl ultrafiltration tubes were bought from Sartorius (Goettingen, Germany). Sterile tangential ultrafiltration columns (MWCO 30 and 100 kDa) were purchased from Spectrum Labs (Los Angeles, USA). Polydiallyldimethylammonium chloride (Poly-DAADMAC) was purchased from Waco chemicals GmbH (Neuss, Germany). Deuterated MTO (dMTO) was bought from Toronto Research Chemicals (Toronto, Canada).

2.2. Synthesis of $SEON^{LA-HSA}$ particles and control particles

$SEON^{LA-HSA}$ particles were produced according to a previously published protocol[11]. Briefly, the SPIONs were synthesized and coated *in situ* with lauric acid. Therefore, iron (II) and iron (III) chloride were dissolved at a molar ratio of 1:2 in ultrapure water and heated to 90 °C in an Argon atmosphere. Under constant stirring, NH_3 solution 25% was added to precipitate the magnetite particles. After addition of excess lauric acid and 15 min of stirring which lead to formation of a brownish colloid the particles were purified by dialysis (molecular weight cut-off: 8 kDa). The purified particles were coated by dropwise addition to a purified human serum albumin solution 20%. Excess non-adsorbed albumin was quantitatively removed using an established tangential ultrafiltration method, leaving an iron to albumin ratio of about 18% (m/m) [15]. The total iron content was determined using the microwave plasma - atomic emission spectrometry (MP-AES). For that purpose, three different aliquots (50 µl) of the respective ferrofluid were dissolved in 950 µl of hydrochloric acid 25%. The iron content was then determined with an Agilent 4200 MP-AES (Agilent Technologies, Santa Clara, CA) using an iron solution as external standard.

Uncoated ($SEON^0$) particles, lauric acid only-coated particles ($SEON^{LA}$) and lauric acid/bovine serum albumin particles ($SEON^{LA-BSA}$) were produced using the same protocol as for abovementioned precursor. Reaction was stopped after precipitation or after lauric acid addition and the samples were purified by dialysis accordingly. For the synthesis of BSA-coated particles BSA solution 20% (m/V) was prepared in ultrapure water. Lauric acid-rich human serum albumin reference was produced by mixing the same human serum albumin solution with a fivefold molar excess of lauric acid, which is the saturation condition for medium-chain fatty acids [16].

2.3. Drug binding studies

Commercially available MTO solution was purified from its salt byproducts using a Phenomenex Strata Phenyl column. The column was conditioned with methanol, 1 ml of MTO solution (2 mg/ml) was eluted and the MTO was washed with at least 10 ml of 1% formic acid solution in water. The MTO was then eluted from the column using 1% formic acid in methanol. The MTO eluate was collected and dried under vacuum, and the MTO was redispersed in ultrapure water. The MTO content of this solution was determined using a previously described HPLC-UV method [17].

The so-prepared salt-free MTO solution was adsorbed to $SEON^0$ and $SEON^{LA-HSA}$ particles (total iron concentrations 2 mg/ml) and to a solution of lauric acid-rich HSA (11.1 mg/ml). After 5 min of equilibration time the particles were either centrifuged for 10 min at 14.000 rpm ($SEON^0$) or purified by ultrafiltration (MWCO 30 kDa) ($SEON^{LA-HSA}$, HSA). Different methods were chosen because the coated particles and HSA are not quantitatively separable from the solvent by centrifugation. The supernatant/filtrate MTO content was determined using HPLC-UV.

2.4. Drug release studies

The dialysis bag model was used to investigate drug release from $SEON^0$, $SEON^{LA-HSA}$ and HSA. Therefore 3 ml of the sample (with added MTO mass of 300 µg) were put into a dialysis tube (MWCO 8 kDa). Under gentle shaking the samples were incubated at 37 °C in 62.5 ml of release buffer. As release buffer an aqueous solution of 0.114 M sodium chloride, 0.003 M potassium chloride, 0.0025 M calcium chloride, 0.001 M potassium dihydrogen phosphate, 0.0008 M magnesium sulphate, 0.024 M sodium hydrogen

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