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Journal of Applied Chemical Research, 11, 2, 51-64 (2017)

Application of Magnetic Polymer Particles Modified with β–Cyclodextrinfor Adsorption of Bovine Serum Albumin

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(Received 14 Sep. 2016; Final version received 15 Dec. 2016)

Abstract

Magnetic polymer particles which were modified by vinyl groups and subjected to polymerization by vinyl β-Cyclodextrin derivative, has been used as adsorbent for sorption and release of bovine serum albumin and the equilibrium and kinetics of the adsorption process were studied. The absorbability and releasing of this protein through the new polymer has been measured by ultraviolet-visible spectroscopy. Effects of various parameters such as pH, contact time and capacity for adsorption of bovine serum albumin have been studied in vitro and the optimum magnitudes were 4, 37.94 mg/g and 5 minutes respectively. The experimental results show that the adsorption of Bovine serum albumin on magnetic particles was affected greatly by the pH andthe adsorption equilibrium isotherm was fitted well by the Freundlich model. Bovine serum albumin was released from magnetic particles in simulated intestinal fluids. It was released up to about 7 h at high speed and then it released slowly up to 30 h.

Keywords: *Bovine serum albumin,Magnetic polymer particles,β-Cyclodextrin,Biocompatible,Biodegradable, Hydrogel.*

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Introduction

Magnetic particles are especially important because of their applications in various areas like biological labeling, targeted drug carriers for cancer therapy, magnetic resonance imaging (MRI), ferrofluids in heat transfer, sensing, magnetic separation, dampers and actuators [1-9]. Large efforts are ongoing to develop drug carrier systems that improve drug performances. The response of magnetic particles to an external field is an appealing feature for their uses in drug delivery [10].

Drug carrier systems modify drug release profile, absorption, distribution and elimination for the benefit of improving patient convenience and compliance. Magnetic particle surface modification provides means for further grafting or conjugating additional functional molecules. For clinical applications these particles have to be biocompatible and selective toward specific targets [11-20]. Surface modification of MPP by various agents and investigation the processes of applying them based on ligand exchange and ligand absorption were recently discussed [21]. However, there is an immense requirement for synthesis of surface modified MPP using modification materials that are convenient as well as biocompatible and stabilized. With this regards, CDs are widely used in the pharmaceutical industry and various CD-based polymers have been authenticated [22]. Electrochemical and spectral study of the interaction between CDs and some pharmaceutical substances was reported previously [23].

CD-containing dendrimeres were, also, prepared as siRNAm shRNA and DNA carriers [24].On the other hand, magnetic particles consisting of bovine serum albumin (BSA) have been shown to be a versatile carrier for drug delivery. These particles show promise as drug delivery systems as a result of their controlled and sustained-release properties and biocompatible with tissue and cells [25]. Among natural polymers, bovine serum albumin, a versatile protein carrier for drug delivery, has been shown to be nontoxic, non-immunogenic, biocompatible and biodegradable and is emerging as versatile carriers for drug targeting [26-27]. BSA is a globular protein (~ 66,000 Da) that is used in numerous biochemical applications due to its stability and lack of interference within biological reactions.

Furthermore, in recent years, this protein plays important roles in many disciplines such as an enzyme stabilizer during purification or for dilution of restriction endonucleases and nucleic acid modifying enzymes. It is also commonly used in DNA and protein labeling experiments as a blocking agent to minimize background [28].In a previous work, we have prepared magnetic

polymer particles (MPP) which were modified first by hydroxyl then by vinyl groups on surface. After that, vinyl β-CD monomer and acryl amide were copolymerized onto the surface of modified magnetic particles to form a hydrogel [29]. This study gives an account of the BSA delivery systems that make use of modified magnetic particles as a BSA carrier. In this article, BSA adsorption and desorption on the surface of this hydrogel particles were conducted in laboratory and in a simulated intestinal fluids. Also the equilibrium and kinetics of adsorption process were studied.

Experimental

Material

Styrene (ST, Merck), divinylbenzene (DVB, Merck) iron oxide (γ-Fe_2O_3**),** oleic acid (OA, Merck), hexadecane (Merck), octane (Merck), 2,2-azobisisobutyronitrile (AIBN, Merck), 2-hydroxyethyl methacrylate (HEMA, Merck), sodium dodecyl sulfate (SDS, Merck), dimethylformamide (DMF, Merck), maleic anhydride (MAH, Merck), acrylamide (AAm, Merck), potassium persulfate (KPS, Sigma-Aldrich) and β-cyclodextrin (β-CD) was obtained from Aldrich Chemical Company. Acetic acid, sodium acetate, hydrochloric acid (HCl, 36 wt.%, Merck), sodium dihydrogen phosphate, disodium hydrogen phosphate were supplied by Merck (Germany) with high purity, Bovine Serum Albumin (BSA, Fraction V, 99%, without fatty acid) was obtained from Sigma Chemical Company and four time distilled water was used throughout the study, All of them were used as received.

Instrumentation

The pH measurements were made with Denver instrument model UB-5 ultra Basic Japan, Ultraviolet- Visible (UV-Vis) Jasco V-530 made of Japan and shaking Incubator 1000 Heidolph Germany were used.

Synthesis method

Synthesis of magnetic polymer particles modified with β-cyclodextrin (MPP-β-CD) was reported in our previous work [29]. Briefly at first a fixed amount of OA was added to ten grams of iron

oxide followed by 120 mL of ammonia solution and the mixture was stirred 3 h at room temperature. The coated iron oxide particles were dispersed in the octane with an iron oxide content of 14 wt. % to form to a ferrofluid. Then the ferrofluid with 0.3 g hexadecane constituted the oil phase was added to solution of SDS. The reaction mixture was heated to 80 °C and the octane evaporated and removed. At the same time, the styrene monomer, DVB, AIBN as initiator and hexadecane were added to a surfactant solution containing amount of SDS and the resulted mixture was stirred for 1 h. Finally, the styrene mini-emulsion and the iron oxide dispersion as obtained above were mixed and stirred. After 3 h, 1 g of HEMA dissolved in 20 mL of water and added to the reaction mixture and polymerization was carried out at 80 °C for 16 h with continual stirring. After the reaction was completed the mixture was washed with methanol. A modified core particles carrying vinyl groups were synthesized by addition of MAH (0.05 mol) in 30 g of DMF-based core particles dispersion and heated at 80 °C. A same value of β-CD-MAH and AAm were added to dispersion of water based particles and use KPS as initiator. The mixture solution was heated at 80 °C for 24 h under the vigorously stirring. Finally, the sediment dispersed and kept in distilled water with iron oxide of 5 wt. %.

Adsorption experiments

The adsorption experiments were carried out by a set of solutions (the volume of each 100 ml) containing 20 mgL^{-1} of BSA. The pH values were adjusted between ranges 3.5-7 with 0.01M acetate buffer and phosphate solutions. Then 0.02g of MPP- β-CD was added to 50 ml of each solution and the bottles were located into a shaker. BSA adsorption was processed for 3 h at room temperature and the amount of BSA remaining in the supernatant was determined by UV-Vis spectrophotometer at the maximum wave length value, which was 278 nm. The optimum pH was then determined as 4 and used throughout further adsorption experiments, which were conducted at various time periods (1, 2, 5, 15, 60 and 120 min) intervals in order to determine the adsorption equilibrium time. The concentration of BSA was determined by UV-Vis spectrophotometer and the saturated adsorption capacity of MPP as a function of contact time was determined.

Isotherm studies

A set of beakers filled with sample solutions (the volume of each 50 ml) containing 5, 10, 20, 50 and 80 mgL^{-1} of BSA was taken, and their pH was adjusted to optimum value 4. Then 10 ml of each solution was mixed with 0.02 g MPP and placed in water bath shaker for 4 h at room temperature. The beakers were then removed from the shaker and each sample was filtered. The content of BSA in MPP was measured by UV-Vis spectrophotometer.

Determination of BSA releasing

To test the desorption of BSA on the MPP, two series of samples, each containing 20 ml BSA aqueous solution of 20 mgL^{-1} were taken. Then 0.02 g MPP and 50 ml pH=7.4 phosphate buffer were added to each sample. One bottle was placed into an incubator and the other was located into a thermostat shaker with temperature set at 37 ºC and the BSA adsorption was processed for 30 h. of the mixtures, 1 ml was taken out from the bottles every hour and the amount of BSA remaining in the supernatants were determined by UV-Vis spectrophotometer. The cumulative release rate-time curve was measures as mentioned above.

Result and discussion

Magnetic polymer particles changed with β- CD have been extensively used with different facets in biotechnology as well as biomedicine fields and the outermost cyclodextrin moiety can act as active sites and particular containers for drugs and biomolecules. The innermost magnetite particles can perceive and reply to an outwardly used magnetic section. A momentous contend in the synthesize of magnetic polymer particles (MPP), specifically for their biological usage, is that the magnetic content of the polymer particles shall be large enough for rapid magnetic separation.

Due to difficulty in dispersion of high concentrations of inorganic hydrophilic particles into droplets of hydrophobic monomers by operations relying on direct monomer polymerization, the magnetic amount in the polymer phase is commonly confined [18, 30]. So, to forbear these difficulties and synthesizenear nano-sized magnetic polymerspherewith high and uniformmagnetic content, a devious procedure relying onminiemulsion polymerization [31–

33]was applied to preparation ofmagnetic polymer particleschangedwith β-cyclodextrin in our previous work [29]. Also the resultedmagnetic polymer particleswere characterized usingFourier Transform Infrared spectroscopy (FTIR), X-ray Diffractometer (XRD), Thermal GravimetricAnalysis (TGA), Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and Vibrating Sample Magnetometer (VSM) [29]. Based on TGA measurements, TEM and SEM micrographs of MPP and MPP-β-CD, we acquired in brief that:

1-The value of core and final particles of iron oxide were 84.42 % and 67.04 % by weight, respectively and the synthesized magnetic particles had high thermal resistant. (about 350 °C).

2- The synthesized core particles (MPP) were spherical with smooth surface and had a size of less than 3 μm.3-The surface of particles has been figured out rough because of polymerization on the surface of MPP after correction of MPP with β-CD (MPP-β-CD), and the size of MPPs was nearly 20 μm after alteration with β-CD.

It is known that BSA is a versatile protein carrier for drug delivery which is non toxic, non-immunogenic, biocompatible and biodegradable. Nanoparticles containing Bovine serum albumin (BSA) performed an emerging role in the expansion of new drug delivery systems and when drug was loaded on magnetic BSA nanoparticles, betterments have been received in the release control [25]**.** In this effort, Bovine serum albumin adsorption on the surface of the hydrogels of magnetic polymer particles (MPP) modified with β-CD was measured at different pH levels and under different contact times. A generally accepted reason for choosing β-CD, as the starting material is that its truncated cone-shaped hydrophobic cavity has a remarkable ability to include various guest molecules. Base upon experimental data, the presence of β-CD onto the surface of magnetic microsphere remarkably increased their protein BSA adsorption profile.

Effect of pH

The pH of the BSA solution plays an important role in the whole adsorption process and particularly on the adsorption capacity. The pH sensitive property was paid more attention to because of the important factor for the drugs' loading and releasing [34]. In the evaluation of calibration curve a linear correlation was found between adsorbance and concentrations of BSA in the range of 0.5-5 mgL^{-1}(R^2 = 0.998) (figure 1). The adsorption of BSA on the hydrogel particles were tested at different pH levels. ^{13}C-NMR and ^{1}H-NMR studies of the β-CD in

alkaline aqueous solutions and atdifferent pH revealed that β-CD does not deprotonated at pH<12 [35-37].According to experimental data the optimum pH range for the sorption of BSA on these particles occurred at pH=4, the point close to the isoelectric point of BSA (figure 2). It is pointing to the fact that β-CD displayed a higher selectivity toward the protonated BSA compared to its zwitterionic form.

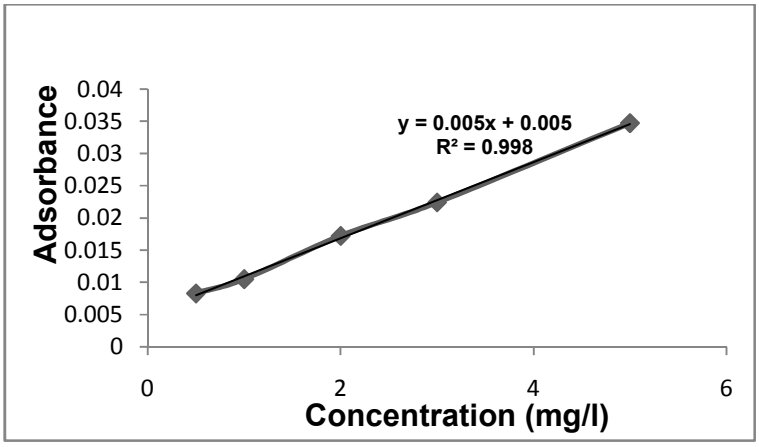

Figure 1. Linear calibration curves of adsorbance BSA.

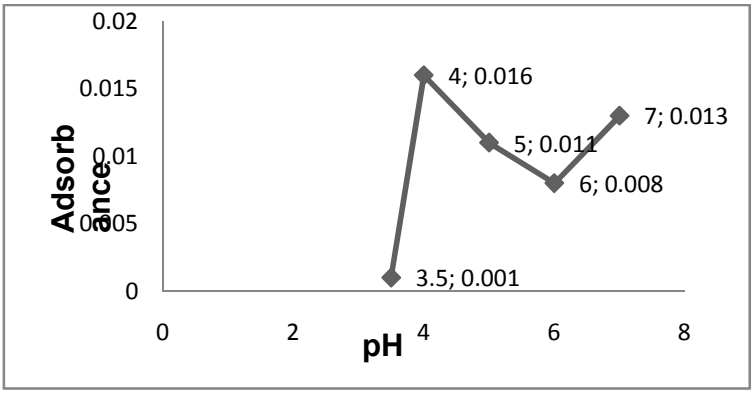

Figure 2. Effect of solution pH on the adsorption of BSA.

Adsorption kinetics

Saturated adsorption capacity evaluation of MPP as a function of contact time was studied and were conducted at various time periods (1, 2, 5, 15, 60 and 120 min) intervals in order to determine the adsorption equilibrium time. Experimental data showed that less than 5 minutes shaking was required for about 86% sorption which this is because of high availability of active sites in adsorbent(figure 3).

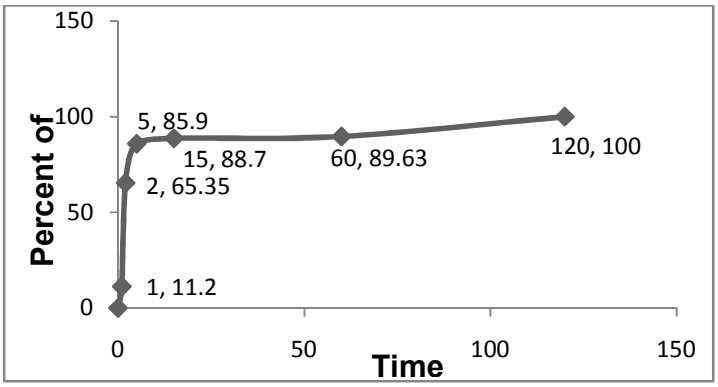

Figure 3. Saturated adsorption capacity evaluation of MPP as a function of contact time.

The amount of BSA at equilibrium Q_e(mg/g) on magnetic polymer particles was calculated from following equation:

$$Q_e=V(C_0-C_e)/W(g)$$

Where C_0 and C_e(mg/lit) are the liquid phase concentration of BSA at initial and equilibrium, respectively, V(l) the volume of the solution, and the W(g) is the mass of MPP used. The sorption capacity of the MPP for BSA was ascertained from the difference between BSA concentration in solution before and after the sorption. The effect of initial concentration of BSA in the solution on capacity sorption of BSA by MPP is indicated in figure 4.

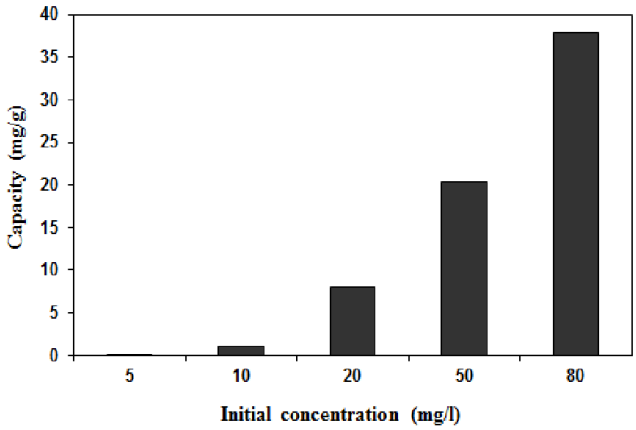

Figure 4. Plot of capacity adsorption of BSA.

Experimental data showed the capacity increased with increasing initial concentration of BSA in the solution and the polymer adsorption capacity (for 1g of polymer in constant temperature) was 37.94 mg/g.

Adsorption isotherms

The amount of BSA adsorbed per unit of MPP is indicated in figure 5 by adsorption isotherm. It is applied for optimized BSA sorption in equilibrium concentration at constant temperature (20°C).The Freundlich is fixed isotherm model for BSA sorption. This isotherm is an experimental isotherm used to define heterogeneous systems in which it is specified by the heterogeneity factor 1/n and Freundlich constant (K_F)with the equation (1):

$$\ln q_e = \ln K_F + 1/n \ln C_e \qquad (1)$$

According to Freundlich model K_F and n values were 1.5×10^{-3} (mg g^{-1}) (L mg^{-1})$^{1/n}$ and 0.25, respectively. The experimental data of this model is based on adsorption on a heterogeneous modified surface [38].

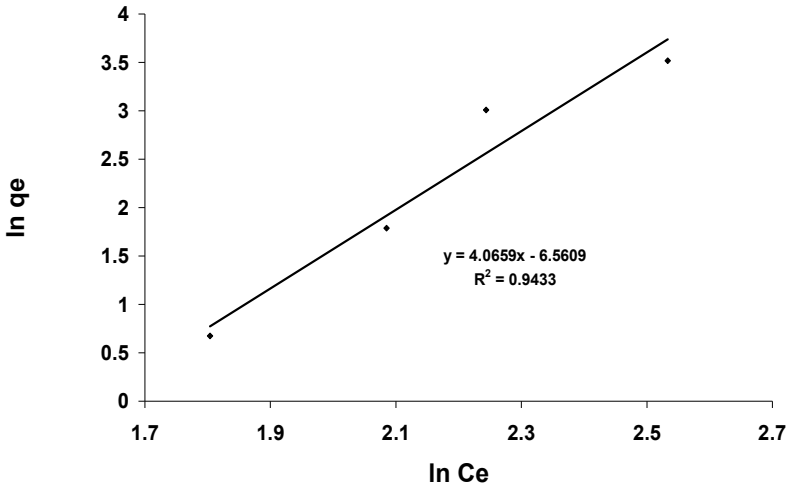

Figure 5. Freundlich plot for the adsorption of BSA onto the particles.

BSA release

BSA release by MPP in simulated intestinal fluid (pH 7.4) is shown in figure 6. Almost, 50% and 40% of the BSA was released in the simulated intestinal fluid in shaking and without shaking mode with steady slope over a period of 30 h at 37°C. This result is indicated the applicability of the work for BSA release in intestinal fluid.

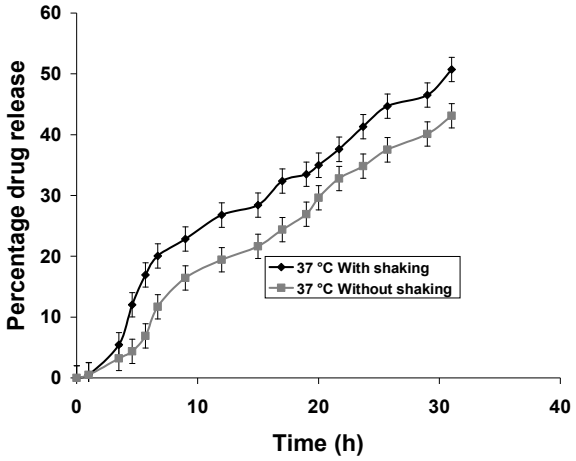

Figure 6.BSA release by MPP in simulated intestinal fluid.

Conclusion

The purpose of this study was to synthesis novel CD-modified magnetic polymer particles for pharmaceutical and biomedical usages. This work is a unique investigation report of sorption and releasing of BSA by CD-modified MPP which proved the practical use of CDs as a BSA carrier in simulated intestinal conditions.Although the interaction of BSA with some selected drugs was previously reported [25], the stability of BSA binding to MPP has never been investigated. This study showed that MPP modified by CD is able to adsorb and desorbed BSA and its adsorption kinetics is highly favorable. The Freundlich is the best fitting isotherm models for the biosorption of BSA and dipole-dipole interaction between MPP and BSA could be clearly suggested as an important factor in adsorbency kinetic.The controlled BSA-release property of these magnetic particles could makes themto be a target of academic researches and this kind of dendrimeres might be successfully employed in the biomedical industries in the coming years.

Acknowledgements

The authors gratefully acknowledge financial and technical supports of the project by the Islamic Azad University, central Tehran branch.

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