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The Study of Albumin Release from Silica/Albumin as a Potential Drug Delivery Carrier

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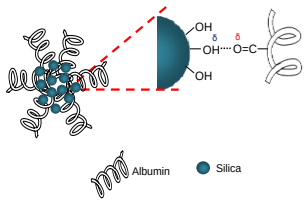
Graphical abstract



Abstract

The drug-delivery field has been an attractive as well as challenging area for research. With the emerging of new formulated drugs and pharmaceutical compounds, development of good drug-delivery system (DDS) is crucially required. This study aims to utilize albumin as the drug template in silica/albumin/drug (S/A/D) system. Prior to designing this system, the interaction between silica and albumin was investigated. It is hypothesized that high interaction between silica and albumin may result in slower drug release over time, which is preferred for a good DDS. Silica and albumin (S/A) materials were prepared by using fumed silica and tetraethyl orthosilicate (TEOS) as the silica precursors. Three different S/A samples were prepared; fumed silica with albumin (FS/A), fumed silica with pre-treated albumin by sodium borohydrate (FS/A-N), and silica sol (TEOS) with albumin (SS/A). *In-vitro* release of albumin in phosphate buffer solution (pH 7) was carried out to examine the interaction between albumin and silica. The concentration of albumin was detected at 280 nm by UV-visible spectrophotometer. All samples were characterized by diffuse reflectance-UV-visible spectrophotometer (DR-UV), Fourier transform infrared spectrophotometer (FTIR) dan thermogravimetric-differential thermal analysis (TG-DTA). DR-UV results show that SS/A exhibited the lowest absorption intensity at 280 nm, which indicates better interaction between silica and albumin. This result was supported by the presence of Si-O stretching band of silanol at 952 cm^{-1} from the FTIR spectrum. Release study of albumin demonstrated that the release of albumin from SS/A was slowest compared to those of FS/A and FS/A-N.

Keywords: Albumin; silica/albumin; high interaction; drug-delivery field

Abstrak

Bidang penghantaran-dadah telah menarik perhatian di samping menjadi bidang kajian yang mencabar. Dengan kemunculan dadah difomulasi dan sebatian farmaseutikal yang baru, pembentukan sistem penghantaran-dadah yang baik adalah amat diperlukan. Kajian ini bertujuan untuk menggunakan albumin sebagai tapak perlekatan dadah di dalam sistem silika/albumin/dadah (S/A/D). Sebelum sistem ini direkabentuk, interaksi diantara silika dan albumin telah disiasat. Dihipotesiskan bahawa interaksi yang tinggi diantara silika dan albumin akan menyebabkan pelepasan dadah yang lebih perlahan dari masa ke semasa, di mana lebih digemari untuk sistem penghantaran-dadah yang bagus. Bahan silika/albumin (S/A) disediakan dengan menggunakan *fumed* silika dan tetraetil orthosilikat (TEOS) sebagai pelopor silika. Tiga S/A sampel yang berbeza telah disediakan; *fumed* silika dengan albumin (FS/A), *fumed* silika dengan albumin pra-rawat dengan natrium borohidrat (FS/A-N), dan silika sol (TEOS) dengan albumin (SS/A). Pelepasan albumin secara *in-vitro* di dalam larutan penimbal fosfat (pH 7) telah dijalankan untuk mengesan interaksi diantara silika dan albumin. Kepekatan albumin telah dilihat pada 280 nm dengan menggunakan spektrofotometer ultra lembayung-cahaya nampak. Kesemua sampel telah dicirikan oleh spektrofotometer pantulan-serakan ultra lembayung-cahaya nampak (DR-UV), spektrofotometer Infra-merah transformasi Fourier (FTIR) dan analisis termogravimetri-pembezaan terma (TG-DTA). Hasil DR-UV mempamerkan bahawa SS/A menunjukkan intensiti serapan yang paling rendah pada 280 nm, di mana interaksi yang lebih baik di antara silika dan albumin telah ditunjukkan. Hasil ini disokong oleh kehadiran jalur regangan silanol Si-O pada 952 cm^{-1} pada spektrum FTIR. Kajian pelepasan albumin menunjukkan bahawa pelepasan albumin daripada SS/A adalah pelepasasn yang paling lambat berbanding FS/A dan FS/A-N.

Kata kunci: Albumin; silika/albumin; interaksi-tinggi; bidang penghantaran-dadah

1.0 INTRODUCTION

Over the past three decades, there has been a rapid growth in the area of drug delivery, especially in searching for new drug delivery systems. Both natural and synthetic carriers have been developed and their applicability in delivering suitable drug has also been extensively performed.

Natural materials such as albumin have a long history in pharmaceutical applications. Albumin has serves as a biodegradable carrier mostly in the form of nanoparticles, for the delivery of numerous anti-cancer drugs [1]. This is due to its excellent biocompability, biodegradability and high stability in blood (1, 2). Besides that, albumin can be found in egg white (ovalbumin), blood serum, milk, animal and plant fluids [3]. An ovalbumin monomer consists of 385 amino acid residues, with molecular weight of 45,000 Da [4]. Its multi-binding site properties are contributed by multiples functional groups present which are amines, carboxylic acids, and cysteine residuals for covalent conjugation with biological molecules and biocompatible polymers [5].

This study is the first attempt to explore the ability of albumin or ovalbumin to act as a drug 'template' in silica/albumin/drug (S/A/D) system. Silica (SiO_2) has been selected as the second carrier or support of this system. Silica nanoparticle and silica-based material have been widely used in drug delivery field as the carrier [6, 7].

Prior to the attachment of the drug, the interaction between silica and albumin need to be addressed. Hence, the purpose of the present work is to investigate the effect of different preparation methods of S/A material towards the release of albumin in phosphate buffer solution (pH 7) at 37 °C. Two type of silica source were used; fumed silica and tetraethyl orthosilicate (TEOS). Sodium borohydrate ($NaBH_4$) was used to denature the secondary structure of albumin in one of the pre-treatment step to prepare S/A material. One hypothesizes that high interaction of S/A may results in slower drug release over time, which is preferred for a good DDS.

2.0 MATERIALS AND METHODS

2.1 Chemicals

Albumin/ovalbumin, from chicken egg white (crystallized and lyophilized, Sigma-Aldrich), fumed silica (CAB-osil®M-5, Fluka), tetraethyl orthosilicate (TEOS, Acros), sodium borohydrate ($NaBH_4$, QRec), curcumin (QRec), ammonia solution (NH_3, 28%, QRec), ethanol (Merck), buffer solution (potassium dihydrogen phosphate, pH 7.00, Merck) and deionised (DI) water were used.

2.2 Characterization Techniques

The FTIR spectra of all samples were collected on a Perkin Elmer Spectrum One spectrometer with 10 scans and resolution of 4 cm^{-1}, in the range of 4000–400 cm^{-1} and potassium bromide (KBr) pellet technique was used. The samples were mixed with KBr in the weight ratio of 1:100. All samples were also recorded by Perkin Elmer Ultraviolet–visible Spectrometer Lambda 900 in the range of 200–800 nm. Amount of albumin and curcumin were detected by using Shimadzu 1800 UV-visible spectrophotometer in the range of 200-800 nm. Thermogravimetric-differential thermal analysis (TG-DTA) was performed using Mettler Toledo TGA-DTA STAR SW.8.10 instrument. The analysis was carried out starting from room temperature to 800 °C with heating rate of 10 °C/min under nitrogen atmosphere.

2.3 Preparation of Silica/Albumin (S/A)

The preparations of S/A are divided into three sections as below. S/A were prepared with the ratio of 25:75 (w/w %).

2.3.1 Fumed Silica/Albumin (FS/A)

First, fumed silica (0.3 g) and albumin (0.1 g) were homogenously ground in a clean mortar. The mixture was then transferred into a clean, dry glass vial (10.5 mL). After that, DI water (5 mL) was added to the vial and the mixture was stirred at room temperature for 5 h. Then, the mixture was transferred into a petri dish covered with perforated aluminium foil. The sample was allowed to dry at room temperature for four days before being collected and kept in a desiccator.

2.3.2 Fumed Silica/Albumin treated with $NaBH_4$ (FS/A-N)

Albumin was first treated with $NaBH_4$ using a previously described method [5]. Albumin (0.1 g) was dissolved in DI water (5 mL) and then $NaBH_4$ (0.004 g) was added to the stirred solution as a reductant. The solution was stirred at room temperature for 1 h and then heated to between 60 and 80 °C until no more gas (H_2) was generated. Albumin was denatured under these conditions and most of its disulfide bonds were converted to sulfhydryl groups [5]. After that, fumed silica (0.3 g) was added into the solution under stirring condition. The mixture was stirred at room temperature for 5 h. Then, the mixture was transferred into a petri dish covered with perforated aluminium foil. The sample was allowed to dry at room temperature for four days before being collected and kept in a desiccator.

2.3.3 Silica sol from TEOS/Albumin (SS/A)

SiO_2 nanoparticles were prepared by hydrolysis and condensation of TEOS in ethanol, in the presence of ammonia as catalyst following previous method [8]. Stöber silica was first prepared by ethanol (1.167 mL), NH_3 (0.195 mL), H_2O (0.450 mL) and the mixture solution was stirred for 5 minutes followed by dropwise addition of tetraethoxysilane (TEOS) (1.116 mL) in ethanol (1.167 mL) by dropwise. The solution was stirred overnight to allow the hydrolysis process. Then the pre-synthesized SiO_2 in colloidal form was mixed with 0.02 g/mL of Alb in water for 5 h. The mixture then was dried at room temperature.

2.4 Release Study

An accurately weighed quantity of S/A samples (50 mg) was added to 30 mL of pH 7 phosphate buffer solutions and stirred gently at 37°C. At different time intervals (0.5, 1.5, 3, 5, 28 and 48 hr), 3 mL of aliquots were removed and replaced with 3 mL of fresh medium. The sample was centrifuged to obtain a clear supernatant and free albumin was detected at 280 nm by UV-visible spectrophotometer.

3.0 RESULTS AND DISCUSSION

Figure 1 shows the DR-UV spectra of S/A samples that have been prepared. The broad absorption peak at ~280 nm is attributed to phenylalanine, tryophan and tyrosine groups in albumin that contains aromatic rings [9]. It should be noted that absorption intensity at 280 nm for SS/A was lower compared to FS/A and FS/A-N. The low intensity might be due to stronger interaction of polymeric SiO_2 containing silanol groups,-Si-OH with the protein.

It is expected that the silanol groups present are attached to the carboxyl (-COOH) and amine (–NH_2) groups that present in albumin via physical adsorption and hydrogen bonding.

It is generally known that fumed silica is insoluble in water. The difference in absorbance can also be explained in terms of chromophore-chromophore interaction in protein. In the case here, chromophores (benzene ring) are stacked in a different arrangement owing to different particle size of SiO_2 prepared. Fumed silica that was used and silica nanoparticle produced in this report each have particle size of 0.2-0.3 µm and ~200 nm, respectively. In addition, smaller particle size provides higher surface area for interaction to occur. Hence, more interfacial interaction was expected to take place between SiO_2 and albumin in SS/A. Figure 2 shows the chemical structure of the side chains in albumin that bear aromatic ring.

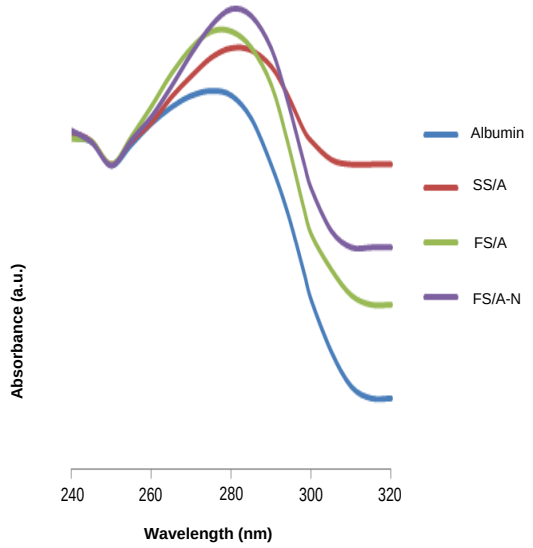


Figure 1 DR-UV spectra of the obtained samples

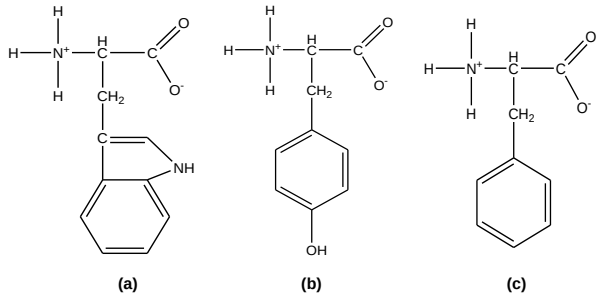


Figure 2 Chemical structure of the protein side chains (a) Tryptophan, (b) phenylalanine and (c) tyrosine

The infrared spectra of the albumin, silica nanoparticle, FS/A, FS/A-N and SS/A are presented in Figure 3(a)-(e). The spectra of albumin (Figure 3(a)) and silica (Figure 3(b)) exhibit the typical vibration bands as reported in the previous literature [3, 10]. Albumin exhibits an intense amide I band at 1649 cm^{-1} which correspond to C=O stretching, while an amide II band at 1541 cm^{-1} is relate to the out-of phase combination of in-plane C–N stretching and N–H bending of amide groups [3]. The presence of amide I band (between 1600 and 1700 cm^{-1}) is mainly associated with the C=O stretching vibration and is directly related to the protein backbone conformation. Significant peak at 1396 cm^{-1} corresponding to COO- of side chains in albumin can also be clearly seen. The broad feature around 3420 cm^{-1} at Figure 3(b) is undoubtedly due to the overlapping of the O-H stretching bands in hydrogen-bonded water molecules and SiO-H stretching of surface silanols hydrogen-bonded to molecular water. The intense band appearing at 1104 cm^{-1} is assigned to Si-O-Si asymmetric stretching vibrations. The symmetric stretching vibration of Si-O-Si is observed at 797 cm^{-1} and its bending mode appears at 471 cm^{-1}. The vibration peak at 952 cm^{-1} was attributable to the Si-O in-plane stretching vibrations of the silanol Si-OH groups. The low energy band at around 560 cm^{-1} is assigned to the defects in SiO_2's network.

It has been clearly demonstrated that the characteristic bands of both silica and albumin are present in the spectra (c-e). However, it is worth noting that there was no observable difference in the FTIR spectra for FS/A and FS/A-N samples. This might be due to less interfacial interaction of fumed silica which was used as the silica source in preparing S/A samples. Referring to Figure B (iv), the absorption peak of Si-O stretching from silanol is present. The existence of the surface silanol groups signifies the hydrophilic nature of these sol–gel materials which are beneficial for protein adsorption. In addition, it was also observed that the vibration bands for C=O stretching at SS/A's FTIR spectrum was shifted to a higher wavenumber compared to native albumin's spectrum. This observation suggest that there was temporary hydrogen bonding between C=O group and silanol surface. In addition, Fenoglio *et al*. in her reviews state that the interaction of a solid with biomolecules is determined by the physico-chemical properties of its surfaces [11]. It has also been suggested that the adsorption of albumin to silica affects the secondary structure of the protein such as helix [12]. Hence, it can be safely deduced that SS/A sample has the best interaction, which is in good agreement with the DR-UV results.

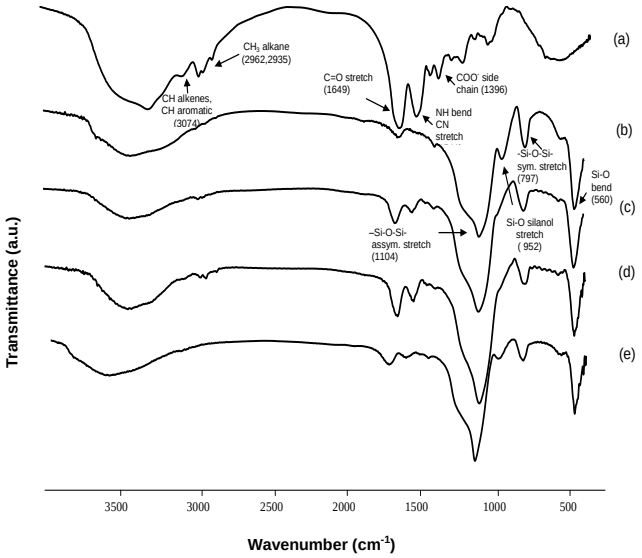


Figure 3 FTIR spectra of obtained samples (a) albumin (b) silica (c) FS/A (d) FS/A-N and (e) SS/A

The thermogravimetric analysis curves for FS/A and SS/A are shown in Figure 4. The weight loss began at 28°C, reached midpoint at 84°C and continued until 150°C. This is caused by the release of water from the samples. The weight loss of sample then continued at 200°C and ended at 380°C, with midpoint of 343°C, due to the decomposition of albumin. It is also notable that the weight loss of the material began at 500°C to 700°C for both samples. In addition, it can be seen that SS/A sample released higher amount of water compared to the FS/A

sample. This observation might be due to more water were absorbed by SS/A sample compared to FS/A and FS/A-N.

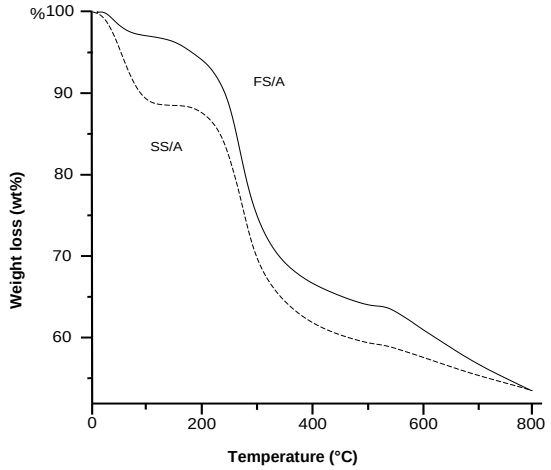


Figure 4 Thermogravimertric curves of SS/A and FS/A

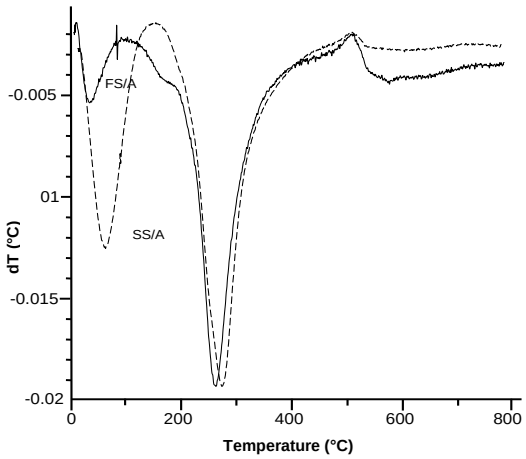


Figure 5 Differential thermal analysis of SS/A and FS/A

Figure 6 shows the release profile of albumin from silica into the pH 7 phosphate buffer solution at 37°C. 50 mg of each S/A sample was used for all the albumin release experiments. For all the samples, the release of albumin follows a two-phase kinetics [13]. In the first phase, the amount of albumin released increase rapidly (burst effect). This burst release implies the desorption of unbonded albumin to silica particles. Another factor that may influence the initial high release is the good water-solubility of albumin in the phosphate buffer solution. The second phase is related to a pseudo-steady state at which the release rate is constant and much slower [13]. It can be seen that after 4 h, the amount of albumin released from each sample was about 40%. However, it is notable that the release of albumin from SS/A was slower compared to FS/A and FS/A-N. For example, over a period of 28 h, the albumin released was ~60% for SS/A, ~72% for FS/A-N and ~80% for FS/A. The albumin release for SS/A was the slowest due to the hydrophilicity of silica from TEOS in the preparation of SS/A. These results proved that the higher interaction of silica and albumin influenced the release rate of albumin. The release of albumin is also affected by the selection of precursors and preparation methods.

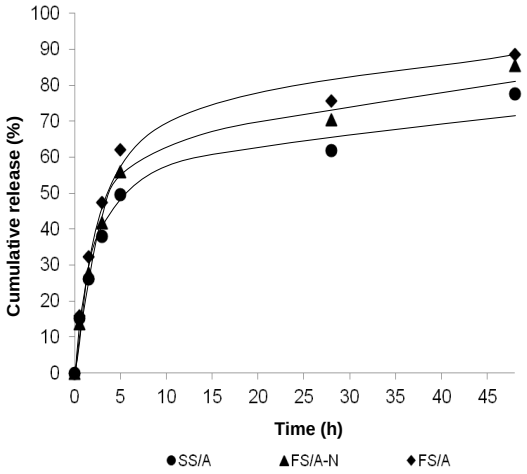


Figure 6 Cumulative release of albumin from S/A samples

On the basis of these results, a model of the possible interaction between silica and albumin is proposed (see Figure 7). This model illustrates the temporary hydrogen bond between the silanol group of silica and the carbonyl group of albumin.

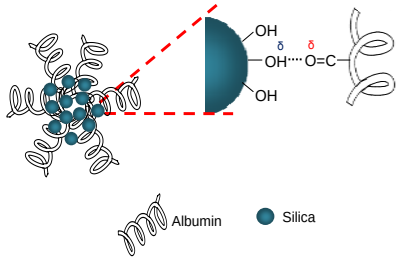


Figure 7 Schematic representation of the interfacial interaction between silica and albumin

4.0 CONCLUSION

In this report, three silica/albumin samples have been prepared. Based on the release profile of albumin, it can be stipulated that the high interfacial interaction of SS/A is contributed by the silica precursor used. High degree of interaction between silica and albumin is expected to increase the potential of this material as a drug-carrier. The application of silica/albumin as a drug carrier will be reported in future papers.

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