Jurnal Teknologi, 49(F) Dis. 2008: 51–68

ADSORPTION CHARACTERISTICS OF BOVINE SERUM ALBUMIN AND RIFAMPICIN ON IMMOBILIZED METAL ION AFFINITY MESOPOROUS ADSORBENTS

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Abstract. Bovine serum albumin and rifampicin adsorption are studied by using MCM-41 and SBA-15 immobilized with metal ion, Cu^{2+}, Ni^{2+} and Co^{2+} during their synthesis as the adsorbents. Batch equilibrium adsorption experiment were carried out to study the effect of the solution pH, types of adsorbents, types of metal immobilized on the mesoporous adsorbents and metal loading concentration on adsorption capacity. Buffered BSA and rifampicin was contacted with the adsorbents, shaken and centrifuged. BSA and rifampicin concentration was determined by UV/Vis spectrophotometer while adsorbate-adsorbent interactions were determined by Fourier Transform Infrared (FTIR). The adsorption capacity of BSA was highest at pH 4 near the pI of BSA at 4.9 while rifampicin uptake was maximum at pH 7 near the pK_{a2} of rifampicin. This is due to electrostatic attraction between proteins and surface of adsorbents. It was found that cationic form of rifampicin exhibited very high degree of adsorption with the cationic exchanger of adsorbents. Immobilization of metal ions into mesoporous framework has proven to increase the adsorption rate of BSA and rifampicin, and metal ions with different concentration also gave some influence to the adsorption capacity.

Keywords: Adsorption; MCM-41; SBA-15; bovine serum albumin; rifampicin

Abstrak. Penjerapan BSA dan rifampicin dengan menggunakan MCM-41 dan SBA-15 yang dimasukkan ion logam Cu^{2+}, Ni^{2+} and Co^{2+} semasa proses sintesis sebagai bahan penjerap telah dikaji. Sistem penjerapan kelompok dijalankan untuk mengkaji kesan pH larutan, jenis bahan penjerap dan ion logam yang serta kepekatan ion logam yang digunakan. BSA dan rifampicin dicampur bersama bahan penimbal, disentuh dengan bahan penjerap, digoncang dan ditapiskan dengan teliti. Kepekatan BSA dan rifampicin diukur menggunakan spektrofotometer UV/VIS manakala tindak balas antara bahan penjerap dan bahan yang dijerap ditentukan menggunakan spektrofotometer pengubah bentuk Fourier Inframerah (FTIR). Keupayaan penjerapan BSA, adalah tertinggi pada pH 4 hampir kepada nilai pI BSA, iaitu pada 4.9 manakala kapasiti penjerapan tertinggi bagi rifampicin adalah pada pH 7 hampir kepada nilai pK_{a2} rifampicin. Ini disebabkan oleh tarikan elektrostatik antara BSA dan rifampicin dengan permukaan bahan penjerap. Rifampicin dalam bentuk kationik didapati mempamerkan penjerapan yang sangat tinggi. Kemasukan ion logam ke dalam struktur bahan penjerap meso didapati telah meningkatkan kadar penjerapan BSA dan rifampicin manakala ion logam dengan kepekatan yang berbeza juga mempengaruhi kadar penjerapan.

Kata kunci: Penjerapan; MCM-41; SBA-15; bovine serum albumin; rifampicin

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1.0 INTRODUCTION

The efficient separation and purification of proteins and antibiotics has been an important prerequisite for the current development in the biomedical and pharmaceutical industries. From all the separation technologies available today for the purpose of separation of delicate bioproducts, those based on affinity interactions are most favoured. One important method is by chromatography and the developments of mesoporous materials as size-selective chromatographic supports which holds a potential for more efficient separations. Chromatographic separations depend on the differential partitioning of proteins or antibiotics between a stationary phase (adsorbent) and mobile phase (buffer). The proper selection of the stationary phase and mobile phase both influence the separation [1 - 3].

There are a few studies in the literature on the adsorption of proteins on siliceous molecular sieve [1, 4 - 6]. Generally, it has been concluded that adsorption capacity is dependent on the pore size of the adsorbent relative to that of the protein or antibiotics, and a pore size slightly larger than the hydrodynamic radius is sufficient to obtain high capacities.

Regarding to this bioproduct separation process, the discovery of the first ordered (where the pores are ordered periodically), mesoporous molecular sieves has sparked interest throughout the scientific community. These materials, possessing pore sizes in the 20-100 Angstroms range, have a wide range of potential applications including shape-selective catalysis and sorption of large organic molecules, chromatographic separations, and uses as host to confine guest molecules and atomic arrays [7]. Mesoporous molecular sieves (MMS), such as the M41S family of materials, have attracted wide attention from researchers since they were first reported by Mobil Corporation in 1991. Their outstanding properties make them promising as catalysts or adsorbents for industrial separation processes. Their high surface area and tuneable pore size with narrow pore size distribution in the mesoporous region, give them substantial potential for size inclusion separation of large molecules such as proteins, which are important in the food and pharmaceutical industries [4].

Two most common representatives of ordered silica are MCM-41 and SBA-15. Both of them possess a well-ordered two-dimensional (2D) hexagonal array of mesopores. MCM-41 materials are usually synthesized in a basic medium in the presence of cationic surfactants such as cetyltrimetylammonium while SBA-15 materials have been synthesized in an acidic medium with the use of commercially available, low cost non-ionic supramolecular templates [8]. MCM-41 may be utilized as an adsorbent since it exhibits both hydrophobic and hydrophilic character depending upon the exact composition and post modification. According to Zhao [9], the removal of hydrocarbons from water, storage of gases, adsorptive xylene separation, and separation of biological and pharmaceutical compound such as protein now seems to be potential areas for developing MCM-41 applications. However, SBA-15 materials show a much higher thermal and hydrothermal stability,

being stable for at least 48 hours in boiling water. Another feature of SBA-15 materials is their unique dual pore system formed by hexagonal arranged cylindrical mesopores with micropores within the walls, which provide connectivity between large pores. In addition, their surfaces may be functionalized by binding organic ligands into the porous structure to enhance their selective adsorption of a target molecule.

Cost and time effective separation of protein and antibiotic in the industry is very crucial to produce high purity of products. Therefore many researches have been done to find the most optimum or the best method to produce high amount of product that will also save time and cost. Regarding to this protein and antibiotic separation, the mesoporous adsorbent used which is MCM-41 and SBA-15 are immobilized with metal ion. Many works have been done studying MCM-41 and SBA-15 modified with metal ion but many of them are used as catalyst [10 - 16]. Therefore the potential of this modified mesoporous adsorbents are used in the adsorption of protein and antibiotic. There are many research using this concept of immobilized metal ion affinity adsorbents using other adsorbents [2, 17 - 19]. Therefore, the combination of immobilized metal ion affinity concept with mesoporous molecular sieves are studied for the adsorption of protein and antibiotic.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Adsorbents

The study was carried out for 2 types of mesoporous molecular sieves which are MCM-41 and SBA-15. These mesoporous molecular sieves were then modified by immobilizing 3 types of metals using the cation exchange method from the metal nitrate solution. The metals immobilized were Cu^{2+}, Co^{2+} and Ni^{2+}. Table 1 shows the general chemicals used for the synthesis of MCM-41 and SBA-15.

Table 1 General chemicals needed for the synthesis of mesoporous adsorbents

Chemicals	MCM-41	SBA-15
Surfactant	Cetyltrimethylammonium Bromide (CTAB)	Pluronic P123 ($PEO_{20}PPO_{70}PEO_{20}$)
Solvent	Deionized water	Deionized water
Silica precursor	Tetraethyl Orthosilicate (TEOS)/Sodium Silicate/Silica Fumed	Tetraethyl Orthosilicate (TEOS)
Mineralizing agents	NH_4OH/ethyl acetate/isopropyl acetate	HCl

2.1.2 Bovine Serum Albumin and Rifampicin

The protein used in this study is bovine serum albumin (BSA) obtained from Sigma Chemical Co, which were used as the model protein. The molecule has the shape of a prolate ellipsoid with dimensions 4.0 × 4.0 × 14.0 nm with an isoelectric point of between 4.7 – 4.9. The antibiotic used in this study was rifampicin which was a gift from Institute of Bioengineering, Zhejiang University, Hangzhou, People's Republic of China.

2.1.3 Buffer Solutions

Phosphate buffer solutions were prepared using phosphoric acid (H_3PO_4), potassium phosphates (KH_2PO_4), di-potassium hydrogen phosphate anhydrous (K_2HPO_4), potassium hydrogen carbonate ($KHCO_3$) and potassium carbonate anhydrous (K_2CO_3). These chemicals were obtained from Mallinckrodt and Fluka, and used without further purification. All aqueous phases were prepared in 0.1 M buffer, which resembles the ionic condition of most fermentation broths and stored in refrigerator at 4 °C when not in used. A series of buffer solutions with pH of 3, 4, 5, 7, 8 and 9 were prepared throughout this experiment. The buffer was adjusted to the desired pH by mixing two solution of different pH. Table 2 shows the two chemical solutions that need to be mixed to obtain the desired pH at certain volume.

Table 2 Buffer solution preparation

Solution	pH
$H_3PO_4 + KH_2PO_4$	2 – 4
$KH_2PO_4 + K_2HPO_4$	4 – 8
$KHCO_3 + K_2CO_3$	8 – 12

2.2 Metal-Modified Mesoporous Adsorbents Synthesis

2.2.1 Metal Modified MCM-41

Initially 2.4 grams of CTAB were mixed with 120 grams of deionized water and the mixture is mixed until it forms a homogenous and dear solution. After about 2 hours, 8 mL of ammonia was added and the mixture was stirred for 5 minutes. Next, 10 mL of TEOS and × grams of respective metal source (Copper Nitrate, Cobalt Nitrate and Nickel Nitrate) were added according to the ratio stated in the journals and then the mixture was stirred overnight.

The next day, the mixture was transferred into a teflon autoclave and aged at 100 °C for 2 to 3 days in the oven. pH control is done at 10.2 everyday by using acetic acid until stable. The final product is then filtered and washed thoroughly with DI water. The filtered sample is spread on a plate and dried in oven at another

100 °C for 24 hours. Finally the sample was calcined at 550 °C at a rate of 1 °C/min for 5 hrs.

2.2.2 Metal Modified SBA-15

Initially, 4 grams of Pluronic P123 are mixed with 30 mL of deionized water and was stirred vigorously for 2 hours. Then, 120 mL of hydrochloric acid 2 M was added at 25 °C and stirred for 1 hour. Next, × grams of respective metal source (Copper Nitrate, Cobalt Nitrate and Nickel Nitrate) were added according to the ratio stated in the journals and then the mixture was stirred for another 1 hour. After that, the mixture were transferred into a teflon autoclave and it was immersed in a silicone oil and stirred at 50 °C. Then, 8.5 grams of TEOS were added drop by drop while stirring for 30 minutes. Slow down the stirring rate to 120 rpm for 20 hours. Then, the samples were aged at 100 °C for 48 hours in the oven without stirring. After aging, the sample were cooled to room temperature, filtered thoroughly using deionized water and then dried in air at room temperature for 1 hour. The sample was then dried in the oven at 80 °C for 12 hours. Finally, calcination was done for 5 hours at 550 °C at a rate of 1 °C/min.

2.3 Characterization of Mesoporous Adsorbents

X-ray diffraction (XRD) was used to identify the crystal phases of modified MCM-41 and modified SBA-15. X-ray diffraction (XRD) is a versatile, non-destructive technique that reveals detailed information about the chemical composition and crystallographic structure of natural and manufactured materials.

2.4 Analytical Procedure

2.4.1 Determination of Concentration

The adsorption rate or the amount of BSA and rifampicin adsorbed onto the mesoporous molecular sieves are determined by measuring the concentration of the bioproducts in the supernatant liquid after adsorption by using UV/VIS Spectrometer.

2.4.2 Spectrum Analysis

Adsorption spectrums for peak wavelengths of protein and antibiotic were obtained by using Lambda 35 UV/Vis Spectrometer (Perkin Elmer, Inc.). Spectrum analysis for bovine serum albumin and rifampicin were given in Figure 1. Meanwhile, Ji [20] reported that the bovine serum albumin was analysed at 280 nm. For rifampicin, peak wavelengths obtained from the experiments were 235.47 and 258.65 nm while literature findings are 237, 255, 334, and 475 nm. The wavelength selected was 257 nm.

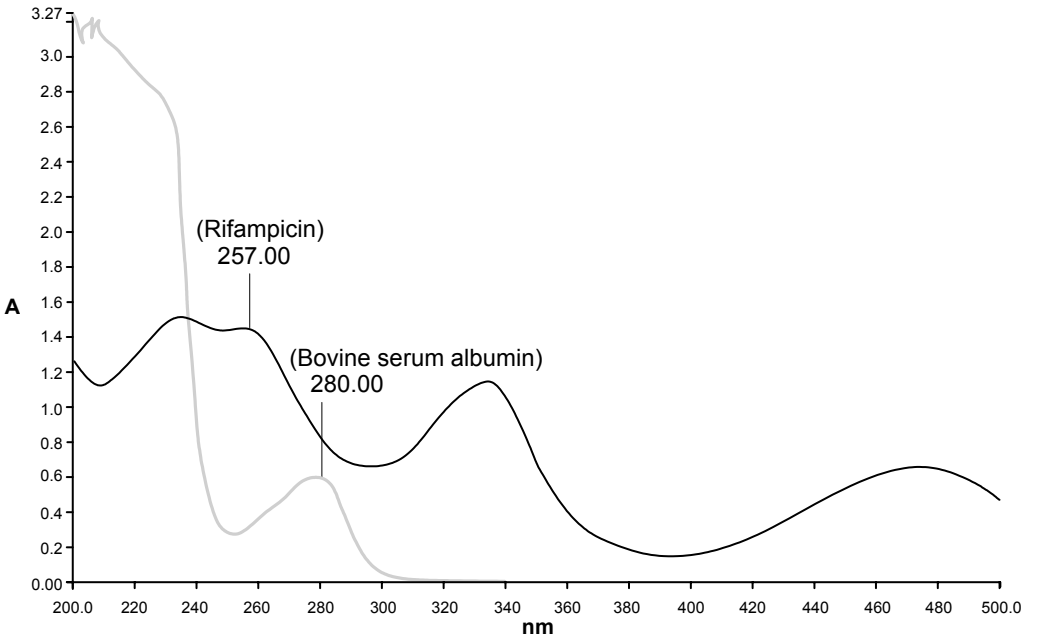

Figure 1 Spectrum analysis in aqueous phase

2.4.3 Calibration of Adsorbance Against BSA and Rifampicin Concentration

Calibration of absorbance against antibiotic and protein concentration was made according to the Bouguer-Lambert-Beer's Law.

$$A = \log_{10} T = \varepsilon bc \tag{1}$$

where A is the absorbance of a particular wavelength and is dimensionless whilst T is the transmittance of optical path length b cm and concentration c mol l^{-1} is a constant of proportionality, called molar absorbtivity.

The calibration factor for BSA and Rifampicin are obtained by plotting the initial adsorption value against protein concentration in aqueous phase. The calibration factors (slope) are tabulated in Table 3.

Table 3 Calibration factor for bovine serum albumin and rifampicin

Sample	Calibration Factor (CF)
Bovine serum albumine	27.39
Rifampicin	20.404

2.5 Protein and Antibiotic Adsorption

Batch adsorption experiments were conducted to obtained equilibrium adsorption data. Bovine serum albumin solutions with concentrations ranging 0.01 mM to 0.30 mM were prepared by dissolving different amounts of bovine serum albumin into deionized water and rifampicin concentrations ranging from 0.01 mM to 0.1 mM were also prepared by using the same method. 10 mg of metal modified MCM-41 and SBA-15 was placed into test tubes containing a mixture of 2 mL of BSA or rifampicin solution and 1 mL of 0.1 M buffer solution at various pH. The samples were continuously shaken vigorously by using a mixer at 293 K for about 6 minutes where it is assumed that equilibrium is reached. Samples were centrifuged (Hettich Zentrifugen, Tuttlingen) for about 10 minutes at 3500 rpm. Centrifugation was made to avoid the interference from scattering particles in the UV-VIS analysis [6]. After centrifugation, a sample of supernatant was withdrawn, and the BSA and rifampicin concentration was analysed by Lambda 35 UV/VIS Spectrometer (Perkin Elmer, Inc) at 280 nm for BSA and 257 nm for rifampicin. The amount of BSA and rifampicin adsorbed onto the metal modified MCM-41 and SBA-15 were calculated based on mass balance. Experimental work was conducted at room temperature, 25 °C.

2.6 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The experiments were carried out using a Perkin Elmer Spectrum 2000 Explorer FTIR (Fourier Transform Infrared Spectroscopy). Fresh metal modified MCM-41 and SBA-15 were ground and then diluted in pure KBr. The sample was pressed to form a pellet. The sample was scanned 10 times per spectrum at 4 cm^{-1} resolution. Infrared spectra for pure BSA and rifampicin sample were also obtained using similar procedure. Sample of adsorbed BSA were prepared by using a mixture of 6 mL of 1 mM buffered protein at pH 5 and 3 mL of buffer solution contacted with 0.5 g modified SBA-15 and rifampicin were prepared using a mixture of 6 mL of 0.1 mM rifampicin, 3 mL of buffer solution and 0.5 g of modified SBA-15. After equilibrium, the samples were chilled at –20 °C and allowed to dry overnight in the Freeze Dryer (Heto FD 4.0). Freeze drying technique was used to minimize the protein and antibiotic denaturation. Infrared spectra for the later samples were obtained by the method mentioned previously.

3.0 RESULTS AND DISCUSSION

3.1 Bovine Serum Albumin and Rifampicin Adsorption Capacity

3.1.1 Effect of pH

Figure 2 shows the effects of solution pH on the adsorption capacity of BSA on pure SBA-15 and metal modified SBA-15. The amount of BSA adsorbed for all SBA-15

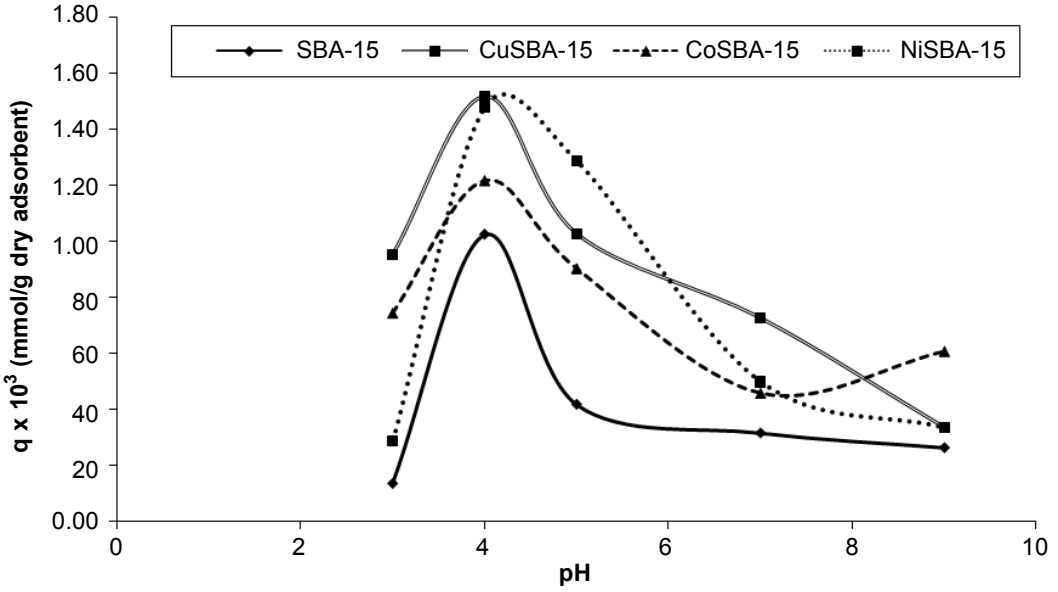

Figure 2 Effect of pH on the adsorption capacity of BSA (0.01 mM) onto pure SBA-15 and metal modified SBA-15

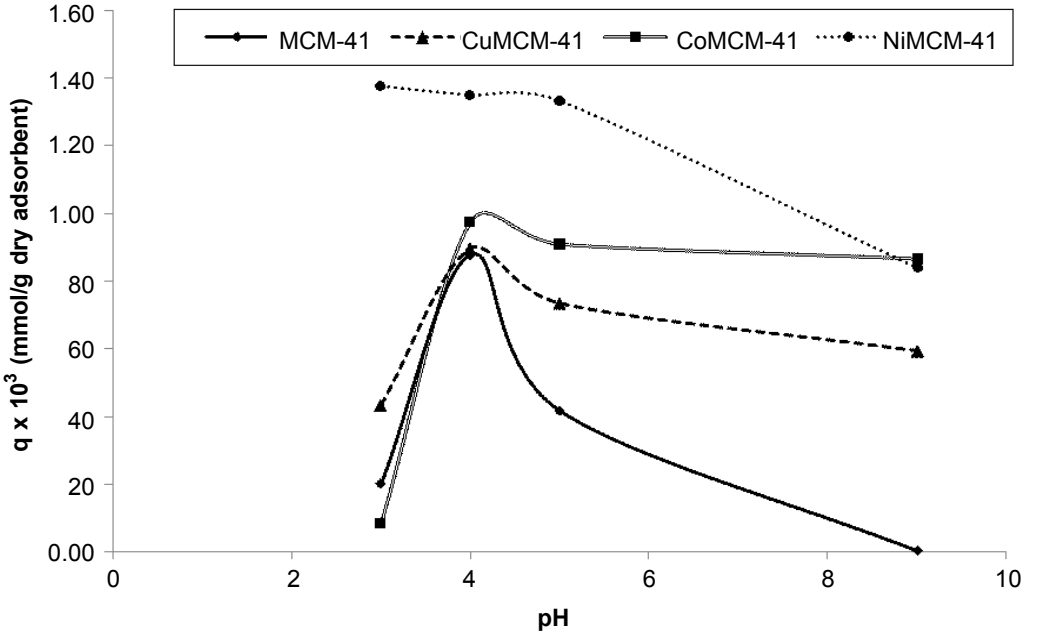

Figure 3 Effect of pH on the adsorption capacity of BSA (0.01 mM) onto pure MCM-41 and metal modified MCM-41

materials reached a maximum adsorption at pH 4 which is close to the pI value which is 4.7 – 4.9.

Meanwhile Figure 3 shows the adsorption capacity of BSA onto pure MCM-41 and the metal modified MCM-41. The adsorption trend shows a significant difference compared to the SBA-15 materials. However most of the adsorption capacities are the highest at pH 4.

BSA is positively charge at pH lower its pI and negatively charged at pH above pI. At a solution pH higher than its pI value, BSA adsorption decreases. Electrostatic repulsion between the negatively charged proteins molecule and the negatively charged of the silanol groups of the adsorbents results in a reduced amount of adsorption. Also, the protein net charge approaches zero near its pI, and the intermolecular interactions are reduced, hence, an adsorbed protein molecules requires less space and allows denser packing of protein. A pronounce effect of the pH towards adsorption capacity was observed as the adsorbed amount increases as the pH increases and reduces as the solution pH exceeds the pI value. The isoelectric point of BSA is 4.7 – 4.9. Meanwhile, the isoelectric point (the point of zero charge, PZC) of the silica surface of the adsorbents is around 2.0 [21], therefore the adsorbent is negatively charged at a pH above 2.0. Parks [22] also reported that the PZC of the alumina-silica of adsorbent is in the range of 0.5 – 3.7, therefore suggests that at pH higher than that value, the adsorbent is negatively charged. According to Kisler [4], the silica surface of MCM-41 has an isoelectric point around 2.0. The interaction between the positively charged protein and the negatively charged adsorbent is one of the dominant driving forces for the adsorption of BSA.

The effect of pH on the uptake of rifampicin by SBA-15 and its metal modified variation is shown in Figure 4. Meanwhile Figure 5 shows the effect of rifampicin loading on MCM-41 and metal modified MCM-41 adsorbents. pH solution in the range of 5 to 8 was identified using phosphate buffer with concentration of 0.1 M. The aqueous phase pH determines the extent of ionization of the rifampicin molecules, thereby affecting their adsorption affinity. Rifampicin is a zwitterions molecule and has two pK_a values which is pK_a 1.7 which is related to 4-hydroxy (anion forming) and pK_a 7.9 which is related to 4-piperazine nitrogen (cation forming). A rifampicin molecule has 16 H-bond acceptors and only 6 H-bond donors. On the other hand, silica is generally reported as having zero point of charge at around 2 ± 0.5 [21] although one siliceous MCM-41 sample was reported having a slightly higher value of 3.2 [23]. Therefore, silica surface will have a net zero charge at around pH 3 and become increasingly negative as the pH increases.

The maximum rifampicin adsorption for CoSBA-15, CuSBA-15 and NiSBA-15 recorded the highest rifampicin uptake at pH 7. From Figure 5, the maximum rifampicin adsorption onto metal modified MCM-41 adsorbents is also recorded at pH 7.

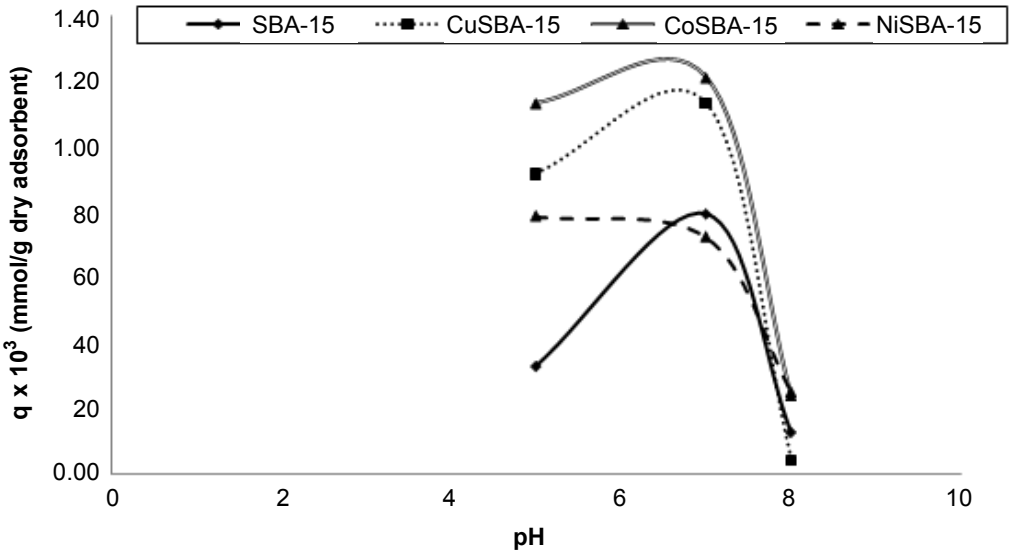

Figure 4 Effect of pH on the adsorption capacity of Rifampicin (0.01 mM) onto pure SBA-15 and metal modified SBA-15

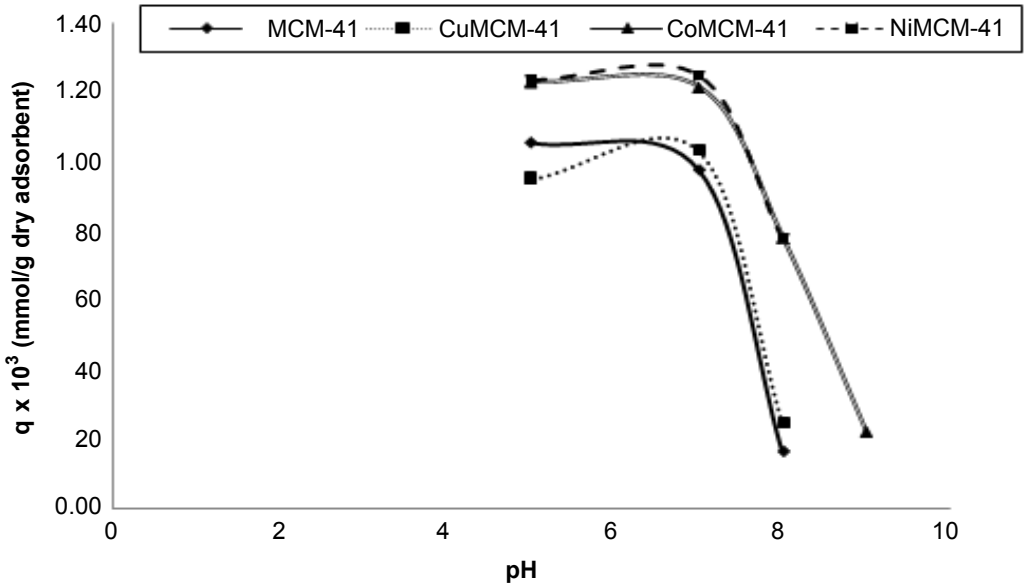

Figure 5 Effect of pH on the adsorption capacity of Rifampicin (0.01 mM) onto pure MCM-41 and metal modified MCM-41

Maximum rifampicin adsorption occurs slightly below the pK_{a2} of the antibiotic which is at pH 7 and diminished rapidly at pH > pK_{a2}. Thus, it is presumes that cationic and zwitterionic rifampicin are adsorbed to the negatively charged silica

surfaces via the protonated piperazynyl group. Similar finding was reported on the adsorption of ofloxacin onto mesoporous silica [24].

3.1.2 Effect of Adsorbents

Figure 6 and 7 illustrate the comparisons of adsorption capacity of BSA using SBA-15 and MCM-41 and the modified mesoporous adsorbents at pH 3, 4, 5 and 7. Figure 6 showed that CuSBA-15 and NiSBA-15 give a good adsorption capacity of

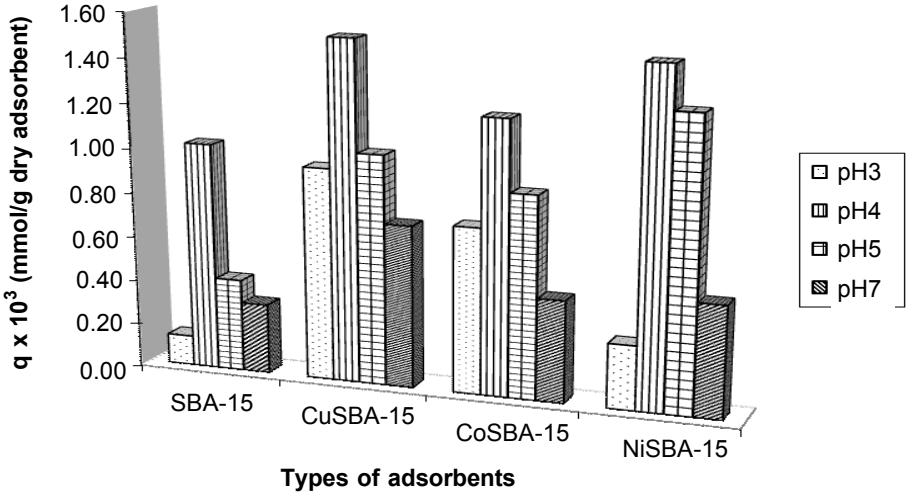

Figure 6 Effect of pure SBA-15 and metal modified SBA-15 on BSA (0.01 mM) adsorption capacity at various pH

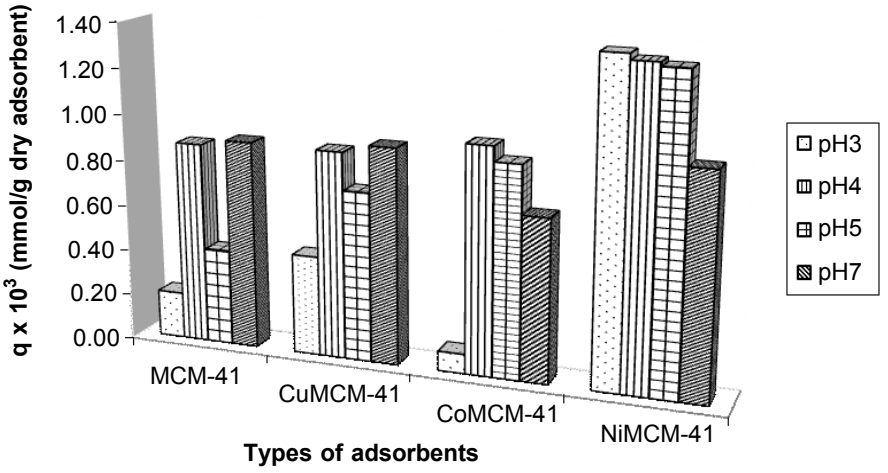

Figure 7 Effect of pure MCM-41 and metal modified MCM-41 on BSA (0.01mM) adsorption capacity at various pH

BSA compared to other adsorbents with the adsorption rate is the highest at pH 4 for all the adsorbents. The highest amount of BSA adsorbed was by CuSBA-15, followed by NiSBA-15 and CoSBA-15. Pure SBA-15 was found to adsorb the least amount of BSA.

As for MCM-41, Figure 7 shows that NiMCM-41 gave the highest adsorption capacity of BSA compared to the other adsorbents at pH 4. The adsorption capacity of MCM-41 was the lowest compared to others.

By comparing the adsorption capacity by using MCM-41 and SBA-15 materials, it can be concluded that the adsorption capacity of BSA onto SBA-15 materials are higher compared to adsorption by MCM-41 materials.

There are several factors influencing the adsorption capacity of proteins. Physical characteristics of adsorbents could increase and reduce the adsorption capacity of proteins. The diameter size of protein should be smaller that the diameter of adsorbent to enable the protein to penetrate into the mesopores of the adsorbent. In general, it has been found that proteins will adsorb in pores that are larger than the hydraulic radius of the protein [1]. Since the pores of MCM-41 and SBA-15 are larger than the dimension of BSA, the pore area can be expected to impact the adsorption capacity. The surface area and pore volume of adsorbent are also important for protein adsorption. High surface area and pore volume will promote adsorption of protein onto adsorbents.

In this study, metal ions Cu^{2+}, Co^{2+} and Ni^{2+} are also immobilized into the framework of mesoporous adsorbents MCM-41 and SBA-15 which have affected the adsorption capacity. The results showed that the adsorption capacity increases by using the metal modified adsorbents compared to the pure form. This separation method utilizes the immobilized metal ion affinity chromatography (IMAC) concept that utilizes the differential affinity of proteins for immobilized metal ions to effect their separation [25]. The histidine residue on the surfaces of the protein becomes the major targets for intermediate metal ions. Sulkowski [26] gave a clear definition of the minimal binding requirements of immobilized intermediate metal ions. He postulated that immobilized Cu^{2+} would bind a protein with a single exposed histidine, Ni^{2+} would bind a protein with two exposed vicinal histidines and Co^{2+} binding requires the presence of at least two adjacent exposed histidines on the surface of a protein.

The adsorption of proteins is based on the coordination between an immobilized metal ion and electron donor groups from the protein surface. The intermediate metal ions used can be considered as Lewis acids. Electron-donor atoms (N, S, and O) present in the chelating compounds that are attached to the chromatographic support are capable of coordinating metal ions and forming metal chelates, which can be bidentate, tridentate, etc., depending on the number of occupied coordination bonds. The remaining metal coordination sites are normally occupied by water molecules and can be exchanged with suitable electron-donor groups from the protein.

Batch experiments were also done to study the effects of different types of metal modified adsorbents on the adsorption capacity of rifampicin. Figure 8 illustrated that CoSBA-15 and CuSBA-15 give a good adsorption capacity for rifampicin, followed by NiSBA-15. The amount of rifampicin uptake by metal modified SBA-15 was also higher compared to the pure SBA-15 adsorbents. This shows that when metal ions immobilized onto SBA-15, it will increase the adsorption capacity.

As for MCM-41 and its metal modified adsorbents, Figure 9 showed that CoMCM-41 and NiMCM-41 give a good and high adsorption capacity for rifampicin. However

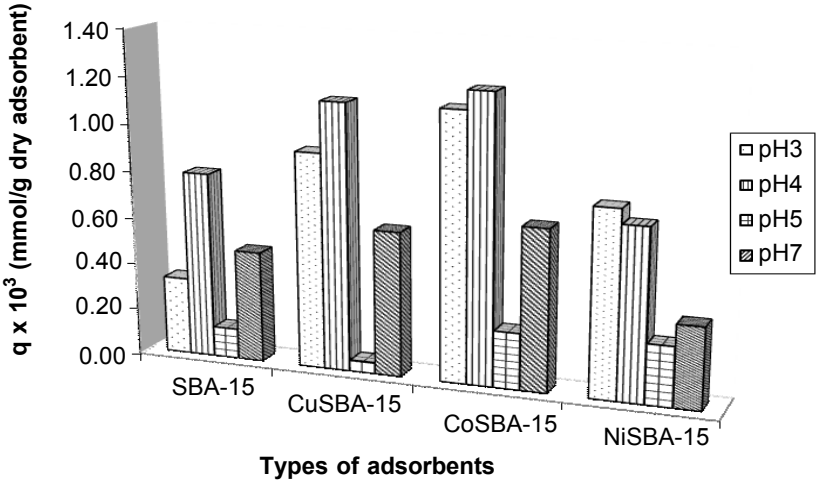

Figure 8 Effect of pure SBA-15 and metal modified SBA-15 on Rifampicin (0.01mM) adsorption capacity at various pH

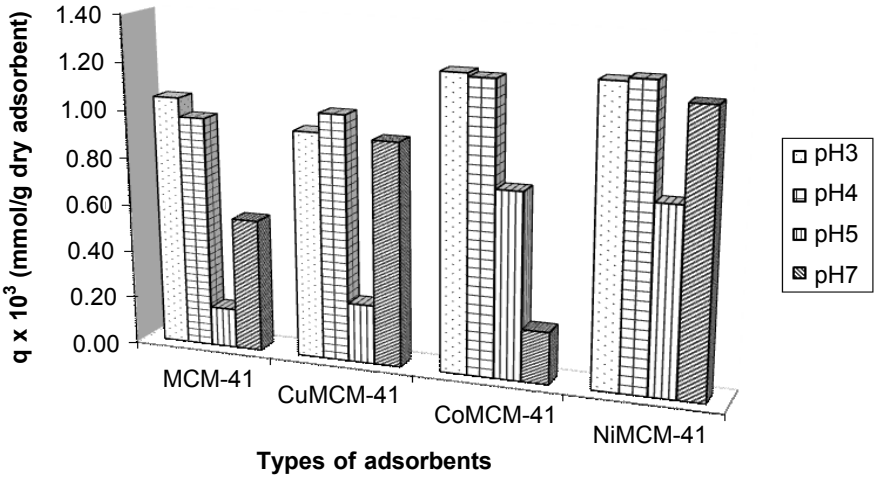

Figure 9 Effect of pure MCM-41 and metal modified MCM-41 on Rifampicin (0.01 mM) adsorption capacity at various pH

the adsorption capacities for CuMCM-41 are slightly higher than the pure MCM-41. Figure 9 also illustrated that MCM-41 immobilized with metal ions increases adsorption capacity of rifampicin compared to the original MCM-41.

The factors that can affect the adsorption capacity of antibiotics by mesoporous molecular sieves are the physical characteristics of the adsorbents which can reduce and increase the adsorption capacity of rifampicin. As a basis, the molecular size of rifampicin molecule should be smaller than the diameter of the adsorbents to allow the antibiotic to penetrate into the mesopores of the adsorbent. Other important characteristics are the surface area and the pore volume of the adsorbents. High surface area and pore volume will promote adsorption of rifampicin onto the adsorbents.

The different types of metal ion immobilized onto the mesoporous adsorbents supports SBA-15 and MCM-41, have proven will increase the adsorption capacity of antibiotic. This type of separation utilizes the concept of immobilized metal ion affinity chromatography (IMAC) combined with adsorption process. IMAC is a separation technique that uses covalently bound chelating compounds on solid chromatographic supports to entrap metal ions, which serve as affinity ligands for bioproducts, making use of coordinative binding of some of the bonds in the rifampicin molecule. The modified mesoporous adsorbents acts as an immobilized metal ion adsorbents which represent the stationary phase and the buffer solution acts as the mobile phase. Rifampicin will adsorb onto the adsorbents through the mobile phase and into the stationary phase.

3.1.3 Effect of Metal Loading

The effect of metal loading or metal ion concentration immobilized onto the structure of MCM-41 is studied and Figure 10 illustrated the effect of nickel ion concentration loaded onto MCM-41 on the adsorption capacity of BSA. The metal ion was immobilized by ion exchange during the synthesis of MCM-41 and the metal ion concentration was varied by molar composition between the metal and the material forming the mesoporous adsorbents. From Figure 10, at initial BSA equilibrium concentration there is a rapid initial rise showing high affinity between BSA and the adsorbent surface. The adsorption capacity of BSA was maximum when adsorbed by NiMCM-41 (0.025 % mole). The amount of BSA adsorbed by NiMCM-41 (0.05 % mole) and NiMCM-41 (0.01 % mole) were increasing lower than NiMCM-41 (0.025 % mole) until the maximum value at higher BSA equilibrium concentration.

There are several reports describing the cooperative mechanism of binding of proteins to IMAC adsorbents due to multipoint interactions between residues on the protein and the immobilized metal ions. As a result of this multipoint adsorption mechanism, the density or concentration of immobilized metal ions plays an important role in regards to the capacity and selectivity of the adsorbents [25].

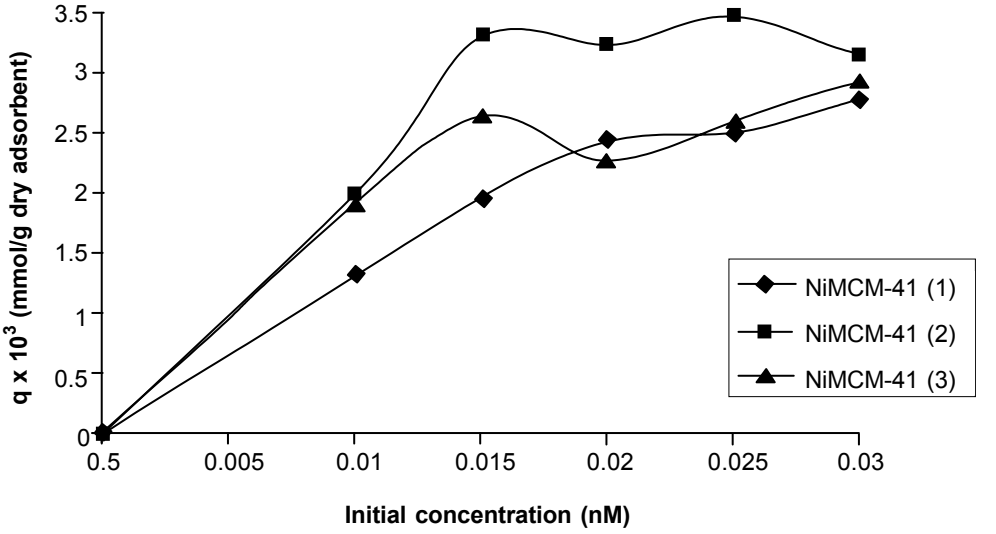

Figure 10 Effect of metal loading percentage in MCM-41 on BSA adsorption [NiMCM-41(1)]:0.01 mole % Ni, [NiMCM-41(2)]:0.025 mole % Ni, [NiMCM-41(3)]:0.05 mole % Ni

The effects of adsorption capacity of rifampicin onto NiSBA-15 with different metal ion concentration are investigated and Figure 11 illustrated this situation. From the figure presented, it can be interpreted that the adsorption capacity of rifampicin by NiSBA-15 (0.05 % mole) are the highest. The adsorption capacity of rifampicin on

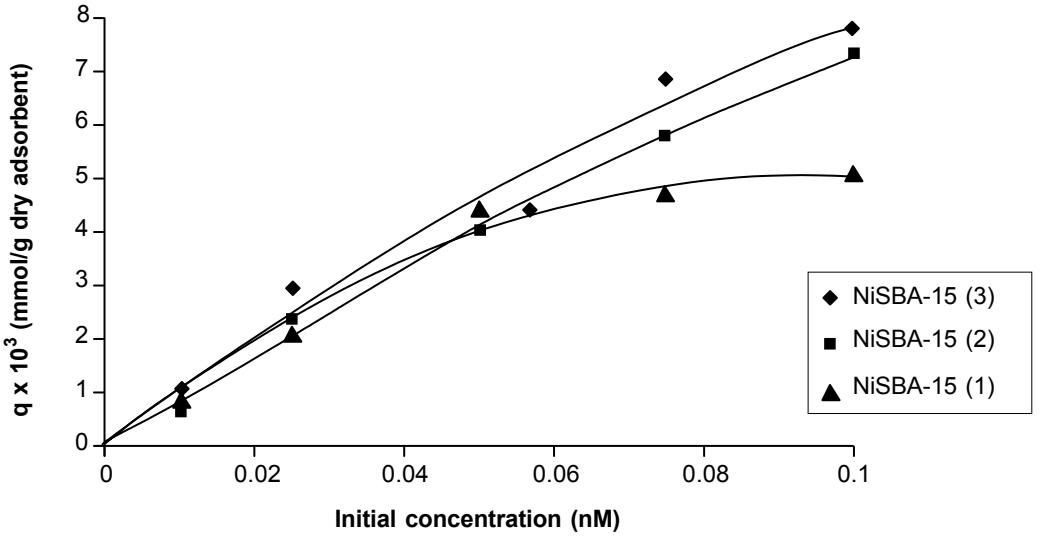

Figure 11 Effect of metal loading percentage in SBA-15 on rifampicin adsorption [NiSBA-15(1)]:0.01 mole % Ni, [NiSBA-15(2)]:0.025 mole % Ni, [NiSBA-15(3)]:0.05 mole % Ni

NiSBA-15 (0.025 % mole) are increasing lower than NiSBA-15 (0.05 % mole), followed by NiSBA-15 (0.01 % mole). The adsorption capacity increases very rapid at the initial concentration of rifampicin showing high affinity of rifampicin towards adsorbents surface and the value increases slowly at higher concentrations. It can be concluded from Figure 11 that NiSBA-15 with the highest metal loading (0.05 % mole) recorded the most rifampicin uptake followed by NiSBA-15 with 0.025 % mole and NiSBA-15 with the lowest metal loading (0.01 % mole) adsorbed the least rifampicin. The density of the immobilized metal ions has proven that it plays an important role regarding to the capacity and selectivity of the adsorbent.

4.0 CONCLUSION

It has been found that the amount of BSA and rifampicin adsorbed on different adsorbents was significantly changed by adjusting the solution pH and increased by the introduction of intermediate metal ion (Cu^{2+}, Co^{2+}, Ni^{2+}) into the pure silica materials. For BSA adsorption, CuSBA-15 at pH 4 adsorbed the most BSA while CoSBA-15 at pH 7 showed good adsorption capacity for rifampicin. Modified SBA-15 and MCM-41 with different nickel ion concentration also can affect and change the adsorption capacity of BSA and rifampicin. The X-ray diffraction results and the FTIR analysis confirms the structure of the modified MCM-41 and SBA-15 and the adsorption of BSA and rifampicin into the adsorbents. The Langmuir isotherm model was found to fit well with the BSA adsorption isotherm while it is revealed that the adsorption of rifampicin fits well the Freundlich adsorption isotherm model instead.

ACKNOWLEDGEMENTS

The corresponding author would to acknowledge Dr. Khairul Sozana Nor Kamarudin and Associate Professor Dr. Hanapi Mat for their guidance and supervision which make him possible to complete this Master's degree research project. Currently, the authors are lecturers from the Faculty of Chemical & Natural Resources Engineering, UTM.

NOMENCLATURE

List of Symbols

A - Absorbance of a particular wavelength
C_e - Equilibrium concentration of rifampicin or BSA
Co^{2+} - Cobalt metal ion
Cu^{2+} - Copper metal ion
Ni^{2+} - Nickel metal ion
N - Nitrogen atom

O - Oxygen atom
pH - Negative logarithmic molar concentration of hydrogen ion, -log $[H^+]$
pI - Isoelectric point
pK_a - Acid dissociation constant
q - Equilibrium BSA or rifampicin concentration in solid phase
S - Sulfur atom
T - Transmittance of optical pathlength
ε - Constant of proportionality, called molar absorbtivity

List of Abbreviations

BSA - Bovine Serum Albumin
CF - Calibration Factor
CTAB - Cetyltrimetylammonium Bromide
FTIR - Fourier Transform Infrared
H_2CO_3 - Carbonic Acid
IMAC - Immobilized Metal Affinity Chromatography
KBr - Kalium Bromate
K_2CO_3 - Potassium Carbonate Anhydrous
K_2HPO_4 - Dipotassium Hydrogen Phosphate Anhydrous
KH_2PO_4 - Potassium Dihydrogen Orthophosphate
$KHCO_3$ - Potasiium Hydrogen Carbonate
MCM - Mobil Catalytic Material
MMS - Mesoporous Molecular Sieves
MW - Molecular Weight
PZC - Point zero charge
SBA - Santa Barbara Amorphous
TEOS - Tetraethyl Orthosilicate
UV/VIS - Ultra Violet Visible
XRD - X-ray Diffraction

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