International Journal of Pharmaceutical and Medical Research
Volume – 5 Issue – 6 December 2017
Website: www.woarjournals.org/IJPMR *ISSN: 2348-0262*

Protein Adsorption on Inorganic Zeolite Materials

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Abstract: ***This study sought to examine the adsorption characteristics of bovine serum albumin (BSA) and cytochrome c (cyt c) on zeolite crystals and membranes. The zeolite materials were prepared by hydrothermal syntheses using different reaction gels to modulate the Brönsted acidity of the microporous structures. In addition, to modulate the surface acidity of the MFI structure, aluminium and vanadium atoms were incorporated into the crystalline framework and the ratios of Si/Al, Si/V, and Al/V were varied changing the chemical composition of the precursor reaction gels. Protein adsorption was studied under different conditions using spectroscopic techniques such as UV-visible and attenuated total reflectance Fourier transform infrared (FT-IR/ATR). This study revealed that zeolite chemical composition and structure influence the kinetics of protein adsorption. The surface of zeolite Na-Y adsorbed a greater amount of BSA and cyt c than the other structures. The percentage of adsorption of BSA depended on the pH of the solution, with adsorption greatest when the pH was near the pI of the protein. The influence of the membrane configuration on the protein adsorption was studied using different zeolite structures and crystallization types. The results suggest that the differences in adsorption capacity depend on the type of hydrothermal crystallization inside the inorganic support.***

Keywords: Protein adsorption, Hydrothermal synthesis, Zeolites, Membranes.

1. Introduction

Protein adsorption on solid surfaces is not only a fundamental phenomenon in basic research, but is also central to novel engineering applications [1-3]. In the nanotechnology field, protein-surface interactions are fundamental for the assembly of interfacial protein constructs, such as advanced functional materials, activators and sensors [4-6]. Moreover, in biomaterials biochemistry, a basic understanding of protein immobilization on new biomaterials like zeolites may facilitate the design of biocompatible structures and devices, since protein adsorption from body fluids is the first stage that can promote or inhibit biocompatibility of implanted devices [7].

In fact, the biocompatibility of inorganic materials depends largely on the ability of the surface to induce denaturation or conformational variations of a specific protein [8]. For example, low-temperature isotropic carbon shows good compatibility with blood: it prevents the activation of blood coagulation proteins by adsorbing and denaturing serum albumin, thereby becoming non-reactive [9].

Since the physicochemical properties of the supports have a very significant effect on the activity of immobilized proteins, it may be useful to study supports that have a well-defined porous crystalline structure, large available surface area per unit volume, high mechanical and chemical durability, thermal stability, microbial resistance, hydrophobicity changing by preparation, and suitability for long-term applications.

Zeolites are inorganic crystalline materials whose basic/acidic character can be modified by varying the Si/Al ratio or introducing different metals (Me) into the crystalline framework to obtain different Si/Me ratios. Moreover, zeolite acidity can be modified exchanging extra-framework metal cations with H^+ [10].

Zeolite membranes constitute a new class of inorganic membranes that do not have the limitations associated with traditional polymeric membranes because their selectivities depend to zeolite structures [11].

Several papers are available on physicochemical aspects of the adsorption of proteins on solid surfaces [12,13], and on some inorganic materials such as silica [14], glass, alumina, and hornblende. However, few studies have examined protein adsorption on zeolite materials [15,16]. We studied the application of zeolite crystals and membranes as adsorbent materials for the immobilization of BSA and observed that the amount of BSA adsorbed on these materials increases when the zeolite crystals are inter-grown for forming a membrane. Furthermore, zeolite crystals and membranes showed a different behaviour versus the same protein. To assess whether the zeolites would behave similarly with different proteins, the study was extended to include the "hard" model protein cyt c. Since the adiabatic compressibility of proteins in aqueous solution consists of the intrinsic compressibility of the molecule, it plays an essential role in their conformational stability and functional properties. Gekko and Hasegawa [17] reported that this protein parameter depends on structural characteristics (such as hydrophobicity, secondary structure and amino acid composition) and it contains direct information on the nature of forces that govern the conformational variation and dynamics of the protein molecules in solution and/or on interfaces. Based on this characteristic, it is possible to classify a highly flexible protein like BSA as "soft protein" (great isothermal compressibility), and a compact protein like cyt c as "hard protein". The present study aimed to analyse the electrostatic influence of zeolite crystals and composite membranes on adsorption of BSA and cytochrome c (cyt c) proteins.

2. Materials and Methods

2.1 Materials and Methods

Zeolite structures were synthesized using fumed silica (99.8% Sigma), colloidal silica (AS-40, 40%, Sigma), tetramethylammonium bromide (TMABr, 25% water solution; Sigma Aldrich), tetrapropylammonium hydroxide (TPAOH, 1 M , Sigma), tetrapropylammonium bromide (TPABr, purum; JANSSEN), tetraethylammonium bromide (TEAOH, 35% water solution, Sigma), sodium hydroxide (Carlo Erba). ammonium fluoride (reagent grade ≥ 98%, Sigma), sodium aluminate (reagent grade ≥ 98%, Jannssen Chimica), and vanadyl sulphate pentahydrate ($VOSO_4 \cdot 5H_2O$, purum, Sigma).

The crystals obtained were used, as seeds, to synthesize new zeolite composite membranes: FAU, BEA and MFI. Organic template ions used for the synthesis of the FAU, BEA and MFI crystals were tetramethylammonium (TMA^+), tetraethylammonium (TEA^+) and tetrapropylammonium (TPA^+), respectively. The resulting gels, after complete homogenization by mechanical agitation, were put into Morey-type, PTFE-lined stainless autoclaves (130 cm^3). MFI-type samples were obtained by hydrothermal synthesis at 170 °C for various times, without stirring. FAU- and BEA-type samples were obtained by hydrothermal synthesis at 100 °C for various times, without stirring. After quenching the autoclaves the products were recovered, filtered, repeatedly washed with distilled water and finally dried at 120 °C overnight. Composite membranes were prepared in the presence of a porous disc support by conducting hydrothermal syntheses using the same chemical compositions and reaction temperatures as for the synthesis of the homologous zeolite crystals. The zeolite crystals and membranes were calcined in air from room temperature up to 570 °C at a heating rate of 1 °C/min. At the end of the heating ramp, the temperature was held constant at 570 °C for 6 h.

Obtained samples were characterized by XRD, SEM and EDX analyses. The power X-ray diffraction patterns were collected using Cu Ka radiation on a Philips Model PW 1730/10 generator equipped with a PW 1050/70 vertical goniometer. The crystal morphologies and sizes were determined by scanning electron microscopy (FESEM) on a FEI – Philips Quanta 200. The amount of aluminium and vanadium in the crystals was determined by atomic absorption (Shimadzu AA-660).

Finally, proteins were adsorbed on these materials, using different amount of protein, pH and temperature values. Protein solutions were prepared both in water and in aqueous buffers.

Cyt c (equine heart, 91.1 % pure) was obtained from Calbiochem-Behring and used as received. Bovine serum albumin (BSA, A-7030, 98-99 % purity) and 1,1'-diethyl-2,2'-carbocyanine chloride (pinacyanol chloride, reagent grade ≥ 99%) were purchased from Sigma (Italy). When not in use, the enzyme was stored under desiccating conditions at 0 °C.

Stainless steel porous thin layer was kindly donated by the Mott Corporation (pore width, 0.2 μm; thickness, 2.0 mm). It was cut into discs with diameters of approximately 250 mm.

2.2 Adsorption Studies

Batch adsorption experiments were carried out by putting the same mass of the different zeolites synthesized in contact with the same protein mass ($mg_{protein}/g_{zeolite}$). The adsorbent crystals and protein solutions were vigorously shaken at different temperatures. The equilibrated samples were centrifuged for 10 min at 38000 g and the supernatant were analyzed.

Protein concentrations before and after immobilization were determined using the Bradford method [18] which employs a UV spectrophotometer (Shimazdu UV-160 A). The test kit was purchased from Biorad (Munich, Germany). The experiments were carried out at selected solution pH values using appropriate buffer. A mass balance was then applied to calculate the protein adsorbed on the zeolite crystals and membranes. Defined calculation of protein immobilized in percentage is as follows:

$$\% \text{ adsorbed protein} = \text{amount of protein adsorbed} / \text{amount of initial protein} \times 100 \quad (2)$$

where the amount of protein adsorbed = total amount of protein in solution before immobilization – total amount of protein in solution (free) after immobilization.

Kinetic experiments were performed using a starting solution with a protein concentration of 1 mg/ml. The percentage of protein solution used in contact with the zeolite crystals ranged from 0.4% to 4%. Naturally, since zeolite structures have micropores that are far smaller than the hydrodynamic diameter of the protein, the adsorption of the biological species occurs solely on the external crystalline surface.

2.3 Protein activity assessment

To verify protein biological activity, BSA and cyt c immobilized onto zeolite crystals and membranes were assayed using Cu(II) sulphate solution and 1,1'-diethyl-2,2'-carbocyanine chloride (pinacyanol chloride), respectively, in the presence of H_2O_2. It is well-known that a Cu(II) sulphate solution can forms a BSA-Cu complex by a specific bond with a residue of the istidine, whereas a mixture containing cyt c and H_2O_2 can generate highly reactive radicals to oxide organic dyes. The decrease in the intensity of the absorption band at 805 nm was used to assay BSA activity, and cyt c activity was assayed at 601 nm [19-21].

3. Results and Discussions

This study analyses the influence of electrostatics on zeolite protein adsorption without modifying the inorganic surface in any way. We prepared by hydrothermal synthesis various zeolite crystals and membranes having FAU, BEA or MFI zeolite structures and different physicochemical characteristics. In this study, we used Bovine serum albumin (BSA) and horse heart cytochrome c (cyt c) as globular protein models to assess adsorption behaviour of these inorganic materials. BSA and cyt c have different isoelectric points, molecular weight, dimension and rigidity. Some of their important physicochemical properties are given in Table I.

Table I. Properties of the model proteins.

Protein	Mol.W. (kDa)	Ad. Compr. ($cm^2 \cdot dyn^{-1}$)	Hydro.diameter (Å)	pI	Secondary structure (Before adsorption)	Function
BSA	66.00 [c]	10.50 [c]	140 x 40*	4.7-4.9 [c]	α-helix (58±2%) β-sheet (22±1%) β-turns (3±1%) Unordered (16±2%)	Carrier protein
Cyt c	12.270 [c]	0.066 [c]	34 x 34^	10.1 [c]	α-helix (40±2%) [#] β-sheet (9±1%) β-turns (14±1%) Unordered (36±2%)	Electron transport

* [22]; ^ [23]; [c] [17]; [#] [24].

The syntheses of Na-Y (FAU), Na-β (BEA), S-1 and V-1 (MFI) zeolites were carried out in alkaline media, while the zeolite samples referred to as V1-ZSM-5, V2-ZSM-5 and V-NH_4F (MFI) were prepared in fluoride gels [25].

Table II provides the molar composition of the precursor gels used to prepare the zeolite crystals in this study.

Table II. Molar composition of the reaction mixtures: $10SiO_2$:*a*$VOSO_4$:*b*R-Br:*c*R-OH:*d*$Al(NO_3)_3$:*p*NH_4F:*q*NaOH:*r*H_2O where a=0.1; $0.9 \le b \le 1.25$; $1.2 \le c \le 5.5$; $0.2 \le d \le 4.6$; p=10.0; $q \le 1$ and $120 \le r \le 570$.

Sample	Structure	a	b	c	d	p	q	r
Na-Y	FAU	-	-	5.5*	4.6	-	0.22	570
V-1	MFI	0.1	-	1.2°	-	-	-	400
V-NH_4F	MFI	0.1	1.25°	-	-	10.0	-	330
V1-ZSM-5	MFI	0.1	1.25°	-	0.5	10.0	-	330
V2-ZSM-5	MFI	0.1	-	-	1.0	10.0	-	330
S-1	MFI	-	0.9°	-	-	-	1.0	125
Na-β	BEA	-	-	3.6°	0.2	-	0.28	120

R =Organic template: ° =Tetrapropylammonium;* =Tetramethylammonium; ° =Tetraethylammonium.

Zeolite composite membranes were prepared using two different crystallization methods to obtain "IN and ON" or "ON" types of zeolite membranes. In particular, S-1 (IN and ON), Na-β (IN and ON, ON) and Na-Y (ON) were prepared using a permanent stainless steel support. Fig. 1a reveals an image of calcined Na-Y composite membrane. Fig. 1b shows the permeation cell used to characterize the defect-free zeolite membranes synthesized in this study.

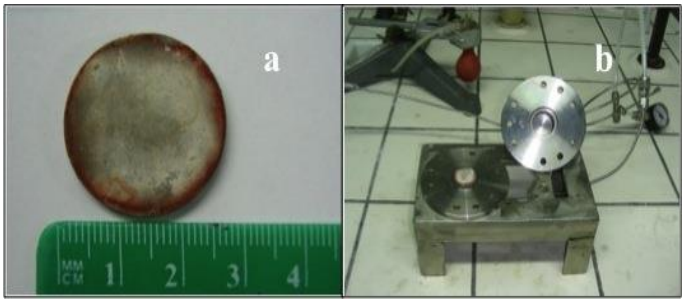

Figure 1. Images of Na-Beta zeolite composite membrane and (a), and the experimental permeation cell used in this work (b).

Fig. 2a and b evidence the FTIR-ATR spectra of BSA adsorbed on the Na-Beta zeolite sample obtained using different incubation times.

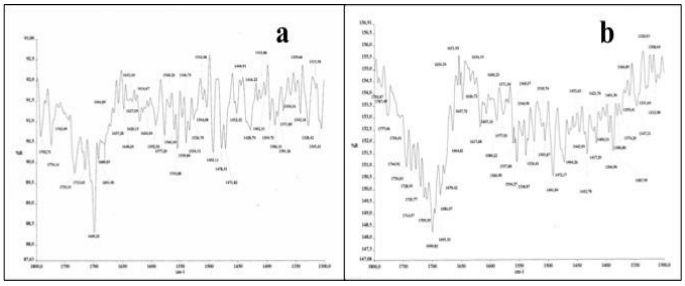

Figure 2. FTIR-ATR spectra of the cyt c adsorbed on the zeolite Na-Beta at two different incubation time (a) t = 0, and (b) t = 48 h.

Attenuated total reflectance (ATR) Fourier Transform Infrared (FT-IR) spectroscopic analysis was used to analyse conformational changes in the proteins as a result of their immobilization on the zeolites. This technique permits acquisition of the entire spectral range without significant interference from water bands, and it is ideal for studying protein adsorption onto polymeric and inorganic supports [26]. In particular, three IR bands (Amide I, Amide II, and Amide III) have been used in the literature to analyse the structure of adsorbed proteins. The Amide I, Amide II, and Amide III bands in native proteins are centered at 1650 cm^{-1}, 1539 cm^{-1}, and 1250 cm^{-1}, respectively. The protein Amide I band arises from the C=O stretching vibrations of the peptide backbone with a small contribution from an out-of-phase C-N stretch and a C-C-N deformation. The exact frequency of these vibrations depends on the nature of hydrogen bonding involving the C=O and NH moieties and it generally appears as a number of overlapping component bands representing α-helices, β-sheets, turns, and non-ordered structures. The Amide II region is related to the C-N stretch and the N-H in-plane bend, but this region is less studied, as its sensitivity to backbone conformations is still not well known.

Specifically, in the first region, bands in the range of 1695 cm^{-1} to 1660 $cm^{-1}$are assigned to the β-sheet; from 1660 cm^{-1} to 1650 cm^{-1} to the α-helix and the possible random structures in cyt c when it is in contact with physiological solutions; from 1650 cm^{-1} to 1640 cm^{-1} to unordered structure; and from 1640 cm^{-1} to 1620 cm^{-1} to β-sheet and turn structures. The Amide I band is known to be extremely sensitive to structural composition and conformational modifications induced by various parameters, such as temperature, oxidation state, ligand binding, and the presence of denaturants. Based on these correlations, one may derive information about the secondary structure of proteins adsorbed on various surfaces from analysis of the Amide I region of the IR spectra, since variations in secondary structure give rise to different vibrational frequencies. This spectroscopic analysis reveals a similar conformational behaviour for both proteins when immobilized. In fact, when following the interaction with the zeolite crystalline material a decrease in the size of the band relative to the α-helix of the protein structure is observed. In addition, both infrared bands relative to the β-sheets and β-sheets and turns of the protein decrease when protein molecules are adsorbed.

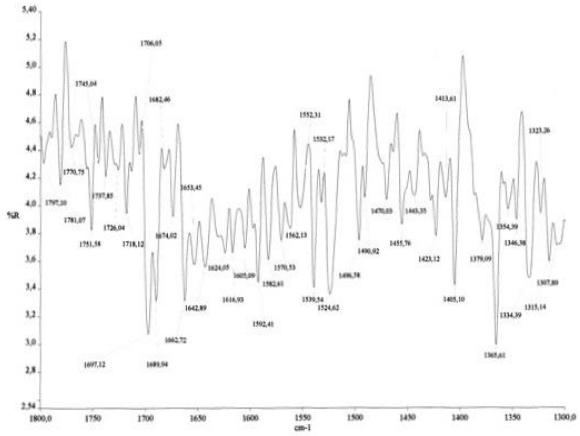

Figure 3. FTIR-ATR spectrum of the cyt c dissolved in phosphate buffer.

Table III shows the evolution of 1%, 5%, 10%, and 50 wt.% synthesized zeolite-containing aqueous suspensions over time. When the solid materials are added to distilled water, the pH of the suspension changes from its initial value to the equilibrium point, or point of zero charge (PZC), within 24 h as a result of the adsorption of H^+ or OH^- ions on the inorganic surface, which is negatively or positively charged. All type of crystalline zeolite structure, have only an interacting chemical group type, which is the silanol group. This group is amphoteric and so it can adsorb H^+ or OH^- as a function of the pH value of the environment. When pH > PZC, the negative charge on the zeolite surface will dominate, but when pH < PZC, the positive charge on the zeolite surface will dominate.

Table III. PZC values for the zeolite crystalline samples used in this work.

Sample	Time (h)	$PZC_{as\text{-}made}$ (wt%)				$PZC_{calcined}$ (wt%)			
		1	5	10	50	1	5	10	50
V-NH4	0	5.72	4.59	4.45	4.53	4.1	4.0	3.90	3.88
	24	6.18	4.55	4.45	4.40	4.02	3.88	3.55	3.55
V1-ZSM-5	0	5.50	4.6	4.36	4.31	3.98	3.68	3.58	3.57
	24	4.68	4.33	4.30	4.2	3.61	3.27	3.28	3.29
V2-ZSM-5	0	5.2	4.50	4.22	50	3.50	3.45	3.40	3.38
	24	4.56	4.25	4.10	4.01	3.30	3.19	3.14	3.14
S-1	0	9.49	10.01	10.21	10.29	9.2	9.80	9.80	9.89
	24	7.70	9.60	9.93	10.01	8.88	9.2	9.5	9.7
Na-β	0	6.55	6.64	7.21	7.33	6.33	6.5	6.81	6.88
	24	6.87	6.89	7.40	7.45	6.5	6.66	6.7	6.7

The structural properties of the composite membranes prepared are listed in Table IV. The structural parameters were obtained from the XRD analysis. The data shows that the PZC values obtained for composite membranes differ substantially from the values for the homologous zeolite crystals with the same chemical composition. This suggests that the stainless steel surface significantly modifies the electrostatic characteristics of zeolite crystals. The PZC value of the stainless steel support obtained in aqueous suspension (50 wt.%) after 24 h was equal to 8.5. Indeed, composite membranes with the same BEA structure, but prepared using two different crystallization methods, have different PZC values.

Table IV. Adsorption of BSA and cyt c on different zeolite composite membranes.

Membrane type	Crystallization type	Zeolite weight (g)	Crystal length (μm)	Membrane $PZC_{calcined}$	Adsorbed BSA (%)	Adsorbed Cyt (%)
S-1	IN and ON	0.47	150	9.20	80	55
Na-β	IN and ON	0.50	12	8.20	89	29
Na-β	ON	0.46	nanocrystals	7.82	79	26
Na-Y	ON	0.11	nanocrystals	8.25	78	44

In this work, the amount of adsorbed protein was measured using the so-called depletion method. Various factors are known to influence the amount of adsorbed protein: these factors include the characteristics of the protein, the solid support surface, and environmental conditions. With regard to the protein, its charge, its structural stability, its compressibility, its dimensions, its amino acid composition, and its three-dimensional conformation need to be taken into consideration. In particular, the compressibility of a protein is an important thermodynamic parameter. It is determined by volume fluctuations and hence, it carries information about the biomolecule's flexibility, which affects its functional properties and its hard or soft behavior (Table I).

With regard to the zeolite material, several factors can affect its Brönsted acidity. In order to make the analysis easier and rationalize the results, this parameter is subdivided into single and total acidity. The first refers to the acidity of single sites. For the same zeolite structure, the acid strength is the result of the local organization, of the chemical composition (the variation of polarity, polarizability, and dielectric constant of the zeolite with the content of atoms different from silicon) [27], of the Si/Al (SAR) ratio of the framework, in addition to the interaction of the silanol group with different species in the channel system [28,29]. For instance, the acidity of a silanol group can be enhanced by the interaction with a strong Lewis acid center (Al^{+3}) or decreased by the presence of fluoride ions in the framework. When comparing different structures, several factors need to be considered, including changes in the angle of the Si-OH-Si bridge imposed by the structure [30,31], the geometry of the pore surrounding the hydroxyl group [32], the different distribution of the $Si(OAl)_x(OSi)_{4-x}$ species in the zeolite framework for topological reasons [28], and confinement effects [33].

The second type of Brönsted acidity, useful for comparing extended zeolite materials like membranes, is related to zeolite PZC values and which is considers the total acidity due to the concentration of hydroxyl groups, their distribution, and their acid strength, in addition to the number of crystalline defects resulting from the hydrothermal synthesis medium. Thus, to a good approximation, the adsorption data can be directly compared for identical zeolite structures prepared using the same method in the same synthesis medium.

The analysis of the protein adsorption on zeolite composite membranes is complicated by the presence of the material used such as mechanical macroporous support. On one hand, it influences the hydrothermal "priming" of the zeolite material to adsorb the protein; on the other hand, it may itself adsorb the protein, thereby affecting the adsorption process and the total amount of the biological species immobilized. Hydrothermal synthesis of zeolite membranes is known to affect the support because its surface provides additional nucleation sites. Moreover, it can selectively restrict the diffusion of the reaction gel species and it can itself dissolve in the synthesis gel, thereby changing the composition of the initial mixture. The support can interact extensively with proteins, as reported in the literature examining the adsorption of various peptide fragments on stainless steel particles and the fouling of bio-medical instruments [34]. The adsorption experiments in the present study were performed on calcined membranes using the same protein/zeolite ratio utilized for homologous crystals. The valuation of membrane PZCs appears to be of particular interest, since these data clearly show that the isoelectric points of the membranes are quite different from those of the crystals. Thus in order to understand the electrostatic interactions between zeolite and protein species, the influence of stainless steel supports must be taken into consideration.Changing the pH in an adsorption experiment simultaneously affects the electrostatic properties of both the protein and inorganic surface. Hence, in order to rationalize the adsorption behaviour on zeolite crystals and membranes, it is necessary to take into account protein and surface charges together, not only the protein charge densities. Moreover, in the case of protein adsorption on zeolite membrane composites, the electrostatic influence of the stainless steel support should be taken into account.

The adsorption kinetics of BSA and cyt c obtained in different acid environment are reported in Tables V and VI, respectively. In both cases, the adsorption was rapid initially and then it gradually levelled off until a plateau region was reached. Approximately 80% of the initial protein was adsorbed within 2 h of contact time in the case of BSA, whereas about 24 h of incubation time was necessary in the case of the cyt c.

Table V. Dynamic behaviour of adsorbed BSA on various zeolite crystals (at 25 °C and pH 7.5).

Incubation Time (min)	Na-Y % adsorbed	V-NH_4F % adsorbed	V1-ZSM-5 % adsorbed	V2-ZSM-5 % adsorbed	Na-β % adsorbed	V-1 % adsorbed	S-1 % adsorbed
0	0	0	0	0	0	0	0
5	45	13	30	16	3	8	28
10	70	12	45	12	10	14	42
15	78	15	51	16	15	23	58
20	84	40	54	42	38	32	74
25	85	43	58	46	40	46	76
30	88	49	62	52	48	50	81
60	90	61	65	64	52	54	82
120	94	68	75	70	53	55	84
180	96	70	80	85	55	56	85
240	98	76	96	98	66	69	98

Table VI. Dynamic behaviour of adsorbed cyt c on various zeolite crystals (at 25 °C and at pH 7.5).

Incubation Time (h)	Na-Y	V-NH_4F	V1-ZSM5	V2-ZSM5	Na-B	V-1	S-1
	% adsorbed	% adsorbed	% adsorbed	% adsorbed	% adsorbed	% adsorbed	% adsorbed
0	0	0	0	0	0	0	0
3	45	13	30	16	3	8	28
6	70	12	45	12	10	14	42
12	78	15	51	16	15	23	58
20	84	40	54	42	38	32	74
24	85	43	58	46	40	46	76
32	88	49	62	52	48	50	81
36	90	55	65	60	52	54	82
40	92	56	69	62	53	55	84
48	93	58	75	63	55	56	85
60	96	65	83	72	60	60	90
72	97	69	87	79	63	64	92
84	98	74	93	86	65	67	95
96	98	76	98	98	66	69	98

For the same zeolite material, the effect of pH on BSA and cyt c adsorption was investigated at three different pH values: 4.8, 6.0, and 10.0. Figure 4 compares the percentage of adsorption using the same zeolite structure after incubation at 25 °C. It is interesting to note that the adsorption capacity of the zeolites for each protein changes substantially when the pH is altered.

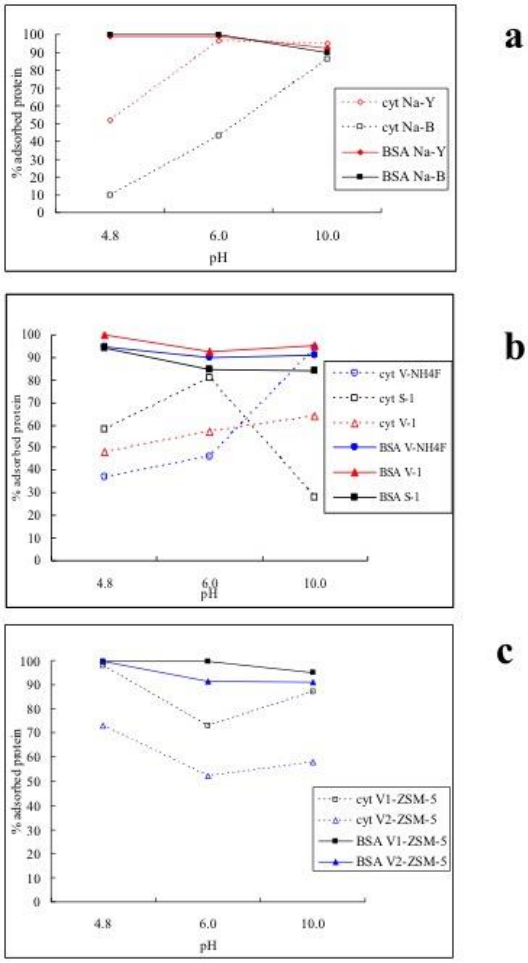

Figure 4. Influence of pH on BSA and cyt c adsorbed on different zeolite supports: (a) Na-Y and Na-Beta, (b) V-NH_4F, S-1 and V-1, (c) V1-ZSM-5 and V2-ZSM-5.

Zeolites adsorb more BSA than cyt c at all pH values, and the adsorption tends to reach steady state faster than with cyt c. The isoelectric point and net charge at pH 7 of BSA are 4.8 and –18, respectively (Peters, 1985), thus the net charge of this protein is negative at pH 10 and pH 6, but it is close to zero at pH 4.8.

4. Conclusions

In this article, the adsorption of two globular proteins on various zeolite structures was studied. These inorganic materials hold tremendous promise as biomaterials [35, 36], but few studies have examined their interactions with biological species. We synthesized Zeolite Na-Y (FAU), Na-β (BEA), S-1, V-1, V-NF_4F, V1-ZSM-5, and V2-ZSM-5 (MFI) crystals using the hydrothermal method in alkaline media and fluoride gels, then we prepared composite zeolite membranes using a permanent stainless steel support. Zeolite composite membranes were prepared using two different crystallization methods to obtain "IN and ON" or "ON" types of zeolite membranes. S-1 (IN and ON), Na-β (IN and ON, ON), and Na-Y (ON) were prepared using a permanent stainless steel support. Protein adsorption onto these materials was studied under various experimental conditions using spectroscopic techniques such as UV-visible absorption and FT-IR/ATR.

This study leads to interesting conclusions about the interactions affecting the adsorption of soft (BSA) and hard (cyt c) model proteins on zeolite materials. The results obtained indicate that albumin adsorbs on both hydrophilic and hydrophobic zeolite surfaces. For cyt c, in contrast, electrostatic interactions seem to be most important in governing immobilization. The study also explores the fact that more BSA and cyt c adsorb on the composite membranes than on the homologous zeolite crystals. This phenomenon was investigated in terms of the PZC values of the crystals and membranes tested.

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