Adsorption of Certain Amino Acids onto Crosslinked Diethylaminoethyl Dextran Microbeads

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ABSTRACT: The adsorption dynamism of certain amino acids [alanine (Ala), serine (Ser), lysine (Lys), proline (Pro), and aspartic acid (Asp)] on crosslinked diethylaminoethyl (DEAE) dextran microbeads was investigated with electronic spectroscopy in aqueous solutions. Among the selected amino acids, Ala-Pro and Ser-Lys showed similar adsorption dynamism, but Asp showed the strongest tendency for adsorption. The interaction dynamism of Asp with crosslinked DEAE dextran microbeads was studied to determine the mechanism of this adsorption. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 1985–1991, 2005

Key words: adsorption; amino acids; diethylaminoethyl dewtran; infrared spectroscopy

INTRODUCTION

Adsorption at various interfaces has concerned scientists since the beginning of this century. This phenomenon underlies a number of extremely important processes of utilitarian significance. The technological, environmental, and biological importance of adsorption can never be in doubt. Practical applications exist in industry and environmental protection that are of paramount importance. The separation methods for mixtures on laboratory and industrial scales increasingly involve the concentration of components at the interface.

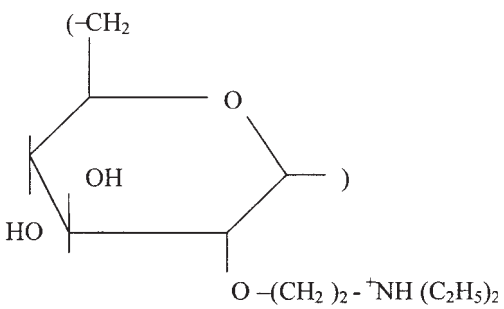
During the last 15 years, new classes of solid adsorbents have been developed, such as activated carbon fibers and carbon molecular sieves, fullerenes and heterofullerenes, microporous glasses, and nanoporous carbonaceous and inorganic materials. Nanostructural solids are very popular in science and technology and have attracted great interest because of their sorption, catalytic, magnetic, optical, and thermal properties. The chromatographic separation of proteins is important not only for analysis but also in large-scale industries such as the food and drug industries.

Dextran-based polyelectrolyte displacers have been successfully employed for the displacement purification of proteins via ion-exchange displacement systems.[1] Yamamoto et al.[2] showed that the adsorption of bovine serum albumin (BSA) on crosslinked *N,N'*-diethylaminoethyl (DEAE) dextran (a weakly basic dextran-type ion exchanger) decreased with decreasing pH. Tsou and Graham[3] studied the effect of the concentration of NaCl in a BSA solution on the isotherm for the adsorption of BSA on DEAE dextran microbeads. Yoshida et al.[4] presented experimental equilibrium isotherms of adsorption of BSA on a crosslinked DEAE dextran ion exchanger with Na^+ and Cl^- ions.

In this work, the interaction dynamism of certain amino acids [alanine (Ala), serine (Ser), lysine (Lys), proline (Pro), and aspartic acid (Asp)] with polycationic crosslinked DEAE dextran microbeads was studied with electronic spectroscopy in aqueous solutions. The interactions of a crosslinked DEAE dextran/Asp system were also investigated with Fourier transform infrared (FTIR) spectroscopy in the solid state.

EXPERIMENTAL

Commercial crosslinked DEAE dextran microbeads were used (Sephadex A-50, Pharmacia Fine Chemicals, Sweden). A schematic representation of the DEAE dextran microbeads follows:



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The amino acids (DL-alanine, L-aspartic acid, L-lysine monohydrochloride, L-proline, and L-serine) were supplied by Merck (Darmstadt, Germany) and were used without further purification. Sorption studies were carried out at a constant temperature of 25°C. The DEAE dextran microbeads were placed in contact with the amino acid solutions, and the solutions were gently mixed.

The spectrophotometric studies of the amino acid solutions were carried out with a Hitachi 100-60 ultraviolet–visible double-beam spectrophotometer (Tokyo, Japan) at room temperature. FTIR spectra were recorded with a Mattson 1000 FTIR spectrophotometer (Cambridge, UK) by the KBr disk method in the 4000–400-cm^{-1} range; 30 scans were taken at a 4-cm^{-1} resolution.

For measuring the microgel swelling, volumetric measurements were taken. For this purpose, a constant weight of crosslinked DEAE dextran microgels was left in the volumetric vessel, and the increase in the volume was followed as a function of time.

The microgels were immersed in distilled water at room temperature. The percentage of swelling (S) was determined as follows:

$$S\ (\%) = V_{\text{swollen}}(\text{mL})/V_{\text{dry}}\ (\text{mL}) \times 100 \qquad (1)$$

where V_{dry} is the initial volume of the microgels at time $t = 0$ and V_{swollen} is the volume of the swollen microgels at time t.

RESULTS AND DISCUSSION

The swelling of the three-dimensional network structure in a suitable solvent (in this study water) is the most important parameter (in particular, the most important parameters are the mass and volume swelling).

Charged groups attached to the polymeric network structure play an essential role in swelling properties. Several attempts at establishing the water concentration of micropores are described in the literature (e.g., by the determination of the difference in the total volumes of wet and dry microspheres or the difference in the total weights with or without blotting). In another work, wet microspheres were filtered, and the change in the filter mass was determined. Another approach for the determination of the solvent content of microspheres is based on their size upon swelling.[5,6] The increase in the size can be determined with static and dynamic light scattering techniques as the swelling of the dextran microbeads, which can be derived from the difference between the volumes of the dry and swollen states.

The presence of hydrogen-bonding groups[7,8] in the polymer matrix causes the network to swell less than a less polar structure. The swelling kinetics of crosslinked DEAE dextran in water were determined, and the equilibrium swelling of DEAE dextran for the microspheres was found to be approximately 105%.

The equilibrium degree of swelling of ionic hydrogels is controlled by three major factors: (1) the mixing of the polymer with a swelling medium (mainly miscibility), (2) the elastic refractive force on the network, and (3) the ionic pressure generation from the mobile counterions onto the charged ions in the network. Because of the low equilibrium swelling value of the crosslinked DEAE dextran microbeads, this matrix can be easily used in column separation applications.[9] The swelling behavior of crosslinked DEAE dextran microspheres at 105% indicates the diffusion of water into the structure; more generally, it is evidence of a water sorption process that exists in the aqueous system first. During the second step, it can be implied that the interaction of amino acids present in the aqueous system with the microbeads displaying polycationic behavior results in an increase in the immobilization and adsorption onto the microspheres. The nature of the interaction dynamics can be determined from the chemical structure of each amino acid, its functional groups, and the probable interaction of these groups with the functional groups of the DEAE dextran structure.

Electronic spectroscopy has been successfully employed[10–12] for following the interaction dynamics of different components,[13–15] and for determining the variation of the concentration in solutions for different sorbent–adsorbent systems during the adsorption period and determining the adsorbed amount in the one-step-further process.

The concentrations of the amino acids adsorbed onto the particles were measured after a certain time until it was confirmed that there was no further adsorption. The adsorption capacities were determined by the measurement of the initial and final concentrations of each amino acid within the reservoir at $\lambda = 193.4$ nm for Ala, $\lambda = 205.0$ nm for Pro, $\lambda = 212.0$ nm for Ser, $\lambda = 213.0$ nm for Lys, and $\lambda = 218.0$ nm for Asp, and with the following formula, the adsorbed phase concentration of the amino acids was calculated:

$$q_{\text{eq}} = V(C_{\text{o}} - C_{\text{eq}})/W \qquad (2)$$

where C_0 and C_{eq} are the initial and equilibrium concentrations of the amino acids in the liquid phase (mg/mL), respectively; q_{eq} is the microbead phase concentration of the amino acids (mg of amino acid/g of crosslinked DEAE dextran microbeads), W is the mass of the microbeads (g), and V is the volume of the solution (mL).

Table I shows the effect of the initial concentration of each amino acid on the equilibrium adsorption. The equilibrium adsorption values remain unchanged for the amino acid systems even at the end of the 4th hour. The interaction dynamics of the amino acids

TABLE I
Adsorption Capacities of Amino Acid/Crosslinked DEAE Dextran Microbeads at 25°C

Amino acid	Initial concentration (M)	Q_{eq} (mg) for amino acid/DEAE dextran
Ala	0.06	0.12
	0.10	0.23
	0.14	0.41
	0.20	0.63
Pro	0.06	0.11
	0.10	0.27
	0.14	0.39
	0.20	0.60
Lys	0.06	0.02
	0.10	0.03
	0.14	0.05
	0.20	0.06
Ser	0.06	0.03
	0.10	0.05
	0.14	0.06
	0.20	0.08
Asp	0.06	0.17
	0.10	0.41
	0.14	0.67
	0.20	0.88

with the crosslinked microspheres and subsequently the adsorption capacity of the microbeads show variations. Maximum adsorption can be observed in Asp, and Ala and Pro follow. For the other two amino acids, Ser and Lys, adsorption is very low.

Before we discuss the interactions between DEAE dextran and the selected amino acids, we should discuss the relative importance of the bonding interactions of these five amino acids in detail, as argued in Patrick's work.[16] On the basis of the strengths of the four types of bonds, the relative importance of the bonding interactions might be expected to follow the same order as their strengths: covalent bonding (>100 kJ mol^{-1}), ionic bonding (20 kJ mol^{-1}), hydrogen bonding (7–40 kJ mol^{-1}), and van der Waals interactions (~2 kJ mol^{-1}).

In fact, the reverse of this order is also true. In most proteins, the most important bonding interactions in tertiary structures are those due to van der Waals interactions and hydrogen bonding, whereas those due to covalent and ionic bonding are the least important.

There are two important reasons for this. Primarily, van der Waals and hydrogen-bonding interactions are much more dominant than covalent and ionic bonding; this can be clearly interpreted only if the numbers and types of amino acids in a typical protein are considered. A disulfide bond is the only covalent bond (binding energy of S—S = 250 kJ mol^{-1}) that can be contributed into a tertiary structure, and only one amino acid, cysteine (Cys), can form such a bond. However, all other eight amino acids [glycine (Gly), Ala, valine (Val), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), Pro, and methionine (Met)] can interact through van der Waals interactions with one another.

On the other hand, only four amino acids [Asp, glutamic acid (Glu), Lys, and Arg] are responsible for ionic bonding, whereas eight other amino acids [Ser, threonine (Thr), Cys, asparagine (Asn), glutamine (Gln), histidine (His), tyrosine (Tyr), and tryptophan (Trp)] interact via hydrogen bonding. Consequently, the number of hydrogen-bonding or van der Waals interactions is much more dominant than the number of possible covalent bonds.

Another important factor that should be kept in mind that is related to tertiary structures is that proteins cannot be considered separately from water systems. Thus, amino acids placed on the surfaces of proteins are thoroughly interacting with water molecules, and because water is a highly polar compound, strong hydrogen bonds are naturally formed. This leads to the expectation that water is highly responsible for the hydrogen bonding during interactions with the aforementioned amino acids.

Water can become positively charged by accepting a proton and may in turn form ionic bonds with Asp and Glu. This would enable further arguments stating that these two acids contain dual functional groups.

Consequently, it is obvious that water has the ability to form hydrogen and ionic bonds with the polar amino acids Ser, Thr, Cys, Asn, Gln, His, Tyr, Trp, Asp, Glu, Lys, and Arg, which are classified as hydrophilic amino acids. All remaining nonpolar amino acids (Gly, Ala, Val, Leu, Ile, Phe, Pro, and Met) may be termed hydrophobic or lipophilic.

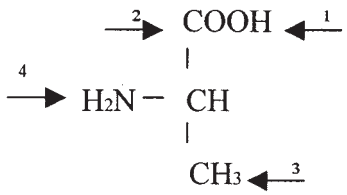
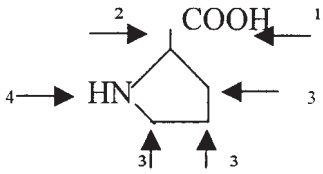
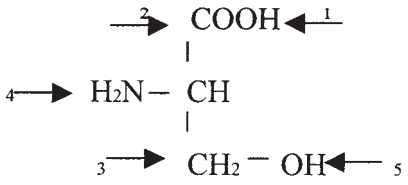
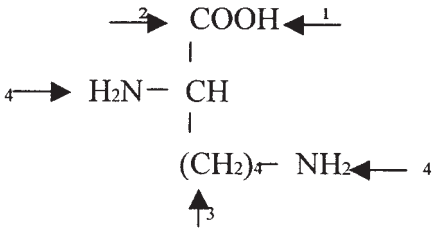
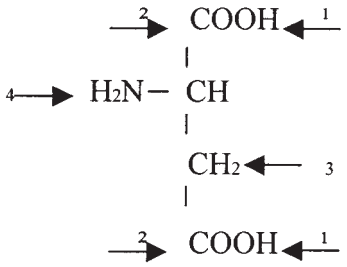
Therefore, the most stable tertiary structure of a protein contains almost all of the hydrophilic groups on the surface, interacting with water molecules, whereas most of the hydrophobic groups are contained in the center, avoiding water and subsequently interacting with each other only

In light of all this information, we can conclude that the number of ionic and hydrogen bonds contributing to the ternary structure is reduced as a result of the formation of ionic and hydrogen bonds between water and hydrophilic amino acids. Thus, the hydrophobic amino acids placed in the center have no choice except to interact with each other, and this leads to overruling hydrophobic interactions within the structure and controls the shape of the protein.

We followed the interaction dynamism of the five amino acids (Ala, Pro, Ser, Cys, and Asp) employed in this study with DEAE dextran microbeads in aqueous solutions. Table II presents the chemical structures and relative bonding competitions.

If the theory discussed for the relative bonding interactions of amino acids with water and DEAE dextran is assumed for our own system, the following

TABLE II
Chemical Structures and Natures of Amino Acids

Amino acid	Structure	Chemical nature
Ala		Nonpolar,hydrophobic
Pro		Nonpolar,hydrophobic
Ser		polar
Lys		ionized
Asp		ionized

1 = an ionic interaction involving the ionized carboxylate group; 2 = a hydrogen bond involving the ketonic oxygen; 3 = a van der Waals interaction involving the methyl and methylene groups; 4 = A hydrogen bond involving the amine group; 5 = A hydrogen bond involving the hydroxyle group.

results may be proposed. Two hydrophobic amino acids, Ala and Pro, are repelled by water because of the hydrophobic interactions within the structure; this leads to the affinity of those two amino acids toward DEAE dextran rather than water. Both amino acids display higher adsorption values in DEAE dextran than the other two hydrophilic amino acids (Ser and Lys), which interact primarily with water and having greater affinity toward water than DEAE dextran. However, DEAE dextran exhibits a polycationic character and may itself be considered hydrophilic. Subsequently, we cannot disregard completely the affinity of the two hydrophilic amino acids toward DEAE dextran as well, though it is very scarce.

As far as the chemical structure is concerned, Asp should be evaluated separately because of the dual

TABLE III
Spectral Characteristics of DEAE Dextran, Asp, and a DEAE Dextran/Asp (0.02*M*) Agglomerate

Frequency (cm^{-1})	Characterization
2900 vb	3409 cm^{-1} O—H stretching (DEAE dextran)
	2931 cm^{-1} O—H stretching (Asp)
1686 sh	C═O stretching (Asp)
1635 s	N—H bending (DEAE dextran)
1601 sh	N—H stretching (DEAE dextran)
1504 s	COO^- stretching (Asp)
1412 s	COO^- stretching (Asp)
1304 s	COO^- stretching (Asp)
1142 s, as	C—O—C stretching (DEAE dextran and Asp)
1034 s, as	C—O stretching (DEAE dextran and Asp)

vb = very broad; sh = shoulder; s = strong; as = asymmetric.

carboxylic acid groups contained in its structure. The occurrence of the maximum molecular interactions and, therefore, maximum adsorption for Asp may thus be explained by this dual carboxylic acid structure.

Although no aggregation was observed with increasing amino acid concentration, significant agglomeration followed with increasing Asp concentration. After the DEAE dextran/Asp aggregates were centrifuged and filtered, the solid precipitates were dried and used for further spectral investigations. Spectral data were detected in two different subgroups. The first group involved the spectral characterization of the most dilute Asp (0.02*M*) DEAE dextran system, as shown in Table III. The second evaluation showed the characteristics for the Asp/DEAE dextran aggregated microspheres with increasing Asp concentration (0.02–0.14*M*).

Spectral detections were followed for the two different groups at two different wavelengths. The initially selected field exhibited the stretching vibrations of O—H and N—H in the range of 2500–3500 cm^{-1} (Fig. 1). The second field was evaluated in the frequency range of 1000–1750 cm^{-1} (Fig. 2).

The OH stretching vibrations of dextran and Asp took place at different frequencies [3409 cm^{-1} for OH stretching vibrations of DEAE dextran and 2931 cm^{-1} for Asp, as shown in Fig. 1(a,b)].

As can be clearly observed in Figure 1, the DEAE dextran/Asp aggregates showed important spectral variations with increasing Asp concentration. These variations are listed in Table IV as OH stretching vibrations.

OH stretching vibrations found because of the acidic character of Asp occurred at a lower frequency than those of DEAE dextran. The band shows an almost asymmetric and broad peak for the aggregate found with the most dilute Asp concentration (0.02*M*). At higher concentrations of Asp, a spectrum has been obtained that shifts more toward the right and is symmetrical [Fig. 1(d,h) and Table IV].

On the other hand, OH stretching vibrations of Asp are given in Table IV according to the increasing Asp concentration. When we compare the original spectrum of Asp [Fig. 1(b)] and that of the aggregate with the highest Asp concentration [0.14*M*; Fig. 1(h)], we can see that the spectrum of the aggregate presents a broader peak. Quantitatively, we can simply say that the Asp content was greater than that of the original spectrum [Fig. 1(b)]; however, because the OH stretch-

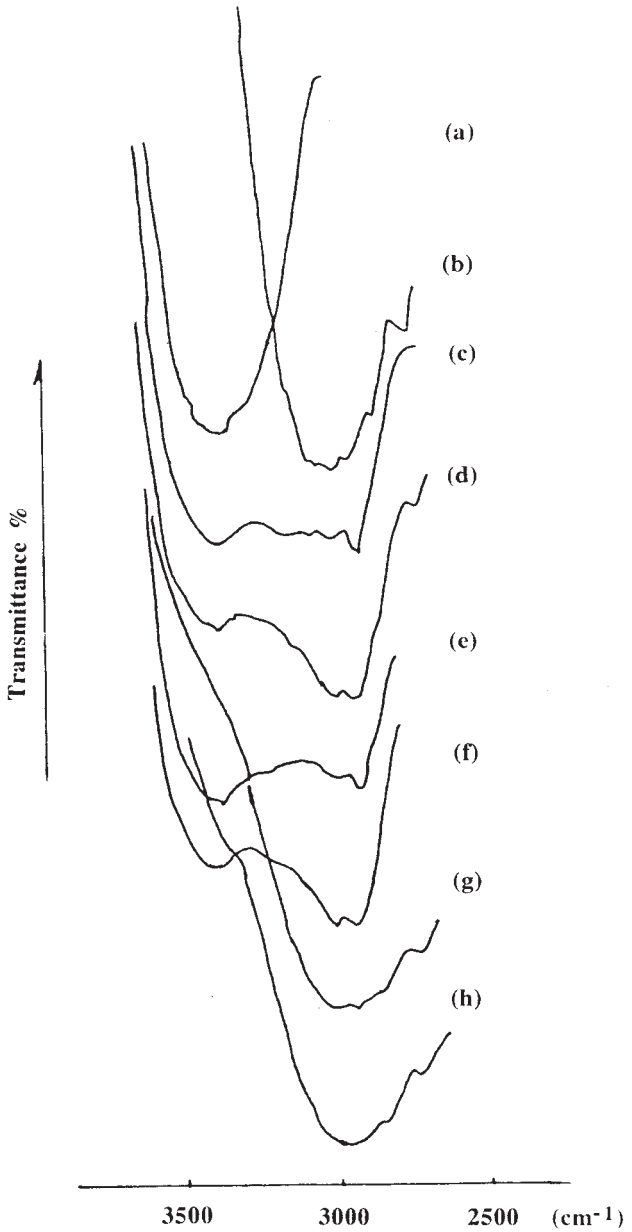


Figure 1 IR spectra of (a) DEAE dextran, (b) Asp, and (c–h) DEAE dextran/Asp agglomerates with (c) 0.02, (d) 0.04, (e) 0.06, (f) 0.08, (g) 0.12, and (h) 0.14*M* Asp.

ing vibrations of DEAE dextran are also involved, we can observe such a broader peak.

The C═O stretching vibration is only present for the Asp structure. The variations in the C═O stretching vibrations are displayed in Figure 2 and Table IV. The band observed at 1686 cm^{-1} as a shoulder for the 0.02*M* Asp concentration has a broader and more asymmetric character with increasing Asp concentration.

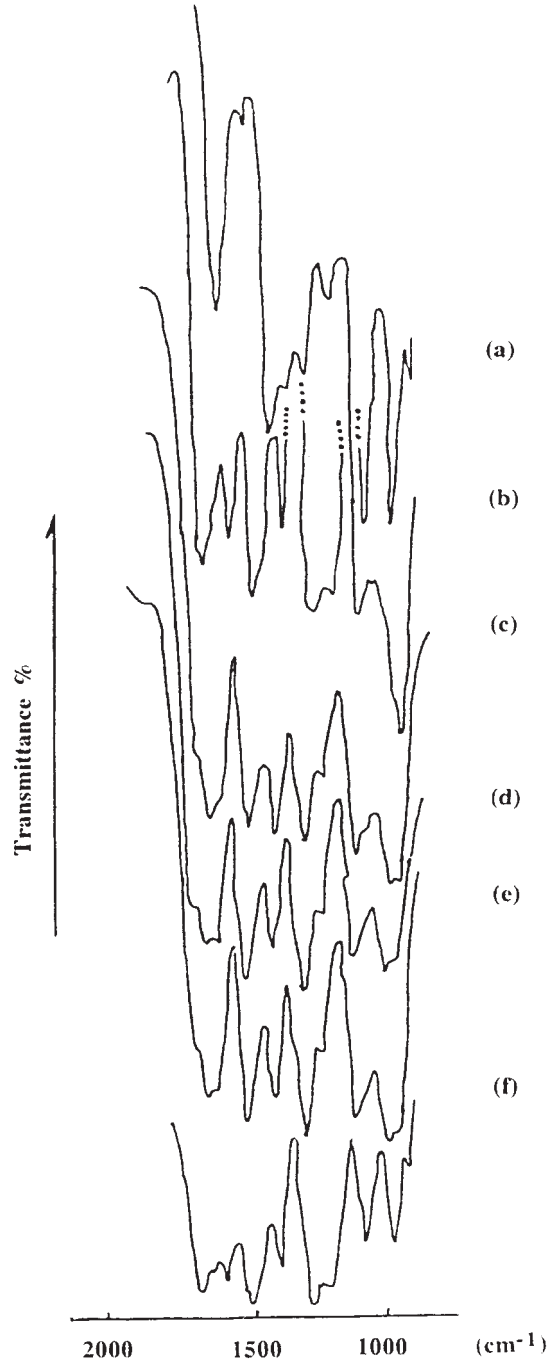


Figure 2 IR spectra of (a) DEAE dextran, (b) Asp, and (c–f) DEAE dextran/Asp agglomerates with (c) 0.02, (d) 0.04, (e) 0.08, and (f) 0.12*M* Asp.

TABLE IV
Spectral Changes of DEAE Dextran/Asp Agglomerates

[Asp] (*M*)	O—H stretching (DEAE dextran)	O—H stretching (Asp, cm^{-1})	C═O stretching (Asp, cm^{-1})
0.02	3409, b	2931, s	1686, sh
0.04	3394, b	3008, 2939, s, ii	1687, w
0.06	3386, b	2931, s	1687, sh
0.08	3402, b, di	3008, 2939, s, ii	1682, sh
0.12	3392, sh	2861–3071, b	1689, m, ii
0.14	3382, sh	2851–3106, b	1689, m

b = broad; s = strong; sh = shoulder; ii = increased intensity; w = weak; di = decreased intensity; m = medium.

N—H bending vibrations occur for both chemical structures. At 1643 cm^{-1}, the N—H bending vibrations of the DEAE dextran structure are characterized, whereas the N—H bending vibrations of Asp can be observed at 1597 cm^{-1}. The intensity of the band observed at 1635 cm^{-1} for the aggregates with 0.02*M* Asp disappears at the highest Asp concentration. Although the original Asp band can be observed at 1597 cm^{-1}, the band for the 0.0.2*M* Asp aggregate shifts toward 1601 cm^{-1} and can be observed as a shoulder. Finally, with increasing Asp concentration, the intensity of the band increases significantly.

The COO— stretching vibration can be observed at 1500–1412 and 1311 cm^{-1} (Table III) for the original Asp; for the DEAE dextran/Asp system, the intensities of these bands increase with increasing Asp concentration.

Consequently, the C—O—C and C—O stretching vibrations of DEAE dextran occur at 1149 and 1010 cm^{-1}, whereas these vibrations for Asp occur at 1134 and 1041 cm^{-1} (Table III). These frequencies appear at almost the same values. As a result of spectral evaluations, no net differentiation has been detected within the Asp concentration (0.02–0.08*M*). However, within this range, the band appearing at 1034 cm^{-1} is not symmetrical at all. At higher Asp concentrations, on the other hand, C—O—C and C—O stretching vibrations are characterized by frequencies of 1134 and 1041 cm^{-1}, and they display symmetrical behavior.

CONCLUSIONS

Our investigation of the competition between five amino acids (Asp, Lys, Pro, Ser, and Ala) for interactions with crosslinked DEAE dextran in aqueous-solution microbeads has resulted in the selection of Asp for the most effective interactions. Asp in DEAE dextran has been proved to be the most adsorbed amino acid by the system; this is supported strongly by spectral evaluations. Increasing the Asp concentration has yielded a higher and more effective adsorption of Asp onto DEAE dextran microbeads. Ser and Lys follow,

with decreasing adsorption in the interaction dynamism of DEAE dextran, whereas Pro and Ala have no significant role in the interaction mechanism. Apart from the five amino acids employed in this study, five other amino acids (Glu, The, Phe, Gly, and Leu) have also been selected for further investigation of the same mechanism in light of knowledge gathered from previous studies.[17]

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