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Preparation and Enantiospecific Binding of Chiral Polyelectrolyte Multilayers: An *In-Situ* ATR-FTIR Study

*Wuye Ouyang, Dietmar Appelhans, Brigitte Voit, Martin Müller**

Summary: Two chiral polyelectrolyte multilayers (PEM) composed of poly(L-lysine) (PLL) and poly(vinylsulfate) (PVS) as well as poly(ethyleneimine-maltose) (PEI-m) and poly(vinylsulfate) and a nonchiral PEM composed of poly(ethyleneimine) (PEI) and poly(vinylsulfate) were deposited on a silica surface using the layer by layer method. For both PEM enantiospecific interaction towards one enantiomer of either L-/D- glutamic acid (L-/D-GLU) or L-/D-ascorbic acid (L-/D-ASC), respectively, was checked under variation of the concentration. Both deposition and enantiospecific interaction were studied by attenuated total reflection Fourier transform (ATR-FTIR) spectroscopy. Preliminary results show a significant enantiospecific preference of D- GLU over L-GLU at PEM of PLL/PVS and of D-ASC over L-ASC at PEM of PEI-m/PVS and no such preference for nonchiral PEM of PEI/PVS. PEM of PLL/PVS shows higher enantiospecifity with increasing L-/D-GLU concentration.

Keywords: chiral surface; enantiospecific interaction; polyelectrolyte multiplayer

Introduction

Enantiospecific surfaces are able to bind one enantiomer (e.g. the L form) of a chiral substance to a higher extent than the other one (e.g. the D form), which is originated in the chiral nature of the surface forming (macro) molecules. Enantiospecific surfaces and layers play an important role for the analytical recognition and preparative separation of chiral drugs in the pharmaceutical industry.[1,2] Most prominent in that respect is the coating of stationary phases used for HPLC by a chiral selector (SO), which can be a chiral reactive polymer or low molecular compound. An example for low molecular chiral SO is R-(-)-N-(3,5-dinitrobenzoyl)-phenylglycine (DNBPG, Chirasep®) on aminated silica gel ('Pirkle phase'[3])[4,5] and one for polymer based on chiral SO is poly(N-acryloyl-L-phenylalanine ethylester) bound to silica gel (Chiraspher®[5]). Weak interactions like π-π, dipole-dipole or hydrogen bonding acting via at least three centers are generally claimed to contribute to the enantiospecific recognition[6] between a chiral selectand (SA) and the chiral SO. However, since such chiral stationary phases (CSP) are very expensive, alternatives are looked for. In that framework a further possible concept for enantiospecific recognition bases on molecular imprinting of chiral SA in a sol/gel matrix,[7] possessing cavities with high steric specifity. Related to that, Kaner et al. reported high enantiospecifity of casted polyaniline films towards L- over D-phenylalanine, exclusively after chiral doting with R-camphorsulfonic acid and dedoting in ammonia solution.[8,9] Up to now the concept of polyelectrolyte multilayers (PEM) for the generation of chiral surfaces was considered only to a low extent. Solely, Schlenoff[10] reported studies on thick PEM membranes consisting of oppositely charged chiral polyelectrolytes (PEL) for the separation of small chiral probes like L- and D-ascorbic acid (L-/D-ASC). Low percent

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Leibniz Institute of Polymer Research Dresden e.V. (IPF), Hohe Straße 6, 01069 Dresden, Germany
Fax: 0049-351-4658 405
E-mail: mamuller@ipfdd.de

selectivity values (see Equation 2) of around 3% were obtained by ATR-FTIR and capillary electrochromatography (CEC).

Herein, we report on first results of related studies using PEM consisting of charged polypeptides and synthetic chiral PEL as chiral SO for enantiospecific binding of L-/D-glutamic acid (L-/D-GLU) and L-/D-ASC as chiral SA. A specific aim of this work is dedicated to the influence of PEL type within PEM on enantiospecifity. In-situ ATR-FTIR spectroscopy was used as the basic analytical method, which has been shown to characterize deposition,[11] molecular composition,[12] conformation, and orientation within PEM[13,14] as well as binding of various probes like small ions,[15] surfactants,[16] and proteins[11,17,18] on a quantitative level.

Experimental Part

Polyelectrolytes, Chiral Probes and Substrates

Poly(L-lysine) (PLL, $M_w = 280.000$ g/mol) and branched poly(ethyleneimine) (PEI, $M_w = 750.000$ g/mol) were obtained from Sigma Aldrich (Deisenhofen, Germany) and poly(vinylsulfate) (PVS, $M_w = 300.000$ g/mol) was obtained from Gelest, Inc. (Morrisville, PA, USA). Poly(ethyleneimine-maltose) (PEI-m) was received by the reductive amination of branched PEI in the presence of excess maltose using a slightly modified method described in literature.[19] L-/D-glutamic acid (L-/D-GLU) and L-/D-ascorbic acid (L-/D-ASC) were obtained from Sigma Aldrich (Deisenhofen, Germany). All commercial samples were used without further purification. PEI, PLL, and PVS were dissolved in 1M $NaClO_4$ solution (Merck, Darmstadt) at $c_{PEL} = 0.01M$. L- and D-GLU were dissolved in Millipore water at different concentrations c_{GLU} resulting in $pH \approx 3.5$. L- and D-ASC were dissolved in Millipore water at a concentration of $c_{ASC} = 0.01$ M and $pH = 4.0$ (adjusted by 1M NaOH). Silicon internal reflection elements (Si-IRE) were used as substrates, which were cleaned with piranha solution (H_2SO_4: $H_2O_2 = 3:1$ (v/v)) for 30 minutes, thoroughly rinsed by water, dried under N_2, and finally placed in a plasma cleaner (PDC-32 G plasma cleaner/sterilizer, Harrick, Ossining, NY, USA) for 3 minutes. Then the clean Si-IRE was fixed in the ATR-FTIR in-situ cell (which will be described in the ATR-FTIR part below) for recording respective ATR-FTIR spectra.

Multilayer Deposition

For PEM-PEI/PVS and PEM-PLL/PVS deposition, 2 ml of the PEL solutions ($c_{PEI} = 0.01M$, 1M $NaClO_4$) were consecutively immersed keeping a final volume of 100 microliter for 15 min above the Si-IRE in the S compartment (see above), respectively. After each adsorption step, the S compartment was rinsed by 2 ml of 1M $NaClO_4$ solution within 15 seconds. After final odd PEM adsorption step glutar dialdehyde solution (0.25% (w/w)) was immersed to crosslink free amine groups of PEI and stabilize the PEM. For PEM-PEI-m/PVS deposition concentrations $c_{PEI\text{-}m} = 0.1$ mg/mL and $c_{PVS} = 0.001M$ in both salt free and 1 M $NaClO_4$ aqueous solution were applied.

Enantiospecific Interaction

For PEM-PLL/PVS the L-/D-GLU concentration was varied in the range $c_{GLU} = 0.001–0.1$ M. L-/D-solutions were immersed above the PEM film starting with the lowest c_{GLU}, followed by rinsing with pure Millipore water and then immersing the next higher concentration followed again by rinsing. The L-/D-GLU solutions were kept in the sample compartment for 20 min. For repetitions of chiral probe binding series on the same PEM film 1M NaCl solution was used to release the adsorbed chiral probes. For PEM-PEI/PVS and PEM-PEI-m/PVS c_{GLU} was 0.05 M at $pH = 3.5$ and c_{ASC} was 0.01 M at $pH = 4.0$.

ATR-FTIR

Both consecutive PEL deposition and enantiospecific adsorption were characterized by *in-situ* attenuated total reflection

Fourier transform (ATR-FTIR) spectroscopy using a commercial ATR-FTIR attachment operated by the single-beam-sample-reference (SBSR) concept (OPTISPEC, Zürich, Switzerland). The ATR- FTIR attachment was installed on the IFS 55 Equinox FTIR spectrometer (Bruker Optik GmbH, Leipzig) equipped with globar source and MCT detector. A homebuilt transparent *in-situ* cell (M.M., IPF Dresden) was used, which sealed a silicon crystal by oval O-rings forming an upper sample (S) compartment, which can be filled with the PEL or chiral probe solution and a lower reference (R) compartment, which can be filled with the solvent. The SBSR concept implies, that single channel spectra $I_{S,R}$ were recorded of both the upper (S) and lower (R) half/compartment of the Si-IRE ($50 \times 20 \times 2$ mm^3) by one IR beam. Normalizing the single-channel spectra according to $A(\nu) = -\log(I_S(\nu)/I_R(\nu))$ resulted in absorbance spectra ($A(\nu)$) with proper compensation of the background absorptions due to the SiO_x layer, solvent, water vapor (spectrometer), and ice on the MCT detector window.

Results and Discussion

Herein, we report on the deposition of enantiospecific polyelectrolyte mulitlayers (PEM), which were formed by consecutive adsorption of the chiral polycation PLL or PEI-m and the polyanion PVS in comparison to the PEM of nonchiral unmodified PEI and PVS. Enantiospecifity was checked by interaction of these PEMs with L-/D-GLU and L-/D-ASC, respectively, as chiral probes.

PEM Deposition

In the following three PEMs consisting of nonchiral PEI/PVS, of chiral PLL/PVS and of chiral PEI-m/PVS are introduced. Generally, for these systems preparation conditions were chosen enabling the most effective deposition, i.e. the largest increase in deposited amount with smallest adsorption steps z.

PEI/PVS

In the Figure 1a *in-situ* ATR-FTIR spectra on the consecutive deposition of PEM-PEI/PVS-z in the presence of 1 M $NaClO_4$ are given. From the bottom to top these spectra reflect the deposited PEL material after the first (PEI, PEM-1), second (PVS, PEM-2) up to the nineth consecutive adsorption step z (PEI, PEM-9). Significantly, an intense band at 1230 cm^{-1} assigned to the stretching vibration of the sulfate group ($\nu(SO_2)$) due to PVS and one at around 1600 cm^{-1} due to the bending vibration of

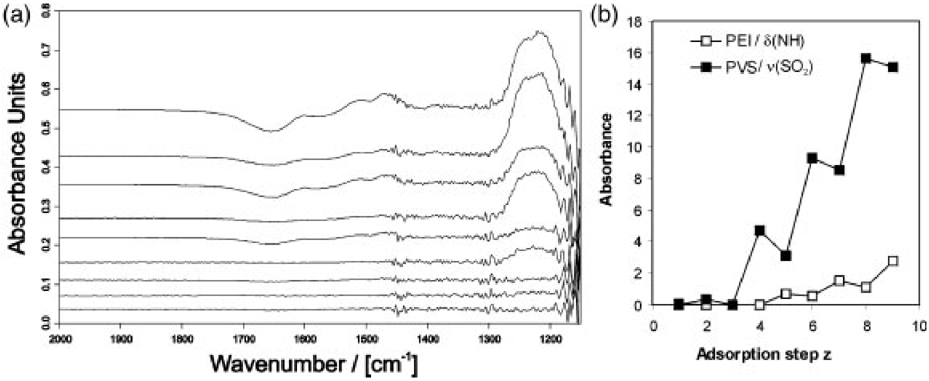

Figure 1.

a). ATR-FTIR spectra on the consecutive adsorption of PEI (0.01 M) and PVS (0.01 M) in the presence of 1M $NaClO_4$ onto the Si-IRE. Spectra of PEM-1 to PEM-9 (from bottom to top) are shown. b). Deposition profile of PEM-PEI/PVS in the presence of 1 M $NaClO_4$ rationalized by the integrated areas of ν(NH) (PEI) and $\nu(SO_2)$ band (PVS) plotted versus z.

the NH group (δ(NH)) due to PEI could be observed. The integrals of these two bands are approximately linear with the respective deposited PEL amounts and were plotted versus the adsorption step z in the Figure 1b. The two different amplitudes were due to the difference in the absorption coefficients for both IR bands. Interestingly, both courses show modulation features with opposite trend: whenever PEI is immersed onto a PEM with outermost PVS layer, the δ(NH) band due to PEI is increased, while the $\nu(SO_2)$ band is decreased and vice versa. From that we conclude a partial release from a given outermost PEL layer by the oppositely charged one to form a soluble complex in the solution above the PEM.

PLL/PVS

Furthermore, in the Figure 2a in-situ ATR-FTIR spectra on the deposition of PEM-PLL/PVS-z in the presence of 1M $NaClO_4$ are shown with increasing adsorption step z = 1–9 from bottom to top. Increasing signals of amide I, amide II, and $\nu(SO_2)$ band at 1645, 1550, and 1230 cm^{-1}, respectively, are visible with increasing z, which are due to the peptide group of PLL and again the sulfate group of PVS. From the wavenumber maxima of the amide I and amide II band it can be concluded that PLL is predominantly in the α-helical conformation, which was already reported.[13,14] This α-helical stabilization is suggested to be originated by specific insertion of perchlorate anions between the ammonium groups of PLL as it was reported by Ebert.[20] The integrals of amide II and $\nu(SO_2)$ band are plotted versus z in Figure 2b. Both bands increase with adsorption step showing again a counterwise modulation feature with increasing z, which can be interpreted as partial release by the oppositely charged respective PEL.

PEI-m/PVS

Finally, in Figure 3a *in-situ* ATR-FTIR spectra of PEM-PEI-m/PVS-z for z = 1–9 are shown. While at 1230 cm^{-1} again the $\nu(SO_2)$ band appeared, a characteristic broad band between 1500 and 1300 cm^{-1} could be identified, which can be assigned to characteristic IR bands of sugars (δ(CH), δ(OH)). Also indicative for PEM deposition is the negative band at around 1640 cm^{-1}, which reflects the removal of water from the Si-IRE surface.[15]

The integrals of $\nu(SO_2)$ (PVS) and δ(CH)/δ(OH) (PEI-m) are plotted in the Figure 3b, from which PEM formation but to a lower extent compared to PEI/PVS and PLL/PVS is evident. Also present but not that pronounced as in the other cases are the counterwise modulation features of PEI-m and PVS uptake, which might be

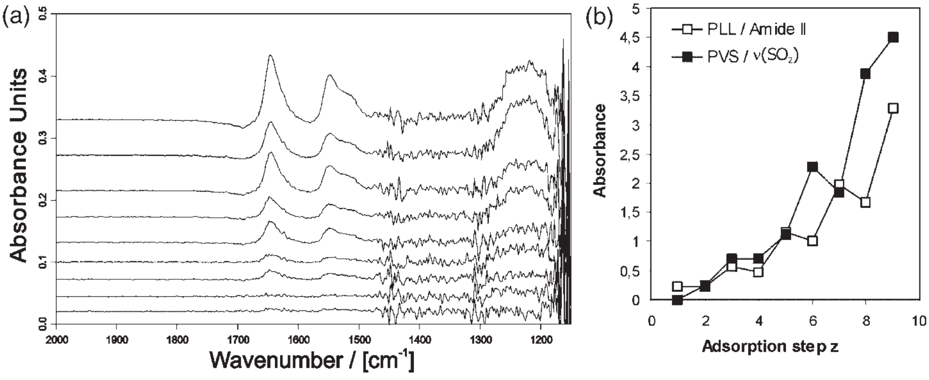

Figure 2.

a). ATR-FTIR spectra on the consecutive adsorption of PLL (0.01 M) and PVS (0.01 M) in the presence of 1M $NaClO_4$ onto the Si-IRE. Spectra of PEM-1 to PEM-9 (from bottom to top) are shown. b). Deposition profile of PEM-PLL/PVS in the presence of 1 M $NaClO_4$ rationalized by the integrated areas of Amide II (PLL) and $\nu(SO_2)$ band (PVS) plotted versus z.

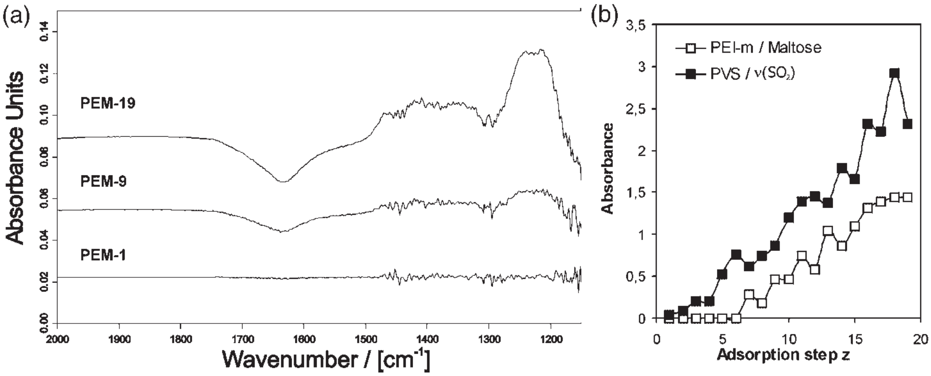

Figure 3.
a). ATR-FTIR spectra on the consecutive adsorption of PEI-m (0.01 M) and PVS (0.01 M) onto the Si-IRE. Spectra of PEM-1, PEM-9 and PEM-19 (from bottom to top) are shown. b). Deposition profile of PEM-PEI-m/PVS rationalized by the integral of combined δ(CH)/δ(OH) (PEI-m) and $\nu(SO_2)$ band (PVS) plotted versus z.

due to the lower deposition level. Since all three studied PEMs are composed of PVS, the integral of the $\nu(SO_2)$ band could serve as a comparable measure for PEM deposition, which is summarized in the Table 1. Based on that the deposited amount decreased in the order PEI/PVS ($NaClO_4$) > PLL/PVS ($NaClO_4$) > PEI-m/PVS. The difference in deposition of PEI/PVS and PLL/PVS could be explained by the high molecular weight of branched PEI forming large coil-like objects under the high ionic strength conditions. Whereas according to previous reports[13,14] α-helical PLL deposits as rodlike objects and there is a considerable amount of nematic order in that assemblies, which causes higher organized but thinner films.

No deposited amount was found for PEM-9 of PEI-m/PVS in the presence of $NaClO_4$ (data not shown) and low but significant deposited amounts were found for PEM-9 and PEM-19 of PEI-m/PVS in the absence of salt. Since the amino groups of PEI-m were found to be mainly disubstituted by ^{13}C NMR characterization we suggest a steric shielding of the charge centers ($^{+}NHR_3$) formed in water by the bulky maltose units. Therefore, in the presence of salt (1 M $NaClO_4$) the steric length scale might exceed the Debye length $\lambda_D \approx 0.3$ nm (i.e. electrostatic reach) and no ion pairs to sulphate groups of PVS can be formed. Whereas, in the absence of salt $\lambda_D > 3$ nm exceeds the steric length scale and stable PEM can be formed by ion pair formation between PEI-m and PVS.

Enantiospecific Interaction

For the measurements related to enantiospecifity the deposited PEM were further stabilized by glutar dialdehyde, since in the deposition profiles indications for PEL release on immersing the oppositely charged one could be obtained, as it was described above. This treatment led to partial crosslink formation between amino groups of PEI, PLL and residual ones for PEI-m, respectively, within the highly entangled PEM internal phase. From immersion

Table 1.
Integrated areas of the $\nu(SO_2)$ band (PVS) for the three studied PEM systems.

PEM	PLL/PVS PEM-9 1 M $NaClO_4$	PEI/PVS PEM-9 1 M $NaClO_4$	PEI-m/PVS PEM-9 1 M $NaClO_4$	PEI-m/PVS PEM-9 no salt	PEI-m/PVS PEM-19 no salt
$\nu(SO_2)$ integral/[cm^{-1}]	5.64	9.15	0 (no signal)	0.86	2.30

experiments with 1 M NaCl solutions, no loss of deposited PEM material was obtained (data not shown).

In the Figure 4a in-situ ATR-FTIR spectra are shown, which are due to the difference between spectra of the PEM-PLL/PVS in contact to L-/D-glutamic acid solutions of various concentrations (0.001M to 0.1M) and the spectrum of PEM-PLL/PVS in contact to pure Millipore water. These spectra reflect the bound amount of L- (solid line) and D-GLU (broken line) at the PEM-PLL/PVS. A highly overlapped line shape in the spectral region between 1750 and 1450 cm^{-1} was observed, which is due to contributions of $\nu(C{=}O)$ around 1710 cm^{-1}, $\nu(COO^-)$ around 1550 cm^{-1}, $\delta(NRH_3^+)$ around 1600 cm^{-1}, and 1500 cm^{-1} of carboxylic acid, carboxylate and ammonium moieties, respectively, all more or less involved in hydrogen bonding one with another or to water.

The sum integrals of these range (1750–1500 cm^{-1}) are plotted versus c_{GLU} in the Figure 4b for the L- and D-form of GLU. After an initial steep rise up to 0.02 M, a linear increase was obtained from 0.02 M up to 0.1 M. Here it has to be noted, that for a concentration of 0.1 M L- or D-GLU the ATR-FTIR method senses also the spectral contribution from the bulk volume phase, as it is shown in the top spectrum of Figure 4a. However this contribution is obviously linear (Figure 4b) to c_{GLU} and can be considered in the following analytical function, which consists of a c_{GLU} dependent Langmuir type and linear term:

$$A = A_0\, c_{GLU}/(B + c_{GLU}) + C\, c_{GLU} \qquad (1)$$

A_0, B, and C denote adjustable parameters. From A_0 the uptake and from $B = k_2/k_1$ the ratio between the rate constants of forward and backward binding reaction can be determined. Qualitatively, comparing the two concentration dependent courses for D-GLU and L-GLU, for high c_{GLU} a significant preference of D-GLU was obtained, which was supported quantitatively by the higher A_0 value for D-GLU ($A_{0,D} = 0.85\ cm^{-1}$) compared to L-GLU ($A_{0,L} = 0.76\ cm^{-1}$) based on Equation 1. For a quantitative evaluation of enantiospecific interaction, the percent enantiospecifity (S_E) can be introduced (Equation (2)), which was used and denoted as "percent selectivity S" therein:[10]

$$S_E = (\Gamma_D - \Gamma_L)/\Gamma_L{}^{*}100\% \qquad (2)$$

Γ_L and Γ_D are the surface concentrations of bound L- and D-enantiomers. In an approximation instead of Γ_L and Γ_D the band integrals A_L and A_D can be used for

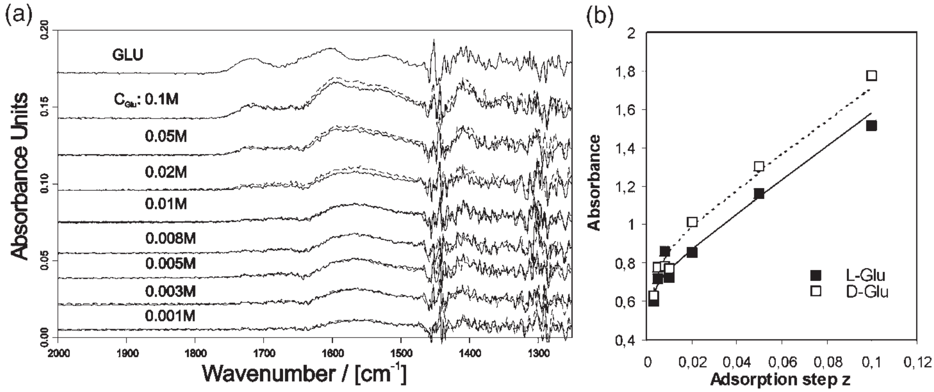

Figure 4.

a). ATR-FTIR difference spectra between PEM-PLL/PVS in contact to L-/D-GLU solutions and the PEM in contact to pure water. The solid line is related to L-GLU, the broken line to D-GLU. The top spectrum is due to a bulk solution of 0.1M L-GLU on the naked Si-IRE surface. b). Sum integrals of the overlapped band (1750–1450 cm^{-1}) due to bound L- and D-GLU at the PEM-PLL/PVS in dependence of c_{GLU}. The data were fitted by analytical function given as Equation (1).

Table 2.
Percent enantiospecifities (S_E) of the three studied PEM systems related to L-/D-glutamic acid (GLU) and L-/D-ascorbic acid (ASC) binding.

PEM	GLU (0.05 M)	ASC (0.01 M)
PEM-PEI/PVS-9	0%	0%
PEM-PLL/PVS-9	$12 \pm 4\%$	$2 \pm 1\%$
PEM-PEI-m/PVS-19	0%	$15 \pm 6\%$

thin PEM films. To characterize now enantiospecifity of a given PEM either A_L and A_D values at various concentrations or $A_{0,\,D}$ and $A_{0,\,L}$ values (see values above) could be taken to insert in Equation (2). Table 2 summarizes found S_E values of the three studied PEM systems for the chiral SA L-/D-GLU and L-/D-ASC.

As expected the nonchiral PEM-PEI/PVS showed no enantiospecifity for L- or D-forms: neither for GLU nor for ASC. However, for PEM-PLL/PVS values of $S_E = 12 \pm 4\%$ for $c_{GLU} = 0.05$ M and of $S_E = 2 \pm 1\%$ for $c_{ASC} = 0.01$ M were obtained. Regarding the latter result Schlenoff[9] found around 3% enantiospecifity for a PEM, which was composed of poly(D-lysine) and poly(D-glutamic acid), towards L-ASC (preference of L over D-enantiomer). Finally, the system PEM-PEI-m/PVS resulted in the highest percent enantiospecifity $S_E = 15 \pm 6\%$ with respect to L- and D-ASC, while no enantiospecifity ($S_E = 0\%$) was found for the same PEM system in contact to L- and D-GLU.

Hence, generally from the Table 2 it can be found, that enantiospecifity is highly dependent on the combination of the chiral SO PEM and the chiral SA probe. Therefore it might be speculated, if a homopolypeptide containing PEM shows more enantiospecifity towards closely related amino acids like GLU compared to sugar derivatives like ASC, while sugar containing PEM might have a higher enantiospecifity towards closer related ASC compared to GLU.

Conclusion

Chiral PEM deposition and their enantiospecific interaction to chiral model probes were studied using *in-situ*-ATR-FTIR spectroscopy. Conditions for an effective deposition of PVS containing PEM using consecutive adsorption were found for three systems: nonchiral PEM of PEI/PVS and the chiral PEMs of PLL/PVS and PEI-m/PVS. The nonchiral PEM did not show enantiospecifity towards the chiral probes L-/D-GLU and L-/D-ASC. Whereas, the chiral PEM of PLL/PVS showed significant enantiospecifity towards D-GLU over L-GLU and the chiral PEM of PEI-m/PVS towards D-ASC over L-ASC. No significant enantiospecifity was found between PLL/PVS and L-/D-ASC and between PEI-m/PVS and L-/D-GLU. PEM of PLL/PVS shows higher enantiospecifity with increasing L-/D-GLU concentration. These first studies contribute to a fundamental understanding of chiral recognition and might help to find alternative concepts in preparative chiral separation technology.

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