# Reversible Switching of Protein Uptake and Release at Polyelectrolyte Multilayers Detected by ATR-FTIR Spectroscopy

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**Summary**: The reversible switching of uptake and release of the proteins lysozyme (LYZ, IEP = 11.1) and human serum albumin (HSA, IEP = 4.8) at the surface attached polyelectrolyte multilayer (PEM) consisting of poly(ethylene-imine) (PEI) and poly(acrylic acid) (PAC) is shown. Protein adsorption could be switched by pH setting due to electrostatic interaction. Adsorption of positively charged LYZ at PEM-6 took place at pH = 7.3, where the outermost PAC layer was negatively charged. Complete desorption was obtained at pH = 4, where the outermost PAC layer was neutral. Additionally the charge state of the last adsorbed PAC layer in dependence of the pH of the medium could be determined in the ATR-FTIR difference spectra by the $\nu(COO^-)$ and $\nu(C=O)$ band due to carboxylate and carboxylic acid groups. Adsorption of negatively charged HSA at PEM-7 was achieved at pH = 7.3, where the outermost PEI layer was positively charged. Part desorption was obtained at pH = 10, where the outermost PEI layer was neutral. PEM of PEI/PAC may be used for the development of bioactive and bionert materials and protein sensors.

**Keywords**: ATR-FTIR spectroscopy; human serum albumin; isoelectric point; lysozyme; multilayer; polyelectrolytes; protein adsorption; surface modification

## Introduction

Protein adsorption at polymer surfaces is an important phenomenon in biomedical, food and pharmaceutical research. On the one hand it is desired for bioactive applications (e.g. uptake of collagenized implants) and on the other it should be prevented for bioinert purposes (e.g clotting on medical devices, membrane fouling) blocking or delaying further bioadhesion cascades. Up to now the correlation between polymer (surface) structure and protein adsorption is not fully solved. In that framework Norde has raised important rules concerning soft and hard properties of the protein and dispersed substrates like polymer latices or inorganic particles [1]. In further fundamental research on this topic often thin films, which

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are accessible by a variety of in-situ analytical techniques, are used to mimick the properties of the surface of the bulk polymer [2, 3]. However, the correlation between the properties of the thin film or other models and the real surface of commercial bulk polymer materials is a difficult task. This has been reflected e.g. by industrial work of Pudleiner and cooperators, aiming at the correlation between thrombogenicity and polymer structure, especially shown for extruded copolymers of polysulfone and polyurethanes with PEO soft segments [4].

Moreover, the thin film itself can be also used in practical applications to modify bulk polymer materials with convenient e.g. mechanical, processing or aging properties. Thereby the given mechanical or other properties of commercial polymers can be preserved and new surface properties can be achieved by simply coating the material or device with a thin often nanoscopic functional layer. Consecutively adsorbed polyelectrolyte multilayers (PEM) introduced by Iler [5] and Decher [6] may be used as a surface modification concept in that respect, since they can be anchored on a variety of materials forming versatile platforms for the exposure of defined structural elements on the modified surface via the outermost polyelectrolyte layer. Such elements could be charged groups, hydrophobic or hydrophilic moieties of blockcopolyelectrolytes or even different conformations of charged polypeptides [7]. Here we report on fundamental studies on reversible pH mediated electrostatic interactions between multilayers consisting of poly(ethyleneimine) (PEI) and poly(acrylic acid) (PAC) and the model proteins lysozyme (LYZ) and human serum albumin (HSA). ATR-FIR spectroscopy is used to monitor the reversible uptake/release of proteins upon modulating the surface charge properties of PEMs. These studies shall demonstrate that PEMs are potentially useful as biomaterial coatings or protein sensor devices.

## Experimental

*Polyelectrolytes, proteins*

All commercial polyelectrolyte (PEL) samples were used without further purification. Poly(ethyleneimine) (PEI, pH = 9) and poly(acrylic acid) (PAC, pH = 4) were dissolved in Millipore water at PEL concentrations $c_{PEL}$ = 0.01 M. The ´as is´ pH values are given in brackets.

Lysozyme (LYZ) and human serum albumin (HSA) were dissolved in phosphate buffer saline (PBS, 1 mg/ml) yielding pH = 7.3. As buffered rinsing solutions PBS (1 mg/ml, pH = 7.3), citrate (CIT, 2 mg/ml, pH = 4) and lysine (LYS, 1 mg/ml, titrated to pH = 10) were applied.

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*Surfaces*

Immediately before the usage the silicon internal reflection elements (IRE) were cleaned in piranha solution (30% $H_2O_2$, 70% $H_2SO_4$) under ultrasonification and rinsed in Millipore water followed by a plasma treatment (PCD 100, Harrick, New York) under reduced pressure.

*Polyelectrolyte multilayers*

PEM-X were prepared in the in-situ-ATR-sorption cell (described below) by consecutively exposing the Si support to solutions of cationic PEI (0.01 M) and to anionic PAC (0.01 M) solutions in defined numbers of cycles X = 6 and 7.

*ATR-FTIR spectroscopy*

The characterization of the deposited PEMs was performed by in-situ-ATR-FTIR spectroscopy, whose principle is given in Fig. 1a. Additionally, the SBSR concept [8], shown in Fig. 1b, was used to obtain well compensated ATR-FTIR spectra. Thereby, the IR beam is guided alternately through the IRE below the sample and the reference compartment, respectively. Typically, the sample compartment is filled with the solution of the adsorbing or binding substance and the reference compartment with the respective solvent. The ATR-FTIR attachment was operated on the IFS 55 Equinox spectrometer (BRUKER-Saxonia, Leipzig) equipped with globar source and MCT detector. Typically, polycation, rinsing, polyanion and protein solutions were injected and cycled through the *in-situ* ATR cell (IPF Dresden) by a peristaltic pump in combination with an addressable valve system (IPF Dresden) operated under computer control as shown in Fig. 1a.

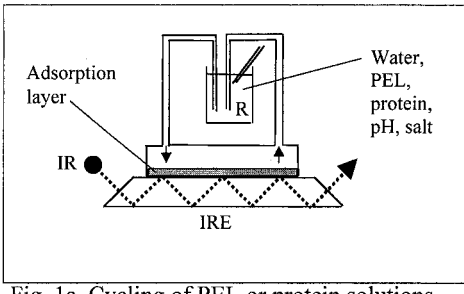


Fig. 1a. Cycling of PEL or protein solutions from a reservoir (R) through the in-situ-ATR-FTIR cell above the Si-IRE.

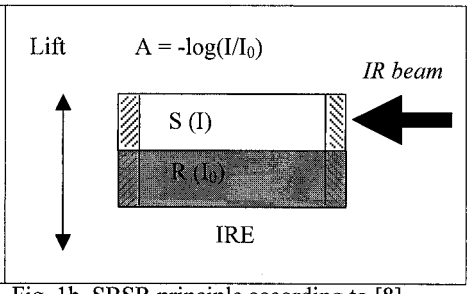


Fig. 1b. SBSR principle according to [8] applied for reproducible spectral background compensation in the in-situ-ATR-FTIR spectra.

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## Results

The polyelectrolyte multilayer (PEM) system composed of PEI and PAC has been developped to become a standard system for reproducible deposition of a defined adsorbed amount and the formation of a laterally homogeneous surface coverage. Both PELs are commercially available. PEM of PEI/PAC have been especially used, to study the influence of electrostatic interactions on protein adsorption [9, 10, 11]. Based on those results here we report on the reversible switching of the charge state of the outermost layer as well as on the related adsorption/desorption cycles at the PEM-6 and the PEM-7 of PEI/PAC.

### 1. Modulation of the charge state of PEM-6

The dissiociation degree and thus the charge density of weak polyacids like PAC is dependent on the pH of the aqueous medium. Therefore the electrostatic interaction between proteins and a polyacid layer can be tuned by the pH setting. In our case the polyacid layer was the terminating PAC layer of a PEM-6 composed totally of three PEI and three PAC layers. In the Fig. 2a in-situ-ATR-FTIR spectra of a PEM-6 of PEI/PAC in contact to citrate buffer (CIT, lower curve) and to phosphate buffer (PBS, upper curve) are shown.

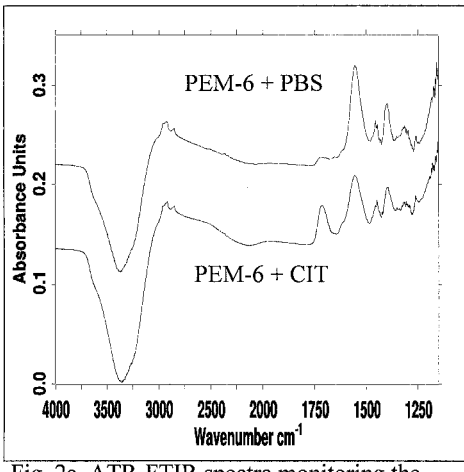


Fig. 2a. ATR-FTIR spectra monitoring the dissociation state of the outermost PAC layer of PEM-6 of PEI/PAC. Upper curve: PEM-6 in the presence of PBS buffer (PBS, pH = 7.3). Lower curve: PEM-6 in the presence of citrate buffer (CIT, pH = 4.0).

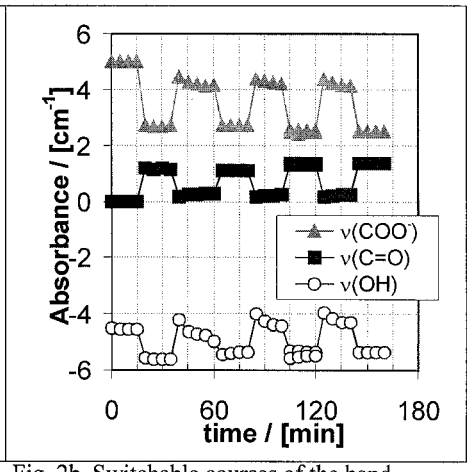


Fig. 2b. Switchable courses of the band integrals of the ν(C=O), ν(COO⁻) and the ν(OH) band (factor: 0.1) of PAC within the PEM-PEI/PAC modulated by the pH of the aqueous medium. The pH was switched between 7.3 (PBS) and 4.0 (CIT) starting with pH = 7.3 (first 15 min).

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Significantly, the pH elevation from pH = 4 to pH = 7.3 caused changes of the ν(C=O) and ν($COO^-$) intensities due to the concentrations of COOH and $COO^-$-groups, respectively. In Fig. 2b the courses of the ν(OH), ν(C=O) and ν($COO^-$) band integrals are plotted in dependence of the time, during which the pH value of the aqueous medium was switched subsequently between pH = 7.3 (pH at the beginning) and pH = 4. In detail the ν(C=O) band is lowered and the ν($COO^-$) band increased at the pH = 7.3 proving elevation of the number of $COO^-$ groups and thus of charge carriers. The opposite is the case at pH = 4: the ν(C=O) is increased and the ν($COO^-$) is lowered proving elevation of the number of COOH groups resulting in diminuation of charge carriers. So with that it can be clearly shown, that the negative surface charge potential can be easily switched by pH setting. As it was shown recently one can also calculate the dissociation degree based on the integated areas A of the ν($COO^-$) and ν(C=O) according to $\alpha_{IR} = A_{\nu(COO^-)} / [A_{\nu(COO^-)} + F * A_{\nu(C=O)}]$ knowing the ratio $F = \varepsilon_{\nu(COO^-)}/\varepsilon_{\nu(C=O)}$ of their absorption coefficients ε [12]. Furthermore the modulated course of the ν(OH) band intensity reflects a certain pH dependent swelling/shrinking behavior of the PEM-6, which was discussed for comparable systems therein [12].

## 2. Reversible electrostatic binding of lysozyme at PEM-6

This effect of switching the charge state of the outermost PAC layer between ´neutral´ and ´negative´ can be used for fundamental studies or applications on the electrostatic binding and release of proteins at PEM composed of PEI/PAC. We have chosen lysozyme (LYZ) as a model protein, since it exhibits an isoelectric point of IEP = 11.1 and has therefore at both applied values of pH = 7.3 and pH = 4 a positive net charge. Hence we expected that changing the pH should have the major influence on the outermost PAC layer as shown in the previous section an a minor one on the net charge state of the protein. ATR-FTIR spectroscopy was used to monitor protein binding and release, since the amide I band is a highly sensitive and diagnostic sensor for proteins. If the concentration in the solution is sufficiently small ($c \leq 1$ mg/ml), the detected amide I signal is only due to the formation of a protein layer on the PEM and can therefore be used for monitoring protein sorption. The ATR-FTIR spectra recorded during exposure of LYZ solution (15 min) to a PEM-6 of PEI/PAC are shown in Fig. 3a, whereby at pH = 7.3 (upper curve) a strong increase and at pH = 4 a strong decrease of the amide I signal can be obtained. For quantification the courses of the amide I band integrals are shown in Fig. 3b, which have been obtained by the follwing two steps:

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(1) LYZ adsorption at pH = 7.3 (PBS buffer)

(2) LYZ release at pH = 4 (CIT buffer) followed by rinsing with PBS.

The modulated uptake of LYZ can be clearly seen, which correlates well with the switching of the charge state of the last adsorbed PAC layer between 'negative' and 'neutral' as it is shown in Fig. 2b. The continuous drop of the adsorbed amount is due to the repeated exposure of the PAC terminated PEM to the same LYZ solution, which was used in the step before and successively depleted by increasing adsorption steps.

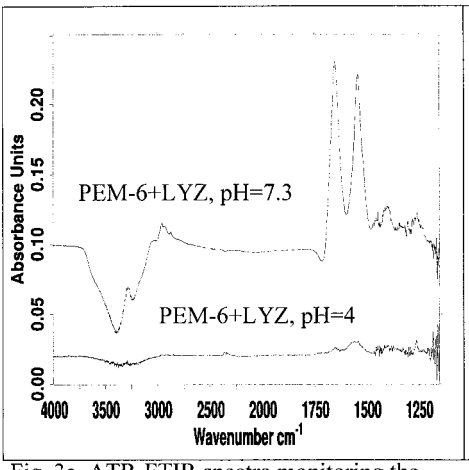


Fig. 3a. ATR-FTIR spectra monitoring the adsorption of LYZ at pH = 7.3 (PBS) (upper curve) and the release at pH = 4 (CIT) (lower curve) at the PEM-6 of PEI/PAC. (Data of the first cycle were taken.)

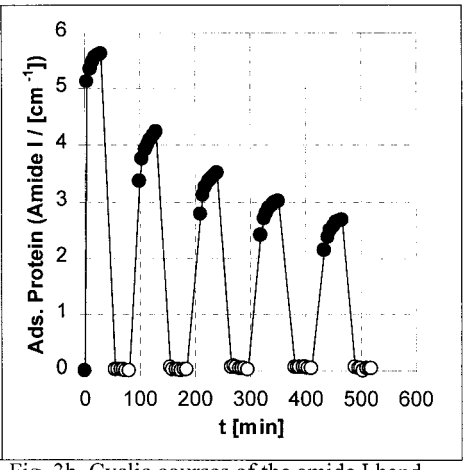


Fig. 3b. Cyclic courses of the amide I band integrals due to the adsorbed LYZ amount at PEM-6 of PEI/PAC modulated by the pH settings: pH = 7.3 (full circles), pH = 4.0 (open circles).

### 3. Reversible electrostatic binding of serum albumin at PEM-7

As another electrostatically interacting system the PEM-7 was generated simply by adsorbing one more PEI layer on top of the PEM-6. In that case the interaction of the acidic protein human serum albumin (HSA) was studied at two pH values, where HSA (IEP = 4.8) has a negative net charge. The ATR-FTIR spectra recorded during exposure (20 min) of HSA solution to a PEM-7 of PEI/PAC are shown in Fig. 4a, whereby at pH = 7.3 (upper curve) a large and at pH = 10 (lower curve) a smaller amide I signal can be obtained. Analogously to Fig. 3b the courses of the amide I band integrals are shown in Fig. 4b, which have been obtained by the follwing two steps:

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(1) HSA adsorption at pH = 7.3 (PBS buffer)

(2) HSA release at pH = 10 (LYS buffer) followed by rinsing with PBS.

Again the modulated uptake of HSA can be clearly seen, which correlates well with the switching of the charge state of the last adsorbed PEI layer between 'negative' and 'neutral'. Unlike the PEM-6 interaction with LYZ in the case of PEM-7 the HSA could not be fully desorbed by pH = 10. Presumably, at pH = 10 the ammonium groups of PEI were not completely deprotonated to neutral primary or secondary amino groups. Hence positively charged groups remained which are able to bind HSA to a certain extent. A further pH increase was not considered, since solutions of pH > 10 could cause damage of the PEM of PEI/PAC as well as of the silicon support. Further studies will be performed in that direction.

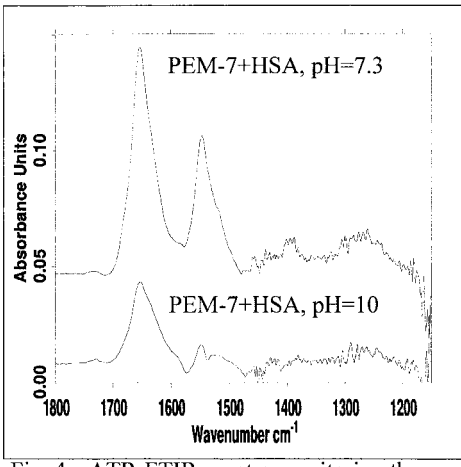


Fig. 4a. ATR-FTIR spectra monitoring the adsorption of HSA at pH = 7.3 (PBS) (upper curve) and the part release at pH = 10 (LYS) (lower curve) at the PEM-7 of PEI/PAC. (Data of the first cycle were taken.)

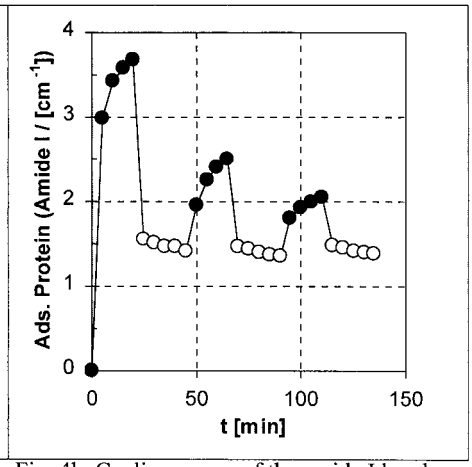


Fig. 4b. Cyclic courses of the amide I band integrals due to the adsorbed HSA amount at PEM-7 of PEI/PAC modulated by the pH settings: pH = 7.3 (full circles), pH = 10 (open circles).

## Conclusion

- PEMs composed of PEI/PAC are switchable platforms for the reversible uptake and release of proteins by electrostatic interaction. They could be switched by the pH of the surrounding aqueous medium. The outermost PAC layer of the PEM-6 is fully dissociated at the pH = 7.3 and approximately neutral at pH = 4, whereas the outermost PEI layer is fully protonated at pH = 7.3 and approximately neutral at pH = 10.

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- This was used to modulate the binding/release of lysozyme (LYZ), which is a basic protein (IEP = 11.1), by electrostatic interaction: At pH = 7 LYZ was adsorbed at a high amount and at pH = 4 nearly all LYZ could be removed from the PEM-6, due to the decreased negative charge of the outermost PAC layer, as it is shown in Fig. 5a.
- Analogously, at the PEM-7 acidic human serum albumin (HSA, IEP = 4.8) could be adsorbed at pH = 7.3 and partly desorbed at pH = 10 due to the charged and the neutral state of the outermost PEI layer, respectively, as iti shown in Fig. 5b.
- The uptake and release of these proteins could be switched in infinite cycle numbers, which makes PEM of PEI/PAC potentially interesting as protein sensors or also as coatings to prevent biosensor membrane fouling [13].

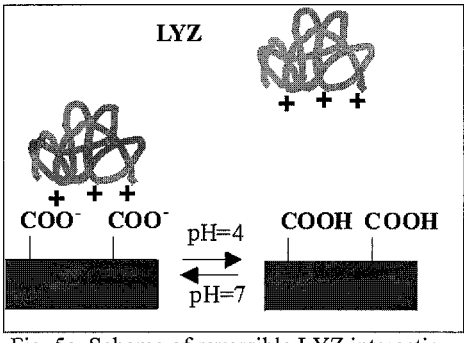


Fig. 5a. Scheme of reversible LYZ interaction to PEM-6 of PEI/PAC.

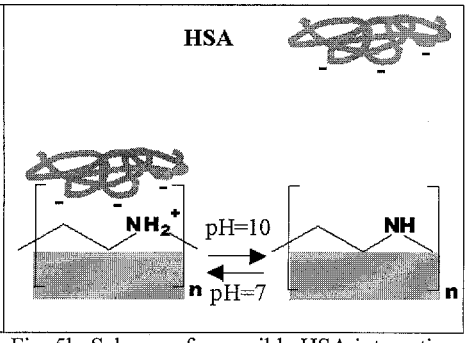


Fig. 5b. Scheme of reversible HSA interaction to PEM-7 of PEI/PAC.

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1. C.A. Haynes, W. Norde, *Coll. Surf. B*, 2, 517 (1994)
2. J.D. Andrade and V. Hlady, *Adv. Pol. Sci.*, 79, 1 (1986)
3. C.-G. Gölander and E. Kiss, *J. Coll. Interf. Sci.*, 121 (1) 240 (1988)
4. R. Dujardin, H. Pudleiner, J. Goossens and U.T. Seyfert, in *'Werkstoffe für die Medizintechnik'*, J. Breme (ed.), pp. 185, DGM-Informationsgesellschaft Verlag, Frankfurt (1997)
5. R.K. Iler, *J. Coll. Interf. Sci.*, 21, 569 (1966)
6. G. Decher, *Science*, 277, 1232 (1997)
7. M. Müller (manuscript in preparation)
8. U.P. Fringeli, in *'Encyclopedia of Spectroscopy and Spectrometry'*, J.C. Lindon, G.E. Tranter, J.L. Holmes (eds.), Academic Press (2000)
9. M. Müller, T. Rieser, K. Lunkwitz, S. Berwald, J. Meier-Haack, D. Jehnichen, *Macromol. Rapid Commun.*, 19, 333 (1998)
10. M. Müller, M. Brišš**o**vá, T. Rieser, A.C. Powers, K. Lunkwitz, *Mat. Sci. Eng. C,* 8-9, 167 (1999)
11. M. Müller, T. Rieser, P. Dubin, K. Lunkwitz, *Macromol. Rapid Commun.*, 22, 390 (2001)
12. M. Müller, *in: "Handbook of Polyelectrolytes and Their Applications"*, S.K. Tripathy, J. Kumar, H.S. Nalwa (eds.), Vol. 1, pp. 293, Stevenson Ranch: American Scientific Publishers (2002)
13. N. Wisniewski, M. Reichert, *Coll. & Surf. B*, 18, 197 (2000)

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