Adsorption of Oligo- and Polypeptides as Model Polyelectrolytes*

Benedict C. Bonekamp, Hendrik A. van der Schee, and Johannes Lyklema

Laboratory for Physical and Colloid Chemistry of the Agricultural University De Dreijen 6, 6703 BC Wageningen, The Netherlands

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The adsorption of oligo- and polylysines on silver iodide and polystyrene latex has been measured as a function of concentration, pH and ionic strength ω. At low pH, polylysine adsorbs in a flat conformation. Loops start to develop if the pH is increased or if ω is raised. The electrolyte effect persists up to very high concentrations. The principles and some results of a new polyelectrolyte adsorption theory are outlined. It explains the experimentally observed features at least semiquantitatively. The segment density profile of a polyelectrolyt adsorbate does not resemble that of an uncharged macromolecule. It depends on the charge on the chain and on ω and under certain conditions it contains a region of negative adsorption.

INTRODUCTION

The study of the adsorption of oligo- and polypeptides at liquid-solid interfaces serves several purposes:

— under some conditions, polypeptides behave as random polyelectrolytes, then they are suitable model substances to investigate the influence of molecular weight, pH, ionic strength and nature of the electrolyte on the adsorption

— oligopeptides adsorption bridges the gap between adsorption of monomers and polymers, or rather between ions and polyelectrolytes. The theory for the adsorption of ions is relatively advanced and a number of equations have been put forward, such as those of Stern and Frumkin-Fowler-Guggenheim (FFG). Polymer adsorption theory is based on statistical considerations and it is useful to cover the overlap range between the two categories

— oligo- and polypeptides are suitable model substances to study the phenomena of steric stabilization and adsorption flocculation

— adsorption of polypeptides provides background information that may be useful for the understanding of protein adsorption. For instance, some polypeptides undergo a helix-coil transition as a function of pH in solution and it is of interest to find out to which extent helices persist after adsorption. Polypeptides being more simple systems than proteins, it is likely that

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theoretical descriptions of any conformational modification can be given in an earlier future for polypeptides than for proteins.

In addition, adsorption of polypeptides has its own interest, for instance the binding of biological debris to soil constituents belongs to this category.

Of these various aspects, we shall in this paper emphasize the role of polypeptides as model polyelectrolytes. The substance under consideration is (mono-, oligo-, poly-) lysine. In aqueous solution the poly-L-lysine molecule is a coil at pH $\lesssim$ 9—10 and a helix above this pH, whereas the corresponding poly-DL-lysine is a coil throughout. We found that under our experimental conditions on the adsorbents silver iodide and polystyrene (PS) latex the adsorbate showed no indication of any presence of helices. One of the arguments is that at pH's above the transition range there is no detectable difference in adsorption behaviour between poly-L and poly-DL-lysine. In this sense, adsorbates of oligo- and polylysines behave as appropriate model substances for random polyelectrolytes.

<div align="center">EXPERIMENTAL</div>

Materials

Poly-L lysine. HBr and poly-DL- lysine. HBr were obtained from Sigma Chemical Cy and used without further purification, except on AgI, where the bromide ion was exchanged against fluoride to avoid precipitation of AgBr. The degrees of polymerization (DP) mentioned in this paper are based on the viscosity-average molecular mass, as given by the manufacturer.

Oligolysines up to DP = 16 were synthesized by a procedure which has been published elsewhere.[1]

Polystyrene latices were prepared emulsifier-free according to the procedure of Furusawa et al[2] except for the following modifications: after preparation, the latex was steam-stripped to remove unreacted monomer; during this latter process, the ionic strength ω was kept at or below the initial value; and thereafter the latex was batchwise subjected to ion-exchange to remove low molecular mass electrolytes, and bring it in the protonated form. It was conductometrically verified that one exchange treatment was sufficient. Further details will be published elsewhere.[3] The surface charge is solely due to sulphate groups.

Silver iodide sols and suspensions were prepared by condensation from KI and AgNO₃ as described by De Wit.[4]

Methods

Concentrations of positively charged polylysines were determined by titration with the potassium salt of polyvinyl sulphate, using toluidine blue as the indicator.[5,6]

Adsorption isotherms were obtained by depletion. If necessary, the adsorbent with adsorbate was centrifuged off. The influence of various factors on the adsorption, such as the state of aggregation of the sorbent, the order of addition and the reversibility were investigated in some detail[3,7] but will not be reported here. The adsorbed amount of polylysine. HBr on polystyrene and polylysine. HF on AgI is expressed in mg m⁻² polylysine. HBr in both cases.
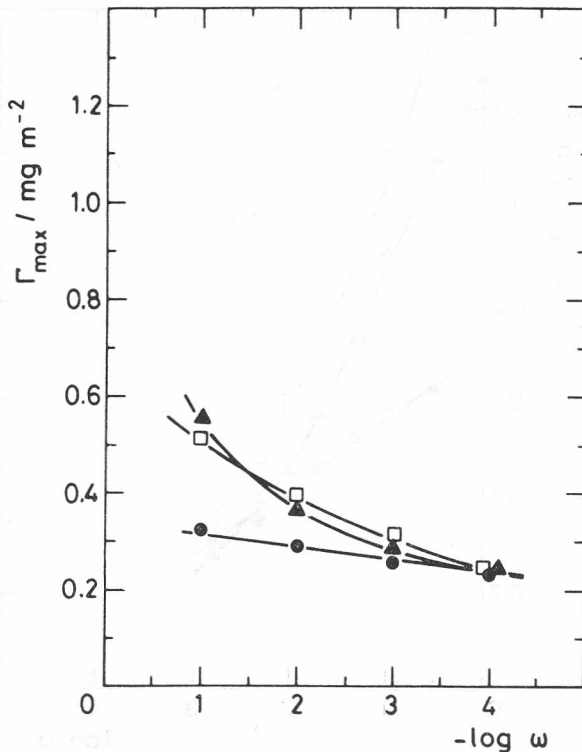
Specific surface areas of the latices were derived electronmicroscopically. The area of AgI dispersions was as a matter of routine obtained from methylene blue adsorption, the cross section of the dye molecule being determined by prior comparison with standard curves.[8]

<div align="center">RESULTS</div>

The primary aim of this paper is to study the polyelectrolyte character of polylysine adsorbates. To that end special attention will be paid to the effects of pH and the ionic strength ω on the adsorption, these variables determining

the charge and the screening of the charge respectively. In order to ensure that certain phenomena are of a general nature, trends on two different adsorbents, AgI and PS latex, will be compared. Pursuing such comparisons, differences of some tens of percents were observed between the absolute values Γ of the adsorbed amounts on the two substrates. The origin of these differences is not yet entirely clear; likely they are due to uncertainties with respect to the specific surface areas, or rather to the areas, available for adsorption of polypeptide. However, for the present issue, these quantitative discrepancies are not of direct concern since the observed trends are very similar.

Polylysine and oligolysine adsorption exhibit a number of characteristics that are in line with present-day experience on adsorption of macromolecules. With increasing DP, from monomers to polymers, the isotherms gradually change from a Langmuir-type to a High-affinity like shape.[9] At the same time, the (pseudo-)plateau increases and the adsorption becomes more irreversible with respect to dilution. The non-desorbability of polymeric adsorbate is not due to a real irreversibility (i. e. it is not a consequence of a frozen non-equilibrium state), but to the extreme dilutions that are required to remove the higher M components from the surface.[10,11] That equilibrium does prevail is evidenced by our experience that polylysine adsorption is reversible with respect to changes in pH and ω. It is therefore fully warranted to subject these measurements to thermodynamic analysis.

The adsorbed amount of a macromolecule is to a large extent determined by segments in loops (and tails to a lesser extent). Such segments remain in contact with the solution and this is essentially the reason why Γ is sensitive to the solvency. For polyelectrolytes, the mutual repulsion between similarly charged groups will prevent the formation of thick adsorbates. On the other hand, if this repulsion is suppressed by electrolytes Γ can become high. Both on AgI and PS latex this trend is indeed observed (Figures 1 and 2). In these figures, plateau values of the isotherms are given. Under the conditions chosen, the polypeptide is positively and the substrate negatively charged. We note, but shall not discuss the difference between the two systems with respect to the magnitudes of Γ. Important for our purpose is that in both cases Γ_{max} increases with increasing ω. At the same time, the adsorption becomes more sensitive to M. This feature is quite comprehensible: polymer adsorption theory[12] predicts an increase of Γ_{max} with M. This increase is largely accounted for by the loops. At low ω (to the right in Figures 1 and 2) the repulsion between chain segments is so strong that no loops can develop, and therefore, under those conditions there is also no effect of M. On the other hand, to the left in the figure, where chain segment repulsion is suppressed, the polyelectrolyte gradually starts to resemble an uncharged macromolecule, exhibiting the characteristic increase of Γ with M.
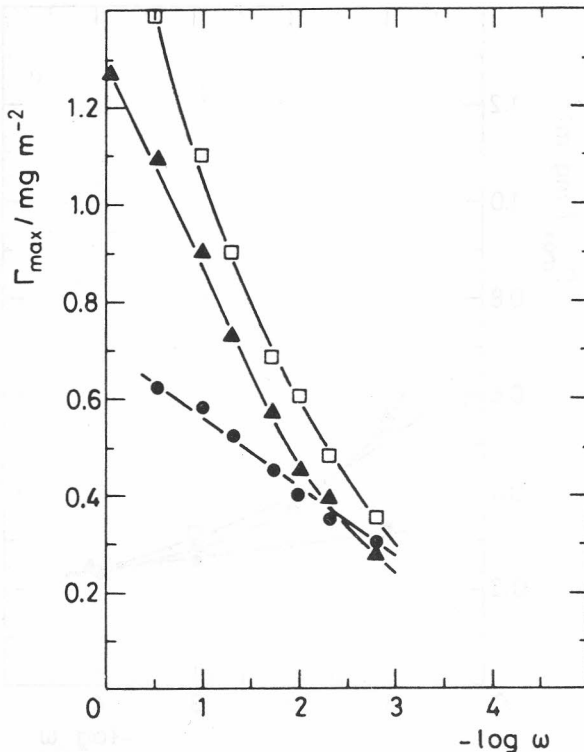

Figure 2. Adsorption of poly-L-lysine on AgI. pAg = 11. T = 20 °C, pH 6 ———●——— DP = 17, ———▲——— DP = 300, ———□——— DP = 2000.

That at low ω polylysine adsorbs more or less as a monolayer, without loops or tails is corroborated by the stability of AgI-sols, covered by a polylysine monolayer: such sols exhibit no steric stabilization whatsoever, their coagulation concentration being very similar to that of naked AgI-sols.

In Figure 2 Γ_{max} continues to rise progressively beyond $\omega \geq 10^{-1}$ M. On PS latex corresponding data are not available but there is no indication of any levelling off (Figure 1). This observation is contrary to experience with colloid stability, which has it that at or below $\omega \sim 10^{-1}$ M all electrocratic sols coagulate because then the diffuse part of the double layer is almost completely compressed ($\varkappa^{-1} \sim 1$ nm). Apparently, for polyelectrolytes at interfaces, the influence of the salt persists above this concentration.

Our observation does not stand alone. For instance, Marra et al[13] reported adsorptions of polystyrene sulphonate on silica which kept increasing up to 3 M NaCl or MgCl$_2$. Soumpasis et al. observed for the same polyelectrolyte an electrolyte influence on the viscosity, also up to very high concentrations[14] and Hlady[15] observed the adsorption of dextrane sulphate ($M \sim 5 \times 10^5$) on calciumoxalate monohydrate in the presence of small amounts of Ca^{2+} to continue to increase with NaCl concentrations up to and beyond 2 M.

Figure 3 relays the influence of the charge on the chain. Polylysines are positively charged at low pH and nearly uncharged at high pH (pK$_o \sim 10$). For the same reasons as expounded above we must therefore expect a drastic rise of Γ_{max} with pH. Experiments confirm this.
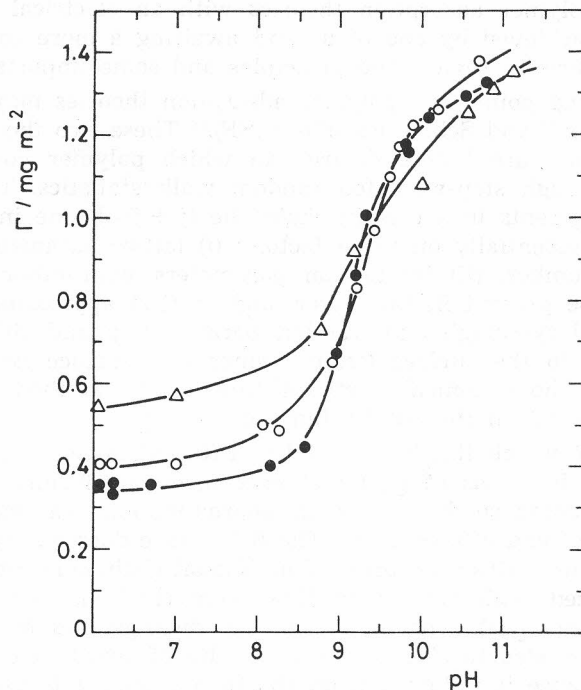

Figure 3. Adsorption of polylysine on PS latex, $\sigma_o = -4.2$ µC cm^{-2}. T = 20 ºC. ———●——— Poly-L-lysine, 0.01 M NaBr DP = 190, ———○——— Poly-DL-lysine, 0.01 M NaBr DP = 240, ———△——— Poly-DL-lysine, 0.1 M NaBr DP = 240.

Similar arguments apply to the effect of NaBr. At low pH and 10^{-2} M NaBr the adsorbate is essentially flat. If ω is increased to 10^{-1} M, loop formation becomes possible and Γ_{max} increases. However, at pH $\sim$ 11 the adsorbate approaches the uncharged state and the influence of NaBr disappears.

It is noteworthy that the difference between poly-L-lysine and poly-DL-lysine is hardly significant, whereas in bulk there is a great difference: poly-DL-lysine is a coil over the entire pH-range investigated, but poly-L-lysine changes its conformation from a coil at low pH to a helix at high pH with a transition range around pH $\sim$ 10, depending on ω. Figure (3) suggests that in the adsorbed state poly-L-lysine is also randomly arranged, or rather, that the distribution of segments is governed by the same entropical and energetic factors that control adsorption of uncharged molecules. Apparently, the cohesive forces in the helix of poly-L-lysine are too weak to persist after adsorption, in contradistinction to those in many proteins.[16,17]

<div align="center">THEORY</div>

Hitherto, no suitable polyelectrolyte adsorption theory existed. The only one available is that by Hesselink[18] but it is inadequate because, among other defects, it assumes an exponential segment density-distance $\varrho(z)$ relationship, thereby following Hoeve's polymer adsorption theory[19]. Although for polymers, this is a reasonable assumption, it is incorrect for polyelectrolytes. Rather, an ab initio theory is needded in which $\varrho(z)$ is computed for varions ω and pH and the most logical approach is to extend one or more of the present day suitable polymer adsorption theories with an electrical term. Recently, this has been achieved by one of us and awaiting a more complete account[7] we shall now briefly review the principles and some important results.

As a starting point, two polymer adsorption theories may be considered, viz. those of Roe[20] and Scheutjens-Fleer (SF).[12] These two theories have much in common. Both are lattice theories in which polymer configurations are formulated through step-weighted random walk statistics. The likeliness to find after j segments in a certain chain the $(j + i)$-th one in a given lattice layer depends essentially on three factors: (i) lattice parameters, such as the coordination number, (ii) interaction parameters with other molecules that may already be present in this layer, and in first approximation quantified through the Flory-Huggins interaction parameter χ and (iii), only for the layer adjacent to the surface (train segments) a surface interaction energy parameter χ^s. The canonical partition function Q_0 is then formulated and maximalized to obtain the equilibrium distribution.

The way in which this is achieved is different in the Roe and SF theory. Roe writes Q_o in terms of $\varrho(z)$ and maximizes the former with respect to the latter. In doing so, he makes an approximation that virtually amounts to the neglect of end effects (tails). The SF picture does not make this simplification, Q_o being written in terms of individual chain conformations, and also being maximized with respect to these conformations. Therefore, the Roe theory gives only $\varrho(z)$, whereas SF theory distinguishes between loops and tails. This 'fine structure' has proved to be of great relevance for steric interaction because it followed from the theory that it is very likely that a number of long tails will be present in most adsorbates under conditions met in practice.

Both the Roe and SF theory have now been amplified with an electrostatic term. If it is done according to the Roe picture, the canonical partition function Q for a polyelectrolyte at a given profile $\varrho(z)$ is obtained from Q_o by multiplication with exp. $(-F_{el}(\varrho)/kT)$, where $F_{el}(\varrho)$ is the electrical free energy of that profile $\varrho(z)$. This free energy can be found, using some charging process, based on a model of the distribution of charges and their interactions. In the present case, a lattice distribution of the charges was assumed and the distribution of small ions was taken to obey Boltzmann statistics. Several charging processes were considered and their equivalence proven, more or less as has been done by Verwey, Overbeek and Casimir in the (simpler) case of double layers around particles.[21] If SF theory is used as the starting point, each segmental weighing factor is multiplied by an electrical term exp. $(-F\psi_i/RT)$ where ψ_i is the potential in layer i; this latter quantity being again found by some model picture.

It appears that for polyelectrolytes the difference between the Roe and SF picture is less pronounced than for uncharged polymers. The reason is that the effect of tails is less important in the charged systems. As the mathematical elaboration is more easy with the Roe picture, we shall give below only results based on this theory. A somewhat more detailed account is given in ref. 13.

Figure 4 gives $\varrho(z)$ for an adsorbed polyelectrolyte. As the distribution is based on a lattice picture, ϱ jumps stepwise and the distance is counted in terms of layer numbers i. In fact, the ordinate gives instead of ϱ the logarithm of the volume fraction φ_i in each layer. The semilogarithmic plot was chosen to accentuate certain qualitative features.

In Figure 4, the upper curve applies to an uncharged adsorbate. The decay is almost linear, which would correspond to an exponential distribution
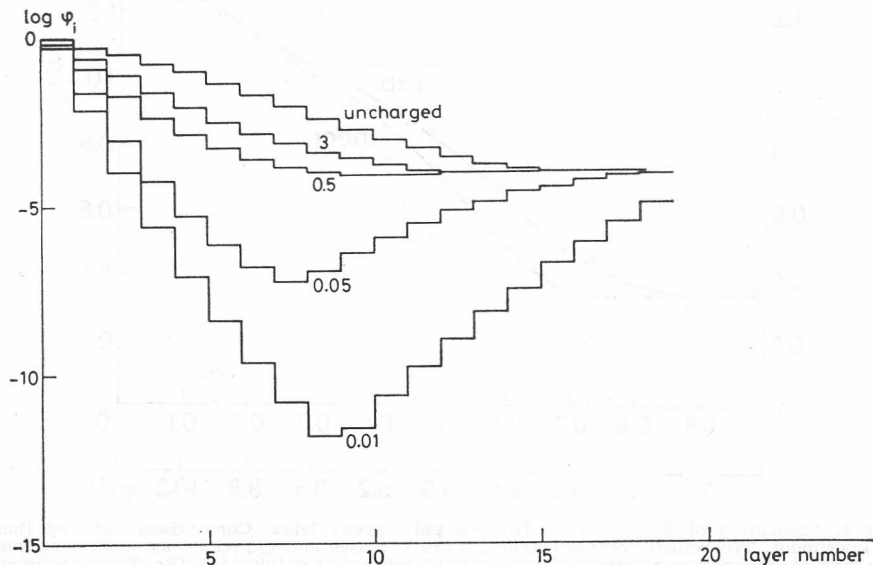

Figure 4. Semilogarithmic concentration profiles for a polyelectrolyte chain of DP = 240 with one charge per Bjerrum length (0.71 nm). $\chi = 0.6$ $\chi_s = 4.85$, $\alpha_{eff} = 0.71$. lattice site length 0.5 nm, $\sigma_o = -0.2$ charges per surface site. The ionic strength ω is indicated.

$\varrho(z)$. The deviations from linearity are a consequence of the presence of tails (for this curve SF theory was used).

Two significant new features deserve attention. The first is that at low ω a region of negative adsorption ($\varphi_i < \varphi_{bulk}$) occurs in the proffiles. Obviously, this is due to the high repulsive potential generated by the polyelectrolyte segments present in the layers adjacent to the surface (Note that due to the logarithmic scale the depths of the minima are exaggerated). The presence of these minima demonstrates the inadequacy of polyelectrolyte adsorption theories with a pre-set $\varrho(z)$.

The second feature of Figure 4 is that electrolytes remain effective in the range above $\omega \sim 0.1$ M. Thus, the theory offers an explanation for our observations in Figures 1 and 2. In our aproach, Boltzmann statistics was employed, implying that the ions were considered point charges. Not very significantly differing results were obtained if the volumes of the ions was taken into account, using Roe's multicomponent adsorption theory[20] so that the conclusion may be drawn that the persistence of electrolyte effects up to very high concentrations is due to the fact that enough volume is available around and between the macromolecular chains to accomodate all ions.

A first comparison between theory and experiment, regarding the influence of pH (that is: the influence of the degree of association α of protons with the $\varepsilon - NH_2$ group of the lysine residues) is given in Figure 5. The theoretical parameters are chosen in such a way as to mimic the experimental system but exact correspondence can not yet be achieved. For instance, it is inherent in the lattice theory in its present form, that equal volumina are assigned to a polymer segment and a solvent molecule, an assumption that can not be
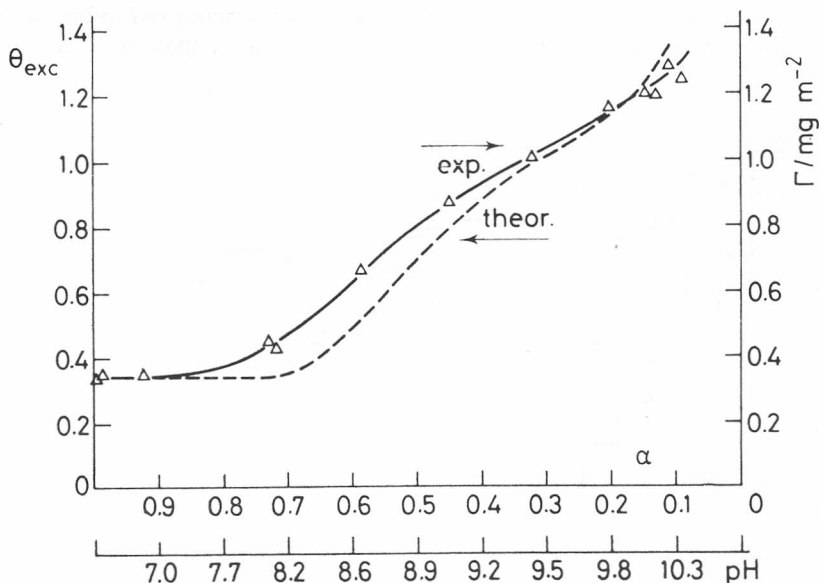

Figure 5. Adsorption of Poly-L-lysine. HBr on polystyrene latex. Comparison between theory and experiment. Treoretical adsorbed amounts are excesses and expressed as equivalent monolayers (one eq. monolayer corresponds with about 1 mg m⁻² of polylysine. HBr. The experimental data are indentical to the corresponding ones of Figure 3. Experimental conditions: DP = 190. σ_0 of latex —4.2 μC cm⁻², $\alpha = 0.71$, $T = 20$ ⁰C Electrolyte, 10^{-2} M NaBr. Theoretical parameters: cubic lattice, $\chi = 0.6$ $\chi_s = 4$ $r = 300$ $\sigma_0 = -0.1$ e/a^2, with $a^2 =$ area per lattice site = 0.3 nm², $\omega = 10^{-2}$.

very appropriate for the relatively bulky polylysine residues. Another difficulty involves the precise definition of a. The effective charge on each lattice site depends not only on pH but also on the extent of counter ion association (or for that matter, on counter ion condensation). According to polyelectrolyte theory the effective charge on a polyelectrolyte chain cannot exceed one unit charge per Bjerrum length, which amounts to ~ 0.71 nm if $\varepsilon = \varepsilon\,(bulk)$.[22]

In the theory underlying Figure 5 it was assumed that $a_{\text{eff}} = 0.71$. A consequence is that the theoretical ϑ_{exc} is constant for values of a above 0.71 (left in the diagram). This value was taken independent of the distance z to the surface. A possible improvement of the theory would be to consider a to vary with z. Considering the various uncertainties and approximations, the correspondence between theoretical and experimental trends is satisfactory but it must be repeated that only trends can be compared because of the reasons given above and because there is also some uncertainty in the absolute value of the adsorbed amount because of the specific surface area problem. At any rate, the analogy between the two curves indicates that the features of $\vartheta_{exc}\,(a)$ are well-established. At low a the measurements become progressively more difficult but there are indications of an increase of ϑ_{exc} due to multilayer formation.

A second illustration gives Figure 6. The theory is capable of explaining the rise of adsorption with increasing ionic strength and it is observed that this effect continues well beyond 10^{-1} M. The theoretical parameters, chosen for this computation differ somewhat from those leading to Figure 5, this difference amounts to a higher affinity of the polypeptide for the surface.

The general conclusion is that the agreement between theory and experiment is sufficiently satisfactory to consider the chosen approach as a promising development, warranting further elaborations.
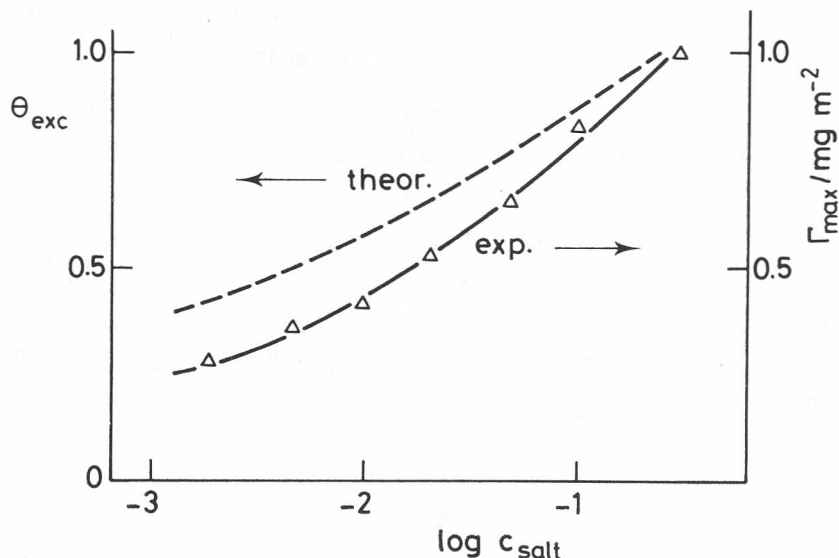

Figure 6. Adsorption of poly-L-lysine on AgI. Comparison between theory and experiment. Theoretical adsorbed amounts are excesses and expresses as equivalent monolayers (one equivalent monolayer corresponds with about 1 mg m^{-2} of polylysine. HBr). Experimental conditions as in Figure 1, except that DP = 300. Theoretical parameters as in Figure 5, except that $\sigma_0 = -2e/a^2$ and $\chi_s = 4.85$.

REFERENCES

1. H. v. d. S c h e e and G. I. T e s s e r, *Colloid & Polymer J.* **261** (1983) 461.
2. K. F u r u s a w a, W. N o r d e, and J. L y k l e m a, *Kolloid-Z. Z. Polymere* **250** (1972) 908.
3. B. C. B o n e k a m p, *Thesis*, Agricultural Univ., Wageningen, Netherlands, in course of preparation.
4. J. N. d e W i t, *Thesis*, Agricultural Univ., Wageningen, Netherlands, (1975).
5. H. T e r a y a m a, *J. Polymer Sci.* **8** (1952) 243.
6. D. H o r n, *Progr. Colloid & Polym. Sci* **65** (1978) 251.
7. H. A. v a n d e r S c h e e, *Thesis*, Agricultural Univ., Wageningen, Netherlands, in course of preparation.
8. B. H. B i j s t e r b o s c h and J. L y k l e m a, *Advan. Colloid & Interfac. Sci.* **9** (1978) 147.
9. H. A. v a n d e r S c h e e and J. L y k l e m a, in *The Effect of Polymers on Dispersion Properties*, Th. F. T a d r o s, Ed., Acad. Press (1982) 81.
10. M. A. C o h e n S t u a r t, J. M. H. M. S c h e u t j e n s, and G. J. F l e e r, *J. Polym. Sci. Polym. Phys. Ed.*, **18** (1980) 559.
11. J. L y k l e m a, *Croat. Chem. Acta* **53** (1980) 353.
12. J. M. H. M. S c h e u t j e n s and G. J. F l e e r, *J. Phys. Chem.* **83** (1979) 1619; **84** (1980) 178.
13. J. M a r r a, H. A. v a n d e r S c h e e, G. J. F l e e r, and J. L y k l e m a, in *Adsorption from Solution* R. H. O t t e w i l l, C. H. R o c h e s t e r, and A. L. S m i t h, Eds., *Acad. Press* (1983) 245.
14. D. M. S o u m p a s i s and K. H. B e n n e m a n, *Macromolecules* **14** (1981) 50.
15. V. H l a d y, private comm.
16. W. N o r d e and J. L y k l e m a, *Croat. Chem. Acta* **45** (1973) 67.
17. W. N o r d e and J. L y k l e m a, *J. Colloid Interfac. Sci.* **71** (1979) 350.
18. F. Th. H e s s e l i n k, *J. Colloid Interfac. Sci.* **60** (1977) 448.
19. C. A. J. H o e v e, *J. Polym. Sci.* **C30** (1970) 361.
20. R. J. R o e, *J. Chem. Phys.* **60** (1974) 4192.
21. E. J. W. V e r w e y and J. Th. G. O v e r b e e k, *Theory of the Stability of Lyophobic Colloids*, Elsevier (1948) Ch. III.
22. G. S. M a n n i n g, *Quart. Rev. Biophys.* **11** (1978) 179.

SAŽETAK

Adsorpcija oligo- i polipeptida kao modela za polielektrolite

B. C. Bonekamp, H. A. van der Schee i J. Lyklema

Mjerena je ovisnost adsorpcije oligo- i polilizina o koncentraciji, pH i ionskoj jakosti na srebro-jodidu i polistirenskom lateksu. Pri niskim vrijednostima pH polilizin se adsorbira u ravnom položaju. Povećanjem pH ili ionske jakosti nastaju petlje. Efekt elektrolita zadržava se sve do visokih koncentracija. Prikazani su temelji i rezultati nove teorije za odsorpciju polielektrolita. Ta teorija objašnjava eksperimentalne nalaze. Gustoća segmenta polielektrolitnog adsorbata nije jednaka gustoći za nenabijene makromolekule, već ovisi o naboju lanca i o ionskoj jakosti. U nekim uvjetima dolazi se u područje negativne adsorpcije.