

The adsorption of weak polyelectrolytes and polyampholytes

an experimental study



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**The adsorption of weak polyelectrolytes
and polyampholytes**

an experimental study

Proefschrift

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
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Plannen mislukken bij gebrek aan overleg, maar door de veelheid van raadgevers komt iets tot stand.

Spreuken 15:22

Stellingen

I

Bij de verklaring van de pH-afhankelijkheid van de adsorptie van PAA op rutiel houden Gebhardt en Fuerstenau ten onrechte geen rekening met de onderlinge repulsie tussen de geladen polymeersegmenten.

Gebhardt, J.E., Fuerstenau, D.W., *Colloids and Surfaces*, 1983, 7, 221.

II

In niet te geconcentreerde zoutoplossingen is de adsorptie van zwakke polyelectrolyten op tegengesteld geladen oppervlakken nagenoeg onafhankelijk van de zoutconcentratie.

Dit proefschrift Hoofdstuk 2

III

Informatie over de conformatie van met spinlabels gemodificeerde polymeren aan grensvlakken mag niet zonder meer geëxtrapoleerd worden naar de conformatie van ongelabelde polymeren.

Dit proefschrift Hoofdstuk 2

IV

De oplosbaarheid van regelmatige oligopeptides met beschermde functionele zijgroepen is tengevolge van de vorming van intermoleculaire waterstofbruggen zeer gering.

Dit proefschrift Hoofdstuk 3

V

De adsorptie van polyamfolyten als functie van de pH op positief en negatief geladen latex vertoont geen symmetrie.

Dit proefschrift Hoofdstuk 4

VI

In veel onderzoek naar de corrosie van de wapening in beton is ten onrechte voorbijgegaan aan het feit dat voor staaloxidatie het beton rond de wapening behalve gecarbonateerd ook voldoende vochtig moet zijn.

Bakker, R.F.M., *Cement*, 1990, 7/8, 24

VII

Er zijn zelfs chemici die foutief veronderstellen dat TAED (tetra acetyl ethyleen diamine) net als EDTA (ethyleen diamine tetra azijnzuur) een rol speelt bij de complexering van metaalionen.

proefschrift *Jan Blaakmeer*

**The adsorption of weak polyelectrolytes and polyampholytes
an experimental study**

Wageningen, 19 oktober 1990

aan mijn ouders
aan Rosa en Nadine

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Introduction

General

Polymers or macromolecules are large molecules consisting of many recurring units (called monomers or segments) usually connected in a linear chain. The segments may be uncharged (neutral polymers) or carry a charge (polyelectrolytes). In strong polyelectrolytes the segment charges are independent of the pH, whereas in weak polyelectrolytes their degree of dissociation varies with the pH.

Synthetically prepared as well as naturally occurring polymers and polyelectrolytes play a very important role in everyday life. Many dissolved polymers have a strong tendency to adsorb, i.e., to accumulate at interfaces. Due to this characteristic polymers can be used both to flocculate (1) and to stabilize (2) colloidal dispersions.

When the polymer dosage is chosen low enough, such that the particles are not fully covered, a long polymer molecule may adsorb onto two or more particles simultaneously, thereby creating flocs which settle much easier than the individual particles. This so-called bridging flocculation is the basis of many separation processes in, for example, mineral technology, waste water treatment, and paper manufacturing. On the other hand, when the particles are fully covered with polymer, stabilization may occur. The polymer then forms relatively thick adsorbed layers inducing repulsion between the colloidal particles. This repulsion is to a large extent determined by entropic factors because the protruding loops and tails hinder each other. However, solvency effects may also play a role; if the solvency is too poor the stability effect is lost because of the collapse of the adsorbed layer. Steric stabilization is extensively used in industrial processes (paints and coatings, magnetic dispersions), in food technology, in pharmaceutical formulations, etc.

There are many other areas where polymers find application. In the field of enhanced oil recovery (3) polymers are dissolved in the injection water in order to decrease its mobility and to improve its efficiency for collecting the oil from the oil-bearing reservoirs. In

order to determine the optimal dosage of polymer, knowledge about how much polymer will be lost by adsorption onto the minerals is important.

Naturally occurring polymers, like proteins and polysaccharides, are important in a wide variety of applications ranging from food processing to paper making and from immobilization of enzymes to artificial implants in the human body. In most of these applications the adsorption behaviour of the polymers is a key issue. The examples given above clearly illustrate the importance of understanding the phenomena that determine the behaviour of polymers at interfaces.

One can divide the synthetic as well as the naturally occurring macromolecules into four groups: **neutral polymers**, **strong polyelectrolytes** with (positively or negatively) charged segments, and **weak polyelectrolytes** with a pH-dependent charge on the segments. Moreover, in many natural and synthetic polymers both positive and negative charges occur. In most cases, both types of charges depend on the pH. For this class of compounds we will use the name **polyampholytes**.

In the next four paragraphs we will discuss - in general terms - the behaviour of these different macromolecules at the solid-liquid interface.

Neutral polymers

The behaviour of neutral macromolecules at interfaces is well documented experimentally as well as theoretically (5). In general it is found that these polymers accumulate at the interface in relatively thick layers. The adsorbed amount depends on the affinity of the polymer segments for the surface, on the solvent quality and on the molecular weight. In a so-called poor solvent the mutual attraction between the segments is stronger than that between segments and solvent molecules. As a result, a polymer coil in a poor solvent is more compact than in a good solvent. This tendency of the macromolecule to contract in a poor solvent also has a large effect in adsorption phenomena: the adsorbed amount from poor solvents is

higher than from good solvents, especially because more and longer loops are formed. However, the thickness of the hydrodynamic layer, which is mainly determined by the tails, is not greatly affected by the solvent quality. A schematic representation of the conformation of dissolved and adsorbed polymer chains is given in Figure 1.

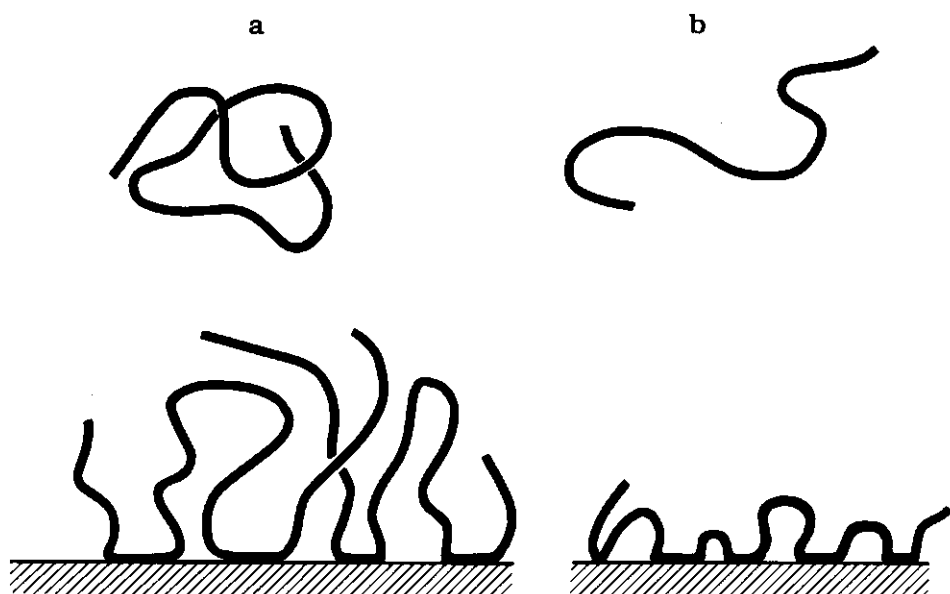


Figure 1. Adsorption of neutral polymers from poor (a) and good solvents (b).

The amount adsorbed from a good solvent is only slightly dependent on the polymer molecular weight. On the other hand, the amount adsorbed from a solvent which is ideally poor (a so-called Θ -solvent) depends linearly on the logarithm of the molecular weight (5).

In aqueous systems, extra effects related to the surface charge may occur. Many types of colloidal particles acquire a surface charge. The surface charge density depends on the concentration of potential determining ions, such as H^+ and OH^- for oxides. Due to the surface charge, a diffuse electrical double layer is formed around the

charged particle. The extension of this double layer depends on the salt concentration.

At low salt concentrations a variation in surface charge density (i.e. the pH) does not affect the adsorption of uncharged macromolecules unless it changes the number of available binding sites for the polymer. This is the case for the adsorption of poly(vinyl pyrrolidone) and poly(ethylene oxide) onto silica (6). However, it has recently been predicted (7) that at high salt concentrations the adsorbed amount may become less when the surface charge is increased. At high salt concentrations the electrical double layer is thin and compensation of the surface charge takes place at relatively short distances from the surface. If the surface is highly charged, the counterion concentration close to the surface is very high, leading to competition between the polymer segments that want to accumulate at the surface and salt ions needed for the compensation of the surface charge. This results in a lower adsorbed amount at higher surface charge densities.

Strong polyelectrolytes

Strong polyelectrolytes have a fixed charge due to strong anionic (e.g., $-\text{SO}_3^-$) or strong cationic (e.g., quaternary ammonium) groups. They will, in general, adsorb preferably onto an oppositely charged surface or, to a lesser extent, on a neutral surface. Adsorption on a surface with the same charge sign occurs only if the electrostatic repulsion between the surface and the charged polymer segments is (over)compensated by the "chemical" affinity of the segments for the binding sites. This is the case, for example, when polystyrene sulfonate is adsorbed onto SiO_2 (8).

The general trends found for strong polyelectrolyte adsorption have been discussed by Cohen Stuart (9). The adsorption behaviour depends strongly on the ionic strength of the solution. At low ionic strength ($c_s \leq 0.2 \text{ M}$), the trends can be compared with those for the adsorption of neutral polymers in an extremely good solvent: low adsorbed amount, flat conformation of the adsorbed polymer, and little dependence of the adsorbed amount on the molecular weight

(see fig. 2b). In this case the intramolecular electrostatic repulsion is high, causing an expansion of the molecule. In solution this leads to a more or less rod-like conformation. In the adsorbed state the conformation is flat with only very small loops, which makes the adsorbed amount virtually independent of the molecular weight. The intermolecular electrostatic repulsions prevent the accumulation of high amounts at the solid/liquid interface.

At high ionic strength ($c_s \geq 1$ M), both the intra- and the intermolecular electrostatic repulsions are small because the charges on the polymer are effectively screened by the salt ions. The adsorption can now be compared to that of neutral polymers. In a poor solvent high adsorbed amounts and thick adsorbed layers are found (see fig. 2a).

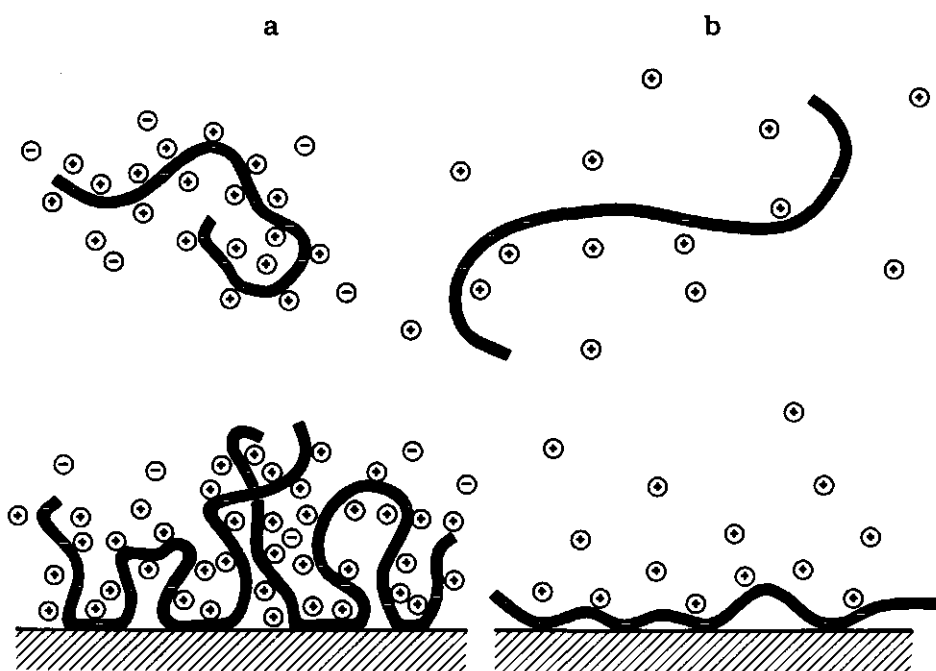


Figure 2. Adsorption of strong polyelectrolytes from high (a) and low (b) salt concentration.

In the progression from low to high ionic strength a gradual transition occurs from a low adsorbed amount with a flat conformation of the adsorbed molecule to a high adsorbed amount and a conformation with many segments in loops. The general trends as mentioned above have been confirmed for the adsorption of polystyrene sulfonate onto uncharged polyoxymethylene crystals (10).

Not only the ionic strength but also the surface charge affects the adsorption behaviour. As a rule, the adsorbed amount is lower on a surface with the same charge sign, and higher on an oppositely charged surface. These trends are easily explained by the electrostatic contribution to the segmental adsorption energy.

Weak Polyelectrolytes

The charge on weak polyelectrolytes is the result of the dissociation of weak acidic groups or the protonation of weak basic groups. This charge will of course depend on the pH of the solution as compared to the pK of the functional group. We will discuss here the situation of a weak polyacid, but for a weak polybase *mutatis mutandis* the same applies. We define K as the dissociation constant of the acidic groups and consider first the situation at very low pH values ($\text{pH} \ll \text{pK}$) and at very high pH values ($\text{pH} \gg \text{pK}$).

At very low pH the macromolecule is neutral and the adsorption behaviour obeys the same rules as discussed in section 1.2. At very high pH all the acidic groups are completely dissociated and the polymer behaves as a strong polyelectrolyte (section strong polyelectrolytes).

At intermediate pH values (around pK) the situation becomes more complex because the degree of dissociation of a polymer segment depends on the local pH, which is a function of the distance from the charged surface (see fig. 3). The concentration of protons close to a positively charged surface will be lower than in the bulk solution.

One would expect that when the pH is increased from low values (where the macromolecule is uncharged) to higher ones, the situation would gradually change from high adsorbed amounts and

thick layers to low adsorption and thin layers for the fully charged polymer at high pH, with the transition zone at a pH around the pK . This is indeed the case for the adsorption onto a neutral surface, but for an oppositely charged surface recently developed theories (7,11) predict a maximum in the adsorbed amount at a pH value just below the pK . Starting at low pH, an increase in the pH leads to a charging of the macromolecule. Therefore, an electrostatic attraction will develop between polymer and surface, resulting in an increased adsorbed amount because the intra- and intermolecular electrostatic repulsions are still small if the pH is not too high. As has been illustrated in Figure 3 the pH, and therefore the degree of dissociation, decreases with increasing distance from the surface. This is the reason that, at relatively low pH, the intermolecular electrostatic repulsion can be neglected. At higher pH more acidic groups in loops and tails will dissociate, giving larger inter- and intramolecular electrostatic repulsions and, consequently, a lower adsorbed amount.

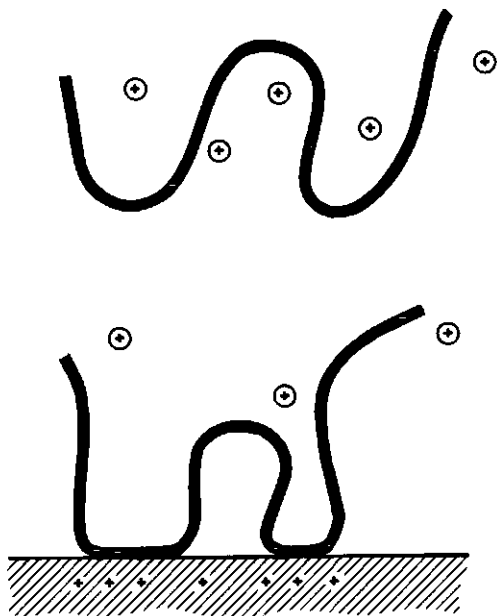


Figure 3. Adsorption of a weak polyelectrolyte at a pH below the pK on a positively charged surface.

Polyampholytes

Polyampholytes are polymers which possess both weak acidic and weak basic groups. These macromolecules have a so-called iso-electric point (i.e.p.). The i.e.p. is defined as the pH at which the net charge on the polymer is zero. An important group of macromolecules with an i.e.p. is that of the proteins. The adsorption behaviour of different proteins is well documented experimentally (12-14). From a theoretical point of view not much is known about the adsorption mechanisms of these complex molecules.

A maximum in the adsorbed amount as a function of the pH has been reported for the adsorption of HPA (human plasma albumin) and BSA (bovine serum albumin) onto negatively charged latex (12, 13). The maximum occurs at pH values just below the i.e.p. of the protein. However, a systematic study with a simple, well-defined polyampholyte is still lacking. Definitively such a study could help to bridge the gap between relatively simple polyelectrolytes, for which the theoretical understanding is rapidly increasing, and proteins for which many experimental data have been collected but where the theoretical basis is still lacking.

An interesting candidate for such a fundamental study would be a macromolecule containing only one kind of acidic and one kind of basic groups with their pK values rather far apart. Such a molecule should have properties characteristic for both simple polyelectrolytes and more complex molecules like peptides and proteins. In particular, such a simple polyampholyte has an i.e.p., a feature which is absent in simple polyelectrolytes, but which is common with proteins. Far from the i.e.p., the molecule is highly charged and can hopefully be described by adsorption models pertinent to strong polyelectrolytes, but close the i.e.p. one expects different behaviour.

Aim of this study

Several papers in the literature deal with the adsorption of weak polyelectrolytes. However, in nearly all cases the materials (polymer, adsorbent) are not sufficiently characterized or the relevant parameters are not accurately controlled. Therefore we decided to carry out a systematic study on the adsorption of weak polyelectrolytes using systems in which all the parameters could be accurately controlled. Moreover, the predicted maximum in the adsorbed amount of weak polyelectrolytes (see section 1.4) reveals an interesting and delicate balance of forces and a careful experimental check of these theories would be most welcome. So far, only one set of measurements on a poorly defined system (15) showed a maximum in the adsorbed amount as a function of the pH, but it is not entirely clear what the origin of the maximum is.

In this thesis we present results of an extensive study of the adsorption of well-defined weak polyelectrolytes on well-defined colloidal particles. The system was chosen such that the surface charge density and the polymer charge could be varied independently. This enables us to compare the outcome with theoretical results by which we hope to gain more insight into the mechanisms that govern the accumulation of weak polyelectrolytes at charged interfaces.

A second part of this work deals with the adsorption of well-defined, simple polyampholytes. For simple polyampholytes no adsorption data are, so far, available. We attempted to synthesize a model polyampholyte to be used for adsorption studies. Unfortunately, only oligomers could be obtained, and we also employed a commercial sample of high molecular weight. Our purpose was to compare the adsorption behaviour with that of strong and weak polyelectrolytes on one hand, and with that of proteins on the other.

Outline of this thesis

In order to determine unambiguously what effect the pH (or, equivalently, the charge on the macromolecule) has on the

adsorption of weak polyelectrolytes it is essential to use an adsorbent with surface properties that are independent of the pH. To this end we synthesized a monodisperse, positively charged polystyrene latex with strong cationic groups. In Chapter 1 we describe this synthesis and check that the positive charge (resulting from quarternary ammonium groups) is indeed independent of the pH in the region of interest ($\text{pH} = 2$ to $\text{pH} = 10$). We carried out adsorption studies with this latex as substrate and poly(acrylic acid) as the adsorptive. This polymer was chosen because of its simple chemical nature. In Chapter 2 the results of the adsorption experiments are described and discussed in the context of recent theory.

As a model polyampholyte (with one type of basic and one type of acidic function) we decided to synthesize a polypeptide with a recurring unit of L-lysyl-L-glutamyl-glycine. It was our aim to prepare a long polymer but this proved to be difficult: we were only able to obtain an oligopeptide with twelve peptide residues. The synthesis is described in Chapter 3.

In Chapter 4, the adsorption of the oligopeptide onto positively and negatively charged latex is reported. For comparison purposes the adsorption of a commercially available random copolymer of lysine and glutamic acid is also studied.

The results of the research described in this thesis on the adsorption of weak polyelectrolytes support the recently developed theories (7, 11). In particular, the maximum in the adsorbed amount as predicted by these theories is also found experimentally. For polyampholyte adsorption some but not all of the trends can be explained in terms of these theories. Clearly, more experimental work has to be carried out to understand these systems in detail.

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Chapter 1

Synthesis of a polystyrene latex with a positive, pH independent surface charge

Abstract

A special azonitrile, N,N'-dimethyl-4,4'-azobis-4-cyano-1-methyl piperidine dinitrate (DACMP.DiN) has been used to synthesize surfactant free polystyrene latices with a relatively high surface charge density which is virtually independent of the pH in the range 4 to 10. The method used leads to very monodisperse particles but it is difficult to obtain reproducible surface charge densities.

Introduction

For adsorption studies of negatively charged polyelectrolytes in our laboratory we needed a dispersion of which the particles are positively charged with a surface charge density that is essentially independent of the pH. The method of Goodwin et al. (1), using azo-N,N'-dimethylene isobutyramidine hydrochloride (ADMBA.2HCl) as the initiator, produces a latex with a positive surface charge density σ_0 , but for this latex σ_0 depends strongly on pH. Potentiometric titrations, conducted in our laboratory, showed that for this latex in 0.1 N KCl $\sigma_0 = 7.5 \mu\text{C}/\text{cm}^2$ at pH 4 and only $1.5 \mu\text{C}/\text{cm}^2$ at pH 8.5, despite the fact that ADMBA is a rather strong base. A reasonable estimate for the pK_a of ADMBA, based upon the analogy with 2-methyl-2-imidazoline (2) is $\text{pK}_a=11$. The strong dependence of σ_0 on pH even if $\text{pH} \ll \text{pK}_a$ is probably due to strong interactions between the charged groups on the latex surface.

With initiators containing a quarternized nitrogen, the surface charge would become more independent of pH. Guthrie (3) synthesized such latices. He prepared his initiators starting from the compound 4,4' azobis-4-cyanopentanoic acid (ACPA). This author used a mixed anhydride route to produce an amide directly from a

carboxylic acid, followed by quarternization of the tertiary amine with methyl iodide. He did not determine the surface charge of his latices. In these latices the charged group is probably at some distance from the surface because of the architecture of ACPA. Therefore we tried to find another quarternized initiator, and finally choose N,N'-dimethyl-4,4'-azobis-4-cyano-1-methyl piperidine dinitrate (DACMP.DiN). This compound has been synthesized by Pankratz and Neuman (4) for their study on decomposition rates and radical production. They reacted N-methyl-4-piperidone with hydrazine sulfate and sodium cyanide in water to form the azine which was isolated and then oxidized to the azo form with bromine. The azo compound was quarternized using methyl iodide and finally ion exchanged to yield DACMP.DiN. The molecular structure of DACMP.DiN is given in Figure 1. Using this initiator we were successful in producing a surfactant free latex with a positive surface charge independent of the pH in the range 4 to 10.



Figure 1. *The molecular formula of DACMP.DiN*

Experimental

Materials

Throughout the experiments deionized (millipore) water was used. The water used for the latex synthesis was boiled to remove carbon dioxide. The initiator for the radical polymerisation of styrene was obtained using the synthesis described by Neuman and Pankratz (3) and was stored in the di-iodide form. Before use, the initiator was transferred into the dinitrate form by adding a stoichiometric amount of silver nitrate to a solution of the initiator followed by filtration to remove silver iodide. Styrene (Baker) was distilled at 15

mm Hg at 39 °C to remove the inhibitor, stored in the dark and used within three days. All the other chemicals were of analytical grade and were used without further purification.

The glassware used for the styrene polymerisation was cleaned rigorously. After cleaning with detergent (rosal liquid, Rogier Bosman) the glass was rinsed seven times with water, three times with ethanol containing potassium hydroxide, five times with deionized water; finally it was dried in an oven. If the glass is cleaned less thoroughly, a foaming reaction mixture is obtained.

Preparation of polystyrene latex

The polymerisation reaction was performed in a 1 l three-necked flask with a total reaction volume of 800 ml as described by Goodwin et al. (1). A typical reaction was carried out in the following manner (see also table 1): 620 ml of water containing the required amount of salt was added to the flask and a glass stirrer with a T-shaped PTFE blade (1x5 cm) fitted into a PTFE guide was then inserted. In all reactions the stirrer was fitted in the same manner with the blade approximately 1 cm from the bottom of the flask. Then the flask was immersed in a thermostatted bath which controlled the reaction temperature within ± 1 °C. Nitrogen was injected into the flask just below the surface of the reaction mixture to remove oxygen and carbon dioxide from the system. Care was taken to minimize evaporation of water from the reaction mixture and to prevent back diffusion of oxygen and carbon dioxide. The stirrer speed was adjusted to 250 r.p.m. After 20 min of nitrogen injection 80 ml of styrene was added. After another 15 min the desired amount of initiator dissolved in water was added quantitatively using 100 ml of water. After 24 hrs the reaction vessel was taken from the thermostat and allowed to cool to room temperature.

Goodwin et al. (1) obtained an empirical equation with which they could predict the latex particle size using the initiator azobisisobutyramidine hydrochloride (ABA.2HCl). The formula relates the particle diameter d (nm) to the polymerisation conditions according to:

$$\log d = 0.384 \left[\log \frac{[M]^{1.099}[I]}{[In]^{0.833}} + \frac{2563}{T} \right] - 0.195 \quad (1)$$

where [M] is the initial monomer concentration, [I] the total initial ionic strength (including the initiator), [In] the initial initiator concentration, and T the absolute temperature. Our experimental conditions were chosen such that Eqn (1) would predict $d=450$ nm. However, the decomposition rates of ABA.2HCl and DACMP.DiN are not the same so that Eqn (1) does not apply to polymerisation with the latter initiator. In practice, we found considerably bigger particles (see table 1), indicating a slower decomposition rate of DACMP.DiN.

Purification of the latex

The latex was filtered through glass wool to remove the coagulum. The glass wool had been boiled out three times with deionized water to remove any impurities.

Unreacted styrene and small oligomers were removed by distillation under reduced pressure at 40°C using a rotary evaporator fitted with teflon sleeves. During the distillation the volume of the latex was kept constant by supplying water. Usually a fifteen fold excess of water had to be distilled before the distillate was free from styrene, as measured spectrophotometrically at 250 nm.

After distillation the mixed bed ion exchange procedure according to Van den Hul and Vanderhoff (5) was used to remove excess electrolyte and to exchange the counterion NO_3^- or Cl^- for OH^- .

Characterization of the latex

The particle size and the particle size distribution were measured using a transmission electron microscope (T400, Phillips). At least 150 particles were sampled. The particle size distribution was characterized by the uniformity coefficient d_{32}/d_{10} , where d_{32} is the volume-surface average diameter and d_{10} the number average diameter.

The surface charge density σ_0 was measured by means of conductometric titrations. In order to investigate the pH-dependence of σ_0 , potentiometric titrations were performed as well. The titrations were conducted using the experimental set up of De Jong et al. (6). Zeta-potentials were determined from streaming potentials according to the procedure described by Van der Put et al. (7), with a slight modification as outlined by Van der Linde et al. (8). The latter authors showed that determining the conductance of the plugs by means of an a.c. conductance measurement at 4000 Hz is to be preferred above the so-called I-V plots (7). A direct a.c. conductance measurement is instrumentally less demanding and is not hindered by electrode polarization which may occur in the procedure used for the I-V plots (9). The streaming potentials were transformed into zeta-potentials according to the theory of Levine et al. (10). Measuring the electrophoretic mobility using a Malvern Zeta-sizer 2 was impossible because the mobility depended on the time elapsed between injection in the cell and the measurement. Occasionally, even charge reversal was found. These effects are presumably due to the adsorption of silicates from the glass wall on the positive latex.

Results and discussion

Three different latices have been synthesized; their characteristics are given in Table 1. The first polymerisation at 80 °C

Table 1. *The conditions for the latex synthesis and results of the characterizations. Concentrations are based on the volume of the aqueous phase (720 ml).*

latex nr	[initiator] (mmol/l)	[NaCl] (mmol/l)	polymeris. temp. (°C)	particle diameter (nm)	standard deviation (nm)	d_{32}/d_{10}	σ_0 ($\mu\text{C}/\text{cm}^2$)	solid content (%)
1	2.7	10	80	834	23.3	1.0015	9.9	8.1
2	2.7	--	70	771	7.5	1.0002	15.9	4.1
3	2.7	--	70	785	7.5	1.0002	9.0	5.5

could not be repeated satisfactorily because for unknown reasons in subsequent attempts a large amount of coagulum was formed. By reducing the temperature and the ionic strength, reproducible results with respect to particle size were obtained but not with respect to the surface charge density. As discussed above, the particle size of latex 1 is much bigger than predicted by eqn. (1) but the formula could be used to choose the reaction temperature when no NaCl was added in order to get approximately the same particle size as in reaction 1.

It is not possible to calculate the conversion rate from the solid content reported in Table 1 because the solid content is determined (by drying for 48 hours at 60 °C) after all the cleaning procedures have been performed. The final volume might not be exactly the same as the reaction volume and also some coagulum was formed during the polymerization.

The surface charge per unit weight was found from conductometric titrations. From the particle size, as measured by electron microscopy, and the specific density of 1.04 g/cm³, as determined by pycnometry, the specific surface area was calculated. Hence, the surface charge density σ_0 (C/m²) was readily obtained.

A typical conductometric titration curve for latex 2 is plotted in Figure 2. The titration curve with HCl (lower curve) and the back titration curve with NaOH (upper curve) are plotted in the same diagram. The HCl curve consists of a weakly descending part (a), a weakly ascending section (b) and a strongly ascending branch (c). The NaOH curve has a strongly descending branch (d) and a strongly ascending section (e). The sections a and b correspond to the replacement of counter ions in the double layer by Cl⁻ ions. Parts c and e reflect the addition of free HCl and NaOH, respectively, and section d corresponds to the replacement of H⁺ by Na⁺.

From the HCl-titration curve it might appear that two different surface groups (parts a and b) contribute to the surface charge, one being strongly basic (negative slope of section a), the other being weakly basic (slightly positive slope of branch b). If weakly basic groups contribute to the surface charge one would expect this also to be evident in the back titration curve with NaOH. Such an effect was

found by Labib and Robertson (11) in conductometric titrations of negatively charged latex with strongly and weakly acidic surface groups. In our experiments, after the latex had been titrated once to a low pH the subsequent conductometric titrations never showed any evidence of a weakly basic surface group. Probably, the slightly ascending section (b) originates from the dissolution of CO_2 in the latex suspension. Because the pH of the purified latex suspension is rather high (pH ≈ 9.5) it is hard to prevent contamination with CO_2 .

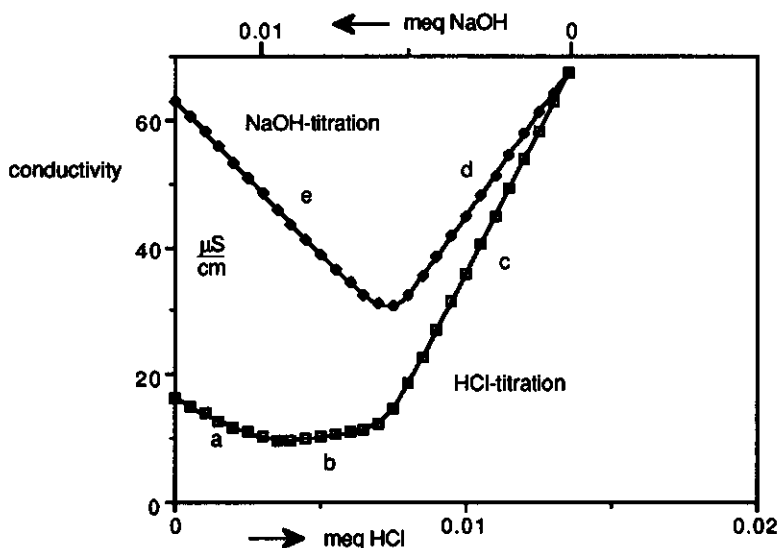


Figure 2. Conductometric titration curves of 0.616 g latex 2 in a total volume of 50 ml. The final point of the titration with HCl is the starting point for the back titration with NaOH.

Once the latex has been at a low pH during the titrations, the CO_2 has disappeared from the solution and since the titrations were performed in a N_2 atmosphere successive titrations were not affected by CO_2 .

For the calculation of the surface charge the extrapolated intersection point of parts b and c was taken as the equivalence point. The intersection point of branches d and e gives the same result. The contamination with CO_2 does not affect the determination

of the surface charge density because at the equivalence point the pH is low, causing CO_2 to escape from the suspension.

Potentiometric titration curves for latex 2 are displayed in Figure 3. In this figure we have plotted two sets of titration curves: 0.6111 g latex 2 in 50 ml 0.01 N KNO_3 (b) and a blank titration of 50 ml 0.01 N KNO_3 (a). From this figure it can be seen that in the pH

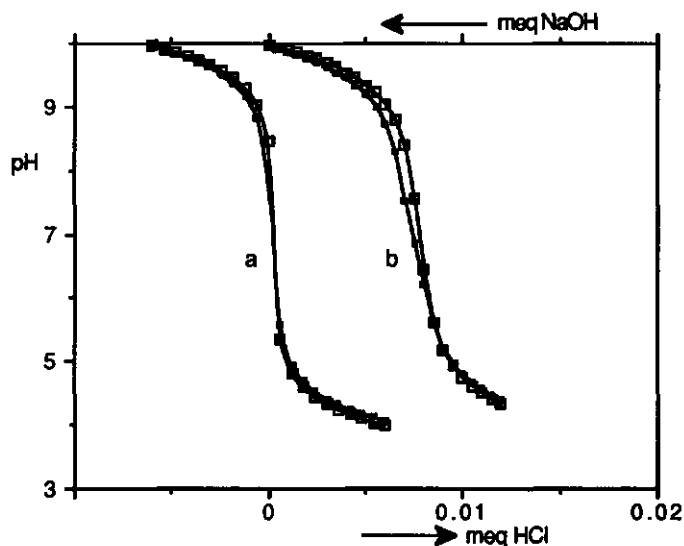


Figure 3. Potentiometric titration curves in 0.01 N KNO_3 (total volume is 50 ml). Curve a is the blank titration of 50 ml 0.01 N KNO_3 , curve b is the the titration of 0.6111 g latex 2. For the back titration curve with NaOH, the scale is the same as for the HCl-titration, with the zero point at the last point of the HCl-titration. The filled symbols correspond to the HCl-titration, the open symbols to the back titration with NaOH.

range 4-10 a small fraction of the surface groups is titrated because the latex titration curve (b) and the blank titration curve (a) are not exactly equidistant. In order to conclude whether the titrability of the surface is due to the interaction between the charged groups on the latex particle surface or can be attributed to the nature of the initiator, we also conducted some potentiometric titrations on solutions containing only initiator (DACMP.D1N) or

tetrabutylammonium nitrate (TBUA.N) as its analogue. These titrations are given in Figure 4. For clarity the titration curve of DACMP.DiN (c) has been shifted 0.005 meq to the right and the curve for TBUA.N (a) 0.005 meq to the left. Surprisingly, as can be

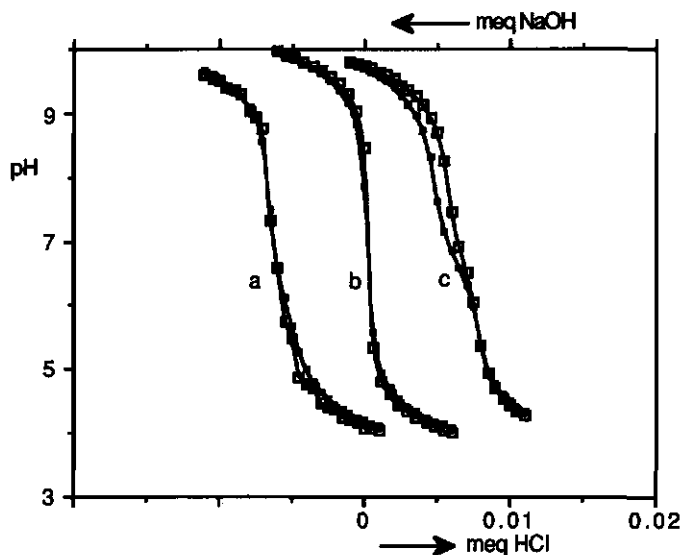


Figure 4. Potentiometric titration curves in 0.01 N KNO_3 (total volume is 50ml). Curve a is the titration of 1.99×10^{-2} meq TBUA.N, b is the blank titration (0.01 N KNO_3) and c is the titration of 4.48×10^{-2} meq DACMP.DiN. Curve a has been shifted 0.005 meq to the left and curve c 0.005 meq to the right. The filled symbols correspond to the HCl-titration, the open symbols to the back titration with NaOH. For the back titration with NaOH, the scale is the same as for the HCl-titration with the zero point at the last point of the HCl-titration.

seen from Figure 4, TBUA.N as well as DACMP.DiN are, in the pH range studied, for a small fraction titratable. From the potentiometric titration curves we calculate that in the pH range 4.5-9.5 a fraction of 9% for TBUA.N, 11% for DACMP.DiN and 30% for the latex surface groups is titrated.

The zeta potentials obtained from streaming potential measurements for latex 1 at constant ionic strength are shown in Table 2. It is obvious that in this pH range the zeta-potential is

independent of the pH. In Figure 5 the zeta potential and the diffuse double layer potential are shown as a function of the ionic strength.

Table 2. Zeta potentials as a function of the pH in 0.01 M KBr.

pH	4.1	5.4	7.8	9.7
ζ (mV)	74.4	74.9	74.9	73.9

The latter was calculated from the Gouy-Chapman theory for flat double layers assuming that the diffuse double layer charge σ_d equals $-\sigma_0$:

$$\sigma_0 = (8\epsilon n k T)^{0.5} \sinh(e\psi_d / 2kT) \quad (2)$$

where ψ_d is the diffuse double layer potential, ϵ the dielectric constant, and n the ionic concentration; the other symbols have their usual meaning. We do not find a maximum in the zeta potential as has

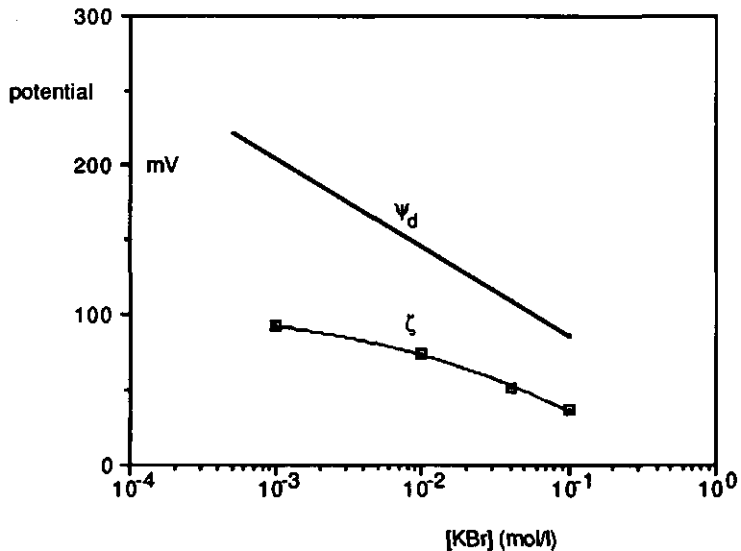


Figure 5. The zeta potential ζ and the diffuse double layer potential ψ_d as a function of the salt concentration, at constant pH (pH=4.5).

been found for negatively charged (persulfate) polystyrene latices by many authors both from streaming potential measurements (9) and from electrophoresis (12-15). Several qualitative explanations have been put forward for such a maximum, most of which have been discussed by Goff and Luner (16). The most likely explanation is the presence of a polyelectrolyte-like hairy layer (17-19).

From the zeta potential we can calculate, again using the Goy-Chapman theory the electrokinetic charge σ_{ek} , i.e. the net charge outside the shear plane. To that end, in Eqn (2) σ_{ek} and ζ are substituted for σ_0 and ψ_d , respectively. In Figure 6 σ_{ek} and σ_0 have been plotted as a function of the ionic strength. From this figure it is clear that the electrokinetic charge becomes smaller with decreasing ionic strength. This can also be concluded from Figure 5 because the

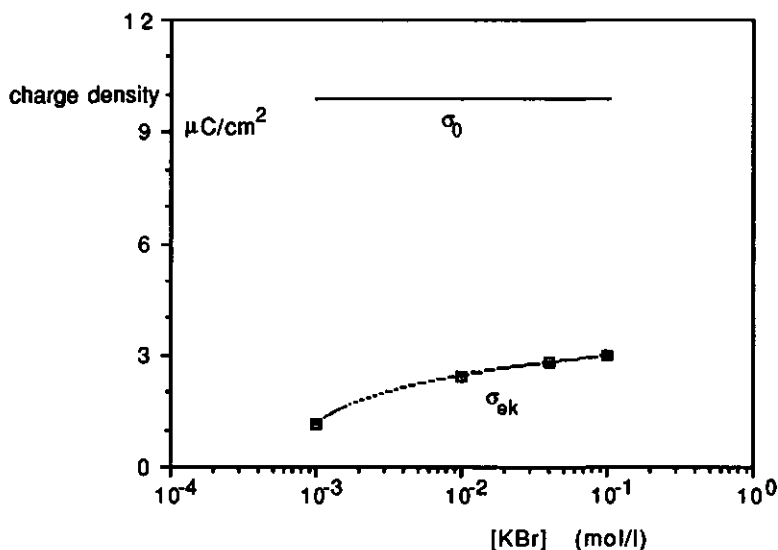


Figure 6. The surface charge density σ_0 and the electrokinetic charge density σ_{ek} , calculated from the zeta potential, as a function of the salt concentration.

zeta potential increases less than ψ_d upon a decrease of the ionic strength. One way to explain this decrease is an outward shift of the shear plane with decreasing salt concentration. Also here, it seems possible that this shift in the shear plane is caused by "hairs" on the

surface, even if there is no maximum in the ionic strength dependence of the zeta potential.

Conclusions

Monodisperse surfactant free polystyrene latex particles with a positively charged surface density which is almost independent of pH in the range 4-10 can be synthesized with N,N'-dimethyl-4,4'-azobis-4-cyano-1-methyl piperidine dinitrate (DACMP.DIN) as the initiator. The synthesis described is reproducible with respect to particle size but not with respect to surface charge density. The zeta potential of the latices is independent of pH. In spite of the fact that one expects the charged groups to be very close to the particle surface, we cannot entirely rule out the possibility of hairs on the surface, based on the behaviour of the electrokinetic charge as a function of the ionic strength.

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Chapter 2

Adsorption of weak polyelectrolytes on highly charged surfaces

Poly(acrylic acid) on polystyrene latex with strong cationic groups

Abstract

We have studied the adsorption of the weak polyelectrolyte poly(acrylic acid) (PAA) on a positively charged polystyrene latex. The latex surface contained quarternary ammonium groups, so that the surface charge was independent of pH. Hence, by changing the pH, only the degree of dissociation of the polymer varied. We found that the adsorbed amount is low at high pH, where PAA is fully charged. With decreasing pH the adsorption increases and passes through a maximum at about 1 pH-unit below the intrinsic dissociation constant pK_0 of the carboxylic groups of the macromolecule. The ionic strength has little effect. The experimental results agree very well with a recent extension (1) of the model of Scheutjens and Fler to polyelectrolyte adsorption.

Introduction

The adsorption of polymeric materials plays an important role in a number of technological processes such as waste water treatment, flotation separations, fine particle recovery by selective flocculation, enhanced oil recovery, etc. (2-5). Much effort has been put into experimental and theoretical work to understand the behaviour of polymers at interfaces. Gradually, the main trends in the adsorption behaviour of neutral polymers and polyelectrolytes on charged and uncharged surfaces are becoming clear.

For the adsorption of neutral polymers one generally finds thick adsorbed layers and long tails with many segments of the adsorbed polymer in loops. In good solvents the adsorbed amount is low and only weakly dependent on chain length. However, in poor

solvents (theta solvents) the adsorbed amount depends linearly on the logarithm of the molecular weight of the polymer. By increasing the ionic strength the adsorption of a neutral polymer may increase because the solvent quality decreases with increasing salt concentration (6,7). Charging the surface has only a minor effect on the interfacial behaviour of uncharged macromolecules. However, the adsorbed amount on a charged surface is less than on an uncharged surface if the number of available sites for binding the polymer decreases on charging the surface. This is the case for the adsorption of poly(vinyl pyrrolidone) and poly(ethylene oxide) onto silica (8). Those polymers adsorb by hydrogen bonding with SiOH groups, the number of which decreases with increasing pH. Another effect of the surface charge on the adsorption of uncharged polymers has recently been predicted theoretically (1). If the surface charge is very high, the accumulation of counterions in the double layer prevents high polymer segment concentrations and decreases the adsorbed amount. As far as we are aware, no experimental study has, as yet, dealt with this effect.

For polyelectrolyte adsorption, we have to make a distinction between strong and weak polyelectrolytes. Some general trends in the adsorption of strong polyelectrolytes, for which the charge does not depend on pH, have been discussed by Cohen Stuart (9). For media with relatively low ionic strength ($c_s < 0.2$ M) and a neutral surface, the charged polyelectrolyte will adsorb in a flat configuration. In this case the adsorbed amount is low and there is no dependence on molecular weight or ionic strength. For media with a relatively high ionic strength ($c_s > 1$ M), the adsorption is a function of the ionic strength and the molecular weight. The trends of the adsorption are, in this ionic strength region, comparable to the adsorption of a neutral polymer. When the surface is oppositely charged, the adsorbed amount of a strong polyelectrolyte depends on the surface charge density, resulting in higher adsorbed amounts for surfaces with higher surface charge densities because of the electrostatic contribution to the adsorption energy of the segments.

For weak polyelectrolytes, the degree of dissociation α depends on the pH. At low pH, where $\alpha = 0$ (poly acid), the situation is similar

as for the adsorption of neutral polymers. In the limit of high pH, where $\alpha = 1$, the adsorption behaviour is like that of strong polyelectrolytes. For intermediate values of the pH, the situation is complex because the degree of dissociation might be a function of the distance from the surface. If the surface carries no charge, the transition between low pH (uncharged groups, thick layers) and high pH (highly charged groups, thin layers at low c_s) is smooth and situated around the pK_0 of the polyelectrolyte. For oppositely charged surfaces some recently developed theories (1,10), which are extensions of the Scheutjens Fler approach (11,12), predict a maximum in the adsorbed amount at a pH value below the pK_0 of the polyacid. These theories also predict that the adsorption of a weak polyelectrolyte on a highly oppositely charged surface depends only weakly on the ionic strength, unless $c_s > 1$ M.

To our knowledge a maximum in the adsorbed amount as a function of the pH has only been reported by Tanaka et al. (13). They studied the adsorption of polymers with weak cationic groups (modified starch) onto bleached kraft pulp. They found a maximum in the adsorbed amount at about 2 pH-units below the pK_0 of their polymers.

In this paper we present experimental results for the adsorption of poly(acrylic acid) (PAA) onto highly charged polystyrene latices with strong cationic groups. We wanted to test some of the theoretical predictions, especially the maximum in the adsorbed amount as a function of the pH, and the weak ionic strength dependence. The latter effect is, at first sight, somewhat surprising because it is known that the salt concentration affects the adsorption of strong polyelectrolytes considerably. In order to avoid unnecessary complications, we decided to use an adsorbent with a pH-independent surface charge and a weak polyelectrolyte that is as simple as possible.

We chose for PAA because it has no hydrophobic side chains. It has been shown that especially those weak polyacids which carry such groups, e.g. poly(methacrylic acid) (14-16), poly(ethacrylic acid) (14), copolymers of maleic acid and alkyl vinyl ethers (17), and copolymers of maleic acid acid and styrene (18), undergo a

conformational change when the degree of deprotonization is increased. It is believed that at low degrees of deprotonization these macromolecules find themselves in a tightly coiled compact conformation (hypercoil). The hypercoil is due to the hydrophobic interactions between the apolar side chains. Upon ionization the hydrophobic microdomains are disrupted and the macromolecule undergoes a conformational change resulting in a more random conformation. For PAA these effects are much weaker. Some authors (19-21) do report a conformational change, whereas others (14-16) state that PAA does not change its conformation on increasing the degree of deprotonization. Since PAA does not have hydrophobic side chains the formation of hydrophobic microdomains is not possible. Presumably, if there exists any conformational change, it must be very subtle. Therefore PAA corresponds quite well to the simple polyelectrolyte presumed in the model. For the intrinsic dissociation constant K_0 (defined as the dissociation constant for an infinitely dilute solution in the limit $\alpha \rightarrow 0$) of PAA Mathieson et al. (19) report, for media with an ionic strength between 0.02 and 0.2 mol/l a value for pK_0 of 4.58. Spencer (22), using the data of Gregor et al. (23), arrives at $pK_0 = 5.33$ for PAA in water and at $pK_0 = 4.52$ in 0.1 M KCl. The dissociation constant pK_a for monomeric acrylic acid is reported as 4.25 (24). It is generally believed that at room temperature PAA is completely soluble in water even at low pH when all the carboxylic groups are protonated. Silberberg et al. (25) studied the temperature dependence of the light scattering of hydrogen bonding polymers. They reported for PAA (molecular weight is 420,000) in 0.2 N HCl a (lower critical solution) θ -temperature of 14 °C.

Theory

Before discussing the experimental data, we give some background of recent theory and a few typical predictions. An earlier version of a polyelectrolyte adsorption theory, in which the small ions are considered as point charges, was published a few years ago (10). A more recent theory, in which the volume of the ions is taken

into account, has been published elsewhere (1). For technical details we refer to these papers.

Physical background

The models as proposed by Böhmer et al. (1) and Evers et al. (10) are extensions of the self-consistent field (SCF) theory of Scheutjens and Fleer (11,12). This theory is based on a lattice with equidistant lattice layers parallel to the surface. Both the electrical and nearest neighbour interaction are averaged over the whole layer (mean field approach). A polymer segment (not necessarily a monomer unit) occupies one lattice site. In the case of the adsorption from a solvent consisting of small molecules, like water, a fixed number of solvent molecules fills one lattice site. These solvent molecules filling one lattice site are considered to behave as a cluster (entity). On adsorption one segment replaces such an entity. All the sites are filled either with a polymer segment, a solvent entity, or (if ions have volume) an ion.

The configuration of a polymer molecule can be modeled as a step weighted walk in the lattice. The weighting factors for each step contain the nearest neighbour contact energy, the electrical potential, and the mixing entropy. The contact energy for interaction between all the components is expressed through the Flory-Huggins χ -parameter. The adsorption energy of a polymer segment (p) and the surface (S) is described by a similar χ_{ps} -parameter*. The mixing entropy accounts for the fact that the probability of a step towards a given lattice layer decreases as the segment concentration in this layer becomes higher.

The additional feature as compared to the Scheutjens-Fleer theory is the electrical potential. In the approach of Van der Schee et al. (26) and Papenhuijzen et al. (27) for strong polyelectrolytes, and in that of Evers et al. (10) for weak polyelectrolytes, the salt ions

*The adsorption energy parameter χ_s as used in previous studies (10-12), expressing the adsorption energy difference between a polymer segment and a solvent entity (o), is given as $-\lambda_1(\chi_{ps}-\chi_{os})$, where λ_1 is the fraction of nearest neighbours in an adjacent layer. For a hexagonal lattice $\lambda_1 = 1/4$. In most cases χ_{os} may be set equal to zero.

are considered to be point charges which occupy no volume. The polymer charges are localized in a plane in the middle of the lattice layer and their charge is smeared out over that plane. The small ions are distributed on both sides of the plane over the whole layer and an electrical double layer is formed. The electrical potentials are now obtained by solving the Poisson-Boltzmann equation. In the recent theory of Böhmer et al. (1), the ions are considered to occupy a lattice site just like polymer segments and solvent entities. All charges are assumed to be in a plane in the middle of a lattice layer. The space between the planes is free of electrical charges. In this case the electrical potential in every plane is obtained by a multi Stern-layer approach. The potential difference between the equidistant planes does depend not only on the charge on each plane, but also on the separation distance d between the planes and on the dielectric constant. Hence, not only the lattice geometry but also the spacing of the lattice layers has to be specified. The dielectric constant in a lattice layer is taken as a linear combination of the dielectric constants of polyelectrolyte, salt, and water.

In the multicomponent theory of Böhmer et al. (1), each type of ion constitutes a separate component. In principle, one should then assign χ_{ij} - and χ_{is} -parameters for the interactions between ions and solvent, ions and polymer segments, and ions and surface. In order to keep the number of parameters as low as possible, all the ions are considered to have identical properties as a solvent entity, except for their charge.

In these models (1,10) for the adsorption of weak polyelectrolytes the degree of dissociation α is not only a function of the pH, but also of the distance z from the surface, since the local electrical potential varies with z .

The most severe drawback of these models for (weak) polyelectrolyte adsorption is the fact that the electrical potential is smeared out over the whole layer. Especially for polymers with a low charge density the local electrical potential will be higher (in the vicinity of a charged segment) or lower (far away from a charged segment) than predicted by these two models. This smearing out of the electrical potential leads in the bulk solution (where the

electrical potential is 0) to a prediction of the titration curve of a weak polyacid which is identical to that of a weak monomeric acid.

Model predictions

In Figure 1 we show three adsorption isotherms at salt concentrations of 0.001, 0.1 and 1 M, respectively, as calculated with the model of Böhmer et al. (1). The excess adsorbed amount θ^{exc} ,

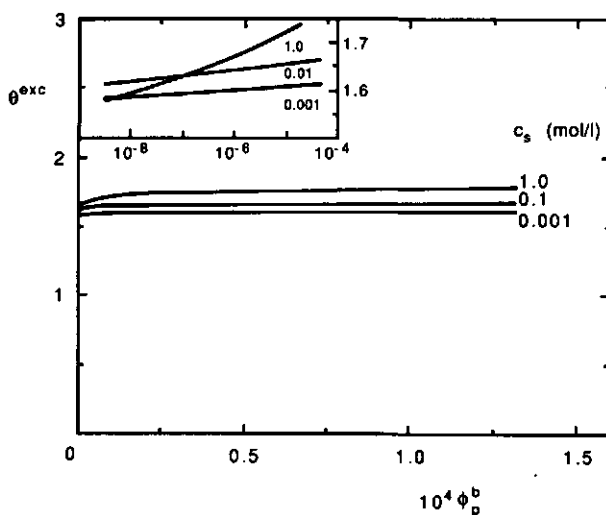


Figure 1. The excess adsorbed amount, expressed in equivalent monolayers as a function of the bulk volume fraction of weak polyelectrolyte ϕ_p^b at different salt concentrations (c_s), as calculated with the model of Böhmer et al. (1). The insert is the adsorbed amount as a function of the concentration polyelectrolyte on a logarithmic scale. The computations were performed with: $\chi_{ps} = -8.0$, $\chi = 0.5$, $r = 500$ segments, $d = 0.6$ nm, $Z = 12$, $pK_0 = 4.25$, $\sigma_0 = 160$ mC/m², and $pH = 4.0$.

expressed in equivalent monolayers, is plotted as a function of the bulk volume fraction ϕ_p^b of polyelectrolyte. In the inset we show the adsorption isotherms with the concentration axis on a logarithmic scale, to illustrate what happens at very low concentrations of polyelectrolyte. The calculations were performed for $pH = 4$, a surface charge density (σ_0) of 160 mC/m², and an intrinsic

dissociation constant pK_0 of 4.25. The values of the adsorption energy (χ_{ps}), the polymer-solvent and polymer-ion interaction parameter (χ), the chain length (r), and the lattice parameters Z and d are given in the legend of Figure 1. For all the computations a value of 20 was chosen for the relative dielectric constant of PAA and the solid, and a value of 80 was taken for all the other components.

The calculated isotherms are of the high affinity type as is generally true for the adsorption of homodisperse polymers and polyelectrolytes. The effect of the salt concentration on the adsorbed amount in the region 0.001-1 M is rather small. This is not only the case for $pH = 4$, but is found to be true for the whole pH range. At the high pH end, where the macromolecule is completely charged, the absolute change in the adsorbed amount is about the same as at $pH = 4$, but since the adsorbed amount at high pH is much lower (as will be illustrated later), the relative effect of the ionic strength at high pH is greater. The computations show that in the ionic strength range from 0.001-1 M with pH values around the pK_0 of the polyacid, the degree of dissociation is adjusted in such a way that the surface charge is fully compensated and sometimes even overcompensated in the first layer (depending on χ_{ps}), resulting in a slightly negative electrical potential in the first layers. Due to this negative potential the pH in the layers adjacent to the surface is lower than in the bulk. As a consequence, the degree of dissociation of the segments in the short loops is very low. Effectively, the particle is virtually neutral with only a very few charged segments in the loops of the adsorbed macromolecule. This explains why salt hardly affects the adsorption at this high surface charge.

From the inset of Figure 1 it can be seen that for very low polyelectrolyte concentrations the adsorbed amount at high salt concentrations is lower than at low c_s , whereas at higher concentrations polyelectrolyte the opposite trend is found. In this example the cross-over point for 0.1 and 1 M salt is situated at $\phi_p^b \approx 1 \times 10^{-7}$. At all salt concentrations the surface charge has to be compensated, either by salt ions or by charged polyelectrolyte segments as discussed above. At low polyelectrolyte concentrations, an increasing fraction of this compensation will be accomplished by

small ions as the ionic strength increases, so that the polyelectrolyte adsorption decreases with increasing c_s . At higher polyelectrolyte concentrations the adsorbed amount is higher and it increases with increasing c_s because the intramolecular electrostatic repulsions are much better screened at high c_s . This explains the cross-over as it occurs in Figure 1. Such a cross-over has also been found experimentally for the adsorption of surfactants which adsorb with the charged headgroup onto an oppositely charged surface (28).

In Figure 2 we have plotted the adsorbed amount as a function of the pH at a bulk volume fraction ϕ_p^b of 1×10^{-4} , which is well within the plateau regions of the individual adsorption isotherms. The results in the upper diagram were obtained using the model of Evers et al. (10) (ions are considered as point charges), the bottom figure was calculated with the model of Böhmer et al. (1) (ions occupy the same volume as a polymer segment). Although the parameters in both figures are somewhat different, we can still compare the trends.

Figure 2a gives three curves, one for the adsorption of a neutral polymer ($\alpha = 0$) and two for the adsorption of a weak polyacid ($pK_0 = 4$) onto an uncharged and onto an oppositely charged surface, respectively. If the small ions occupy no volume, the adsorption of a neutral polymer ($\alpha = 0$) is not affected by the surface charge. At low pH all three curves merge to the same adsorbed amount. This is to be expected because at this extreme, a weak polyacid is uncharged and should behave like a neutral polymer. Upon increasing the pH, the adsorption of the weak polyacid onto an uncharged surface becomes lower. When the polyelectrolyte is charged by increasing the pH, the inter- and intramolecular electrostatic repulsions oppose the accumulation of polyelectrolyte. Only in the first layer, where segments experience the attractive interaction of the surface does such accumulation occur. This results in a flat configuration of the adsorbed polyelectrolyte as is illustrated by the calculated segment density profiles (profiles not shown). In this manner one molecule will occupy more surface area and the adsorption will be less than when a molecule adsorbs in a conformation with long loops and tails. For the adsorption of a weak polyelectrolyte onto an oppositely

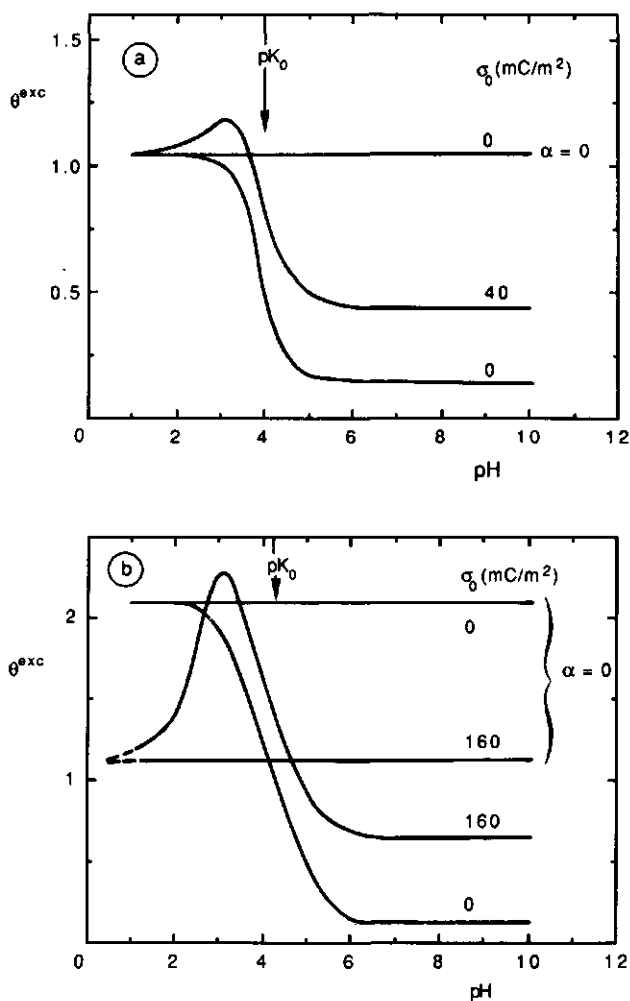


Figure 2. The excess adsorbed amount as a function of the pH for a neutral polymer ($\alpha = 0$) and a weak polyelectrolyte at different surface charge densities. The top figure (a) has been calculated with the model of Evers et al. (10) (salt ions have no volume, see text). Parameters: $\chi_{ps} = -2.0$, $\chi = 0.5$, $r = 500$ segments, $d = 1.0$ nm, $Z = 12$, $\phi_p^b = 1 \times 10^{-4}$, and $c_s = 1.0$ M. The bottom diagram (b) has been computed according to the model of Böhmer et al. (1) (salt ions occupy one lattice site, see text). Parameters: $\chi_{ps} = -8.0$, $\chi = 0.5$, $r = 500$ segments, $d = 0.6$ nm, $Z = 12$, $\phi_p^b = 1 \times 10^{-4}$, and $c_s = 0.1$ M.

charged surface the behaviour is quite different. Starting at low pH, an increase in the pH first leads to an increase in the adsorbed amount, subsequently followed by a decrease just as for adsorption onto an uncharged surface. The initial increase is due to the electrostatic attraction between surface and polyelectrolyte, giving an additional electrostatic contribution to the adsorption energy. At the pH values where this increase occurs, the degree of dissociation of the segments not in contact with the surface is very low, so there is hardly any inter- or intramolecular electrostatic repulsion between the segments in the loops or tails. The segments in contact with the surface are, at this pH, dissociated to a much larger extent than the segments in the loops or tails, i.e., some H^+ is released upon adsorption. Therefore, there is an extra attraction between surface and polyelectrolyte while there is hardly any extra repulsion. When the pH is increased further the electrostatic repulsion increases and as a result the adsorption will decrease for the same reason as in the case of an uncharged surface. The adsorption of a weak polyacid on an oppositely charged surface is always higher than on a neutral surface due to the electrostatic contribution to the adsorption energy.

Figure 2b shows similar curves for the model where salt ions have their own volume. In the limit of low pH the curves for weak polyacids merge with the curves for neutral polymers, as is to be expected. An obvious difference between Figures 2a and 2b is that in the latter case an increase in the surface charge density reduces the adsorption of an uncharged polymer. The computed segment- and ion-density profiles show that this reduction is caused by the presence of salt ions at the interface, necessary for shielding the (very high) charge on the surface. The volume occupied by the salt ions is no longer available for the polymer and as a result the adsorption becomes less (salt exclusion). This salt exclusion effect occurs only at high surface charge densities, where a considerable fraction of the sites in the first layer are filled with salt ions. To our knowledge such a large effect of the surface charge density on the adsorption of a neutral polymer has never been reported experimentally. It will be difficult to obtain such experimental results

because one would have to be able to vary the surface charge without affecting the chemical nature of the surface. Nevertheless, Koopal (29), studying the adsorption of poly(vinyl alcohol) onto AgI, reports a small decrease in the adsorbed amount upon increasing the pAg from 5.6 to 11 (changing the surface charge density from 0 to -35 mC/m^2). He attributes this difference in the adsorbed amount to inaccuracies in the specific surface area of AgI at pAg = 5.6. However, it could also be explained by the salt exclusion effect.

The pH-dependence of the adsorption on an uncharged surface follows similar trends in Figures 2a and 2b; the lower adsorbed amount at high pH in Figure 2b is mainly due to the lower salt concentration. On the other hand, the adsorption maximum on a highly charged surface is much more pronounced in Figure 2b. This is caused by the fact that charged polyelectrolyte segments and salt ions compete for shielding the surface charge. Already at low pH this competition is won by the polymer segments because of their adsorption energy. Moreover, the degree of dissociation of the adsorbed segments adjusts itself in such a way as to effectively screen the surface charge.

In Figure 3 we illustrate the effect of the chain length on the excess adsorbed amount. This figure has been computed with the model of Böhmer et al. (1). We have plotted θ^{exc} as a function of chain length for three pH values and for a neutral and a highly oppositely charged surface, respectively. At pH = 6.5 the polyacid is almost completely dissociated and the adsorbed amount does not depend on the molecular weight, as has been found for strong polyelectrolytes by Van der Schee et al. (26) and Papenhuijzen et al. (27). Increasing the surface charge density at this pH leads to an increase in the adsorbed amount due to the extra electrostatic attraction between surface and polyelectrolyte. For the other extreme (pH = 1.01), the polyacid is virtually uncharged and behaves like a neutral polymer. Here the adsorbed amount depends linearly on the logarithm of the molecular weight, as demonstrated by Scheutjens and Fleer (11) for the adsorption of a neutral polymer from a theta solvent. At this pH

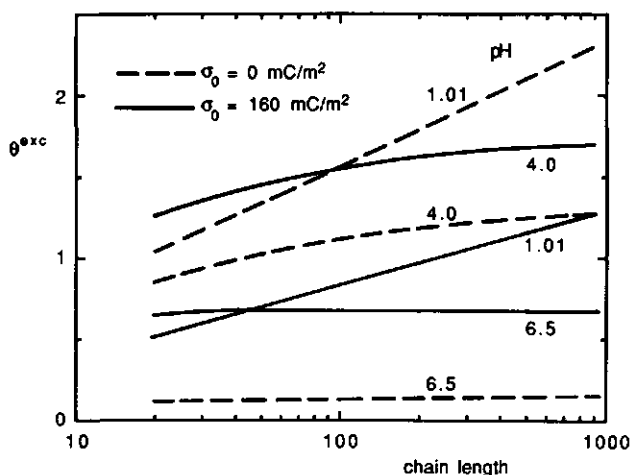


Figure 3. The dependence of the excess adsorbed amount on the chain length, at three pH values and two surface charge densities, respectively, as predicted by the model of Böhmer et al.. Parameters: $\chi_{PS} = -8.0$, $\chi = 0.5$, $d = 0.6$ nm, $Z = 12$, $\phi_p^b = 1 \times 10^{-4}$, $pK_0 = 4.25$, and $c_s = 0.1$ M.

an increase in the surface charge density results in a decrease in the adsorbed amount due to the salt exclusion effect. At pH = 4 an intermediate situation is found, with a weaker chain length dependence than at pH = 1.01, and a higher adsorption when the surface carries an opposite charge.

Experimental

Latex

We used polystyrene latex with a positive pH independent surface charge as the adsorbent. The preparation of this latex has been described elsewhere (30). The properties of the latices used are given in Table 1.

Table 1. The properties of the latices that have been used in the adsorption studies. The variables d_{32} and d_{10} are the volume-surface average diameter and the number-average diameter, respectively, and σ_0 is the surface charge density.

latex no	particle diameter (nm)	standard deviation (nm)	d_{32}/d_{10}	σ_0 (mC/m ²)
1	834	23.3	1.0015	100
2	771	7.5	1.0002	160

Poly(acrylic acid)

Poly (acrylic acid) (PAA) in the sodium form was purchased from Polysciences Inc. (Warrington, Pa., USA.). The molecular weight distributions of the samples are rather broad, as can be seen from Table 2. The molecular weights reported in Table 2 are as given by the manufacturer and were determined by gel permeation chromatography.

Table 2. The characteristics of the PAA samples used in the adsorption experiments. M_w and M_n are the weight and number average molecular weights, respectively.

PAA sample	M_w	M_n	M_w/M_n
1	18,100	12,800	1.41
2	70,900	39,400	1.80

Conductometric titrations of PAA revealed that the samples as obtained from the manufacturer contained some excess of NaOH, because the initial part of the first titration curve with HCl showed a rather steep decay of the conductivity on adding HCl. Successive titrations showed the normal behaviour of a weak (poly) acid with a strong base. From the back titration curve the concentration of PAA is obtained in meq/l. This concentration can be converted to ppm using a monomer molecular weight of 73. The concentration of PAA determined in this way was less than that based on dry sample

weight, even after correction for the excess amount of NaOH. The discrepancy could be reduced by drying the PAA in an oven at 80 °C. So we concluded that Na-PAA is hygroscopic, and we decided to use the conductometric titration method for the determination of PAA in the stock solutions. All PAA concentrations in this paper are expressed in ppm (mg/l) based on PAA in the acidic form.

Adsorption isotherms

The adsorption experiments were performed in 10 ml polycarbonate centrifuge tubes. Before the solutions were pipetted into the centrifuge tube they were adjusted to the required ionic strength and pH. Measurements with the single particle optical sizer as described by Pelssers (31) revealed that it was necessary to sonicate the latex suspension for 20 minutes to get a suspension with only singlet particles.

First 2 ml of the latex suspension (ca 0.6 m²) was pipetted into a tube, followed by the desired volume of a KNO₃ solution and the required amount of the PAA stock solution. The total volume in the centrifuge tubes was 8 ml. Then the tubes were capped, vigorously shaken, and slowly rotated end-over-end for 24 hours. Preliminary experiments had shown that after rotating for 24 hours the adsorbed amount did not increase any more. An almost clear supernatant was obtained by centrifuging for 20 min at 20,000 rpm (Beckman centrifuge, JA21 rotor). Then the pH of the supernatant was measured and a latex free solution was obtained by filtering the supernatant through an acrodisc filter assembly (Gelman sciences, 0.2 µm). The concentration of PAA in the latex free solution was determined and the adsorbed amount Γ (in mg/m²), was calculated from the mass balance.

Determination of residual PAA concentrations

During our experiments we had to change our method for determining the equilibrium concentrations PAA twice. At low ionic strength (0.001 M) we used a colourimetric colloid titration procedure, which is based upon a stoichiometric complex formation between a polyanion and a polycation. This did not work satisfactorily

at an intermediate ionic strength (≈ 0.01 M). Here we used a total organic carbon analyzer (TOC). At high ionic strength (≥ 0.1 M) the TOC determination was not reliable so we here used a simple acid base titration procedure.

Colourimetric titration

Colloid titrations can be a suitable means of determining concentrations of positively or negatively charged polyions as long as they combine stoichiometrically with an oppositely charged polyelectrolyte (32,33). The method as outlined by Terayama (32) was used for measuring unknown concentrations of PAA in media of low ionic strength (0.001 M). To a PAA solution an excess of positively charged poly(diallyldimethylammonium chloride) was added. The excess of this polycation was titrated back with potassium poly(vinyl sulfate) to a colourimetric end-point with toluidine blue as the indicator. Using a calibration curve, concentrations of PAA could be determined with an accuracy of 2 % down to a concentration of a few ppm. For media with a ionic strength higher than 0.001 M it was difficult to determine the equivalence point in the back titration step because the colour change was not distinct. Probably the complexes formed between PAA and poly(diallyldimethylammonium chloride) are not sufficiently stable at higher salt concentrations.

Total organic carbon analyzer

For the determination of PAA in media of higher ionic strength we used an automatic total organic carbon (TOC) analyzer (TOC Sin II, Phase Separation, Queensferry, U.K.). This apparatus measures the total organic carbon content after combustion of the sample to CO_2 and H_2O in a furnace at 1100°C , followed by drying of the gas stream and subsequent reduction of CO_2 to CH_4 by H_2 in the presence of a catalyst at 200°C . The amount of CH_4 is determined by a flame ionisator. Before the sample is combusted, any CO_2 from the atmosphere is removed by acidifying the sample, followed by deaerating. Using a calibration procedure, PAA could be accurately determined in media of medium ionic strength (≤ 0.01 M). However,

at higher ionic strength (≈ 0.1 M) the apparatus was not reliable. The malfunctioning of the analyzer was due to plugging of the tubing by salt, especially of the pipe that entered the furnace, and to contamination of the catalyst.

Potentiometric titration

For the measurements at 0.1 M KNO_3 we developed a simple titration procedure. The pH of a 5 ml aliquot of the solution to be analyzed was adjusted to just below $\text{pH} = 4$. Then the amount of titrant (5×10^{-3} N NaOH) necessary to raise the pH from 4 to 7 was measured. With a calibration procedure PAA could be determined. The calibration curve (amount of titrant needed to raise the pH from 4 to 7 versus [PAA]) showed that approximately 70% of the carboxylic groups are titrated in the pH range 4 to 7 . We chose to start at $\text{pH} = 4$ because otherwise the blank titration required too much titrant, and we stopped at $\text{pH} = 7$ to prevent interference of CO_2 .

Results and discussion

The experiments were designed to check the major trends discussed in the theoretical section. Special attention was paid to the pH-dependence of the adsorbed amount (where the theory predicts a maximum at $\text{pH} < \text{pK}_0$ at high opposite surface charge density) and the effects of salt concentration, chain length, and surface charge.

In Figure 4 we give some experimental adsorption isotherms for the PAA sample of $M_w = 18,100$ onto latex with $\sigma_0 = 100$ mC/m² in 0.001 M KNO_3 , at three different pH values. The adsorbed amount, expressed in mg/m², is plotted as a function of the equilibrium concentration of PAA in ppm, measured by means of the colourimetric colloid titration procedure. It is obvious from this figure that in the region around pK_0 (4.5 for PAA) the pH has a large effect on the adsorbed amount, as predicted theoretically (see fig. 2). The adsorption isotherms are of the high affinity type but they are not quite as sharp as in the theory. This is probably due to the polydispersity of the polyelectrolyte sample, as suggested by Cohen

Stuart et al. (34). It appears from Figure 4 that the isotherms become less sharp when the pH decreases. This can be attributed to the fact that the adsorption depends more strongly on the molecular weight at a low pH (see figure 3), which gives rise to more pronounced polydispersity effects.

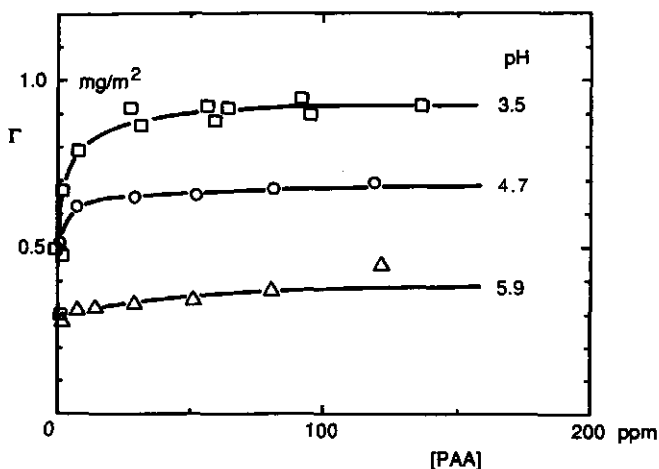


Figure 4. Experimental adsorption isotherms of PAA ($M_w = 18,100$) onto a cationic latex with $\sigma_0 = 100 \text{ mC/m}^2$ in 0.001 M KNO_3 , at three pH values (the equilibrium concentrations of PAA are expressed in ppm (w/w)).

Our adsorption data may be compared with trends reported in the literature. The adsorption of PAA onto rutile (TiO_2) and hematite ($\alpha\text{Fe}_2\text{O}_3$) has been studied by Gebhardt and Fuerstenau (35). They also found a large decrease in the adsorbed amount upon increasing the pH from 4 to 8. They explained this solely on the basis of the decrease in surface charge density on increasing the pH, thereby neglecting the increase of the charge on the polyelectrolyte chain. Our results, with a constant σ_0 , show how important the effect of the polyelectrolyte charge is on the adsorbed amount. Foissy et al. (36) studied the adsorption of PAA onto anatase (TiO_2) and reported a high adsorbed amount at $\text{pH} = 2.8$ and a very low adsorbed amount at $\text{pH} = 9.8$. There is a big difference between the results of Gebhardt

and Fuerstenau (35) and Foissy et al. (36). The latter authors found an adsorption density which was about 7 times higher, although the molecular weight of their samples was much lower. Another difference is that Foissy et al. (36) still found adsorption at $\text{pH} = 9.8$, whereas Gebhardt and Fuerstenau (35) did not measure any adsorption above $\text{pH} = 7$. The plateau region of the adsorption isotherms as measured by Gebhardt and Fuerstenau (35) is reached at the same equilibrium concentration of PAA as in our case, whereas in the experiments of Foissy et al. (36) the plateau region is reached at equilibrium concentrations that are 10 times higher. If these large differences in the adsorption of PAA onto rutile and anatase are real, they must be mainly due to a different chemical affinity (different χ_{ps}) of PAA for the two crystal forms of TiO_2 because the electrokinetics of rutile and anatase are virtually the same (37,38).

The experimental pH-dependence of the adsorption of PAA on positively charged latex is shown in Figure 5 and compared with the theoretical predictions. The adsorbed amount was measured at an equilibrium concentration of PAA of 100 ppm, which is in the (semi)plateau of the adsorption isotherms. The points in this graph are the experimentally determined adsorbed amounts for the adsorption of the $M_w = 70,900$ PAA sample onto latex with a surface charge density of 160 mC/m^2 in 0.1 M KNO_3 ; for these points the left hand scale (Γ in mg/m^2) applies. The final concentrations of PAA were measured by means of the potentiometric titration procedure. The drawn curve gives the theoretical excess adsorbed amount θ^{exc} (right hand scale, in equivalent monolayers) as predicted by the model of Böhmer et al. (1), with values for the variables as given in the legend.

The qualitative agreement between theory and experiment is excellent. The overall shape is identical for the theoretical and experimental curves, with both showing a maximum at a pH somewhat below pK_a . There is a pH difference, of the order of 0.5 in the exact position of the maximum, but the main trends are fully accounted for in the theoretical model.

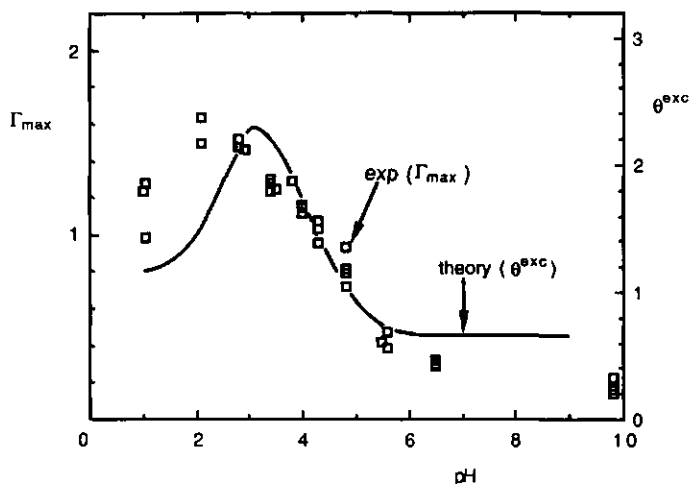


Figure 5. The adsorbed amount Γ as a function of the pH for the adsorption of PAA, ($M_w = 70,900$) onto a cationic latex with $\sigma_0 = 160 \text{ mC/m}^2$ in 0.1 N KNO_3 . The experimental points (corresponding to the left hand scale in mg/m^2) were measured at an equilibrium concentration of 100 ppm (w/w) . The drawn curve is the theoretical excess adsorbed amount θ^{exc} (right hand scale, in equivalent monolayers). This curve was computed according to the model of Böhmer et al. (1), using $\chi_{ps} = -8.0$, $\chi = 0.5$, $r = 500$ segments, $d = 0.6 \text{ nm}$, $Z = 12$, $\phi_p^b = 1 \times 10^{-4}$, $pK_0 = 4.25$, $\sigma_0 = 160 \text{ mC/m}^2$, and $c_s = 0.1 \text{ M}$.

The theoretical curve predicts a slightly sharper maximum than was obtained experimentally. A reason for this might be the way in which water is accounted for in the model. For calculating the degree of dissociation of the polyelectrolyte segments the local water concentration is taken into account. Because both segments and salt ions occupy one lattice site, the volume fraction of water in the first layer becomes very low at high surface charge densities, resulting in a rather low degree of dissociation. As a consequence, more salt ions are needed for screening the surface charge, leading to a lower adsorbed amount at low pH than is found experimentally.

For a more quantitative comparison one should relate the experimental scale (mg/m^2) to the theoretical one (equivalent monolayers). For such a comparison one needs an estimate for the conversion of monolayers to mg/m^2 , and it is very difficult to find an

exact conversion factor. Assuming that one polymer segment is identical to one monomer and using a reasonable molecular model, one finds that a fully packed monolayer would correspond to about 0.4 mg/m^2 , whereas Figure 5 suggests a value of 0.7 mg/m^2 . The agreement is reasonable, especially considering the fact that we did not try to optimize the theoretical parameters (lattice spacing, chain length, interaction parameters χ and χ_{ps}). At present, there is not enough information to make such a best fit. However, we may already conclude that the correlation between the SCF theory in which the electrostatic interactions are incorporated and experiment is semi-quantitative. The available experimental data cannot, at present, distinguish between the model in which ions have their own volume (1) and the model in which the ions are treated as point charges (10). For such a comparison, more detailed information would be needed.

There are only a very few examples in the literature where a maximum in $\Gamma(\text{pH})$, as in Figure 5, is obtained. Tanaka et al. (13) report for the adsorption of cationic polymers (modified starch) onto bleached kraft pulp such a maximum in the the adsorbed amount. For different cationic polymers, it is found at a pH where approximately 3% of the active groups in the bulk solution are ionized. The cationic groups on the polymer are a result of a chemical modification of the macromolecule and the degree of substitution is always lower than 0.8. Since the chemically modified groups will have different interaction energies, it is not yet possible to give a theoretical interpretation for these experiments. Also, the characteristics for their adsorbent are not known. Nevertheless it is noteworthy that the maximum appears at low degrees of ionization, well below pK_0 .

Wang et al. (39) and Durand-Plana et al. (40) have studied the adsorption of a cationic copolymer as a function of the fraction τ of cationic monomeric groups in an otherwise uncharged polymer. Wang et al. (39) adsorbed this polymer onto silica, whereas the latter authors used a sodium montmorillonite clay as the adsorbent. At $\tau = 1\%$ these authors found a maximum in the adsorbed amount. For these copolymers it is very likely that the different monomers have different interaction energies with the surface and with the solvent

and therefore the present theoretical models can not be applied to these experiments. An additional problem is that the mean field approach might break down for a polymer chain with only 1% strong cationic groups that behave as discrete charges. Notwithstanding the uncertainties in the detailed theoretical interpretation, the fact that a maximum is found at a low fractional polymer charge is an interesting feature.

In Figure 6 we show two adsorption isotherms at $\text{pH} = 4.0$ for the adsorption of PAA ($M_w = 18,100$) onto a highly charged surface at two salt concentrations. In agreement with theory (see fig. 1) there

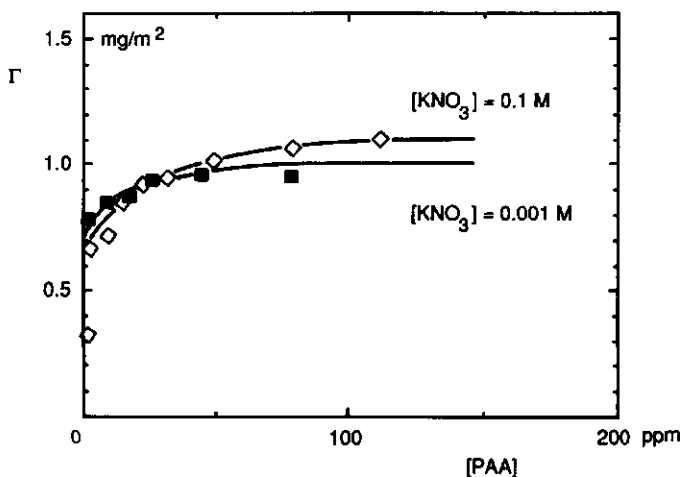


Figure 6. The effect of the ionic strength on the adsorption isotherms of PAA ($M_w = 18,100$) onto latex ($\sigma_0 = 160 \text{ mC/m}^2$) at $\text{pH} = 4.0$.

is only a small effect of the ionic strength on the adsorption. At low equilibrium concentrations the adsorption from the low ionic strength medium is slightly higher, whereas at higher concentrations the reverse is observed. Such a cross-over point is also predicted by theory. The theoretical cross-over appears at a much lower concentration than the experimental one. This is probably related to the shape of the isotherm, which for the experimental, polydisperse system is much more rounded than for

monodisperse polyelectrolyte. It is noteworthy that the theoretically predicted and the experimentally determined cross-over points appear at the same relative adsorption, i.e., at the same fraction of the plateau in the isotherm. In any case, the most important conclusion is the weak ionic strength dependence for the adsorption of weak polyelectrolytes, both theoretically and experimentally, in strong contrast with the behaviour of a polyelectrolyte with strong groups. As explained in the theoretical section, the reason is the high degree of dissociation of groups close to the surface (leading to charge compensation) and the low charge of groups in loops and tails.

Upon increasing the ionic strength from 0.001 M to 0.1 M KNO_3 , the plateau values of the adsorption isotherms increase with approximately 9 %. The calculations for these experimental conditions (fig. 1) predict also a small increase. The agreement is within experimental error. The computations also show that the absolute amount by which the adsorption increases on increasing the ionic strength is almost independent of the pH. This implies that at a high pH, where the adsorbed amount is low, the relative increase in the adsorbed amount is much larger.

We are not aware of any paper dealing with the adsorption of a weak polyelectrolyte onto a highly oppositely charged surface as a function of the ionic strength. Meadows et al. (41) report for the adsorption of 90 % hydrolyzed polyacrylamide (weak polyacid) onto a latex with weak cationic groups a strong dependence of the adsorbed amount on the ionic strength. Cafe et al. (42), studying the adsorption of radio-labeled PAA onto BaSO_4 -crystals at pH values where the crystals are slightly negatively charged, found a small increase in the adsorbed amount upon increasing the ionic strength. Wright et al. (43) investigated the adsorption of spin-labeled PAA onto BaSO_4 -crystals prepared in a slightly different manner. They report a large increase in the adsorbed amount with increasing ionic strength. The usual assumption in studies of the adsorption of spin- or radio-labeled macromolecules is that the label does not affect the behaviour of the polymer. This assumption may not be valid because it has been shown (39, 40) that for a polymer which has a very low

affinity for a negatively charged surface the introduction of only 1 % strong cationic groups is enough to get a large adsorbed amount. This might be a reason why Cafe et al. (42) report different adsorption results for spin- and radio-labeled PAA.

The molecular weight dependence of the adsorbed amount in 0.1 M KNO_3 at pH = 4 is illustrated in Figure 7. In this figure we have plotted two adsorption isotherms for $M_w = 70,900$ and $M_w = 18,100$, respectively, onto latex with $\sigma_0 = 160 \text{ mC/m}^2$. The adsorption at this

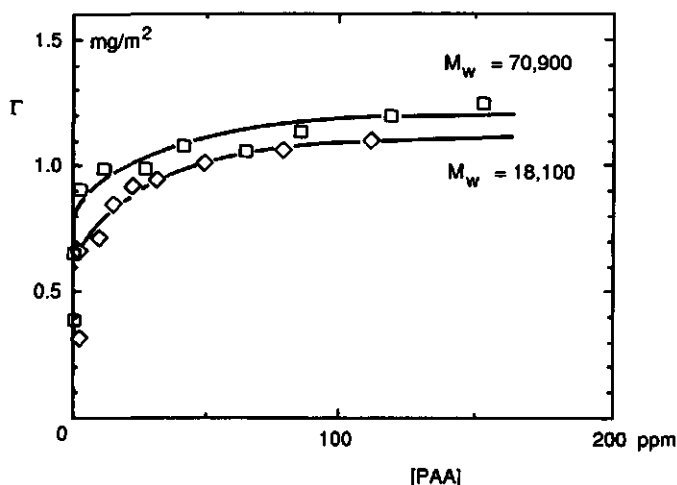


Figure 7. The effect of molecular weight on the adsorption isotherms of PAA onto latex ($\sigma_0 = 160 \text{ mC/m}^2$) in 0.1 N KNO_3 at pH = 4.0.

pH increases only slightly upon increasing the molecular weight. In the plateau region the increase is about 10 % from $M_w = 18,100$ to $M_w = 70,900$. Theoretically (fig. 3) approximately the same increase is predicted. Foissy et al. (36) report for the adsorption of low molecular weight PAA samples ($M_w = 700$, 2000, and 4000, respectively) onto anatase at pH = 2.8 a much larger dependence of the adsorbed amount on molecular weight. This is easily understood because at this pH PAA behaves more like a neutral polymer. Moreover, at low molecular weights the dependence of the adsorption on chain length is most pronounced. These two effects are demonstrated in Figure 3.

Our last experimental result deals with the effect of the surface charge. The dependence of the adsorption of PAA on the surface charge density of the latex is shown in Figure 8, for $M_w = 18,100$ in 0.001 M KNO_3 at $\text{pH} = 4$. Two latices were used, with surface charge densities of 100 and 160 mC/m^2 , respectively. It is evident that by increasing the surface charge density the adsorption increases, in this case by about 12% . The computations for this situation with the model of Böhmer et al. (1) predict an increase that is of the same order of magnitude. Again the agreement is satisfactory.

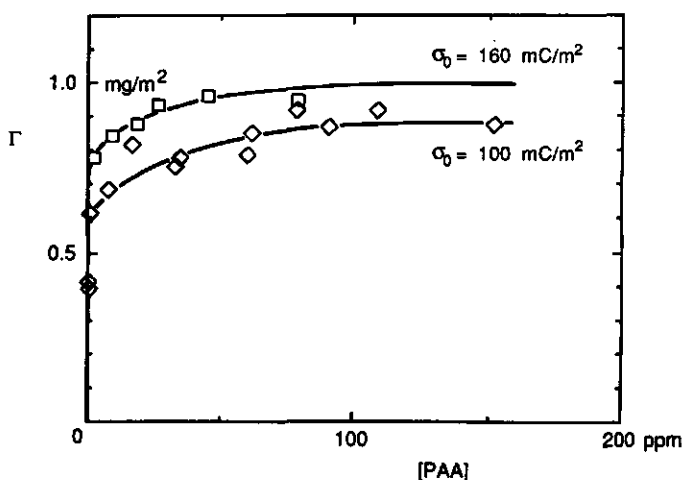


Figure 8. The effect of the surface charge density on the adsorption isotherms of PAA ($M_w = 18,100$) onto latex in 0.001 M KNO_3 at $\text{pH} = 4.0$.

In the theoretical section we stated that the surface charge is already compensated in the first layer. On the basis of this statement one would expect the adsorption to increase roughly proportional to the increase in the surface charge density. However, one has to bear in mind that at the same pH in the bulk solution the degree of dissociation of segments in contact with a highly charged surface is higher than that of segments in contact with a surface having a lower charge. Therefore, upon increasing the surface charge density only a small increase in the number of train segments is needed to compensate the surface charge in the first layer.

Conclusions

The adsorption of poly(acrylic acid) (PAA) onto a highly charged polystyrene latex with strong cationic groups depends strongly on the pH. When the plateau values of the adsorption isotherms are plotted as a function of the pH, a maximum is obtained at a pH which is about 1 unit below the pK_0 of the polyacid. The measurements were used to test a theoretical model (1) which describes the adsorption of weak polyelectrolytes. The experimental maximum in the adsorbed amount is found close to the pH where the theory predicts the maximum. The maximum arises because of two opposing trends. With increasing pH there is a higher electrostatic contribution to the segmental adsorption energy, but as the polyelectrolyte groups become progressively dissociated the increasing repulsion between these groups opposes the accumulation of polyelectrolyte near the surface.

The effect of the salt concentration on the adsorbed amount at $pH = 4$ is very low. This is also predicted by the computations which show that the polymer segments in contact with the surface are dissociated to such a degree that the surface charge is already compensated, or even slightly overcompensated, in the first layer. The segments in the loops and tails are dissociated to a much lower degree. So in effect, with the bulk solution as reference point, one sees a nearly neutral particle. Therefore, salt will hardly affect the adsorption. It is interesting to note that adsorption isotherms at two different ionic strengths intersect each other at a low polyelectrolyte concentration, both experimentally and theoretically.

Not only the dependences of the adsorption on the pH and on the ionic strength are well predicted by theory, but also the effects on the adsorption of the molecular weight of the macromolecule and the surface charge density of the adsorbent. We can conclude that the adsorption of PAA onto a positively charged latex is described semi-quantitatively by recent SCF theories, in which the electrostatics are incorporated.

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Chapter 3

Towards the synthesis of defined oligomers of H-Lys-Glu-Gly-OH and H-Ala-OH

Implications of the insertion of N^{α} -(2,4-dimethoxybenzyl)amino acids in oligomers

Abstract

The synthesis of the model compound Aloc-Ala-Ala-Dma-Ala-Ala-OMe¹⁾ has been described as an illustration of the fact that a large steric group is capable of disturbing the regularity of a peptide backbone. Application of such a group greatly enhances the solubility, but the function alters the properties of the intermediates to such an extent that the use of the group in the synthesis of long Lys-Glu-Gly oligomers had to be given up. Consequently, the solubility of the growing protected peptide chain became progressively less and the synthesis was terminated with Z-[Lys(Boc)-Glu(OBu^t)-Gly]₄-OMe, at the dodekapeptide level.

Introduction

We were interested in a very well-defined polyampholyte, which would resemble naturally occurring polyampholytes such as proteins as much as possible. This polyampholyte was to be used in adsorption experiments (1) as a more advanced model of proteins than the simple poly(amino acids) like polylysine which have been used so far. The adsorption behaviour of simple polyelectrolytes has been studied both experimentally (2-6) and theoretically (7,8) but such macromolecules lack the essential property of an isoionic point.

1) Abbreviations and symbols used in this communication are in accordance with the recommendations of the IUPAC-IUB Joint Commission for Chemical and Biochemical Nomenclature as given in *Eur. J. Biochem.* **138**, 9-37 (1984). Further abbreviations: Dma = N-2,4-dimethoxybenzyl-L-alanine, Dmg = N-2,4-dimethoxybenzylglycine, and Sar = sarcosine.

It was therefore decided that studying the adsorption behaviour of polyampholytes might improve the understanding of the phenomenon of protein adsorption, which plays an important role in everyday life. We chose to synthesize a well-defined oligomer of H-Lys-Glu-Gly-OH. To this end we synthesized the tripeptide derivative Z-Lys(Boc)-Glu(OBu^t)-Dmg-OMe, which then could be doubled to a hexapeptide derivative by means of an active ester coupling. The hexapeptide could again be coupled to a dodecapeptide, etc. It is well known that when an active ester coupling procedure is envisaged, racemisation of the chiral α -C atom of the C-terminal amino acid will occur upon activation with dicyclohexylcarbodiimide. Because we wanted a very well-defined conformation using a synthesis route in which activation of the C-terminal amino acid with dicyclohexylcarbodiimide had to be used, we preferred to use glycine (which has no chiral α -C atom) as the C-terminus.

The synthesis of long defined oligomers of simple neutral amino acids is often hampered by the occurrence of association. This phenomenon was encountered earlier during the synthesis of antigens containing the sequence -Leu-Val-Val-Val-Gly- (9) and, in another instance, during the attempted synthesis of oligomers of alanine (10) which had to be given up, since oligomers with chain lengths exceeding five to six L-alanyl residues are hardly soluble (even in trifluoro acetic acid). The phenomenon of association appeared to be correlated with the presence of the monotonously recurring amide bond in the peptide backbone, suggesting a predominant role of *intermolecular* hydrogen bonds in the association. The presumed hydrogen bonding depends on the nature of the solvent and has been monitored (11) by observing the presence or disappearance of the diagnostic carbonyl stretch vibration of the amide I band in the IR spectra. The association is likely to be augmented by the also monotonously positioned hydrophobic side chains which tend to unite *intermolecularly* by hydrophobic interaction (12) and which affect the solubility negatively.

In the synthesis of oligomers of the tripeptide H-Lys-Glu-Gly-OH the same problem was expected to turn up, since the side chains

of the monomer, although highly polar in the end-product, would require temporary protection during the synthesis. Unfortunately, the protective functions currently in use (*tert*-butyloxycarbonyl for amino protection and *tert*-butyl for carboxyl protection), strongly reduce the polarity and endanger the solubility.

In order to reduce problems resulting from aggregation, it was presumed that (a reversible) perturbation of the regularity of the molecular backbone would hamper the association and thus increase the solubility.

The best way to affect the peptide backbone appeared to be alkylation of the N^α-nitrogen atom with an acid labile function. Introduction of the 2,4-dimethoxybenzyl function (13,14) changes the nature of an amino acid profoundly, and since hydrogen bonding is impossible, *intermolecular* aggregation will be hampered. The dimethoxybenzyl group has been used to this end (15-17) and led to two defined soluble oligomers of leucine and glycine. In these compounds, N-2,4-dimethoxybenzyl-L-leucine was introduced and inserted between trileucylglycine and dileucylleucine. Only one perturbing group per six unperturbed peptide bonds (15) appeared sufficient to endow the obtained product with a much better solubility. Application of the function in the synthesis of an alanine oligomer is summarized in Scheme 1.

When a glycine derivative with an N-2,4-dimethoxybenzyl moiety was used, the familiar peptide reactivity/stability changed dramatically: on removing the N-benzyloxycarbonyl function from Z-Glu(OBu^t)-Dmg-OMe by hydrogenation, a diketopiperazine was formed immediately. Even when the Dmb group was replaced by CH₃ (sarcosine) a diketopiperazine was formed very rapidly.

The present article describes the application of N-2,4-dimethoxybenzylglycine in the attempted synthesis of H-[Lys-Glu-Dmg]_n-OH and of N-2,4-dimethoxybenzylalanine in the successful synthesis of Aloc-Ala-Ala-Dma-Ala-Ala-OMe as a model compound for further oligomerization. First we shall describe the synthesis of the model compound Aloc-Ala-Ala-Dma-Ala-Ala-OMe, as an illustration of the enhancement of the solubility by alkylation of one nitrogen atom (summarized in Scheme 1). Then we will discuss the application of

the (ar)alkyl functions in the attempted synthesis of Lys-Glu-Xxx derivatives and end with the synthesis of tetra-[Lys-Glu-Gly]-OMe, Scheme 2. Eventually, the application of an (ar)alkyl group in the latter compound had to be given up for the reasons given above.

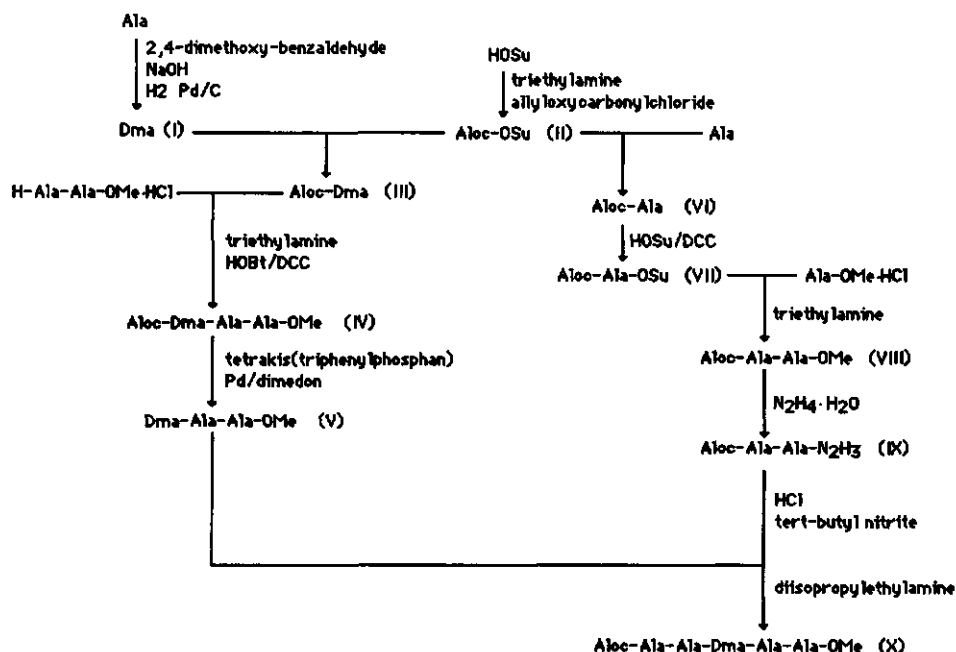
Experimental

Reaction progression was measured by means of thin layer chromatography using pre-coated fluorescent silica gel plates (Merck, 60F-254). Three elution systems were used: A) chloroform/methanol (9 : 1), B) chloroform/methanol/acetic acid (95 : 20 : 3), and C) n-butanol/acetic acid/water (4 : 1 : 1). Peptides with free amino groups were detected by staining with ninhydrine. Peptide bonds were detected by spraying with the TDM reagent as described by Von Arx et al. (18). The presence of the 2,4-dimethoxybenzyl moiety was revealed by spraying with 10 % H_2SO_4 in methanol and heating, which causes purple spots. The compound Aloc-Ala-Ala- N_2H_3 was detected by spraying with a mixture of equal amounts of ferric chloride (15 % in water) and potassium hexacyanoferrate (1 % in water). Prior to colouring, the spots were observed by their fluorescence quenching at 254 nm.

Melting points were determined with a Tottoli apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at concentrations of ca. 1 % (w/v). Amino acid analyses were performed with a modified Jeol J.L.C.-6 analyzer with samples hydrolyzed in sealed evacuated ampoules at 110-115 °C for 24 hrs. in 5.7 M hydrochloric acid (Merck, suprapur). A Bruker 360 MHz spectrometer was used for recording ^1H -NMR spectra in which tetramethylsilane served as the internal standard. The values of the physical constants are reported in an appendix to this chapter.

Synthesis of Aloc-Ala-Ala-Dma-Ala-Ala-OMe

The outline of the synthesis of this model compound is given in Scheme 1.



Scheme 1. The route followed for the preparation of the *N*^α-alkylated amino acid Dma and its insertion into the model compound Aloc-Ala-Ala-Dma-Ala-Ala-OMe.

Dma, 2,4-Dimethoxybenzyl-L-alanine (I). The procedure of Quitt et al. (19) was modified to prepare this compound. To a solution of 2,4-dimethoxy-benzaldehyde (3.32 g, 20 mmoles) in methanol (40 ml), an aqueous solution of L-alanine (1.78 g, 20 mmoles, in water (15 ml)) and sodium hydroxide (5 ml 4 N, 20 mmoles) were added. The homogeneous solution was subjected to catalytic hydrogenation for about 24 h in the presence of palladium on charcoal (600 mg, 10 %). The suspension was then filtered, the filtrate was concentrated *in vacuo* to remove most of the methanol, the residue was diluted with a little water and the resulting basic solution was extracted with

diethylether, to remove small amounts of 2,4-dimethoxybenzyl alcohol. The aqueous layer was neutralized (pH 6) with 1 N hydrochloric acid, inorganic salts and a trace of alanine were removed by countercurrent distribution in an apparatus equipped with 25 ml elements using the system butanol/acetic acid/water = 4 : 1 : 5; 80 transfers, $K = 1.01$, $r_{\max} = 40$. The contents of the tubes 28-52 were collected, concentrated to about 30 ml, diluted with water and freeze-dried to give the N-alkylated alanine. Yield: 3.78 g (79 %) $^1\text{H-NMR}$ (D_2O): CH_3 , d, (3H), δ 1.50 ppm; αH , q, (1H), δ 3.64 ppm; OCH_3 , s, (6H), δ 3.86 ppm; CH_2 , s, (2H), δ 4.17 ppm; o,m-protons, d, (2H), δ 6.61 ppm; m-proton, s, (1H), δ 7.29 ppm.

Aloc-OSu, 1-(Allyloxycarbonyloxy)succinimide (II). N-Hydroxysuccinimide, (100 mmoles, 11.5 g) was dissolved in acetonitrile (100 ml), triethylamine (14 ml, 100 mmoles) was added and the mixture was cooled to 0 °C. A solution of allyloxycarbonyl chloride (20,21), (100 mmoles, 12.05 g) in acetonitrile (100 ml) was added while stirring. Triethylammonium chloride separated as a white precipitate. Following the addition of the chlorocarbonate, the reaction mixture was stirred for an additional 15 min. and then filtered. The filtrate was concentrated to give an oil, which was next dissolved in ethyl acetate and extracted with water. The crude compound may be washed with potassium hydrogen sulfate to remove residual base if necessary. The neutral organic extract was dried, and evaporated to give an oil which crystallized slowly in the refrigerator. The crystalline compound melted unsharp at about 25 °C. $^1\text{H-NMR}$ (CDCl_3): $\text{CH}_2\text{-CH}_2$ (succinimido), s, (4H), δ 2.80 ppm; $\alpha\text{-CH}_2$ (allyl), d, (2H), δ 4.76 ppm; $\gamma\text{-CH}_2$ (allyl), t, (2H), δ 5.40 ppm; $\beta\text{-CH}$ (allyl), m, (1H), δ 5.96 ppm.

Aloc-Dma, N-Allyloxycarbonyl-N-2,4-dimethoxybenzyl-L-alanine (III). Dma, (2.39 g, 10 mmoles) was suspended in a mixture of acetonitrile and water (4 : 1, v/v), an excess of Aloc-OSu (2.09 g, 11 mmoles) was added and the apparent pH was adjusted to 8.5 using triethylamine. Then the solution was evaporated, diluted with water, acidified to pH 2.5 using potassium hydrogen sulphate and extracted with ethyl acetate. The dried extract left a colorless oil upon evaporation, weighing 2.62 g (81 %). $^1\text{H-NMR}$ (CDCl_3): CH_3 (alanine),

d, (3H), δ 1.39 ppm; CH₃ (methoxy), s, (6H), δ 3.80 ppm; α -H (alanine), q, (1H), δ 4.27 ppm; CH₂ (benzyl), s, (2H), δ 4.49 ppm; α -CH₂ (allyl), d, (2H), δ 4.63 ppm; γ -CH₂ (allyl), t, (2H), δ 5.24 ppm; β -CH (allyl), m, (1H), δ 5.93 ppm; arom. o,m-protons, d, (2H), δ 6.43 ppm; m-proton, s, (1H), δ 7.20 ppm.

Aloc-Dma-Ala-Ala-OMe, N-Allyloxycarbonyl-N-2,4-dimethoxybenzyl-L-alanyl-L-alanyl-L-alanine (**IV**). Aloc-Dma (2.52 g, 7.9 mmoles) was dissolved in dimethylformamide (39 ml) and H-Ala-Ala-OMe · HCl (ref.22) (1.82 g, 8.70 mmoles) was added. Subsequently, triethylamine (1.22 ml, 8.7 mmoles) and N-hydroxybenzotriazole (1.57 g, 10.3 mmoles) were dissolved in the mixture, which was then cooled. Neglecting the precipitated triethylammonium hydrochloride, dicyclohexylcarbodiimide (1.71 g, 8.30 mmoles) was added to the well stirred suspension and the reaction vessel was kept in the refrigerator for about 18 h. Following filtration, the filtrate was concentrated, diluted with water, the separated apolar phase was taken up in ethyl acetate and extracted with water, 0.2 M potassium hydrogen sulphate solution, water, hydrogen carbonate solution, water and finally with concentrated sodium chloride solution. The dried extract (sodium sulphate) was evaporated to leave an oil, which crystallized spontaneously; yield, 3.1 g (84 %). ¹H-NMR (CDCl₃): CH₃ (alanine), broad m, (9H), δ 1.33 ppm; CH₃ (ester), s, (3H), δ 3.73 ppm; CH₃ (methoxy), s, (3H), δ 3.80 ppm; CH₃ (methoxy), s, (3H), δ 3.84 ppm; α -H (alanine), broad m, (3H), δ 4.18 ppm; CH₂ (benzyl), s, (2H), δ 4.49 ppm; α -CH₂ (allyl), d, (2H), δ 4.61 ppm; γ -CH₂ (allyl), t, (2H), δ 5.27 ppm; β -CH (allyl), m, (1H), δ 5.92 ppm; arom. o,m-protons, d, (2H), δ 6.50 ppm; m-proton, s, (1H), δ 7.16 ppm. Elem. Anal.: calcd. for C₂₃H₃₃N₃O₈: (479.54), C 57.61, H 6.94, N 8.76; found: C 57.0, H 7.0, N 8.9; Mass: 480 (M+1)⁺.

Dma-Ala-Ala-OMe, N-2,4-dimethoxybenzyl-L-alanyl-L-alanyl-L-alanine methyl ester (**V**). The method of Kunz et al. (23,24) was used to remove the Aloc function. The foregoing protected tripeptide ester (**IV**, 0.632 g, 1.32 mmoles) was dissolved in tetrahydrofuran (10 ml) and the vessel was flushed with argon. Tetrakis-(triphenylphosphan)-palladium (150 mg, 0.13 mmoles) was added

and dimedon (1.4 g, 10 mmoles) was added as a nucleophile; the mixture was left for about 30 min and then dried *in vacuo*. The residue was taken up in ethyl acetate and extracted with aqueous acetic acid solution. The pH of the aqueous extract (3.4) was then raised to 10 and the product extracted with diethylether. The dried extract left 0.29 g (56 %) of a white solid. $^1\text{H-NMR}$ (CDCl_3): CH_3 (alanine), broad m, (9H), δ 1.35 ppm; α -H (N-terminal) q, (1H), δ 3.23 ppm; CH_2 (benzyl), d, (2H), δ 3.63 ppm; CH_3 (ester), s, (3H), δ 3.72 ppm; CH_3 (methoxy), s, (3H), δ 3.80 ppm; CH_3 (methoxy), s, (3H), δ 3.82 ppm; α -H (alanine), broad m, (2H), δ 4.51 ppm; arom. o,m-protons, d, (2H), δ 6.41 ppm; m-proton, s, (1H), δ 7.08 ppm.

From the water layers another compound, also containing alanine and the dimethoxybenzyl moiety, presumably zwitterionic $^+\text{H}_2\text{-Dma-Ala-Ala-O}^-$ (0.21 g, 41 %) was isolated; it is significant that the corrected yield for the condensation is thus near quantitative.

Aloc-Ala, Allyloxycarbonyl-L-alanine (VI). Protected alanine lacking the additional benzyl moiety was obtained from alanine (3.30 g, 37 mmoles) using the same procedure as given for Aloc-Dma. The compound did not crystallize, the yield amounted to 5.31 g (83 %). $^1\text{H-NMR}$ (CDCl_3): CH_3 (alanine), d, (3H), δ 1.47 ppm; α -H (alanine), q, (1H), δ 4.36 ppm; α - CH_2 (allyl), d, (2H), δ 4.60 ppm; γ - CH_2 (allyl), t, (2H), δ 5.29 ppm; β -CH (allyl), m, (1H), δ 5.93 ppm; OH (carboxyl), s, (1H), δ 8.97 ppm.

Aloc-Ala-OSu, Allyloxycarbonyl-L-alanine succinimidyl ester (VII). The foregoing product (35 mmoles, 6.06 g) was dissolved in acetonitrile (70 ml). 1-hydroxysuccinimide (4.37 g, 38 mmoles) was added and the clear solution obtained was cooled with ice. Dicyclohexylcarbodiimide (36 mmoles, 4.72 g) was added when the solution reached the temperature of the bath, the vessel was kept at 0 °C for 1 h and then left in the refrigerator during the night. Dicyclohexylurea (7.93 g, 98 %) precipitated out. Following filtration, the filtrate was evaporated to leave 9.28 g of a solid. Recrystallization of the crude material from isopropyl alcohol afforded 8.12 g (86 %). $^1\text{H-NMR}$ (CDCl_3): CH_3 (alanine), d, (3H), δ 1.60 ppm; $\text{CH}_2\text{-CH}_2$ (succinimido), s, (4H), δ 2.83 ppm; α - CH_2 (allyl), d, (2H), δ 4.60; α -H

(alanine), q, (1H), δ 4.80; γ -CH₂ (allyl), t, (2H), δ 5.29 ppm; β -CH (allyl), m, (1H), δ 5.95 ppm.

Aloc-Ala-Ala-OMe, Allyloxycarbonyl-L-alanyl-L-alanine methyl ester (VIII). The foregoing product (3.70 mmoles, 1.00 g) and alanine methyl ester hydrochloride (3.70 mmoles, 0.52 g) were dissolved in dimethylformamide (7 ml) and then triethylamine (3.7 mmoles, 0.518 ml) was added. Triethylammonium hydrochloride precipitated and was removed by filtration after a reaction period of 30 min. The filtrate was concentrated, and diluted with water; the separated apolar phase was taken up in chloroform and extracted with water, the crude extract was processed as described above for Aloc-Dma-Ala-Ala-OMe (water layers were reextracted with chloroform). The combined chloroform extracts were dried with sodium sulphate and evaporated to give a solid, which was recrystallized from ethyl acetate, yield 0.50 g (52 %). ¹H-NMR (CDCl₃): CH₃ (alanine), d, (6H), δ 1.44 ppm; CH₃ (ester), s, (3H), δ 3.78 ppm; α -H (alanine), broad m, (2H), δ 4.34 ppm; α -CH₂ (allyl), d, (2H), δ 4.59; γ -CH₂ (allyl), t, (2H), δ 5.29 ppm; β -CH (allyl), m, (1H), δ 5.92 ppm; NH, s (1H), δ 6.58 ppm; NH, s (1H), δ 6.64 ppm.

Aloc-Ala-Ala-N₂H₃, Allyloxycarbonyl-L-alanyl-L-alanine hydrazide (IX). The protected dipeptide ester Aloc-Ala-Ala-OMe (250 mg, 0.97 mmoles) was dissolved in methanol (15 ml) with gentle heating and hydrazine hydrate (0.14 ml, 2.91 mmoles) was added. At room temperature, the solution deposited part of the desired product in about 16 hrs. The crystallization was completed by concentrating the reaction mixture and subsequent cooling; 245 mg (98 %) were obtained.

Aloc-Ala-Ala-Dma-Ala-Ala-OMe, Allyloxycarbonyl-L-alanyl-L-alanyl-N-2,4-dimethoxybenzyl-L-alanyl-L-alanyl-L-alanine methyl ester (X). The foregoing product, Aloc-Ala-Ala-N₂H₃ (64.5 mg, 250 μ mol) was dissolved in dimethylformamide (2.5 ml) by gentle heating and the solution was then rapidly cooled in a previously prepared cooling bath (-15 °C). At this (internal) temperature, hydrochloric acid in ethyl acetate (0.7 mmole, 0.28 ml, 2.5 M) and *tert*-butyl nitrite were added in the given order. During the course of 15 min the amount of detectable hydrazide [Fe³⁺/KFe(CN)₆-test] decreased to

zero, indicating the absence of starting material. All of the hydrochloric acid present was neutralized using diisopropylethylamine (0.70 moles, 0.118 ml), and the amino component Dma-Ala-Ala-OMe was added. The mixture was left in the refrigerator for 16 hrs. Following the isolation indicated for Alloc-Dma-Ala-Ala-OMe, only 22 mg (3.5 μ mol, 14 %) was isolated; m.p. 140 °C (decomposing). The product dissolved well in methanol and ethanol, and almost as well in diethylether and in ethylacetate.

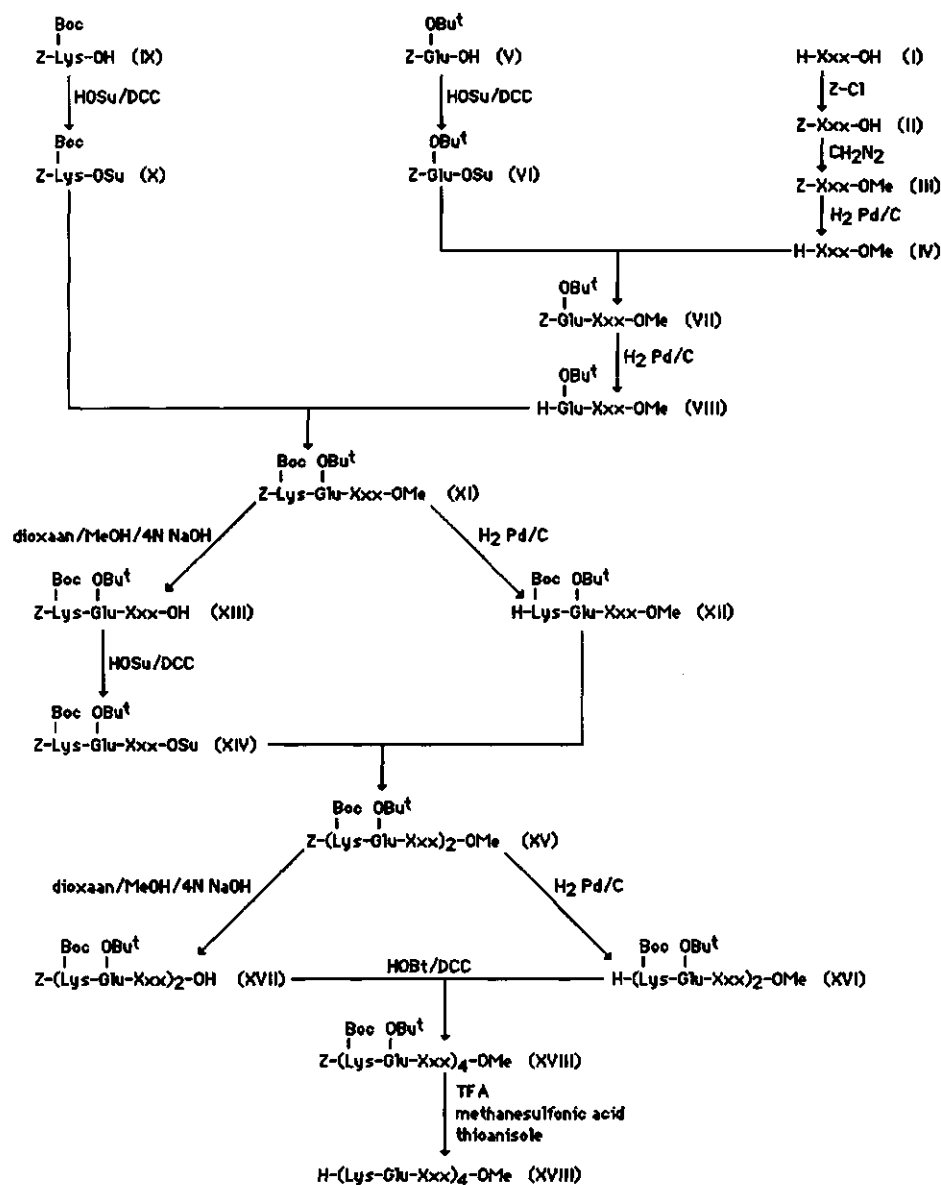
Synthesis of H-[Lys-Glu-Xxx]_n-OMe (Xxx = Dmg, Sar, or Gly).

The outline of the synthesis of oligo(Lys-Glu-Xxx) is given in Scheme 2. The symbol Xxx in this scheme stands for Gly, Dmg, or Sar. Only a few experiments were carried out with sarcosine and therefore there is no section on Xxx = Sar. The effect of Sarcosine will be reported in the discussion section. The compounds Z-Lys(Boc)-OH, and Z-Glu(OBu^t)-OH were obtained from Bachem, Bubendorf, Switzerland.

Oligomers with Xxx = Dmg

To prevent loss of the dimethoxybenzylresidue the methylester of Dmg was not prepared by esterification in acidic medium but rather in neutral medium by treatment of an N-protected form of the compound with diazomethane; sarcosine was esterified in the same way (scheme 2).

H-Dmg-OH, N-2,4-dimethoxybenzylglycine (I). This compound was prepared according to a modification of the procedure described by Quitt et al. (19). To a solution of 2,4-dimethoxybenzaldehyde (16.6 g, 100 mmoles) in MeOH (200 ml), glycine (7.5 g, 100 mmoles) and aqueous NaOH (100 ml, 1 N) were added. The desired product was obtained by reduction with hydrogen using palladium on charcoal (600 mg, 10 %) as a catalyst. After a reduction period of 12 h it was shown to be necessary to replace the catalyst, and another aliquot of 2,4-dimethoxybenzaldehyde (4.15 g, 25 mmoles) had to be added to complete the reaction. After another 24 h all of the glycine had



Scheme 2. The route for the preparation of oligo *H*-Lys-Glu-Xxx-OH, Xxx was represents either Dmg, Sar, or Gly.

reacted; the catalyst was removed by filtration and the filtrate was concentrated. The residue was diluted with water, acidified with a sodium hydrogen sulfate solution to give pH \approx 3 and then extracted with diethylether to remove dimethoxybenzylalcohol. The water layer was then made slightly alkaline (pH \approx 9) by the addition of sodium hydrogen carbonate, and again extracted with diethylether. After neutralization (pH \approx 6) the product was extracted into the diethylether layer. Then, the solvent was evaporated and zwitterionic **I** (21.4 g, 95 %) was obtained.

Z-Dmg-OH, N^{α} -benzyloxycarbonyl- N^{α} -2,4-dimethoxybenzylglycine (**II**). This compound was prepared by the addition of benzyloxycarbonyl chloride, (13.6 ml, 95 mmoles) to a cooled solution (0 - 5 °C) of **I** (22.5 g, 95 mmoles) in 1 N NaOH (200 ml). After the reaction was finished, the mixture was extracted with diethylether. The desired product was obtained, following acidification of the reaction mixture (pH \approx 2), by extraction with diethylether, drying of the extract and evaporization of the solvent. The resultant compound (an oil) weighed 27.3 g (80 %).

Z-Dmg-OMe, N^{α} -benzyloxycarbonyl- N^{α} -2,4-dimethoxybenzylglycine methyl ester (**III**). To a solution of **II** (18 g, 50 mmoles) in diethylether (100 ml) diazomethane was added dropwise till a small excess (ca 5 %) persisted. The excess was removed by the addition of a few drops of acetic acid. Residual acetic acid was removed from the crude reaction product by extraction with KHCO_3 (10 % in water). The desired product (16.6 g, 89 %) was obtained by evaporation of the dried ether layer.

H-Dmg-OMe, N^{α} -2,4-dimethoxybenzylglycine methyl ester (**IV**). The foregoing product (**III**, 26.1 g, 70 mmoles) was dissolved in methanol (200 ml) and subjected to neutral hydrogenation at atmospheric pressure, using palladium on charcoal (10 %), and shaking the suspension in a closed apparatus as indicated by Schwyzer and Rittel (25). When the uptake of hydrogen ceased, the progression of the deprotection reaction was monitored by thin layer chromatography. (If small amounts of the starting material were still present, the suspension was filtered and again subjected to the catalytic hydrogenation using fresh catalyst). Then the filtrate was

evaporated and to prevent ring closure the residue was directly used in the next step. The yield was very good: 16.4 g (98 %) were obtained as a chromatographically pure oil.

Z-Glu(OBu^t)-OSu, N^α-Benzyloxycarbonyl-O^γ-*tert*-butyl-L-glutamic acid succinimidyl ester (VI), and **Z-Lys(Boc)-OSu**, N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxycarbonyl-L-lysine succinimidyl ester (X). The proper N-protected amino acid derivatives (25 mmoles) were dissolved in acetonitrile (60 ml). To the solution N-hydroxysuccinimide (HOSu, 3.2 g, 27.5 mmoles) was added, the mixture was cooled (0 °C) and N,N'-dicyclohexylcarbodiimide (DCC, 5.7 g, 27.5 mmoles) was dissolved in the mixture. After about 15 min at 0 °C the reaction mixture was put in the refrigerator (4 °C) for about 3 hours and filtered to remove precipitated DCU (dicyclohexylurea). The filtrate was concentrated and the activated compound was crystallized from the proper aprotic solvent, to prevent transesterification. The total yield amounted to ca 80 % in each case.

Z-Glu(OBu^t)-Dmg-OMe, N^α-benzyloxycarbonyl-O^γ-*tert*-butyl-L-glutamyl-N^α-2,4-dimethoxybenzylglycine methyl ester (VII). The activated glutamic acid derivative VI (12 g, 50 mmoles) was dissolved in DMF (100 ml). To this solution H-Dmg-OMe (14.3 g, 60 mmoles) was added. Then the reaction mixture was allowed to stand at room temperature for three hours and subsequently was evaporated *in vacuo*. The residue was dissolved in ethylacetate and the solution was extracted with water, sodium hydrogen sulfate, water, sodium hydrogen carbonate, water, and saturated sodium chloride. Following evaporation of the dried (Na₂SO₄) extract, VII (27.6 g, 99 %) was obtained.

H-Glu(OBu^t)-Dmg-OMe, O^γ-*tert*-butyl-L-glutamyl-N^α-2,4-dimethoxybenzylglycine methyl ester (VIII). Hydrogenation of (VII) was readily performed in MeOH, using palladium on charcoal (10 %) as the catalyst, but on checking the reaction progression with TLC (thin layer chromatography) no free amine could be detected in the reaction mixture. Moreover, the compound formed by the hydrogenation reaction did not react with an active ester. Even hydrogenation at a controlled pH (using a pH-stat set at pH = 5 and

distributing HCl) did not give rise to the formation of a hydrochloride salt.

Oligomers with Xxx = Gly

H-Gly-OMe.HCl, Glycine methyl ester monohydrochloride (IV). The general method of Brenner (26) was used to prepare compound IV. The product (20 g) was recrystallised from methanol (50 ml), since it was shown chromatographically that it still contained some glycine.

Z-Glu(OBu^t)-Gly-OMe, N^α-Benzyloxycarbonyl-O^γ-*tert*-butyl-L-glutamylglycine methyl ester (VII). To a solution of the succinimidyl ester VI (8.7 g, 20 mmoles) in dimethylformamide (175 ml) compound IV (2.5 g, 22 mmoles) was added. The reaction was initiated by the addition of diisopropylethylamine as a sterically hindered, non-nucleophilic base. The reaction mixture was set aside for three hours at room temperature, and subsequently evaporated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was extracted with water, sodium hydrogen sulfate, water, sodium hydrogen carbonate, water and saturated sodium chloride solution. Following evaporation of the dried (Na₂SO₄) extract, compound VII (7.9 g, 97 %) was obtained.

H-Glu(OBu^t)-Gly-OMe, O^γ-*tert*-butyl-L-glutamylglycine methyl ester (VIII). The foregoing product (VII) was subjected to neutral hydrogenation using palladium on charcoal (10 %) at atmospheric pressure as described for the preparation of H-Dmg-OMe (Xxx = Dmg). Finally the filtrate was evaporated and to prevent ring closure the residue was directly used in the next step. Typically, from VII (10.2 g, 25 mmoles) in MeOH (150 ml), the free ester (6.8 g, 99 %) was obtained.

Z-Lys(Boc)-Glu(OBu^t)-Gly-OMe, N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxycarbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycine methyl ester (XI). Using the procedure described above for the the synthesis of VII, compound XI was prepared from the succinimidyl ester X (9.5 g, 20 mmoles) and the dipeptide derivative VIII (6.0 g, 22 mmoles) using DMF as the solvent. Yield 11.8 g (95 %).

H-Lys(Boc)-Glu(OBu^t)-Gly-OMe, N^ε-*tert*-butyloxycarbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycine methyl ester (**XII**). The free tripeptide ester was obtained by hydrogenolysis as described for H-Dmg-OMe. The hydrogenation of **XI** was much slower however and the catalyst had to be replaced more often as compared to the hydrogenation of the dipeptide ester **VII**. Nevertheless 7.0 g (11 mmoles) of compound **XI** afforded 5.3 g (95 %) of the free peptide ester **XII**.

Z-Lys(Boc)-Glu(OBu^t)-Gly-OH, N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxycarbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycine (**XIII**). Compound **XI** (7.3 g, 11.5 mmoles) was dissolved in 66.5 ml of a mixture of dioxan : methanol = 14 : 5 and hydrolysed by the addition of 4 N sodium hydroxide (3.5 ml, 14 mmoles). The hydrolysis reached completion during mixing. The solution was then neutralized and evaporated to remove the organic solvents. The residue was diluted with water and acidified with citric acid, whereupon an oil precipitated. The heavy layer was extracted into ethyl acetate and washed with water until neutral (pH = 5). To remove traces of neutral impurities, the acid was again dissolved in sodium hydrogen carbonate solution, the water phase again extracted with ethyl acetate and acidified. The product was isolated as indicated above. The yield amounted to 6.5 g (91 %).

Z-Lys(Boc)-Glu(OBu^t)-Gly-OSu, N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxycarbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycine succinimidyl ester (**XIV**). This succinimide ester was obtained from (**XIII**) using the general procedure as exemplified above in the preparation of the active esters **VI** and **X**. In this case we had to use isopropylalcohol for recrystallization. Although an alcohol was used no transesterification took place. Compound **XIII**, 6.2 g (10 mmoles) yielded 6.5 g (90 %) of pure **XIV**.

Z-[Lys(Boc)-Glu(OBu^t)-Gly]₂-OMe, N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxy-carbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycyl-N^ε-*tert*-butyloxy-carbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycine methyl ester (**XV**). This hexapeptide derivative was prepared by acylation of compound **XII** with the active ester **XIV** as described above for the synthesis of **VII**. On evaporation of the reaction mixture the concentrated residue could not be dissolved in a two phase mixture

of ethyl acetate and water unless a small quantity of butanol was added. The acylation of **VII** (4.7 g, 9.4 mmoles) with **XIV** (6.3 g, 8.7 mmoles) in DMF (90 ml) afforded **XV** (6.6 g, 70%), using the extraction procedure for a neutral compound. The protected hexapeptide could be isolated by trituration of small quantities of the organic phase with diethylether.

H-[Lys(Boc)-Glu(OBu^t)-Gly]₂-OMe, N^ε-*tert*-butyloxycarbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycyl-N^ε-*tert*-butyloxycarbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycine methyl ester (**XVI**). This product was prepared by the hydrogenation of **XV**. In this case we could not use the closed apparatus that had been used formerly, since the hydrogenation reaction was very slow. Compound **XV** (3.3 g, 3.0 mmoles) was therefore dissolved in a large quantity of MeOH (250 ml) and H₂ was bubbled through the suspension as it was being stirred. In the course of two days, during which the catalyst had to be replaced four times, the reaction reached completion; the yield of compound **XVI** amounted to 2.3 g (80 %).

Z-[Lys(Boc)-Glu(OBu^t)-Gly]₂-OH, N^α-Benzyloxycarbonyl-N^ε-*tert*-butyloxy-carbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycyl-N^ε-*tert*-butyloxy-carbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycine (**XVII**). This compound was prepared by hydrolysis of the fully protected hexapeptide ester **XV** as described above for the synthesis of **XIII**. Thus compound **XV** (3.3 g, 3.0 mmoles) was dissolved in a mixture of dioxane and methanol (14 : 5) to which 2 ml DMF was added. Hydrolysis was obtained by the addition of 4 N NaOH (1 ml, 4 mmoles). Dissolution and extraction of the product could be enhanced by the addition of a few drops of butanol to the ethyl acetate (the extraction solvent). The yield of the reaction was 2.9 g (90 %).

Z-[Lys(Boc)-Glu(OBu^t)-Gly]₄-OMe, N^α-Benzyloxycarbonyl-tri[-N^ε-*tert*-butyloxy-carbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycyl]-N^ε-*tert*-butyloxycarbonyl-L-lysyl-O^γ-*tert*-butyl-L-glutamylglycine (**XVIII**). This tetrameric tripeptide derivative was obtained by dissolution of amine **XVI** (2.3 g, 2.4 mmoles) and acylation with the peptide acid **XVII** (2.6 g, 2.4 mmoles) in DMF (50 ml). Then hydroxybenzotriazol (0.4 g, 2.7 mmoles) was added and the solution was cooled on ice.

Dicyclohexylcarbodiimide (0.56 g, 2.7 mmole) was dissolved in the mixture and the reaction vessel was stored overnight at 4 °C. The next day the product was obtained by dilution with water, drying and trituration with ethylacetate. The desired compound (3.2 g) was obtained in 66 % yield.

H-[Lys-Glu-Gly]₄-OMe, tetra[L-lysyl-L-glutamylglycyl] methyl ester (**XIX**). The benzyl and tert-butyl derived protecting groups were removed by acidolysis by dissolution of the fully protected dodecapeptide derivative ester **XVIII** (3.2 g, 1.58 mmole) in a mixture of TFA (trifluoro acetic acid, 30 ml), thioanisole (3 ml) and methanesulfonic acid (3 ml). After 2 hours the reaction mixture was added dropwise to a stirred volume of 500 ml of diethylether whereupon the oligopeptide precipitated readily. After filtration the crystals were washed twice with diethylether. The anions present in the product were exchanged for acetate ions by dissolution of the product in 0.1 N AcOH (acetic acid) and filtration of the solution through a weak basic ion exchanger in the acetate cycle. The filtrate was lyophilized and the dodecapeptide ester (2.3 g) was obtained as an acetate in about 92 % yield.

Characterization of H-[Lys-Glu-Gly]₄-OMe

Thin layer chromatography could not be used for verification of the purity of the obtained compound since the R_f values in all elution systems were virtually 0.

It was indicated by ¹H-NMR spectra that in the synthesized compound the amino acids Lys, Glu, and Gly occurred in a ratio of 1 : 1 : 1. It was also evident from the recorded spectra that the oligopeptide contained at least two molecules of AcOH per oligopeptide molecule. Also the methyl ester was shown to be present in the synthesized dodecapeptide.

The prepared oligopeptide was also subjected to an amino acid analysis. The results of this analysis indicated a relative occurrence of the amino acids Lys, Glu, and Gly of 0.57 : 0.95 : 1.00. This implies that the amino acids Glu and Gly appear equimolar in the synthesized compound. The method used for the determination of the

concentrations of amino acids in the hydrolyzed sample is not reliable for determining the concentration of Lys. On the basis of this result we can not conclude that Lys appears less than Glu or Gly in the synthesized compound.

The molecular weight of the synthesized product could not be determined by the mass spectrometers that were available to us because the molecular weight was too high. Instead the molecular weight was measured by means of vapour pressure osmometry on the aqueous solution using a Knauer vapor osmometer. With this method a number averaged molecular weight of 266.7 g/mol was obtained. Assuming that each oligopeptide molecule in solution splits off five AcO^- ions, a molecular weight of $(5 + 1) \times 266.7 = 1600$ was calculated for the oligopeptide. On the basis of the structural formula (including five AcOH molecules) one calculates a molecular weight of 1588 which corresponds nicely to the measured molecular weight of 1600. The assumption that the compound contains five AcOH molecules per oligopeptide molecule is very reasonable since one oligopeptide molecule contains five free amino groups and the product was lyophilized from 0.1 N AcOH.

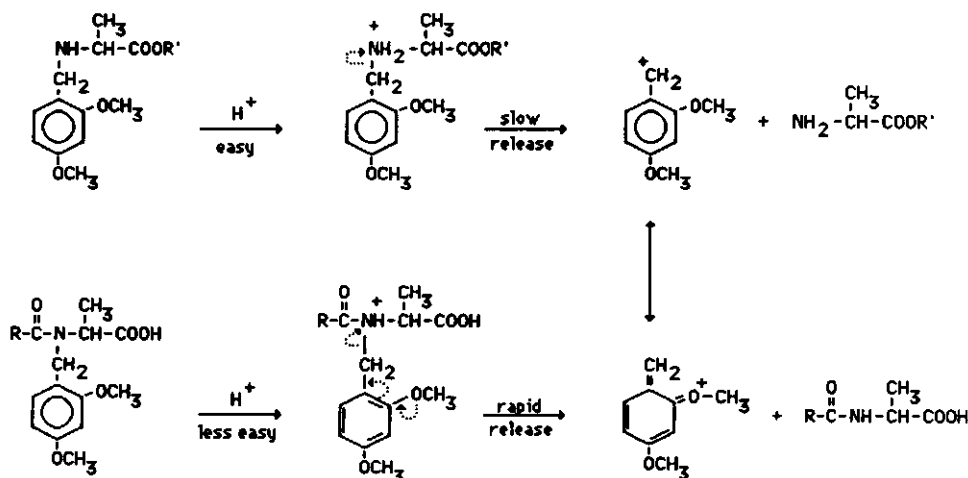
Discussion

It is evident from the good solubility of the model compound Aloc-Ala-Ala-Dma-Ala-Ala-OMe that the 2,4-dimethoxybenzyl function is capable of disturbing the regularity of the molecular backbone of tetra-L-alanyl-L-alanine methyl ester. The latter compound is hardly soluble in any solvent (10) whereas the pentapeptide with only one 2,4-dimethoxybenzyl function dissolves very well in methanol and ethanol.

It is surprising to note that the reaction in which the allyloxycarbonyl function is attached to Dma is performed in almost the same yield as the reaction in which this function is attached to Ala (81 % versus 83 %). This precludes any care for a hampered access to the amino group in Dma. The reaction in which the Aloc function is removed from Aloc-Dma-Ala-Ala-OMe is performed in a relatively low yield (56 %). Also the last reaction, in which the

pentapeptide derivative is formed by means of an azide coupling gives a discouraging low yield (14 %).

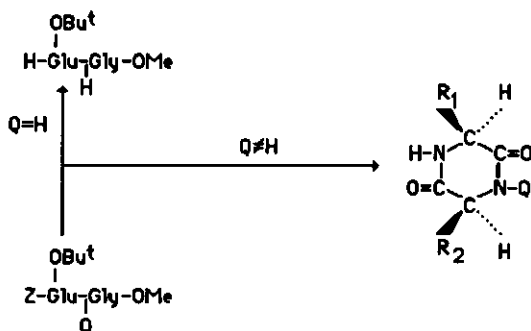
The aim of this endeavour was to arrive at the construction of a synthon enabling the preparation of the hitherto elusive peptide containing about 20 (or more) L-alanine residues. By optimizing the reaction procedure one should be able to reach a higher yield in the final coupling reaction. It now seems that application of the 2-(methylsulfonyl)ethyloxycarbonyl group (Msc) for amino protection (27) and NH-NH-Trt as a carboxyl protecting function, which can be converted into an activation, would have been the more promising route. Both functions improve the solubility and are easily removed under conditions compatible with the maintenance of the chirality and of the dimethoxybenzyl group (base for Msc and 80 % AcOH, 20 min for Trt). Care should be taken when a Trt function is removed by treatment with acid when Dma is not the N terminal amino acid. In this case the 2,4-dimethoxybenzyl function is more acid labile as illustrated in Scheme 3.



Scheme 3. The N-2,4-dimethoxybenzyl group is less labile in acidic media if the amino group must leave as a stronger Lewis base.

The synthesis of the tripeptide derivative Z-Lys(Boc)-Glu(OBu^t)-Dmg-OMe was not accomplished due to the immediate ring closure

of H-Glu(OBu^t)-Dmg-OMe on hydrogenation of the dipeptide ester Z-Glu(OBu^t)-Dmg-OMe. No ninhydrine positive compound(s) were formed, and the product obtained was resistant to acylation with an active ester. The only explanation for these phenomena is that hydrogenation introduced an immediate formation of a diketopiperazine (see scheme 4). Since in the formation of the



Scheme 4. Diketopiperazine formation on hydrogenation of the dipeptide derivative Z-Glu(OBu^t)-Xxx-OMe (Xxx = Dmg, Sar, and Gly). In this scheme, Q can be 2,4-dimethoxybenzyl, methyl, and H. Only in the situation that Q = H is the ketopiperazine formed slowly enough to allow for further acylation.

diketopiperazine the methyl ester must have been the leaving group, it was decided to synthesize the compound Z-Glu(OBu^t)-Dmg-OH according to the procedure described above for the synthesis of Z-Glu(OBu^t)-Dmg-OMe. Hydrogenolysis of the acid led once more to ninhydrine negative compounds. It is by no means clear how the supposed aminolysis of a *carboxylic* group takes place.

To exclude the possibility that a strong steric effect of the 2,4-dimethoxybenzyl group on the conformation of the molecule would cause the free amino function to reside in very close proximity to the terminal carboxylic group we inserted sarcosine (N-methylglycine) in the position of the Dmg residue. The dipeptide derivative Z-Glu(OBu^t)-Sar-OMe was readily prepared according to the procedure described above for the synthesis of VII. But on hydrogenation of this compound no ninhydrine positive compounds were found, most

likely because a diketopiperazine was formed again. A way around this diketopiperazine formation might be the synthesis of the tetrapeptide derivative Z-Lys(Boc)-Glu(OBu^t)-Dmg-Gly-OMe. In this case a tripeptide instead of a dipeptide has to be hydrogenated and it is very unlikely that a diketopiperazine will be then formed.

In the preparation of Z-Lys(Boc)-Glu(OBu^t)-Gly-OMe no problems were encountered and every reaction step was performed in a relatively high yield. However, when we wanted to double the dodecapeptide derivative **XVIII** (scheme 2) the solubility of this compound was too low to be able to remove the Z function by means of hydrogenation.

From the results of the characterization of H-[Lys-Glu-Gly]₄-OMe and from the results of the reaction progression measurements by means of thin layer chromatography during the synthesis, we conclude that the final product is indeed the oligopeptide.

Conclusions

(1) The preparation of peptide chains consisting of identical aminoacyl or peptidyl residues as building blocks leads relatively soon to aggregating oligomers, certainly if their side chains have an apolar character or tend to form hydrogen bonds.

(2) It has been shown that the application of a large steric group (2,4-dimethoxybenzyl) reversibly attached to 1 out of 5 alanine nitrogens greatly enhances the solubility of the pentapeptide. In the presence of such a large function the formation of *intermolecular* hydrogen bonds is much more difficult, resulting in a much better solubility.

(3) The introduction of structural modifications in the peptide backbone appears to be connected with large variations in the structural properties and in the reactivity of the constructed modified peptide chain. Crystallinity decreased, but the solubility increased. A clear disadvantage is the instability of the free base arising on the neutral hydrogenation of compounds of the type Z-Glu(OBu^t)-Xxx-OMe (Xxx = Dma or Dmg). Diketopiperazine formation was immediate.

(4) The synthesis of oligo-(Lys-Glu-Gly) could not be extended beyond the dodeca-peptide derivative, due to the low solubility of the fully protected peptide derivative.

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Appendix The physical constants of the reaction products in the synthesis of the model compound Aloc-Ala-Ala-Dma-Ala-Ala-OMe and of the oligopeptide Lys-Glu-Xxx (Xxx can be Dmg, Sar, or Gly). The abbreviations have the following meanings: m.p. = melting point, R_f = retention factor in TLC for the solvent systems: (A) Chloroform - methanol = 9 : 1, (B) chloroform - methanol - acetic acid = 95 : 20 : 3, (C) n-butanol - acetic acid - water = 4 : 1 : 1.

Name	m.p. (°C)	$[\alpha]_D^{22}$ (°)	solvent	R _{fA}	R _{fB}	R _{fC}
SCHEME 1.						
Dma (I)	215-216	1.3	water			
Aloc-OSu (II)	≈ 25					
Aloc-Dma (III)					0.78	
Aloc-Dma-Ala-Ala-OMe (IV)	116-119	-35.5	EtOAc			
Dma-Ala-Ala-OMe (V)	119-122				0.32	
Aloc-Ala (VI)					0.67	
Aloc-Ala-ONSu (VII)	99-103	42.9	acetonitril			
Aloc-Ala-Ala-OMe (VIII)	191-192	-50.1	EtOAc			
Aloc-Ala-Ala-N ₂ H ₃ (IX)	225-230	-15.6	DMF			0.36
Aloc-Ala-Ala-Dma-Ala-Ala-OMe (X)	140					
SCHEME 2.						
H-Dmg-OH (I)						0.46
Z-Dmg-OH (II)						0.78
Z-Dmg-OMe (III)				0.18		0.80
H-Dmg-OMe (IV)						0.44
Z-Glu(OBu ^t)-Dmg-OMe (VII)				0.79		
H-Sar-OH (I)						0.14
H-Sar-OMe. HCl (IV)						0.24
Z-Glu(OBu ^t)-Sar-OMe (VII)				0.91		
Z-Glu(OBu ^t)-Sar-OH						0.73
H-Gly-OH (I)						0.17
H-Gly-OMe.HCl (IV)	168 - 169					0.30
Z-Glu(OBu ^t)-OSu (VI)	103 - 104	-33	MeOH	0.70		
Z-Glu(OBu ^t)-Gly-OMe (VII)				0.74		
H-Glu(OBu ^t)-Gly-OMe (VIII)	62 - 64	-16.7	MeOH	0.27		
Z-Lys(Boc)-OSu (X)	95 - 98	-20.8	MeOH	0.72		
Z-Lys(Boc)-Glu(OBu ^t)-Gly-OMe (XI)	97 - 102	-24.7	MeOH	0.78		0.86
H-Lys(Boc)-Glu(OBu ^t)-Gly-OMe (XII)	< 60	-9.2	MeOH			0.32
Z-Lys(Boc)-Glu(OBu ^t)-Gly-OH (XIII)	73 - 75	-21.0	MeOH	0.11		0.79
Z-Lys(Boc)-Glu(OBu ^t)-Gly-OSu (XIV)	71 - 74	-27.1	MeOH	0.69		
Z-[Lys(Boc)-Glu(OBu ^t)-Gly] ₂ -OMe (XV)	167 - 178	-21.8	MeOH	0.58		
H-[Lys(Boc)-Glu(OBu ^t)-Gly] ₂ -OMe (XVI)	94 - 97	-20.7	MeOH	0.15		
Z-[Lys(Boc)-Glu(OBu ^t)-Gly] ₂ -OH (XVII)	97 - 104	-15.8	MeOH	0		0.64
Z-[Lys(Boc)-Glu(OBu ^t)-Gly] ₄ -OMe (XVIII)						0.84
H-[Lys-Glu-Gly] ₄ -OMe (XIX)	155-162	-19.6	MeOH			

Chapter 4

The adsorption of polyampholytes on negatively and positively charged polystyrene latices

Abstract

The adsorption of two polyampholytes (a random copolymer of D-glutamic acid and D-lysine, and a well defined tetramer of L-lysyl-L-glutamyl-glycine) onto positively and negatively charged latex was studied as a function of the pH and the ionic strength. The adsorbed amount proved to be almost independent of the salt concentration. The pH dependence was found to follow the same trends on negatively charged and positively charged latex. At low pH, where the polyampholytes are positively charged, a high adsorbed amount was found irrespective of the sign of the surface charge. At high pH, where the macromolecules are negatively charged, no adsorption was measured, not even with the positive latex. This is probably due to the very good solubility of the polyampholytes at this pH. Electrophoretic mobility measurements revealed that even at very low concentrations of polyampholyte, charge reversal of the particles occurred.

Introduction

The interfacial behaviour of neutral, flexible polymers is governed by three main variables: molecular weight, solvent quality, and energy of interaction between the monomer units and the substrate. The adsorption of this class of compounds is therefore relatively simple and extensive research efforts over the last 25 years have led to a well developed theoretical picture (1). In contrast, the adsorption of charged macromolecules depends on at least three more variables and is, due to this complexity, less well understood. However, some progress has been made recently. From a

fundamental point of view, the simplest charged macromolecule is the strong polyelectrolyte. Several experimental (2-4) and theoretical studies (5) have been devoted to the adsorption of strong polyelectrolytes. The basic effects of electrostatic interactions and the concomitant variables like salt concentration and surface charge density are becoming clear. A logical extension is to allow for dissociation equilibria, i.e., weak polyelectrolytes. Evers et al. (6), and more recently Böhmer et al. (7), developed extensions of the polymer adsorption theory of Scheutjens and Fleer (8,9) to this end, and we carried out a detailed experimental study of such a system (10). Proteins are also charged macromolecules, but their adsorption behaviour, although well-documented empirically, is not at all understood at the same level of detail. Greater understanding is an important goal in view of the vital role of these molecules in numerous biological and biotechnological processes.

A protein molecule is a very complex macromolecule consisting of many (up to twenty) different amino acids. Depending on the pH, some of these amino acids can be positively charged and some are negatively charged. The adsorption of proteins onto different adsorbents has been studied in detail and also much is known of the behaviour of specific peptides in water (11-13). However, there have been no attempts to develop a model capable of describing the adsorption of such complex molecules. In several experimental studies a synthetic poly(amino acid) like polylysine (14,15) was chosen as a plausible model for a protein. However, such homopolymeric peptides do not possess the essential property of an isoionic point. We therefore decided to study polyampholytes since these would serve better as models for the adsorption behaviour of proteins. Polyampholytes have the advantage over weak polyelectrolytes that they contain both acidic and basic groups but are not as complex as proteins.

We decided to study the adsorption of two polyampholytes, both containing lysine and glutamic acid, on positively and negatively charged polystyrene latex. Essential variables are the ionic strength and the pH. At pH below the pK of the acidic group and above the pK of the basic group one should be able to compare the adsorption of

the polyampholyte with that of simple polyelectrolytes because in these pH regions one type of charge dominates. At intermediate pH values the dependence of the adsorption on the electrostatic interactions will be more complex because here the ampholyte molecule contains both positive and negative charges. One may expect that in this pH region the behaviour of polyampholytes would show some trends that can be compared with those of a more complex molecule like a protein.

Materials and methods

Latices

For the adsorption experiments we used a negatively and a highly positively charged monodisperse polystyrene latex. The properties of the two latices, designated as PS+ en PS-, respectively, are given in Table 1. The preparation of the positively charge latex has been described elsewhere (16). The negatively charged latex was

Table 1. *The characteristics of the latices used in the adsorption studies. The particle diameters were measured by electron microscopy and the surface charge σ_0 was determined by conductometric titration.*

latex	particle diameter (nm)	specific surface area (m ² /g)	σ_0 (mC/m ²)
PS+	785	7.28	90
PS-	650	8.79	-25

prepared according to the method of Goodwin et al. (17). It has been shown (18) that the surface charge of these latices, when freshly prepared, increases with ionic strength. However, once the latex particles have been subjected to a high ionic strength medium, the surface charge becomes independent of the salt concentration. In order to obtain reproducible surface properties we followed the recommended procedure (18) to add 0.1 M KCl to the negatively

charged latex, and leave it at this ionic strength for 24 hrs. Then most of the ions were removed by dialysis, followed by the mixed bed ion exchange procedure as described by Van den Hul et al. (19) to displace the remaining salt ions and to replace all the counter ions by H^+ .

Polyampholytes

Two different polyampholytes were used. One was a random copolymer of 60 mole % D-glutamic acid and 40 mole % D-lysine.HBr, purchased from Sigma chemical company. This polymer has a peptide backbone in which each α -carbon carries either a $-(CH_2)_2COOH$ or a $-(CH_2)_4NH_3^+Br^-$ side group, in the overall ratio 3 : 2. The molecular weight of this polymer reported by the manufacturer is 24,000 as determined by viscometry and 20,000 as measured by low angle laser light scattering. Using the well known Henderson-Hasselbalch equation we calculated for this random copolymer an isoelectric point (i.i.p.) of 4.9. For these calculations we assumed a pK value of 4.6 for the γ -carboxylic group of glutamic acid and a value of 9.6 for the pK of the ϵ -amino-function of lysine. The charge of this polyampholyte as a function of the pH (as calculated with the Henderson-Hasselbalch equation) is shown in Figure 4, below. The polymer was found to be insoluble in the pH range 4.1 - 5.7, as shown by turbidity measurements.

The other macromolecule was a well defined tetramer of L-lysyl-L-glutamyl-glycine: $(Lys-Glu-Gly)_4$. Its structural formula can be represented as:

$H-(NH-CHR_1-CO-NH-CHR_2-CO-NH-CH_2-CO)_4-OCH_3$, with $R_1 = -(CH_2)_4NH_4^+Ac^-$ and $R_2 = -(CH_2)_2COOH$. The synthesis of this molecule has been reported elsewhere (20). As a result of the synthesis all the ϵ -amino functions are in the acetate form, and the terminal carboxylic group is methylated. A glycine residue had to be inserted because otherwise racemisation of the α C-atom of glutamic acid would have occurred during the synthesis. Using the same pK values as above and a value of 7.8 for the α -amino group of lysine we calculated an i.i.p. of 8.4 for this macromolecule. This oligopeptide was soluble at all pH values.

Adsorption isotherms

Measurements with the SPOS (single particle optical sizer) as described by Pelssers (21) revealed that it was necessary to sonicate the latex suspension for 20 min. to obtain only singlet particles.

Prior to pipetting the latex and polyampholyte solution into a 2 ml polystyrene tube they were adjusted to the required ionic strength and pH. In a typical experiment 0.7 ml latex suspension (having a area of ca 0.2 m²) was pipetted into the tube first, followed by the desired volume of a KCl solution of the same ionic strength and pH. Finally, the required volume of the polyampholyte solution was added, giving a total volume of 1 ml. The tubes were capped, vigorously shaken by hand, and slowly rotated end-over-end. After 24 hrs. the pH was measured and an almost clear supernatant was obtained by centrifuging for 30 min. in a table centrifuge at 5600 rpm (5000 g). The residual concentration of polyampholyte in the supernatant was measured and the adsorbed amount Γ (mg/m²) was calculated from the mass balance.

Determination of the residual concentration of polyampholyte

Concentrations of polyampholyte were measured by the method described by Samejima et al. (22,23). This procedure is based on the fact that ninhydrine condensates with aldehydes and primary amines to yield highly fluorescent ternary products. In the standard procedure a fluorescent complex was obtained by mixing 2.0 ml of a 0.2 M phosphate buffer (pH = 7), 0.2 ml of an aqueous solution of ninhydrine (50 mM), 0.1 ml of phenylacetaldehyde in ethanol (10 mM), and 0.1 ml sample solution. This mixture was incubated in a waterbath at 60 °C for 60 min. A Perkin Elmer LS-5 luminescence spectrometer was used to measure the fluorescence. The excitation was carried out at 393 nm and the fluorescence was measured at 488 nm.

With this method it was possible to determine concentrations (Lys-Glu-Gly)₄ as low as 10 ppm with an accuracy of 5 %, using only a 0.1 ml sample. For measuring the concentration of the random copolymer with the same accuracy we had to double the sample volume to 0.2 ml.

Zeta potential measurements

A Malvern zetasizer II was used for obtaining the electrophoretic mobility of charged polystyrene latex particles in the presence of low concentrations of polyampholyte. The zeta potentials were calculated from the electrophoretic mobilities by means of the *Helmholtz-von Smoluchowski* equation.

Results and discussion

Adsorption isotherms of $(\text{Lys-Glu-Gly})_4$ onto negatively charged latex at $\text{pH} = 4$ and salt concentrations of 0.1 and 0.001 M, respectively, are reported in Figure 1. In this plot the adsorbed amount,

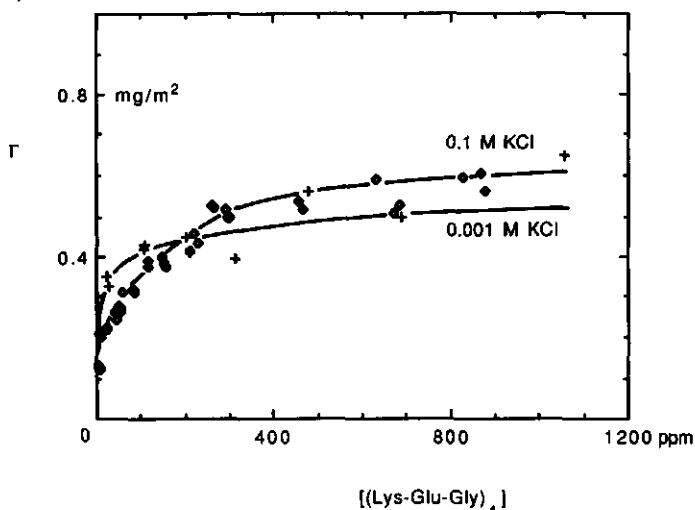


Figure 1. Adsorption isotherms of $(\text{Lys-Glu-Gly})_4$ onto negatively charged latex at $\text{pH} = 4$, in 0.001 and 0.1 M KCl.

expressed in mg/m^2 , is given as a function of the equilibrium concentration of ampholyte. It is clear from this figure that the adsorption of this molecule is not of the high affinity type as is generally found for the adsorption of homodisperse polymers. This is most likely due to the fact that the adsorbing species consists of only 12 monomers, which is not long enough to give this molecule the

characteristic adsorption behaviour of long polymers. It is also evident from Figure 1 that salt has only a minor effect on the adsorbed amount. At low equilibrium concentrations the adsorption is higher in a low ionic strength medium, whereas at higher final concentrations the reverse order is observed. This cross-over, which also showed up in the adsorption of poly(acrylic acid) onto positively charged latex, can be fully explained by the role of the electrostatic interactions (10). At low polyampholyte concentrations, the adsorbed amount is lower in high ionic strength as compared to low c_s because the potassium ions play a greater role in the compensation of the surface charge when their concentration is higher. At higher polyampholyte concentrations the adsorbed amount at high salt concentrations is higher than with low salt content because the inter- and intramolecular electrostatic repulsions are screened more efficiently.

When the pH is lower than 5 all the amino groups are fully charged and one can consider the ampholyte as a special kind of weak polyelectrolyte with a fixed (pH independent) positive charge and a variable (pH dependent) negative charge. It has been shown both theoretically (7) and experimentally (10) that the adsorption of a weak polyelectrolyte onto an oppositely charged surface hardly depends on the ionic strength in the region 0.001 - 0.1 M. The theoretical model calculations showed that the effect of the salt concentration on the adsorption of weak polyelectrolytes is small because the charge in the first layer adjusts itself in such a way that the surface charge is just compensated. Looking from the bulk solution, one then sees an almost neutral particle so that salt will hardly affect the adsorption: the adsorbed amount is mainly determined by the surface charge. Probably, the same mechanism operates in the adsorption of an ampholyte: the charge in the first layer is adapted by changing the degree of dissociation of the carboxylic groups so as to compensate the surface charge. Hence, the adsorption of this polyampholyte is independent of the salt concentration for the same reason as for weak polyelectrolytes on oppositely charged surfaces. This explanation is supported by the electrophoretic mobility measurements, which show that a slight

charge reversal occurs on adsorption of this macromolecule (see fig. 7, below). When all the carboxylic groups are dissociated and all amino functions protonated, we have at an adsorbed amount of 0.5 mg/m^2 a total net positive charge of 5 mC/m^2 due to the terminal amino group. This corresponds to an available area of 0.44 nm^2 per amino acid residue. From molecular models, assuming an essentially flat conformation, we estimated that, on an average, one amino acid residue will occupy 0.43 nm^2 . This means that an adsorbed amount of 0.5 mg/m^2 corresponds to one monolayer.

We also attempted to measure the adsorption of the oligopeptide onto the PS+ latex but the reproducibility of the results was poor. Nevertheless it was clear from these experiments that the maximum adsorbed amount on this latex at $\text{pH} = 9.5$ is about half the adsorbed amount onto the PS- latex at $\text{pH} = 4.0$ (see also fig. 3 for the results of the adsorption of the random copolymer on both latices). Probably this low adsorbed amount (ca 0.2 mg/m^2) was the reason for the very poor reproducibility of these experiments.

The effect of the pH on the adsorption of $(\text{Lys-Glu-Gly})_4$ onto negatively charged latex in 0.1 M KCl is reported in Figure 2. In this graph we have plotted the adsorbed amount as a function of the pH at equilibrium concentrations of ca 200 ppm . This final concentration is not completely sufficient to reach the plateau value of the adsorption isotherms, as is evident from Figure 1. However, this concentration was chosen because in this concentration region the most accurate measurements were possible.

When, starting at low pH, the pH is increased the adsorption increases slightly, subsequently followed by a strong decrease. The latter decrease is readily understood: when the pH increases the number of positively charged amino groups decreases and the number of negatively charged carboxylic groups increases, making it electrostatically less favourable for the molecule to adsorb onto a negatively charged surface. When the pH becomes high enough the (chemical) adsorption energy is no longer sufficient to overcome the electrostatic repulsion between the surface and the adsorbing molecule and the adsorbed amount drops to zero.

At very low pH all the carboxylic groups are uncharged and all the amino groups are positively charged, resulting in a strong electrostatic attraction between surface and adsorbing molecule, whereas at the same time there is a considerable inter- and intramolecular electrostatic repulsion. As a consequence the adsorbed molecules will be in a flat conformation. With an increase of the pH some of the carboxylic groups become deprotonated and the inter- and intramolecular electrostatic repulsions become less. Still, the electrostatic attraction between surface and adsorbing species is sufficient. In this situation the conformation of the adsorbing ampholyte molecules will become less flat and more molecules can accumulate at the interface before the surface charge is compensated. This explains the initial increase of Γ with pH.

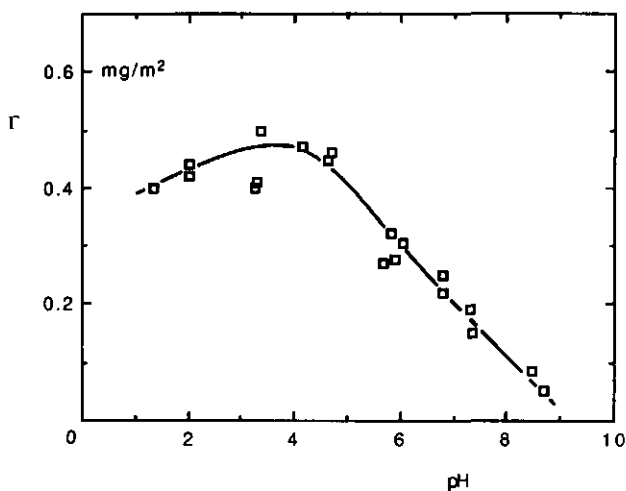


Figure 2. The adsorbed amount of $(\text{Lys-Glu-Gly})_4$ onto negatively charged latex as a function of the pH, at a final concentration of 200 ppm. The ionic strength ($\text{KCl} + \text{HCl}$ or $\text{KCl} + \text{NaOH}$) was 0.1 M.

Also for weak polyelectrolytes (on oppositely charged surfaces) a maximum in $\Gamma(\text{pH})$ has been reported, both theoretically (7) and experimentally (10). However, the mechanism is slightly different in this case because, for polycarboxylic acids, there is no fixed positive charge. The basic point here is that with increasing pH the

electrostatic component of the adsorption energy increases. This effect is, at relatively low charge densities of the polyelectrolyte, stronger than the increasing inter- and intramolecular electrostatic repulsions, which would decrease the adsorbed amount. At still higher pH the latter repulsions dominate, leading to a decreasing (but not vanishing) adsorbed amount.

We can now compare the results of the adsorption of weak polyelectrolytes with the adsorption of this polyampholyte. Blaakmeer et al. (10) studied the adsorption of poly(acrylic acid) (PAA) onto a positively charged latex. At high pH (positive surface, negative polyelectrolyte) the adsorption is low, but it increases with decreasing pH, i.e., as the charge on the polyelectrolyte decreases. A similar effect occurs in the left hand side of Figure 2, with the signs of the charge on adsorbate and adsorbent reversed. In the case of adsorbing PAA a much stronger dependence of the adsorbed amount on pH is found. Presumably, the adsorption of PAA is more affected by pH-dependent electrostatic interactions than the adsorption of this polyampholyte; in the latter case these interactions depend much less on pH because of the (at low pH) constant charge of the amino groups. The right hand side of Figure 2 (negative surface, negative polyampholyte) can not be compared with the adsorption of PAA onto oppositely charged latex because in the latter case the PAA and the adsorbent have opposite charges at all pH values. It may be noted that a maximum as in Figure 2 is commonly found for proteins; in this case it is situated at pH values just below the i.i.p. of the protein for adsorption on a positive surface and just above the i.i.p. for adsorption on a positive surface (12,24,25).

The adsorption isotherms of the random copolymer onto the negatively charged latex at pH = 4 in 0.001 and 0.1 M KCl, respectively, are displayed in Figure 3. In this graph we also give the adsorption isotherm onto the positively charged latex at pH = 9.7 in 0.1 M KCl. Again it is evident that the adsorption of the random copolymer on the negatively charged latex hardly depends on the ionic strength. Upon increasing the ionic strength from 0.001 to 0.1 M, the adsorption increases only slightly, as was also the case for the adsorption of (Lys-Glu-Gly)₄ (see fig. 1). These adsorption isotherms

show much more the high affinity behaviour which is so characteristic for polymer adsorption than the adsorption isotherms for $(\text{Lys-Glu-Gly})_4$. This is due to the fact that we now have a much longer molecule. That the isotherms are not completely high affinity is probably due to polydispersity, as has been proposed by Cohen Stuart et al. (26). The cross-over point that was found for the ionic strength dependence of the adsorption of $(\text{Lys-Glu-Gly})_4$ (fig. 1) is now absent. However, it is quite possible that it would occur at a rather low polyelectrolyte concentration, in the region where measurements are inaccurate or impossible. For protein adsorption the effect of the salt concentration on the adsorbed amount has also been reported to be small (12,25).

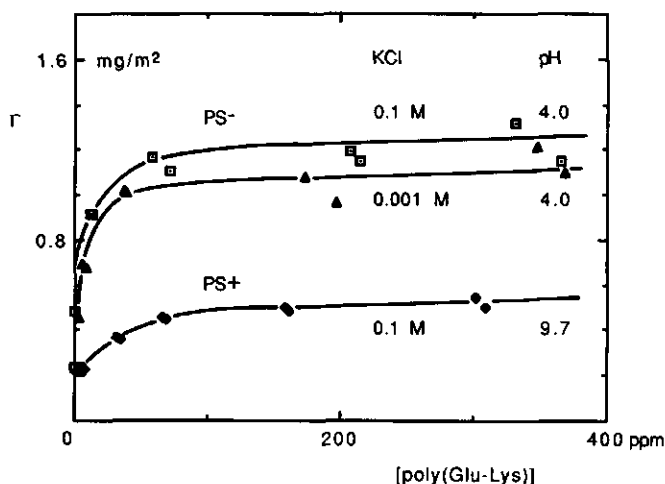


Figure 3. Adsorption isotherms of the random copolymer on negatively charged latex at $\text{pH} = 4.0$ (at two ionic strengths) and on positively charged latex at $\text{pH} = 9.7$.

The adsorbed amount of the random copolymer is about twice as high as that of $(\text{Lys-Glu-Gly})_4$. This is also most likely due to the much higher molecular weight of the random copolymer. This adsorbed amount is too high to allow all the molecules to lie flat on the surface and as a consequence loops and tails must exist.

Before we discuss the difference in the adsorption of the polyampholyte onto PS- and PS+ we want to focus on the fact that the sign and the value of the charge on the polyampholyte depend strongly on the pH. This is important because the charge determines all the electrostatic interactions (between surface and adsorbing molecule, and between charged amino acid residues). In Figure 4 we present a calculated titration curve of the random copolymer, using

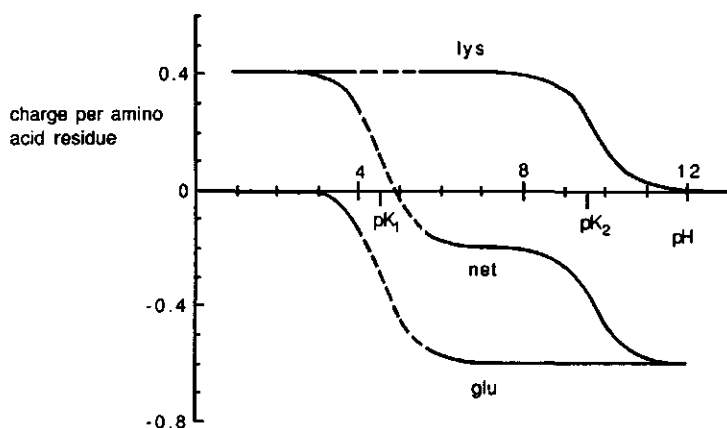


Figure 4. The charge on the random copolymer as a function of the pH, calculated with $pK_1 = 4.6$ (ϵ -carboxylic group) and $pK_2 = 9.6$ (γ -amino group). The charge of the lysine, the glutamic acid residues, as well as the total charge is expressed as charge per amino acid residue. In the pH region where the polyampholyte is not soluble the curves are drawn discontinuously.

the pK values as given in the section on materials and methods, and neglecting the effect of electrostatic interactions between charged segments on their dissociation. The charge on the molecule due to charged lysine and glutamic acid residues as well as the total charge on the molecule is expressed as charge per amino acid residue. Clearly, three pH regions in which the charge on the molecule is constant can be distinguished: at $pH < 3$ the molecule is positively charged, in the region $6 < pH < 8.5$ it has a low negative charge, and for $pH > 11$ it is highly negatively charged.

The adsorbed amount of the polyampholyte on the PS+ latex at pH = 9.7 is less than half the adsorbed amount on PS- latex. At pH = 9.7 the negative charge is about the same, or only slightly higher than the positive charge at pH = 4 and one would expect the adsorbed amount onto the PS+ latex to be as high or higher than the adsorbed amount on the PS- latex at pH = 4, because the absolute value of the surface charge density of the positive latex is much higher (+90 versus -25 mC/m²). Yet the adsorbed amount is much lower at pH = 9.7. A possible explanation is that the intramolecular electrostatic repulsions are higher at pH = 9.7 because the amino function is separated by 4 CH₂-groups from the molecular backbone whereas the carboxylic group is only 2 CH₂-groups away from the molecular backbone. As a consequence the positive charges are, on average, further apart than the negative charges. This results in higher intramolecular electrostatic repulsions when the molecule is negatively charged (high pH) than when the molecule is positively charged. This last argument is in line with the results of Goren et al. (27,28) who investigated the viscometric and potentiometric behaviour of poly(Lys-Ala-Glu) for conformational studies. They concluded that in the charged state glutamic acid at pH \approx 10 is a stronger α -helix disturber than lysine at pH \approx 3. From this it follows that at pH \approx 10 the volume of a polyampholyte molecule is larger than at pH \approx 3, supporting the conclusion that the intramolecular electrostatic repulsions are higher when glutamic acid residues are charged than when lysine residues are charged.

Throughout their papers Goren et al. (27-29) assume that all the amino acids have the L-form. However their polyampholyte was synthesized using an active ester coupling procedure (29). It is well known that this method leads to racemisation of the chiral α C-atom of the carboxylic end terminal amino acid. Because of this racemisation they have probably studied a mixture of polyampholytes with glutamic acid in both the D- and L-form. An amino acid in the D-form in a peptide chain with all the other amino acids in the L-form acts as an α -helix disturber. Therefore at all pH values the optimal α -helix conformation is already disturbed by some glutamic acid residues in the D-form and the distortion resulting from either

charged lysine or glutamic acid residues is partly masked by the former effect. We suspect that if all the amino acids in the polyampholyte used by Goren et al. (27,28) would have had the same form their results would have supported their conclusions even stronger.

In Figure 5 we show the adsorbed amount of the random copolymer onto PS- and PS+ latex as a function of the pH, in 0.1 M KCl. The adsorbed amount is again determined at a final

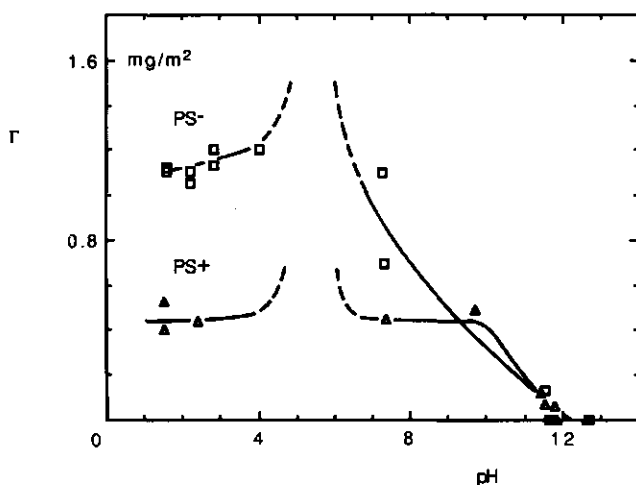


Figure 5. The adsorbed amount of the random copolymer onto negatively and positively charged latex as a function of the pH, at an equilibrium concentration of 200 ppm in 0.1 M KCl.

concentration of 200 ppm, which is in the plateau region of the adsorption isotherms (see fig. 3). In the pH range 4 - 6.5 we have no data because the molecule is not soluble in this pH region. The most striking feature shown by this plot is that the adsorption is high at low pH and low at high pH for the adsorption on positively as well as on negatively charged latex. One would expect the trends for the adsorption of the polyampholyte onto PS- and PS+ to be opposite. If the electrostatic interactions between surface and ampholyte would dominate, the adsorbed amount at high pH would be low on PS- (which is found experimentally) and high on PS+ (in contrast with

fig. 5). At low pH the opposite trend would apply, which is confirmed by experiment. Since it can be safely excluded that the PS⁺ loses its charge by hydrolysis at high pH, the very low adsorption of negatively charged polymer onto PS⁺ is an unexpected, yet real effect. As a possible explanation, we suggest that at high pH the adsorption is almost completely determined by the solution properties of the polymer and hardly by the surface characteristics. We return to this point below. Since at low pH there is adsorption of the positively charged macromolecule onto the positive latex there must also be a strong nonelectrostatic attraction between surface and adsorbing species. At low pH the electrostatic repulsion between surface and adsorbing macromolecule can be minimized by adapting the conformation of the macromolecule in such a manner that the charged amino groups are as far from the surface as possible.

For the adsorption of the random copolymer onto the negatively charged latex there appears to be an obtuse maximum in the adsorbed amount as a function of the pH. This can be explained with the same arguments as for the adsorption of (Lys-Glu-Gly)₄ onto PS⁻ latex. An additional argument would be that the surface acts as a nucleus for incipient phase separation, a feature which is well-known for polymer adsorption just below θ -conditions. For that reason there might also be a maximum in the adsorbed amount on PS⁺, but we do not have enough experimental points to support this conjecture.

It should be noted that for the adsorption on PS⁻ at pH = 7 a relatively large adsorbed amount is still found, even though at this pH the polymer is already negatively charged (see fig. 4). This is another argument for a strong nonelectrostatic attraction between surface and adsorbing molecule. The adsorption at pH = 7 onto PS⁻ (negative surface, negative polymer) can be compared with the adsorption onto PS⁺ at pH = 2 (positive surface, positive polymer). The adsorbed amount in the former case is higher than the adsorbed amount in the latter situation. This is probably due to the lower charge on the polyampholyte at pH = 7 (see fig. 4), leading to lower intramolecular and surface-adsorbate repulsions.

To explain the lack of adsorption of the random copolymer at high pH on PS⁺, despite the opposite charges in this case, we

invoked the solution properties of the polymer as a possible factor. To check whether the polyampholyte behaves differently in solutions of low and high pH, we performed some viscosity measurements at 25 °C, using a Fica viscosimeter. In Figure 6 we plot the specific viscosity η_{sp} as a function of the pH for a 1000 ppm solution of the polyampholyte in

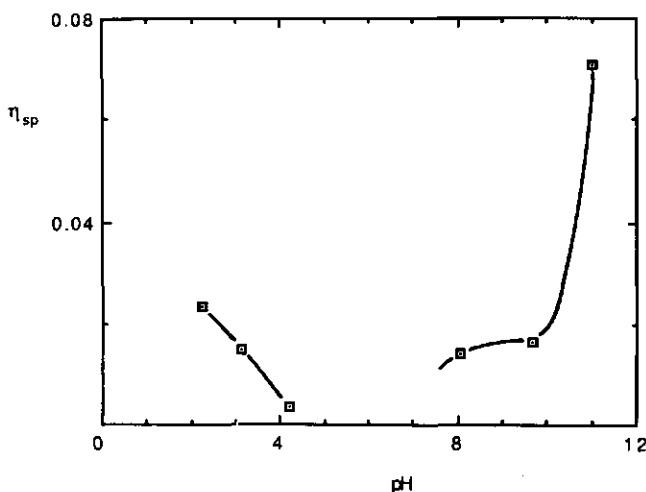


Figure 6. The reduced viscosity of a 1000 ppm solution of the random copolymer in 0.01 M salt as a function of the pH at 25.0 °C.

0.01 M salt. It is obvious that the viscosity behaviour at high pH is different from that at low pH. With decreasing pH (starting at pH = 4) only a small increase in the viscosity occurs. With increasing pH (starting at pH = 8) a large increase in the viscosity takes place around pH \approx 10. This implies that the volume of the molecule increases much more at high pH than at low pH, indicating a better solubility at high pH. The pH at which this large increase is found corresponds with the pH value at which the adsorption strongly decreases (see fig. 5) and with the pH at which the polyampholyte becomes strongly negatively charged (see fig. 4). This could explain why the opposing force against adsorption at pH > 10 becomes stronger. Still, it is not easily understood why the strong electrostatic driving force for adsorption on PS⁺ does not lead to at

least some accumulation at the surface. At present, we can give no plausible explanation.

At this point it is perhaps useful to make a comparison with some adsorption data for proteins. For several proteins it has been found that the trends in the dependence of the adsorbed amount on the pH are opposite for adsorption onto positively and negatively charged surfaces (12,30). Both HPA (human plasma albumin) (12) and BSA (bovine serum albumin (30) on negatively charged latex show a maximum in $\Gamma(\text{pH})$ at a pH below the i.i.p. of the protein, with a rather low adsorbed amount at high pH. For the adsorption onto positively charged latex the maximum was found at a pH above the i.i.p., with in that case a low adsorbed amount at low pH. For the adsorption of HPA and RNase (bovine pancreas ribonuclease), Norde et al. (31) showed, by titrating the adsorption complex, that even on a negative surface the charged carboxylic groups are in closer contact with the surface than the amino functions. They explained this surprising effect by the large volume of the carboxylic group as compared to the amino group, causing a larger polarizability and a weaker hydration (i.e., a lower affinity for the solvent).

In the discussion of the pH dependence of the adsorption of both polyampholytes onto PS+ and PS- as given above, it was assumed that the surface charge densities are completely independent of the pH. This may not be completely valid because at very low pH ($\text{pH} < 2$) the surface charge on PS-, resulting from sulfate groups, might become slightly less. This could give an alternative explanation for the decrease in the adsorbed amount on PS- at very low pH. At very high pH ($\text{pH} > 11$) the surface charge on PS+, resulting from quarternary ammonium groups, might decrease somewhat. This effect is not able to account for the strong decrease in the adsorbed amount of the random copolymer onto PS+ at high pH: there would still be a strong electrostatic attraction between surface and negatively charged polyampholyte molecule, even if the high value of σ_0 would be reduced by, say, a factor of 2.

In Figures 7, 8, and 9 the results of the electrophoretic mobility measurements are reported. In these three graphs the zeta-potential as calculated from the electrophoretic mobility (according

to the Helmholtz-Smoluchowsky equation taking 8.9×10^{-4} Pas for the viscosity and 6.92×10^{-10} CV $^{-1}$ m $^{-1}$ for the dielectrical constant, respectively) is plotted as a function of the initial concentration polyampholyte. The difference between final and initial concentrations polymer in the experiments is negligible (below 0.1 ppm) because in this case the latex suspension is very dilute.

In Figure 7 the zeta-potential of the negatively charged latex at pH = 4 has been plotted as a function of the initial concentration (Lys-Glu-Gly) $_4$ in 0.1 and 0.001 M KCl, respectively. It is evident that

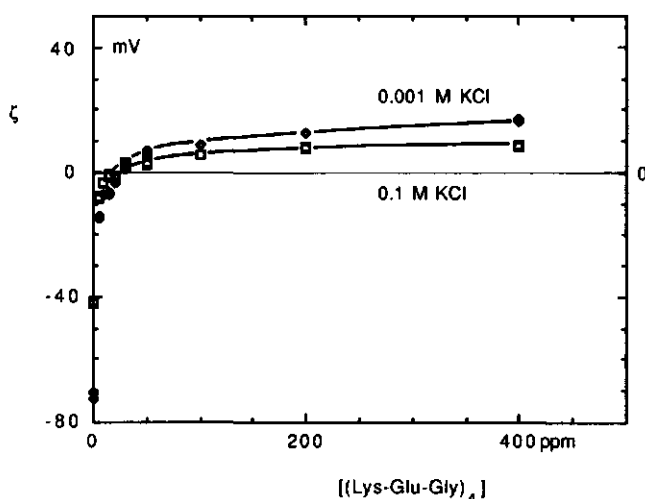


Figure 7. The zeta-potential of negatively charged latex as a function of the initial concentration (Lys-Glu-Gly) $_4$ at pH = 4, in 0.001 and 0.1 M KCl.

adsorption of this molecule at this pH results in a slight charge reversal of the latex particle. The zeta-potential is very sensitive to low adsorbed amounts of polyampholyte. It is also obvious that ζ increases much faster with increasing concentrations ampholyte than the adsorbed amount (see fig. 1). At low ionic strength the change in ζ is slightly larger because the charges are screened to a smaller extent leading to a cross-over point. Since, at low ionic strength $|\zeta|$ is larger than at high ionic strength, and since ζ reverses sign, the two curves must intersect.

For the zeta-potential measurements in the presence of low concentrations of random copolymer a strong change in the electrophoretic mobility occurs even at concentrations where no adsorption was measurable. The zeta potential of the negatively charged latex at pH = 4 as a function of the initial concentration of random copolymer in 0.001 and 0.1 M KCl, respectively, has been plotted in Figure 8. In this graph we observe the same trends as for the adsorption of (Lys-Glu-Gly)₄: charge reversal of the latex particle,

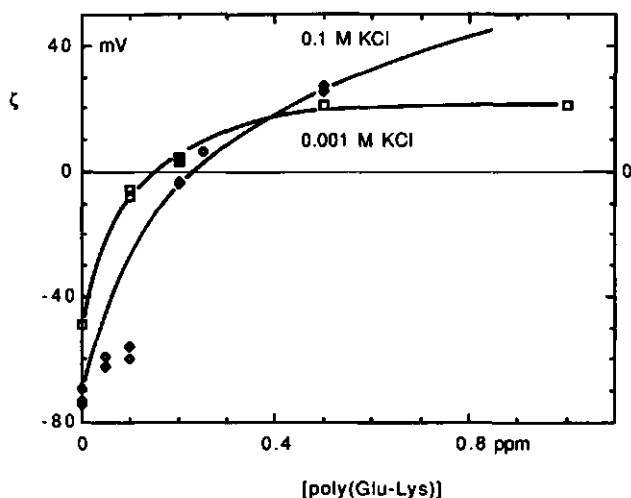


Figure 8. The zeta-potential of negatively charged latex as a function of the initial concentration of random copolymer at pH = 4, in 0.001 and 0.1 M KCl.

a stronger dependence of ζ on the concentration than of the adsorbed amount on the concentration, and a larger change in ζ at low ionic strength. As compared with Figure 7 the charge reversal is more pronounced, and ζ is more sensitive to low polyampholyte concentrations. The latter effect is probably due to the greater high affinity type adsorption of the random copolymer as compared to the adsorption of (Lys-Glu-Gly)₄ (compare fig. 1 and fig. 3). The stronger reversal of ζ in the case of the random copolymer can be explained by the higher adsorbed amount, with a greater positive charge

accumulated in the loops and tails as compared to the adsorption of (Lys-Glu-Gly)₄.

The results of the electrophoretic mobility measurements on the positive latex as a function of the concentration of random copolymer at pH = 10 are given in Figure 9, for 0.1 and 0.001 M KCl. Again we find charge reversal and a stronger dependence of ζ on the concentration as compared to that of the adsorbed amount, and a larger change in ζ at low salt concentrations. In this case the cross-over point is situated at such low concentration that it seems to lie on the abscissa.

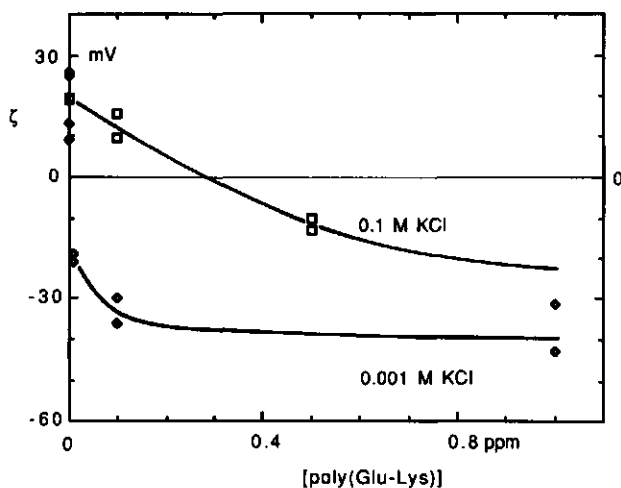


Figure 9. The zeta-potential of positively charged latex latex as a function of the initial concentration of random copolymer at pH = 10, in 0.001 and 0.1 M KCl.

Conclusions

The adsorption of (Lys-Glu-Gly)₄ onto negatively charged latex is not of the high affinity type because the molecular weight is too low. For a random copolymer D-Glu-D-Lys, high affinity type adsorption isotherms were measured. The adsorption of both molecules was found to be virtually independent of the ionic strength. There appeared to be a slight maximum in the adsorbed

amount at a pH of 4, both on negatively and on positively charged latex.

The dependence of the adsorbed amount of the polyampholyte on the pH proved to be independent of the charge sign of the latex: high adsorbed amount at low pH and no adsorption at high pH. This trend is easily understood for a negative adsorbent, where the adsorbed amount is high for opposite charge signs of adsorbate and adsorbent, and is low or zero when the charges have the same sign. However, for the positive latex, adsorption takes place when the polyampholyte is positively charged (pointing to a strong nonelectrostatic adsorption energy), and no adsorption is found when the polymer has a high negative charge. Apparently, in this case the strong attraction between surface and polyampholyte is not sufficient for adsorption of the polyelectrolyte from a very good solvent.

The electrophoretic mobility measurements showed that adsorption of the random copolymer leads to charge reversal when the latex and the polyampholyte have opposite charge signs. The zeta potential depended much more strongly on the polymer concentration than the adsorbed amount.

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Summary

The objective of this study was to collect systematic data on the adsorption behaviour of weak polyelectrolytes and polyampholytes. The measurements were performed on well-defined systems in order to be able to compare the results with the recently developed theories of Evers et al. and Böhmer et al. for the adsorption of weak polyelectrolytes. The adsorption of polyampholytes was studied in order to help bridge the gap between the theoretically well understood behaviour of polyelectrolytes at interfaces, and the adsorption characteristics of proteins, which are, so far, mainly experimentally documented.

In order to compare experiment with theory it is essential to be able to vary the surface charge and the polymer charge independently. In Chapter 1 we describe the synthesis of a monodisperse, positively charged polystyrene latex with fixed surface charge. The positive charge results from quarternary ammonium groups and proved it to be independent of the pH in the range 4-10, as shown by streaming potential measurements on plugs.

In Chapter 2 the adsorption data of poly(acrylic acid) onto this positively charged latex are reported and compared with theory. The agreement between theory and experiment is remarkably good. The theoretically predicted maximum in the adsorbed amount as a function of pH is fully confirmed experimentally. The maximum occurs because of two opposing trends. With increasing pH, the increased negative charge on the polyacid leads to a stronger attraction between surface and polyelectrolyte groups and, thus, to a higher adsorption. On the other hand, the higher repulsion between the segments opposes accumulation of polymer near the interface. At relatively low pH the latter effect is small and the electrostatic contribution to the adsorption energy gives high adsorbed amounts. At higher pH, the intersegmental repulsion leads to a decreased adsorption.

It was found experimentally that the adsorption is almost independent of the salt concentration. This is also predicted by

theory. The computations showed that the segments in contact with the surface are dissociated to such a degree that the surface charge is just balanced. The segments in loops and tails are dissociated to a much lower degree. The effective charge of the colloidal particle plus the adsorbed polymer layer is therefore small, so that salt will hardly affect the adsorbed amount. The adsorbed amount increases slightly with increasing molecular weight of the polyelectrolyte and with increasing surface charge. These trends also correspond to theoretical predictions; the agreement is again semi-quantitative.

A next step was the adsorption of simple polyampholytes. Because well-defined homodisperse polyampholytes are not commercially available, we decided to synthesize such a macromolecule ourselves. The synthesis of a model polyampholyte is described in Chapter 3. The starting point for the polymerization was the tripeptide L-lysyl-L-glutamyl-glycine, with fully protected amino- and carboxylic functions. Coupling of two tripeptide molecules to a hexapeptide, two hexapeptide molecules to a dodecapeptide, etc. had to be attained by activation of the carboxylic terminal amino acid (c-terminus) with dicyclohexyl carbodilimide. It is known that this activator causes racemisation of the c-terminus. Therefore glycine was chosen as the c-terminus. The controlled coupling had to be given up at the dodecapeptide level due to the extremely low solubility of the fully protected peptide derivative.

In Chapter 4 adsorption studies are described with this dodecapeptide (L-lysyl-L-glutamyl-glycine)₄ and with a commercially available random copolymer of lysine and glutamic acid, both on positively and on negatively charged polystyrene latices. It was found that the dependence of the adsorption on pH was the same for adsorption on the positively and on the negatively charged latex: high adsorbed amounts at low pH and virtually no adsorption at high pH. This behaviour is easily understood for the negatively charged latex: at low pH the positively charged macromolecule adsorbs easily on the negative surface, whereas at high pH the molecule and surface repel each other. However, for the positively charged latex one would expect just the reverse so that the observation is difficult to understand. A possible explanation is that at low pH the "chemical"

affinity of the adsorbate for the adsorbent is larger than the electrostatic repulsion, while at high pH the very good solubility of the polyampholyte counteracts the electrostatic attraction with the surface to such an extent that the adsorption is only small.

In conclusion, the adsorption behaviour of weak polyelectrolytes with only one type of charged group is now understood reasonably well, and the agreement between theory and experiment is excellent. For polyampholytes with anionic and cationic groups in the molecule there is no symmetry in the behaviour with respect to positive and negative surfaces. Since, for purely non-specific electrostatic interactions, such symmetry must exist, other factors such as solubility or formation of internal ionic bonds within the polyampholyte must be held responsible for the observations. The latter conclusion may be important for the understanding of proteins at interfaces.

Samenvatting

De adsorptie van zwakke polyelectrolyten en polyamfolyten

een experimenteel onderzoek

Het doel van het in dit proefschrift beschreven onderzoek was om systematisch informatie te verzamelen over het adsorptiegedrag van zwakke polyelectrolyten en polyamfolyten en deze gegevens te vergelijken met theoretische modellen.

Polyelectrolyten en polyamfolyten behoren tot de groep van polymeren. Polymeren zijn lange moleculen opgebouwd uit aan elkaar gekoppelde bouwstenen, de monomeren. Het aantal soorten monomeren waaruit een polymeer is opgebouwd kan variëren van één (homopolymeer) tot twintig (bijv. eiwitten). Polyelectrolyten zijn polymeren waarbij de monomeren een zijketen hebben die een geladen groep bevat. Voor zwakke polyelectrolyten is die lading afhankelijk van de zuurgraad van de oplossing (pH). Polyamfolyten hebben sommige groepen die als functie van de pH positief en andere groepen die als functie van de pH negatief geladen kunnen zijn. Het adsorptiegedrag van polymeren speelt in veel industriële processen een belangrijke rol bij het stabiliseren of destabiliseren van kolloïdale suspensies.

De adsorptiemetingen voor zwakke polyelectrolyten zijn uitgevoerd aan goed gedefinieerde modelsystemen om de resultaten te kunnen vergelijken met de recentelijk ontwikkelde theoriën van Evers *et al.* en Böhmer *et al.* Het adsorptiegedrag van goed gedefinieerde polyamfolyten is bestudeerd omdat dit kan helpen de kloof te vernauwen die bestaat tussen het theoretisch goed verklaarbare gedrag van polyelectrolyten aan grensvlakken enerzijds en de hoofdzakelijk experimenteel gekarakteriseerde adsorptie van complexe polymeren zoals eiwitten anderzijds.

Om de theorie aan het experiment te kunnen toetsen is het noodzakelijk om de lading van het polyelectrolyt onafhankelijk te kunnen variëren van de oppervlaktelading. Om dit te realiseren beschrijven we in Hoofdstuk 1 de synthese van een monodispers polystyreenlatex met een constante oppervlaktelading. De positieve lading op het oppervlak wordt veroorzaakt door quarternaire ammoniumgroepen, afkomstig van de initiator die gebruikt is voor het polymeriseren van styreen. Met stromingspotentiaalmetingen aan propen is aangetoond dat in het pH-traject van 4 tot 10 de oppervlaktelading niet verandert.

In Hoofdstuk 2 worden de resultaten van de adsorptiemetingen van polyacrylzuur op deze positief geladen latices gerapporteerd en vergeleken met de theorie. De overeenkomst tussen de experimenten en de theorie is verassend goed. Het theoretisch voorspelde maximum in de geadsorbeerde hoeveelheid als functie van de pH wordt experimenteel volledig bevestigd.

Het maximum wordt veroorzaakt door twee elkaar tegenwerkende mechanismen. Met toenemende pH leidt de groter wordende negatieve lading op het polymeer tot een sterkere electrostatistische attractie tussen oppervlak en polymeer, resulterend in een grotere geadsorbeerde hoeveelheid. Bovendien zijn de geladen segmenten beter in staat om te concurreren met de tegenionen die de lading van het oppervlak compenseren. Aan de andere kant leidt de toenemende lading op het polyzuur tot een hogere inter- en intramoleculaire repulsie die de accumulatie van polyelectrolyt juist tegenwerkt. Bij een relatief lage pH overheersen de twee eerst genoemde effecten waardoor de adsorptie toeneemt met toenemende pH. Bij een hogere pH begint de electrostatistische repulsie tussen de geladen segmenten een grotere rol te spelen, en neemt de adsorptie weer af.

Experimenteel is gevonden dat in niet te geconcentreerde zoutoplossingen de adsorptie zo goed als onafhankelijk is van de zoutconcentratie. Dit gedrag wordt door de theorie bevestigd. De berekeningen tonen aan dat de segmenten welke in contact zijn met het oppervlak een dusdanige dissociatiegraad hebben dat de oppervlaktelading juist gecompenseerd wordt. De segmenten in de

lussen en staarten hebben een veel lagere dissociatiegraad. De netto lading op het kolloïdale deeltje is daardoor klein en als gevolg daarvan beïnvloedt zout bij ionsterktes kleiner dan 0.1 M de adsorptie bijna niet.

De geadsorbeerde hoeveelheid neemt in geringe mate toe met toenemend molekuulgewicht van het polyzuur en met toenemende oppervlaktelading. Deze waarnemingen worden door de theorie semi-kwantitatief bevestigd.

De volgende stap in het onderzoek was de adsorptie van eenvoudige polyamfolyten. Omdat goed gedefinieerde homodisperse polyamfolyten niet commercieel verkrijgbaar zijn hebben we besloten zelf zo'n macromolekuul te synthetiseren. In Hoofdstuk 3 is de synthese van het model-polyamfolyt beschreven.

Het uitgangspunt voor de polymerisatie was het tripeptide L-lysyl-L-glutamyl-glycine met volledige beschermde amino- en carboxylgroepen. Gecontroleerde polymerisatie was mogelijk door het koppelen van twee tripeptides tot een hexapeptide, twee hexapeptides tot een dodecapeptide, etc. In de koppelingsreacties is dicyclohexyl carbodiimide gebruikt om het eindstandige aminozuur met de vrije carboxylgroep te activeren. Omdat bekend is dat deze activatie leidt tot racemisatie van het betreffende aminozuur is gekozen voor glycine als het aminozuur met de eindstandige carboxylgroep. De polymerisatie moest opgegeven worden op het dodecapeptide niveau omdat de volledig beschermde peptide-derivaten zeer slecht oplosbaar waren.

In Hoofdstuk 4 rapporteren we het adsorptiegedrag van dit dodecapeptide $[(L\text{-lysyl-L-glutamyl-glycine})_4]$ en van een commercieel verkrijgbaar 'random' copolymeer van lysine en glutaminezuur op zowel positief als op negatief geladen polystyreen latex. Voor het adsorptiegedrag op de tegengesteld geladen latices werd een identieke afhankelijkheid van de pH gevonden: een hoge geadsorbeerde hoeveelheid bij lage pH en bijna geen adsorptie bij hoge pH. Voor adsorptie op de negatief geladen latex is dit eenvoudig te begrijpen: bij lage pH adsorbeert het positief geladen macromolecuul makkelijk op het negatief geladen oppervlak terwijl bij een hoge pH de negatieve ladingen elkaar afstoten. Voor de

adsorptie op het positief geladen oppervlak zou men het tegenovergestelde effect verwachten. Daarom zijn deze waarnemingen moeilijk te begrijpen. Een mogelijke verklaring is dat bij lage pH de chemische affiniteit van het macromolekuul voor het oppervlak groter is dan de electrostatische repulsie, terwijl bij hoge pH het dan zeer goed oplosbare polyamfolyt weinig neiging vertoont om te adsorberen, ondanks de electrostatische attractie, waardoor de adsorptie gering is.

Samenvattend kunnen we zeggen dat het adsorptiegedrag van zwakke polyelectrolyten met slechts één soort geladen groep goed te begrijpen is en dat de overeenstemming tussen experiment en theorie semi-kwantitatief is. Polyamfolyten (molekulen met kationgene en aniongene groepen) vertonen geen symmetrisch gedrag met betrekking tot de adsorptie op negatief en positief geladen oppervlakken. Aangezien voor processen, die alleen bepaald worden door niet-specifieke electrostatische interacties zo'n symmetrie moet bestaan, moeten andere factoren zoals oplosbaarheid of de vorming van ionogene bindingen binnen het macromolekuul verantwoordelijk zijn voor deze waarnemingen. Deze laatste conclusie kan belangrijk zijn om het gedrag van eiwitten, die nog weer gecompliceerder zijn dan polyamfolyten, aan grensvlakken te begrijpen.

Curriculum Vitae

Jan Blaakmeer werd geboren op 3 mei 1959 te Slootdorp. Via Mavo, Havo, Hogere Landbouwschool en met behulp van de regeling wederzijdse doorstroming HBO Universitair onderwijs werd in 1979 aan de Landbouwhogeschool (nu Landbouwuniversiteit) te Wageningen begonnen met de studie Moleculaire Wetenschappen.

Binnen deze studierichting werd de fysisch-chemische oriëntatie gevolgd met afstudeervakken bij de vakgroepen Fysische en Kolloïdchemie, Bodemkunde en Plantevoeding, en Moleculaire Fysica. De stage werd vervuld bij het "Department of materials, science, and engineering" aan de "University of Florida" in Gainesville, USA. In november 1985 studeerde hij cum laude af waarna hij van 1 februari 1986 tot 1 februari 1989 als wetenschappelijk assistent gewerkt heeft bij de vakgroep Fysische en Kolloïdchemie van de Landbouwuniversiteit te Wageningen. In deze periode is het experimentele werk, beschreven in dit proefschrift, verricht.

Vanaf 1 juni 1989 is hij als chemicus in dienst bij ENCI Nederland BV te Maastricht

Nawoord

Het in dit proefschrift beschreven onderzoek is niet alleen het werk van de promovendus maar is het resultaat van een gezamenlijke inspanning.

Promotor en leider van de polymeergroep Gerard Fler heeft mij wegwijs gemaakt in de polymeeradsorptie. Aan zijn motiverende en stimulerende aanpak, ook in de eindfase van het schrijven en aan zijn kritisch oog en vermogen om scherp te kunnen formuleren heb ik veel te danken.

Bij de vele vragen en problemen van experimentele aard en met het lezen van de verschillende manuscripten heeft Martien Cohen Stuart mij met raad en daad geholpen.

Op het gebied van de organische chemie heeft Dick de Bie mij geholpen met het synthetiseren van de initiator voor de latexbereiding. Aan de faculteit voor organische chemie van de Katholieke Universiteit Nijmegen heeft Frits Tesser mij ingewijd in de beginselen van de eiwitssynthese. Zonder zijn grote experimentele vaardigheid en theoretische kennis zou ik de synthese van het polyamfolyt al veel eerder opgegeven hebben. Theo Tjisse-Klasen heeft in het kader van zijn doctoraalstudie de synthese van Aloc-Ala-Ala-Dma-Ala-Ala-OMe voor zijn rekening genomen.

Maarten Bakkenes en Ans Hofman hebben een belangrijke bijdrage geleverd aan het experimentele werk met betrekking tot de adsorptie van respectievelijk polyelectrolyten en polyamfolyten.

Marcel Böhmer heeft mij met raad en daad bijgestaan bij het runnen van de door hem ontwikkelde software.

Gert Buurman heeft ervoor gezorgd dat de plaatjes in het proefschrift er netjes uitzien en Roy Davison heeft, wat het Engels aangaat, de puntjes op de i gezet.

De goede samenwerking met de werkplaats, het secretariaat en de amanuenses alsmede de onderhoudende koffiepauzes hebben positief bijgedragen aan het werkklimaat.

Tot slot wil ik iedereen bedanken voor de prettige en leerzame tijd die ik heb doorgebracht bij de vakgroep Fysische en Kolloïdchemie.