

New polyelectrolyte complex particles as colloidal dispersions based on weak synthetic and/or natural polyelectrolytes

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Abstract. This study aims to evidence the formation of stable polyelectrolyte complex particles as colloidal dispersions using some weak polyelectrolytes: chitosan and poly(allylamine hydrochloride) as polycations and poly(acrylic acid) (PAA) and poly(2-acrylamido-2-methylpropanesulfonic acid – co – acrylic acid) (PAMPSAA) as polyanions. Polyelectrolyte complex particles as colloidal dispersion were prepared by controlled mixing of the oppositely charged polymers, with a constant addition rate. The influences of the polyelectrolytes structure and the molar ratio between ionic charges on the morphology, size, and colloidal stability of the complex particles have been deeply investigated by turbidimetry, dynamic light scattering and atomic force microscopy. A strong influence of polyanion structure on the values of molar ratio n^-/n^+ when neutral complex particles were obtained has been noticed, which shifts from the theoretical value of 1.0, observed when PAA was used, to 0.7 for PAMPSAA based complexes. The polyions chain characteristics influenced the size and shape of the complexes, larger particles being obtained when chitosan was used, for the same polyanion, and when PAMPSAA was used, for the same polycation.

Keywords: *nanomaterials, polyelectrolyte complex, weak polyelectrolyte, dynamic light scattering, atomic force microscopy*

1. Introduction

Oppositely charged polymers form polyelectrolyte complexes (PEC) after mixing of their solutions [1]. PECs are of interest due to their facile preparation and responsiveness to environmental stimuli. Moreover, using water as a solvent, PECs are attractive for biomedical applications. Examples include controlled drug delivery systems, enzyme and DNA carriers, surface modification of medical implants, membranes for cell culture and growth, biosensors, and nanostructured materials [2–5].

At relatively low concentrations and when one of the components is taken in excess, PEC formation can lead to stable colloidal dispersions [6–12]. The characteristics of the polyelectrolyte components

(molecular weight, nature of ionic groups, charge density, and architecture) and the solvent (ionic strength, pH) determine the internal structure of the particles. The level of aggregation is dominantly controlled by the concentration of the polymer solution. PECs as colloidal dispersions are interesting for industrial applications as coatings, auxiliary products in paper manufacturing, and for special purposes in biotechnology and medicine [13–15]. When weak polyelectrolytes are involved in PEC preparation, the molar ratio between charges when neutral complex particles were obtained is strongly influenced by the pH value of polyions solutions, conferring an amphoteric character to the PEC aggregates [16]. The amphoteric behavior of the PEC

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particles, namely the sign of net surface charge, is determined by the positive or negative charges on the chain loops and chain ends on the particle surface.

Chitosan (CS) is a natural amino-polysaccharide (copolymer of β -(1-4) linked 2-acetamido-2-deoxy-D-glycopyranose and 2-amino-2-deoxy-D-glycopyranose) produced by deacetylation of chitin from crustaceans' exoskeletons and fungal cell walls [17]. Sustainable interest in the biomedical application of chitosan to discourage bacterial adhesion and implant infection is stimulated strongly by its non-toxicity [18], biodegradability [19], and strong antibacterial properties [20]. It has been already shown that polyelectrolyte complexes and multilayers based on chitosan, by virtue of their biocompatibility, have valuable biomedical applications such as membranes for dialysis, packaging, coatings and wounds dressing, polyelectrolyte complex beads for controlled delivery of proteins, drugs, vaccines [21–28]. Promising results were obtained in application of chitosan based nonstoichiometric PECs as flocculants [29].

Our previous studies dealt with the preparation of PECs nanoparticles as stable colloidal dispersions using strong synthetic polyanions and synthetic or natural (CS) polycations. Also, the factors (polyions structure and molar mass, molar ratio between charges, addition order, titrant addition rate) which influence particles size, morphology, and storage stability were investigated [30–33]. This study aims to obtain PECs as colloidal dispersion using some weak polyelectrolytes: CS and poly(allylamine hydrochloride) (PAH) as polycations and poly(acrylic acid) (PAA) and poly(2-acrylamido-2-methylpropanesulfonic acid – co – acrylic acid) (PAMP-SAA) as polyanions. PAA and PAH, which have identical polymer backbones and differ only in the ionic side group, were chosen as model weak polymers for PEC formation. The influence of polyelectrolytes structure and the molar ratio between ionic charges on the complex particles morphology, size, and colloidal stability have been deeply investigated by turbidimetry, dynamic light scattering (DLS) and atomic force microscopy (AFM).

2. Materials and methods

2.1. Materials

CS sample was kindly provided by Yaizu Suisankagaku Ind. (Shizuoka, Japan), as powder and was used without further purification. The viscometric average molar masses of CS sample, $M_v = 80\,000$ g/mol, was estimated using Equation (1) [34]:

$$[\eta] = 6.95 \cdot 10^{-4} \cdot M_v^{0.81} \quad (1)$$

The intrinsic viscosity of CS solution in 0.3 M $\text{CH}_3\text{COOH} - 0.2$ M CH_3COONa (1:1, v/v) was measured with an Ubbelohde viscometer at $25 \pm 0.1^\circ\text{C}$.

The deacetylation degree was determined by two methods:

- by potentiometric titration, according to ref. [35], dissolving CS in a known excess of HCl and performing potentiometric titration with 0.1 N NaOH, using an all-purpose titrator 716DMS Titrino, Metrohm (Herisau, Switzerland), equipped with a dosing unit and a combined glass electrode;
- from $^1\text{H-NMR}$ spectra recorded on Bruker NMR model Avance DRX 400 (400 MHz) spectrometer (Bruker, Switzerland). The CS sample was dissolved in D_2O and, to avoid the overlap between the signals of acetic acid and acetyl groups, some drops of HCl have been added to solubilize the polymer. The deacetylation degree was calculated as described before by Hirai *et al.* [36].

A deacetylation degree of about 82.5% was determined by both methods.

PAH, purchased from Aldrich (code no: 283215), with $M_w = 15\,000$ g/mol, was used as received. PAA with a molar mass $M_w = 70\,000$ g/mol was synthesized and purified according to ref. [37]. The copolymer PAMP-SAA, which contains 55 mol% of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and 45 mol% of acrylic acid (AA), was synthesized and purified according to ref. [38]. The intrinsic viscosity of the copolymer, determined in 1M NaCl at 25°C , was $[\eta] = 0.72$ dl/g.

2.2. Preparation of polyelectrolyte complexes as colloidal dispersions

CS solution was prepared in 1 vol% acetic acid solution, and 0.02 M NaCl. PAH and polyanions

solutions, were prepared by dissolution of appropriate amounts in 0.02 M NaCl aqueous solution. The concentration of polycations solution was $5 \cdot 10^{-4}$ M and that of polyanions solutions was $5 \cdot 10^{-3}$ M. Dispersions of PECs were prepared at room temperature, by mixing the solutions of oppositely charged polyelectrolytes in appropriate proportions. The amount of polycation was kept constant within a complex series, while the amount of polyanion was varied according to the desired mixing molar ratio, n^-/n^+ . The polyanion solution was added dropwise to the polycation solution, under magnetic stirring. A constant addition rate of 3.8 ml polyanion/(ml polycation·h) was used as optimum addition rate, taking into account the complex morphology and the preparation speed, according to our previous investigations on the formation of PECs as colloidal dispersion from synthetic polycations and polyanions [30–33]. After mixing, the formed dispersions were stirred 60 min and were characterized after 24 h, if other conditions were not specified.

2.3. Characterization methods

Polyelectrolyte and potentiometric titrations were performed with the particle charge detector Müték PCD 03 (BTG Instruments GmbH, Herrsching, Germany). The particle charge detector includes a Teflon piston, which moves up and down in a cylindrical Teflon cell containing the polyelectrolyte solution in the slit between the cylinder and piston. Displacement of the ion cloud around the polymer chains which are stuck on the porous wall of Teflon cylinder creates the streaming potential (measured in mV) at the electrode pair located in upper and bottom parts of the cell. The measured streaming potential is linearly correlated with the charge density of polyelectrolytes and it becomes zero in case of charge neutrality. The concentration of the charged groups of each solution was evaluated by titration with a standard solution of a strong oppositely charged polyelectrolyte, poly(sodium ethylenesulfonate) or poly(diallyldimethyl-ammonium chloride), with a concentration of 10^{-3} M. The concentration of the charged groups in the examined solution was calculated from the amount of standard solution needed to reach the zero value of the streaming potential. All measurements were made at room temperature. Potentiometric titration was

carried out between pH 2 and 10, using 0.1 M NaOH and HCl, respectively.

The turbidity of the complex dispersions was characterized by the optical density at $\lambda = 500$ nm (OD_{500}), with a Lambda 900 spectrometer (Perkin-Elmer Co. Ltd., Massachusetts, USA), using deionized water to establish the baseline. At this wavelength, the used polyelectrolytes do not absorb. Optical density results were expressed as the average of at least three independent measurements.

DLS measurements of PEC dispersions were carried out using a Zetasizer 3000 (Malvern Instruments, Worcestershire, UK) equipped with a 10 mW He-Ne laser (633 nm) as light source. The measurements were performed at a scattering angle of 90° , and the reported results are the average of two DLS independent measurements. The samples were kept at a constant temperature of 25°C during all experiments. DLS measures the light diffusion in particle dispersions, which can be interpreted using the Stokes-Einstein equation (Equation (2)) to yield the particle average hydrodynamic diameter, D_h :

$$D_h = \frac{k_B T}{3\pi\eta_s D} \quad (2)$$

where k_B is the Boltzmann constant, T is the absolute temperature (298 K), η_s is the dynamic viscosity of the solvent (for water 0.8872 cP), and D is the z-average translational diffusion coefficient. To get information about the size distribution of PEC particles, the size polydispersity was also included in the interpretation.

The shapes of PEC particles were examined by means of a SPM Solver PRO-M AFM (NT-MTD Co. Zelenograd, Moscow, Russia), using a high resolution ‘Golden’ silicon NSG10/Au/50 cantilever with an Au conductive coating. The topographic images were obtained in tapping mode and were repeated on different areas of the same sample. Prior to use, the silicon wafers substrates were carefully cleaned in two steps: first in ‘piranha solution’ followed by intensive rinsing with deionized water and second with the mixture $\text{NH}_4\text{OH}/\text{H}_2\text{O}_2/\text{deionized water}$, at 70°C , in ultrasonic bath, intensively rinsed with water and finally dried under a nitrogen flow. The clean silicon wafers substrates were immersed in PEC dispersions, identical with those used for DLS, for 20 min, then three times each

1 min in distilled water and finally air-dried at room temperature (in a dust-free environment) for 48 h.

3. Results and discussion

3.1. Potentiometric titration of polyelectrolytes

Although the value of streaming potential depends on numerous factors and can not be used as an absolute value (in the same way as ζ -potential), the method of colloidal titration with streaming potential detection is useful to determine the point of zero charge (pzc) in solutions of polyelectrolytes and colloidal dispersions. The pzc was considered to be the numeric value of pH where the streaming current is zero mV. When the pH is lower than the pzc value, the system is below the pzc . Below the pzc , the acidic water donates more protons than hydroxide groups, and so the adsorbent surface is positively charged. Conversely, above pzc the basic water donates more hydroxide groups than protons and the adsorbent surface is negatively charged. Figure 1 shows the potentiometric titrations curves for all polymers involved in this study, in the presence or absence of NaCl.

As it is already known, the net charge on the weak polyelectrolyte molecules is affected by pH of their surrounding environment. The polyanions used in this study contain carboxyl groups. Below pzc , the carboxyl group can undergo protonation ($-\text{COOH}_2^+$) at either the carbonyl oxygen or at the hydroxyl oxygen, and a small positive potential was obtained. Above pzc , located at pH 1.8 for PAMPSAA and 2.2 for PAA (Figure 1), the presence of hydroxyl

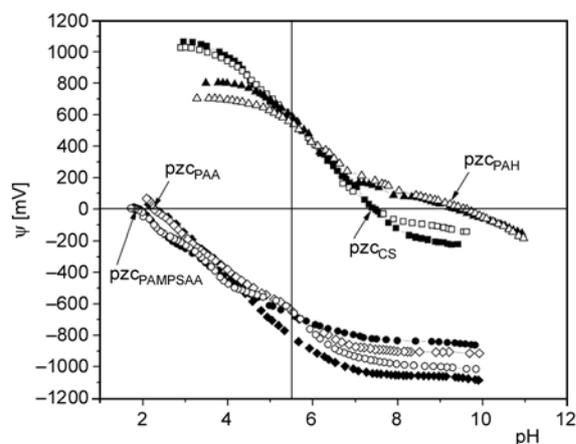


Figure 1. Potentiometric titration of CS (square), PAH (triangle), PAA (diamond), PAMPSAA (circles) in salt-free aqueous solution (close symbols), and 0.02 M NaCl aqueous solution (open symbols); pzc = point of zero charges.

ions induced the dissociation of carboxyl groups ($-\text{COO}^-$). Thus, above the pzc the polyanions had negative streaming potential. At pzc the ionization degree of polyanions is zero; e.g., the ionization degree of PAA at pH = 2.2 is 0.007, taking into account that pKa of PAA is close to 4.3.

On the other hand, below pzc , in the presence of hydronium ions, the primary amino groups ($-\text{NH}_2$) of the polycations PAH and CS can be protonated ($-\text{NH}_3^+$), leading to pzc around pH 7.4 for CS and 9.2 for PAH. Primary amines have protic hydrogens and therefore possess a small degree of acidity. Thus, above pzc a proton from amine group can be dissociated ($-\text{NH}$), due to the strongly basic medium and a negative streaming potential is obtained.

In the presence of a small amount of low molecular weight salt (0.02 M NaCl), no significant changes in the shape of titration curves are observed, and the pzc remains almost unchanged (Figure 1).

Taking into account the results obtained by potentiometric titration, for PECs preparation the pH value of polyions solutions was adjusted to 5.5, to ensure a similar ionization degree of the complementary polyelectrolytes, suggested by the complementary streaming potential values at this pH: ~ 600 mV for polycations and ~ -600 mV for polyanions.

3.2. Preparation and characterization of PECs

3.2.1. Turbidimetric titration

The formation of colloidal PECs between all studied polyelectrolyte pairs was followed firstly by turbidimetric titration, OD_{500} , as a function of the mixing ratio n^-/n^+ , i.e., the molar ratio between anionic and cationic units (Figure 2).

It is well known that the turbidity values reflect the influence of some parameters that characterize the PECs dispersions such as concentration, size, shape and polydispersity [6–8, 14, 30, 31]. Therefore, the increase of OD_{500} values with the increase of molar ratio between charges n^-/n^+ , observed in Figure 2, could be assigned also to the increase of the sizes and polydispersities of the complex particles.

As it is already known, the values of molar ratio n^-/n^+ corresponding to neutral PECs particles ($(n^-/n^+)_n$) is strongly influenced by the structure of the complementary polyelectrolytes, namely by their structural ability to intrinsic compensate their ionic

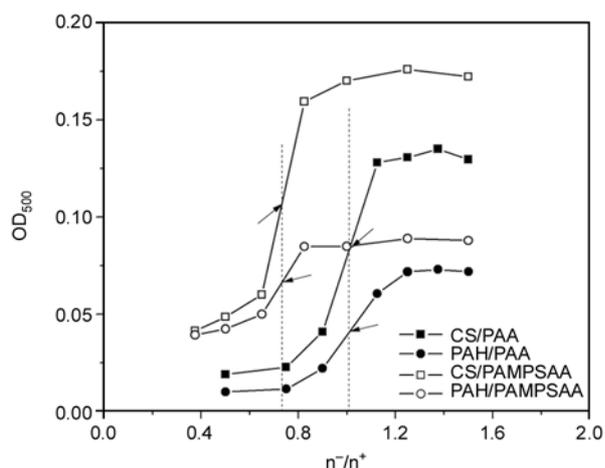


Figure 2. OD_{500} values as a function of the molar ratio between charges, n^-/n^+ . The values are the mean of three independent experiments that deviated 0–7%. The continuous lines are ‘hand-drawn’ lines.

charges (polymer/polymer ion pairs) [39]. The values of molar ratio $(n^-/n^+)_n$ was considered as the abscissa value of the rising curve corresponded to the one-half of the maximum of OD_{500} . As Figure 2 shows, neutral complex particles were obtained at a molar ratio between charges of around $n^-/n^+ = 1$, when PAA was the polyanion, and around $n^-/n^+ = 0.7$ when PAMPSAA was used, irrespective the polycation structure. A deviation of the endpoint of polyelectrolyte interactions to lower values of $(n^-/n^+)_n$ was also observed for other studied systems [31] and was ascribed to the difficulties in the intrinsic charge compensation between the complementary polyions.

For the same polyanion, higher values were obtained when CS was the polycation, along the whole range of molar ratio, n^-/n^+ (Figure 2). These results may be ascribed to the lower flexibility of CS chain (which has a semi-rigid chain unlike the synthetic polycation which is flexible), which probably induced the formation of particles with higher size, reflected by higher OD_{500} values. For the same polycation, the OD_{500} values were higher when PAMPSAA was used, comparative with PAA.

3.2.2. Dynamic light scattering

DLS is a suitable method to monitor the formation of PEC particles as a function of the mixing molar ratio n^-/n^+ . Figure 3 shows the DLS profiles on the PEC formation between CS and PAH, as starting polycations, and PAA and PAMPSAA as added polyanions.

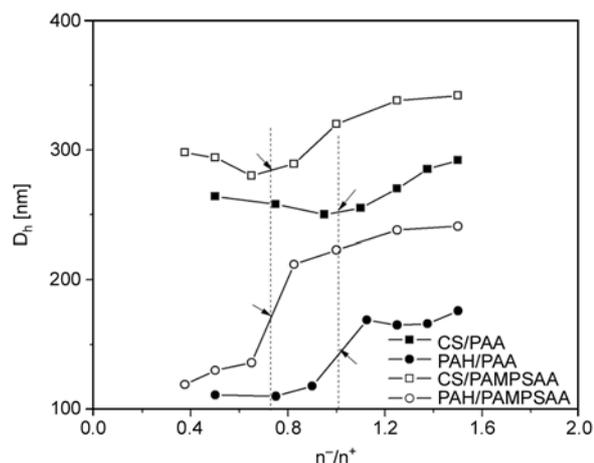


Figure 3. D_h of the PEC dispersions as a function of the molar ratio between charges (n^-/n^+). The values are the mean of three independent experiments that deviated 0–7%. The continuous lines are ‘hand-drawn’ lines.

The shape of the DLS curves was clearly influenced by the complementary polyelectrolytes structure. Thus, for PAH based complexes, D_h values slowly increased up to about 0.9 and 0.6 when PAA and PAMPSAA was used, respectively, abruptly increased around $(n^-/n^+)_n$, and remains almost constant after that. For CS based PECs, a slight decrease in the particle sizes was evident when CS was in excess, i.e. from 300 to 280 nm when PAMPSAA was used and from 265 to 248 nm when PAA was the added polyanion (Figure 3). This behavior suggests that below $(n^-/n^+)_n$ the addition of polyanions allows both the formation of new particles, evidenced by the slow increase of the OD_{500} values (Figure 2), and also their rearrangement toward more compact structures. At values of molar ratio n^-/n^+ higher than that corresponding to $(n^-/n^+)_n$, the inter-particles aggregation took place, leading to the monotonous increase of the complex particles sizes. It seems that this kind of behavior is characteristic for CS based systems, as it was previously observed for PECs prepared with other polyanions [29, 33, 40].

DLS results confirm the values of molar ratio $(n^-/n^+)_n$, observed in Figures 2, at $n^-/n^+ = 1$, when PAA was the polyanion, and around $n^-/n^+ = 0.7$ when PAMPSAA was used, irrespective the polycation structure. When PAMPSAA was used as polyanion, the particle sizes after the molar ratio $(n^-/n^+)_n$ were higher than the sizes of the PEC formed with PAA as polyanion, irrespective of polycation nature

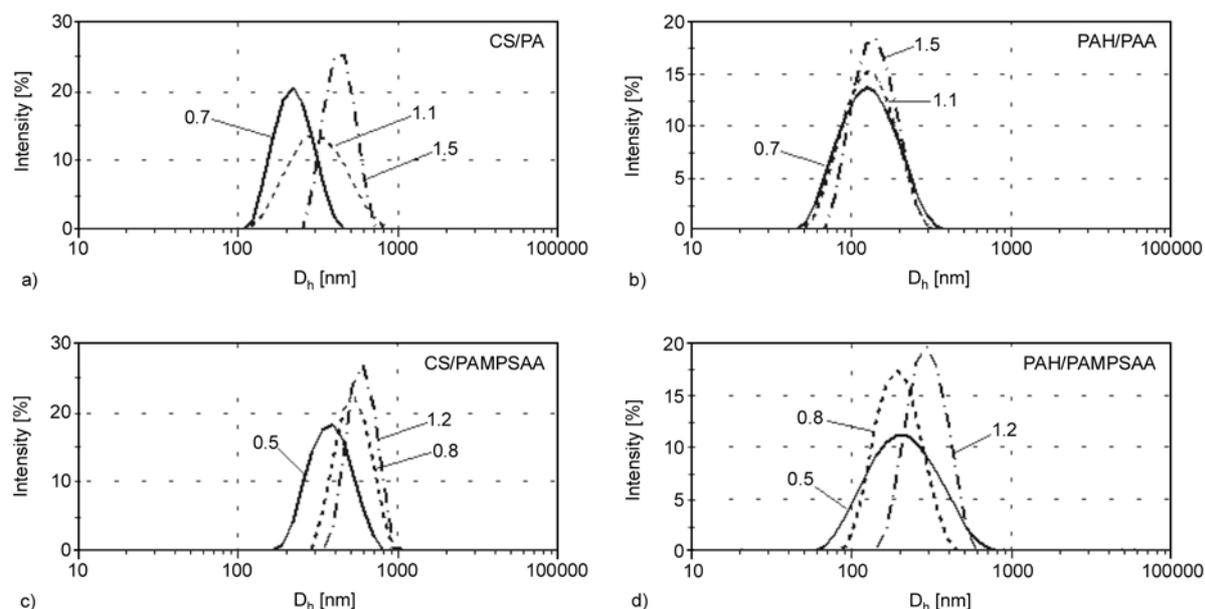


Figure 4. The particle size distribution analysis by intensity, obtain by DLS, for all studied systems and some selected molar ratios between charges

and molar ratio between charges. The copolymer contains 55% AMPS units, with the ionic group attached by a longer spacer to the macromolecular backbone, with high flexibility, and a higher ability to compensate the ionic charges of the polycation. Thus, probably part of the AA and CS ionic sites are hindered, and remain extrinsic compensated by small ions, leading to values of molar ratio $(n^-/n^+)_n$ lower than 1.0.

To evidence the influence of polyions structure on the size and polydispersity of PECs, the intensity of particle size distribution, determined by DLS, was also represented for all studied systems and some selected molar ratio between charges (Figure 4).

As Figure 4 shows, unimodal size distribution was observed for all studied systems, irrespective of the molar ratio between charges. At the same time, high values of polydispersity were observed at values of molar ratio n^-/n^+ below and around $(n^-/n^+)_n$, the highest values being obtained for the PAH/PAMPSAA pair. Moreover, a small decrease of the intensity of particle size distribution at $n^-/n^+ = 1.2$ was evident for all studied samples. This behavior suggests that the secondary aggregation by inter-particles interactions was not so fast and took place only when the polyanion was in excess, leading to a monotonous increase of the complex particles sizes (Figure 3).

3.2.3. Atomic force microscopy

In Figure 5, the AFM images of the PEC dispersion formed between all the studied polyions, at a molar ratio between charges of 1.2 are presented.

As Figure 5 shows, the adsorption of PEC particles on silicon wafers surface, resulted in an inhomogeneous mixture of smaller and bigger particles, for the studied samples. This is not surprising since the PEC particles showed a high polydispersity, evidenced by DLS measurements (Figure 4). Moreover, the AFM images confirm the results presented in Figure 3, namely bigger particle sizes can be observed for PECs prepared with CS as compared to PAH for the same polyanion (Figures 5a and c), and with PAMPSAA as compared to PAA for the same polycation (Figures 5b and d).

The structure of polyanion strongly influenced the PECs particles shape: with PAA almost spherical particles were formed, but with PAMPSAA an irregular shape is evident, irrespective of polycation structure, probably due to the irregular compensation of the copolymer ionic sites.

The profiles of some selected particles of each sample (marked with a white line in Figures 5a–d) are presented in Figure 6.

Even if the interaction with the silica surface could induce conformational changes in the individual PEC particles, due to electrostatic interactions dur-

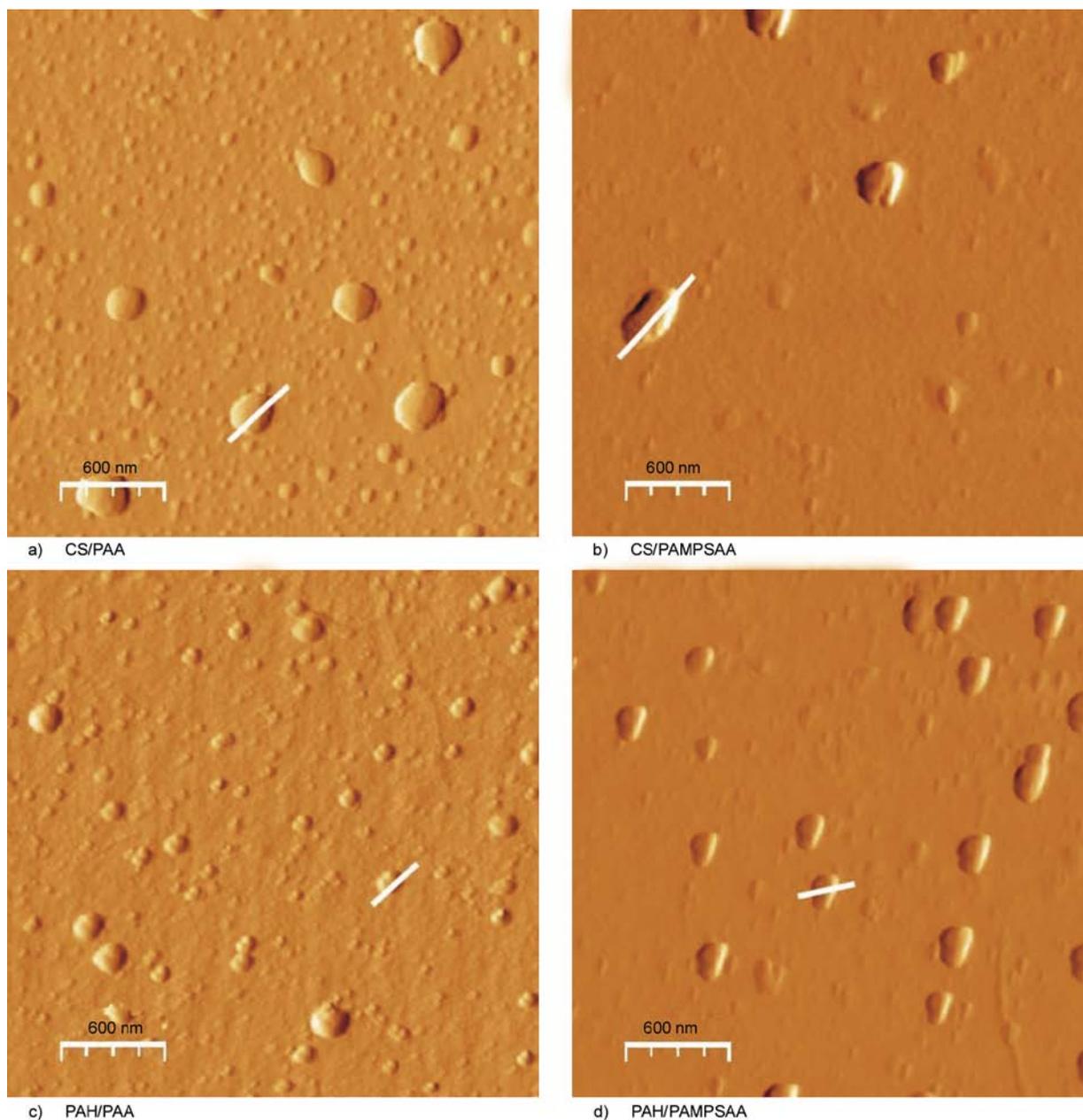


Figure 5. Tapping mode amplitude AFM images of the complex particles at a molar ratio between charges of 1.2, adsorbed on the silicon wafers

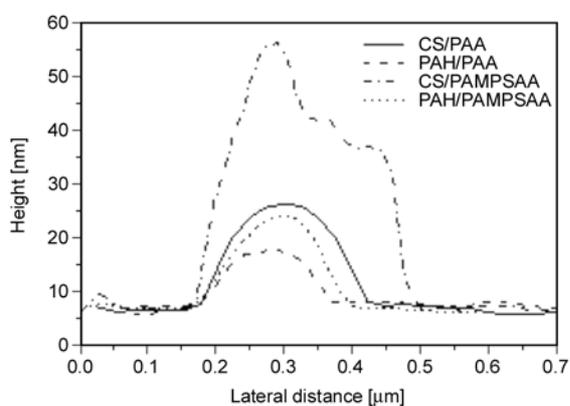


Figure 6. AFM profiles of some selected PEC particles (see white lines labeled particles in Figure 5) adsorbed on silicon wafers

ing adsorption, after the air-drying procedure, the PECs particles still display 3D structures, the values of particles height increasing with their diameter, irrespective of the polyion pair involved in their preparation. Moreover, the values of the particles height were in agreement with the hydrodynamic diameter values (Figure 3), the smallest values being obtained for PAH/PAA complex particles and the highest for CS/PAMPSAA particles.

3.3. Colloidal stability

When the complexation takes place between polyelectrolytes having significantly different molecular weights, weak ionic groups, in nonstoichiometric mixing ratios and under dilute conditions, the reaction mixture develops a colloidal, Tyndall effect. This behavior is ascribed to PECs structure, consisting of a neutral core surrounded by the polyelectrolyte in excess, which stabilizes PECs particles against aggregation, to provide practical nanoscale and microscale products [2, 6, 9, 11]. The excess polyelectrolyte dictates the conditions for stability in different medium conditions. A very important characteristic of the PECs as colloidal dispersion is their colloidal stability. Storage colloidal stability of PECs is very sensitive to the structure of the complementary polyions, to the molar ratio between charges, i.e., the complex is closer or further to the molar ratio $(n^-/n^+)_n$, to the polyions addition order, especially when the complementarities of oppositely charged polyions is disturbed by their structure, and very much to the titrant addition rate, as it was previously demonstrated for PECs prepared with strong polyanions [31–33]. Therefore, the particle sizes, D_h , measured after 24 h from preparation were compared with those measured after two weeks of storage at room temperature ($\sim 22^\circ\text{C}$), without stirring (data not shown here). A high colloidal stability, i.e., low changes in the particle sizes, was found for PAH based PECs, along the whole range of investigated molar ratios between charges. As concerns the CS based PECs, when the polyanion is in excess the values of the particle sizes only slightly changed, the differences being more significant when CS was in excess, compared with those measured after 24 h, the increase of the particles size being in the range of 15–30 nm. These differences can be ascribed to the possible interactions between the primary aggregates, accompanied by rearrangements of chains and the formation of compact particles with higher sizes.

4. Conclusions

Two polyanions, PAA and a copolymer of AMPS with AA, and two weak polycations, PAH and CS, were used in the preparation of some PEC dispersions. Turbidimetry, DLS and AFM have been used as complementary methods to describe the charac-

teristics of the PEC dispersions as a function of the polyions structure and the ratio between charges.

The value of molar ratio $(n^-/n^+)_n$ was clearly influenced by the polyanion structure, and was located at about 1.0 and 0.7 when PAA and PAMPSAA were respectively involved in the complexes preparation, irrespective of the polycation structure. The deviation of the endpoint of polyelectrolyte interactions to lower values of $(n^-/n^+)_n$, in the case of PAMPSAA based PECs, can be ascribed to the presence of AMPS groups, which probably hindered the AA groups, conducting to a partially intrinsic compensate of carboxylic groups by the small counterions. The AFM images indicated that the adsorbed particles prepared with a molar ratio between charges of $n^-/n^+ = 1.2$, appeared as compact particles, their size and shape being strongly influenced by the complementary polyelectrolytes structure.

A high colloidal stability was found for PAH based PECs, on the whole range of investigated molar ratios between charges, and after the $(n^-/n^+)_n$ for the CS based PECs.

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Abbreviations

AFM	atomic force microscopy
CS	chitosan
D_h	hydrodynamic diameter
DLS	dynamic light scattering
n^-/n^+	the molar ratio between anionic and cationic units
$(n^-/n^+)_n$	the molar ratio between anionic and cationic units when neutral complex particles were obtained
OD_{500}	optical density at $\lambda = 500$ nm
PAA	poly(acrylic acid)
PAH	poly(allylamine hydrochloride)
PAMPSAA	poly(2-acrylamido-2-methylpropanesulfonic acid – co- acrylic acid)
PI	polydispersity index
PEC	polyelectrolyte complexes
pzc	point of zero charge

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