

## Nanoparticles formed by complexation of poly-gamma-glutamic acid with lead ions

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Received 16 December 2006; received in revised form 4 June 2007; accepted 24 September 2007

Available online 29 September 2007

### Abstract

The present investigation describes the preparation and characterization of novel biodegradable nanoparticles based on complexation of poly-gamma-glutamic acid ( $\gamma$ -PGA) with bivalent lead ion. The prepared nano-systems were stable in aqueous media at low pH, neutral and mild alkaline conditions. The particle size and the size of the complexes were identified by dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements. It was found that the size of the complexes depended on the pH and concentrations of  $\gamma$ -PGA and lead ions. Particle sizes measured by TEM revealed that at low concentrations, nanosized particles were formed, however, at high concentrations of  $\gamma$ -PGA and lead ions, the formation of large aggregates with a broad size distribution was promoted. The size of individual particles was in the range of 40–100 nm measured by TEM. The results from the DLS measurements showed that the low and high pH values in mixtures with high concentrations of  $\gamma$ -PGA and  $\text{Pb}^{2+}$  ions favored the growth of large complexes. The  $\gamma$ -PGA nanoparticles, composed of a biodegradable biomaterial with high flocculating and heavy metal binding activity, may be useful for various water treatment applications.

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**Keywords:**  $\gamma$ -PGA; Lead binding; Nanoparticles; Complexation

### 1. Introduction

Effective methods that will allow a reduction of industrial water consumption are becoming increasingly important. One such method is enhanced ultrafiltration using polymers to remove metal ions from diluted wastewater streams [1,2]. Biomacromolecules have an important role in a wide range of industrial fields including water treatment [3,4]. For separation of toxic heavy metal ions, including lead ions, several natural polymers have been investigated [5,6]. The most valuable properties of these biopolymers are their biocompat-

ibility, biodegradability, and flocculating activity for metal ions [7,8].

Flocculation is a process that enhances the agglomeration or collection of smaller floc particles into larger, more easily sedimentable particles through gentle stirring by hydraulic or mechanical means. The addition of flocculating agents may promote the formation of flocs. Flocculating agents that are usually used for water treatment can be categorized into three major groups, namely, inorganic flocculants [9], organic synthetic polymer flocculants [10,11], and natural biopolymer flocculants [12,13]. Among these flocculants, organic synthetic polymers are widely used, because they are inexpensive and effective. However, the use of these flocculants may give rise to environmental and health problems, because some of them are not completely biodegradable, and the intermediate products of their degradation are toxic for humans [14]. To solve these problems, biodegradable flocculants have been

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investigated to minimize the risk for the environment and humans.

Flocculation of polyelectrolytes in the presence of bivalent ions is an important process, and is widely used in water treatment technologies. For separation of toxic heavy metal ions, natural poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA) and other natural polymers have been investigated [3,5]. The aggregate size distribution in flocculants was studied and it was found that it mainly depended on the pH and concentration of the electrolytes [15].

$\gamma$ -PGA is a water soluble, biodegradable, edible and nontoxic polyanion [16,17]. The potential applications of  $\gamma$ -PGA and its derivatives have been used in a wide range of industrial fields such as cosmetics, food, medicine, and water treatment [18,19]. Poly- $\gamma$ -glutamic acid consists of repetitive glutamic acid units connected by amide linkages between  $\alpha$ -amino and  $\gamma$ -carboxylic acid functional groups. The secondary structure of PGA has been described as an  $\alpha$ -helix in a water solution. The naturally produced  $\gamma$ -PGA usually contains nearly equal amounts of D- and L-units and the molecular weight of the polymer is usually high (up to 1.2 million). The ratio of the two optical isomers can partially be controlled by technological means so that polymers with varying degree of stereoregularity can be made [20].  $\gamma$ -PGA is different from other proteins, in that glutamate is polymerized via the  $\gamma$ -amide linkages, and thus is synthesized in a ribosome-independent manner.

The present investigation reports the formation of complexes of poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA) with bivalent lead ions. The solubility and size of these nanoparticles in the dried and swollen states will be described and discussed. In aqueous solution the average size of the particles varies strongly depending on pH and the concentrations of  $\gamma$ -PGA and  $\text{Pb}^{2+}$ . The stable nanoparticles were visualized by TEM measurements. The  $\gamma$ -PGA–lead complexes may form more or less stable colloids in aqueous media depending on the pH, and the concentrations of  $\gamma$ -PGA and lead ions in the mixture. Because  $\gamma$ -PGA is a poly-acid and has good flocculating activity, high affinity and binds toxic heavy metal ions, such as lead, it may be useful for treating wastewater.

To characterize large flocs that precipitate under certain conditions, density measurements have been conducted. Thermodynamic properties of the systems at different pH and  $\gamma$ -PGA/ $\text{Pb}^{2+}$  compositions have been surveyed by turbidity experiments, the sizes of the particles and complexes in aqueous solutions have been determined by means of dynamic light scattering (DLS). The TEM results on the systems in the dry state disclosed the existence of large clusters when the level of  $\gamma$ -PGA and  $\text{Pb}^{2+}$  ions in the mixture is high.

## 2. Materials and methods

### 2.1. Chemicals and materials

Poly- $\gamma$ -glutamic acid ( $M_w = 1.2 \times 10^6$ ) was prepared in our laboratory by using the biosynthetic methods described earlier [21,22]. Briefly,  $\gamma$ -PGA was produced by bacterial fermentation using *Bacillus licheniformis* strain ATCC 9945a on fermentation

medium “E” at 37 °C. After incubation, the contents of the culture fluids were centrifuged and filtered through a 0.22  $\mu\text{m}$  pore size nitrocellulose membrane under pressure to remove the bacterial biomass. After the addition of 2½ volumes of ice cold 95% acetone to the filtrate, the precipitated polypeptide was separated by centrifugation. The  $\gamma$ -PGA was redissolved in and was dialysed exhaustively against distilled water, finally the polypeptide solution was freeze-dried. Lead(II)-nitrate was purchased from Sigma–Aldrich Co., Hungary, and was used as received without further purification.

### 2.2. Synthesis of $\gamma$ -PGA nanoparticles with lead ions

$\gamma$ -PGA solution ( $c = 6$  mmol, pH 2.6) and  $\text{Pb}(\text{NO}_3)_2$  solution ( $c = 3.125$  mmol, pH 2.3) were produced and used for preparation of  $\gamma$ -PGA nanoparticles by lead ion complexation.  $\text{Pb}(\text{NO}_3)_2$  solution was added to the  $\gamma$ -PGA solution dropwise. The mixture was diluted to 50 ml and the pH was adjusted to the desired pH value with 0.1 M sodium hydroxide solution. The reaction mixture was stirred at room temperature. Formation of  $\gamma$ -PGA particles with bivalent lead ions at diverse stoichiometric ratios and concentrations were made according to the described reaction conditions summarized in Table 1.

### 2.3. Characterization of the samples

#### 2.3.1. Density measurements

The density measurements were carried out using a DMA 5000 digital densitometer from A. Paar GmbH, Graz, Austria. The precision in the measured density values is better than  $\pm 1.0 \times 10^{-5}$  g/cm<sup>3</sup>. All experiments were performed at 25 °C.

#### 2.3.2. Turbidimetry

The transmittances of  $\gamma$ -PGA/ $\text{Pb}^{2+}$  mixtures of different composition and pH were measured with a temperature controlled Helios Gamma (Thermo Spectronic, Cambridge, UK) spectrophotometer at a wavelength of 500 nm. At this wavelength, strong signals are obtained from the samples. The trend of the turbidity is the same also at other wavelengths. The apparatus is equipped with a temperature unit (Peltier plate) that gives a good temperature control ( $25 \pm 0.05$  °C) over an extended time. The turbidities ( $\tau$ ) of the samples can be determined from the following relationship:  $\tau = (-1/L) \ln(I_t/I_0)$ , where  $L$  is the light path length in the cell (1 cm),  $I_t$  the transmitted light intensity, and  $I_0$  is the incident light intensity. The results from the spectrophotometer are presented in terms of the turbidity.

Table 1

Reaction conditions for the formation of  $\gamma$ -PGA nanoparticles by lead ion complexation

Sample	$\gamma$ -PGA solution	$\text{Pb}(\text{NO}_3)_2$ solution
PGA-Pb 1 (3–1)	25 ml (3 mM)	16 ml (1.0 mM)
PGA-Pb 2/2 (1.5–2.5)	12.5 ml (1.5 mM)	8 ml (0.5 mM)
PGA-Pb 3 (0.75–0.25)	6.25 ml (0.75 mM)	4 ml (0.25 mM)
PGA-Pb 2/1 (1.5–1)	12.5 ml (1.5 mM)	16 ml (1.0 mM)
PGA-Pb 2/3 (1.5–0.25)	12.5 ml (1.5 mM)	4 ml (0.25 mM)

### 2.3.3. Transmission electron microscopy (TEM)

A JEOL2000 FX-II transmission electron microscope was used to characterize the size and morphology of the dried  $\gamma$ -PGA nanoparticles. For TEM observation, the nanoparticles were prepared from the reaction mixture, where the pH of the solution was 3.0. The sample for TEM analysis was obtained by placing a drop of the colloid dispersion containing the  $\gamma$ -PGA nanoparticles onto a carbon-coated copper grid. It was dried at room temperature and examined using a TEM without any further modification or coating.

### 2.3.4. Dynamic light scattering (DLS)

The beam from an argon ion laser (Lexel laser, model 95), operating at 514.5 nm with vertically polarized light, was focused onto the sample cell through a temperature-controlled chamber (temperature controlled to within  $\pm 0.05$  °C) filled with refractive-index-matching silicone oil. The sample solutions were filtered through 5  $\mu$ m filters (Millipore) directly into pre-cleaned 10 mm NMR tubes (Wilmad Glass, USA) of highest quality.

The light-scattering process defines a wave vector  $q = (4\pi n/\lambda) \sin(\theta/2)$ , where  $\theta$  is the scattering angle and  $n$  is the refractive index of the medium. The value of  $n$  was determined for all samples at  $\lambda = 514.5$  nm by employing an Abbé refractometer.

In the present study, the full homodyne intensity autocorrelation function  $g^2(t)$  was mostly measured at a scattering angle of  $90^\circ$  with an ALV-5000 multiple- $\tau$ -digital correlator. If the scattered field obeys Gaussian statistics (as for all the solutions considered in this study) the measured correlation function  $g^2(t)$  can be related to the theoretically amenable first-order electric field correlation function  $g^1(t)$  by the Siegert relationship  $g^2(t) = 1 + B|g^1(t)|^2$ , where  $B$  is an instrumental parameter. The correlation functions were recorded in the real time “multiple- $\tau$ ” mode of the correlator, in which 256 time channels are logarithmically spaced over an interval ranging from 0.2  $\mu$ s to almost 1 h.

For the  $\gamma$ -PGA particles and the  $\gamma$ -PGA/Pb<sup>2+</sup> complexes, the decays of the correlation functions were always found to be bimodal [23–25] initially a single exponential, followed at longer times by a stretched exponential:

$$g^1(t) = A_f \exp\left(\frac{-t}{\tau_f}\right) + A_s \exp\left[-\left(\frac{t}{\tau_{se}}\right)^\beta\right] \quad (1)$$

with  $A_f + A_s = 1$ . The parameters  $A_f$  and  $A_s$  are the amplitudes for the fast and the slow relaxation mode, respectively. Analyses of the time correlation functions of the concentration fluctuations in the domain  $qR_h < 1$  ( $R_h$  is the hydrodynamic radius) have shown [22–24] that the first term (short-time behavior) on the right-hand side of Eq. (1) is related to the mutual diffusion coefficient  $D_m$  ( $\tau_f^{-1} = D_m q^2$ ). In this study, the samples probably consisted of single particles and aggregates due to complexation. The fast relaxation mode monitors the diffusion of single particles and some small clusters of particles. In this work, the second term (long-time feature) was also found to be diffusive and this slow relaxation mode was expected to be associated

with the diffusion of large complexes. Since the principal aim of this study was to monitor the size (hydrodynamic radius) and distribution of sizes of the species under various conditions of pH and  $\gamma$ -PGA/Pb<sup>2+</sup> compositions, we have chosen to perform most of the measurements at a fixed scattering angle ( $90^\circ$ ) and to analyze the relaxation results in terms of hydrodynamic radii ( $R_{h,f}$  and  $R_{f,s}$  determined from the fast and the slow relaxation mode, respectively), calculated via the Stokes–Einstein relationship. The variable  $\tau_{se}$  is some effective relaxation time, and  $\beta$  ( $0 < \beta \leq 1$ ) is a measure of the width of the distribution of relaxation times. The mean relaxation time is given by

$$\tau_s = \frac{\tau_{se}}{\beta} \Gamma\left(\frac{1}{\beta}\right) \quad (2)$$

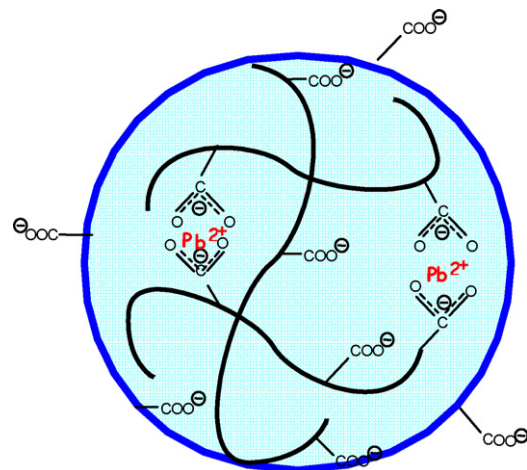
where  $\Gamma(\beta^{-1})$  is the gamma function of  $\beta^{-1}$ .

In the analysis of the correlation function data, a non-linear fitting algorithm (a modified Levenberg–Marquardt method) was used to obtain best-fit values of the parameters  $A_f$ ,  $\tau_f$ ,  $\tau_{se}$ , and  $\beta$  appearing on the right-hand side of Eq. (1).

## 3. Results and discussion

### 3.1. Formation of nanoparticles

The carboxylic groups of linear  $\gamma$ -PGA chains were cross-linked with lead ions, and formed stable nanoparticles (Scheme 1). Complexation was observed and separated spherical particles or aggregates were obtained depending on pH and the  $\gamma$ -PGA/Pb<sup>2+</sup> composition. The size and the stability of the particles depended on the pH and the concentrations of  $\gamma$ -PGA and Pb<sup>2+</sup> ions. In neutral and alkali media, the carboxylic groups are deprotonated, and the repulsive electrostatic forces between the negatively charged parts affect the physicochemical properties of these particles. In acidic media,  $\gamma$ -PGA is an uncharged macromolecule, and this may lead to smaller sizes of the  $\gamma$ -PGA/Pb<sup>2+</sup> particles unless the concentrations of the components in the mixture are sufficiently high to form intermolecular complexes.



Scheme 1. Schematic illustration of  $\gamma$ -PGA-nanoparticles formed through the interaction with lead ions.

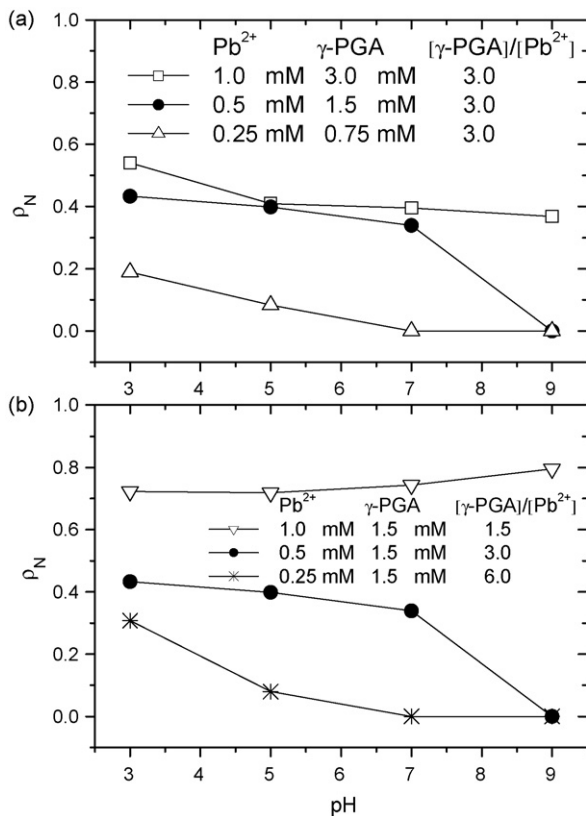


Fig. 1. Effects of pH on the normalized density at the compositions of the mixture indicated.

### 3.2. Solubility and sedimentation of particles

To characterize this process, we have carried out density measurements. Depending on the pH value and the concentrations of  $\gamma\text{-PGA}$  and  $\text{Pb}^{2+}$  ions, turbid dispersions were observed and huge aggregates formed that precipitated. To quantitative the aggregation and precipitation processes, we have carried out density measurements on samples with sedimented particles ( $\rho_{\text{sed}}$ ) and on mixtures without precipitation ( $\rho_{\text{m}}$ ). In Fig. 1, a normalized density  $\rho_N = (\rho_{\text{m}} - \rho_{\text{sed}})/(\rho_{\text{m}} - \rho_{\text{w}})$ , where  $\rho_{\text{w}}$  is the water density, is plotted as a function of pH for  $\gamma\text{-PGA}/\text{Pb}^{2+}$  mixtures at a fixed ratio and at different compositions. In this plotting procedure,  $\rho_N = 0$  suggested that no precipitate was formed, whereas a value of  $\rho_N = 1$  indicated that all the material has sedimented. In the case of the lowest concentrations of  $\gamma\text{-PGA}$  and  $\text{Pb}^{2+}$  ions (Fig. 1a), precipitation occurred at lower values of pH, where the polymer is uncharged and probably the addition of bivalent ions makes the solubility power of the solvent weaker for the polymer. A similar but stronger trend was observed for the  $\gamma\text{-PGA}\text{-Pb}$  2/2 mixture, which suggested that the higher concentrations of the components strengthened the tendency to form large complexes that sediment. For the sample with the highest concentrations ( $\gamma\text{-PGA}\text{-Pb}$  1) of the components, pronounced precipitation was observed even at high pH values. The reason is probably that in this case enhanced interchain complexation of the  $\gamma\text{-PGA}$  chains with lead ions occurred at low pH with the formation of large clusters that sediment.

When the concentration of  $\gamma\text{-PGA}$  was kept constant in the mixture (Fig. 1b), an interpolymer complexation accompanied with precipitation occurred at low pH and low  $\text{Pb}^{2+}$  concentration. At the highest level of  $\text{Pb}^{2+}$  addition, a large fraction of sedimented material was observed over the whole pH range. Finally, the density measurements of the precipitates were consistent with visual inspection of the samples.

### 3.3. Turbidimetry

Results from the turbidity measurements and the DLS experiments, were only presented for systems that exhibited weak or no precipitation. The systems were studied at equilibrium conditions to obtain reproducible results, and to acquire a consistent picture from the employed experimental techniques. With the experimental methods we have used, it is difficult to monitor the precipitation kinetics of the systems.

Fig. 2 shows the pH dependence of the turbidity at a fixed (3.0)  $\gamma\text{-PGA}/\text{Pb}^{2+}$  ratio (Fig. 2a) and for mixtures with a constant  $\gamma\text{-PGA}$  concentration (Fig. 2b). The general trend that appears in Fig. 2a is that the value of the turbidity rises with increasing pH, and this effect becomes stronger when the concentrations of the components increased. This is compatible with the idea that interpolymer associations were formed under acidic conditions, and this behavior should have been strengthened as the concentrations of  $\gamma\text{-PGA}$  and  $\text{Pb}^{2+}$  increased. At a constant  $\gamma\text{-PGA}$  concentration (1.5 mM), a higher level of  $\text{Pb}^{2+}$  addition lead to a pronounced increase of the turbidity at low pH values, which suggests that higher  $\text{Pb}^{2+}$  concentrations promote interchain associations because of poorer solvent power.

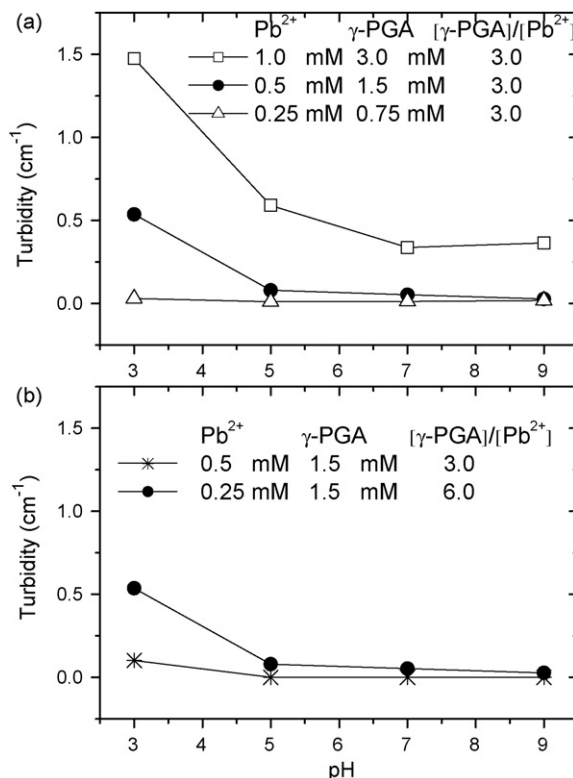


Fig. 2. Effects of pH on the turbidity at the mixture conditions indicated.

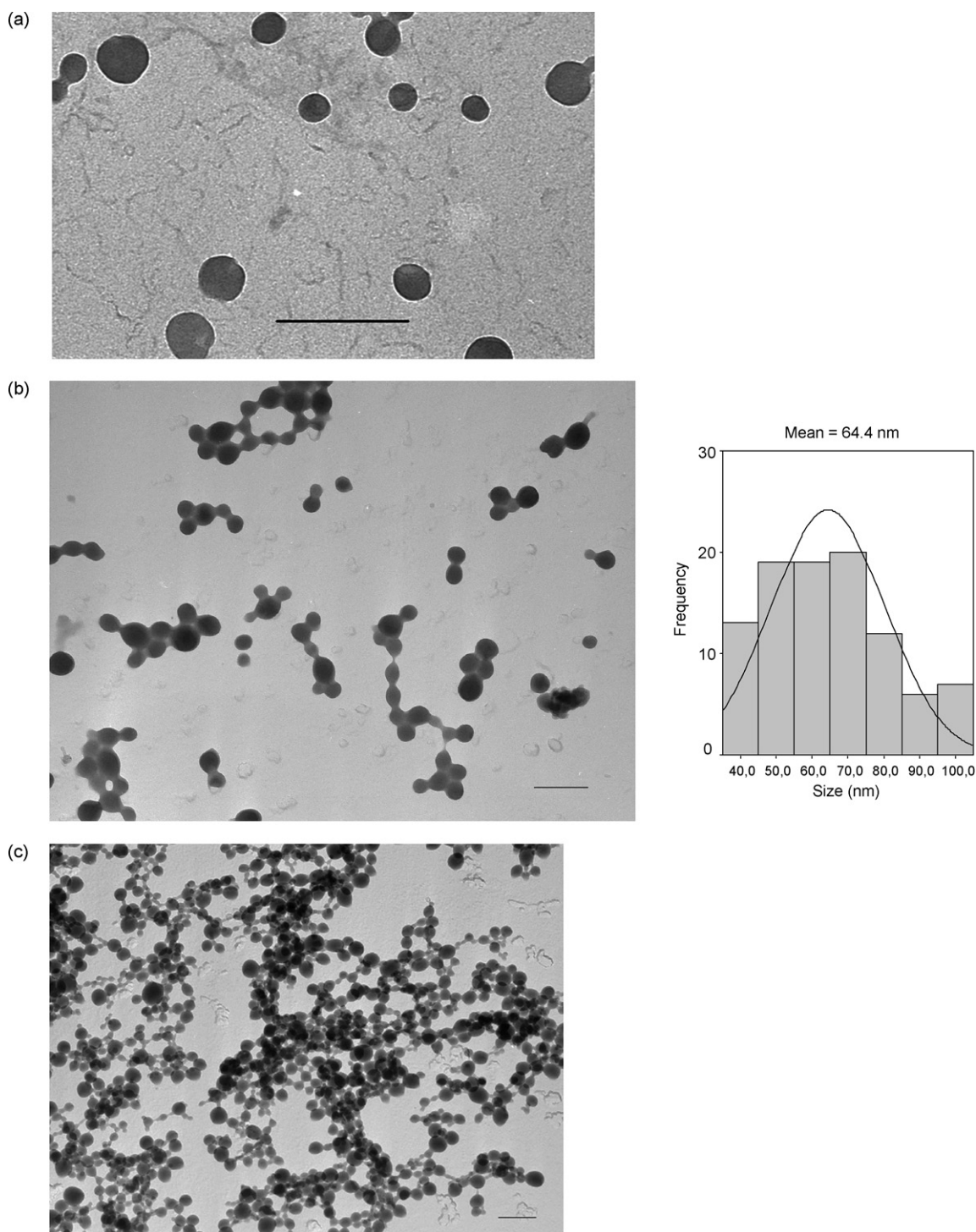


Fig. 3. (a) TEM image of  $\gamma$ -PGA nanoparticles formed in sample PGA-Pb 3 by bivalent lead ions. The bar in the figure is 200 nm. (b) TEM image of flocs of  $\gamma$ -PGA nanoparticles formed in the sample PGA-Pb 2/2 by bivalent lead ions. The bar in the figure is 200 nm. The size distribution of the species is given in the histogram to the right. (c) TEM image of complexes of  $\gamma$ -PGA nanoparticles formed in the sample PGA-Pb 1 by bivalent lead ions. The bar in the figure is 200 nm.

#### 3.4. Particle size by TEM

TEM micrographs in Fig. 3 confirmed the nanosize and the structure for dried systems of  $\gamma$ -PGA particles in the presence of  $\text{Pb}^{2+}$  ions. By increasing the level of  $\gamma$ -PGA and  $\text{Pb}^{2+}$  ions in the dried sample, a broad distribution of aggregates with various sizes were observed. This can probably be ascribed to interpoly-

mer complexation of the polymer with lead ions. We note that very large clusters of particles can be observed, which indicates that higher concentrations of the components favor the formation of huge flocs. At still higher concentrations of the components, giant flocs with connectivity resulted. These findings clearly suggest that high concentrations of  $\gamma$ -PGA and  $\text{Pb}^{2+}$  promote the growth of large complexes.

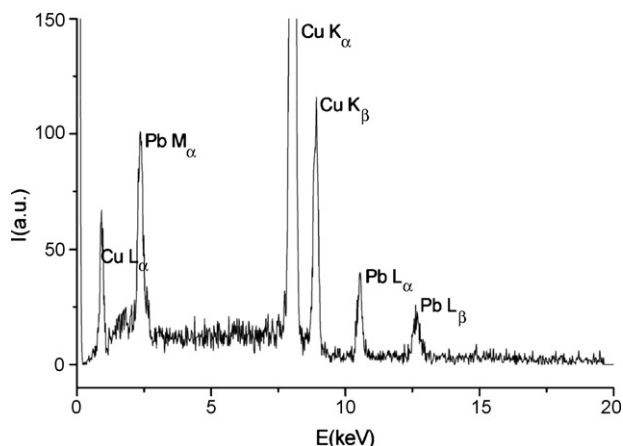


Fig. 4. Spectrum from elemental analysis of the sample PGA-Pb 1.

Elemental analysis determined the heavy metal content of nanoparticles. Fig. 4 shows the high lead content of dried sample, of the nanoparticles. The spectrum confirmed that a complex of the polymer with lead ions was formed. The copper content of the elemental analysis sample resulted from contamination by the copper grid used to support the samples.

### 3.5. Dynamic light scattering

Fig. 5 shows the pH dependences of the hydrodynamic radii derived from the fast ( $R_{h,f}$ ) and the slow ( $R_{h,s}$ ) relaxation mode,

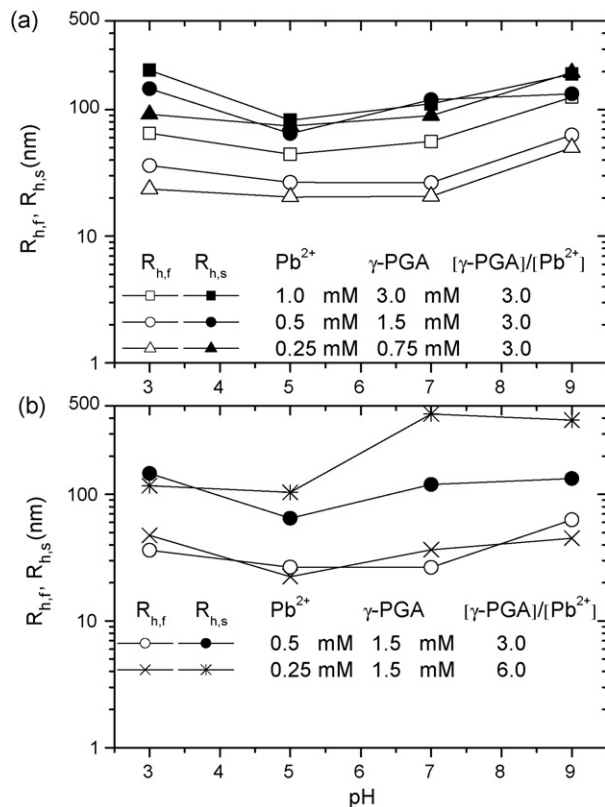


Fig. 5. Effects of pH on the hydrodynamic radii from the fast ( $R_{h,f}$ ) and the slow ( $R_{h,s}$ ) relaxation modes at the compositions of the mixture indicated.

for  $\gamma$ -PGA/Pb<sup>2+</sup> mixtures. These studies were done with a fixed value (Fig. 5a) of the ratio and at different values (Fig. 5b). At a constant ratio, the value of  $R_{h,f}$  increased as the concentration of the components were raised, and the upturn of  $R_{h,f}$  at low pH was most pronounced at the highest concentration of the components. These findings confirmed that low pH and high levels of  $\gamma$ -PGA and Pb<sup>2+</sup> favored the formation of intermolecular associations. At high pH, the growth of  $R_{h,f}$  was stronger at the highest concentration of the components, which probably reflected the formation of oppositely charged intermolecular  $\gamma$ -PGA/Pb<sup>2+</sup> complexes. The parameter  $R_{h,f}$  monitored some average size of single particles and small particle clusters. This means that the growth of  $R_{h,f}$  indicated that the number of small aggregates in the system increased. The values of  $R_{h,s}$  were significantly higher than the corresponding values of  $R_{h,f}$  (cf. Fig. 5a). A prominent feature was the strong upturn of  $R_{h,s}$  at low pH when the concentrations of  $\gamma$ -PGA and Pb<sup>2+</sup> were high, which again suggested that acid conditions promoted the growth of large clusters.

The results in Fig. 5b revealed that  $R_{h,f}$  was almost independent of pH. The effect of moderate Pb<sup>2+</sup> additions on the value of  $R_{h,f}$  (the average value of  $R_{h,f}$  is approximately 40 nm) was modest. The behavior of  $R_{h,s}$ , which reflects the size of the large species, was quite different especially at the low lead ion concentration. If the proportion of lead ions is very small, the lead ions cannot react with the carboxylic groups optimally at higher values of pH. This is expected to lead to a result in which an increase in pH will give rise to the formation of large-scale

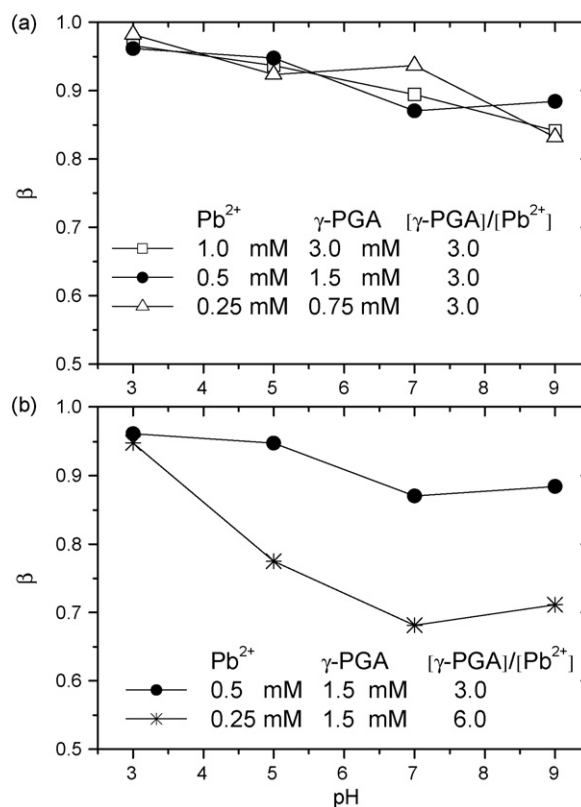


Fig. 6. Effects of pH on the exponent  $\beta$  at the compositions of the mixture indicated.

complexes that have a broad distribution of sizes. At higher levels of lead ions, intermolecular complexes between  $\gamma$ -PGA and  $Pb^{2+}$  can be formed at higher pH, and this was reflected by the enhanced values of  $R_{h,s}$ . At low pH, poorer thermodynamic conditions (lower solvent power) may yield larger association complexes.

Fig. 6 shows the effects of pH and  $\gamma$ -PGA/ $Pb^{2+}$  concentrations on the parameter  $\beta$ , which characterizes the width of the distribution of relaxation times of the correlation function. In the case of a constant  $\gamma$ -PGA/ $Pb^{2+}$  ratio,  $\beta$  was virtually independent of the concentrations of  $\gamma$ -PGA and  $Pb^{2+}$  over the considered pH range. The decrease of  $\beta$  with increasing pH indicated a broader distribution of particle sizes, which may be attributed to the growth of the complexes at higher values of pH. In the case of a constant  $\gamma$ -PGA concentration, a marked drop of  $\beta$  with increasing pH was evident at the lowest  $Pb^{2+}$  concentration, which reflected a great difference in the sizes of the complexes formed at a deficiency of lead ions. At a higher level of  $Pb^{2+}$  ions, the modest decrease of the value of  $\beta$  reflected complexes with a rather narrow size distribution. The decrease may indicate growth of the number of intermolecular complexes.

#### 4. Conclusions

In this work, the complexation between  $\gamma$ -PGA and  $Pb^{2+}$  ions at different concentrations of the components and at various pH values was studied using several experimental methods. Depending on the concentrations of the polymer and the lead ions, flocculation and sedimentation of large complexes may occur. High concentration of the components and low pH values promoted the formation of huge aggregates. A quantitative account of the amount of complex precipitation in the systems was obtained from the density measurements. The turbidity experiments support the hypothesis that high concentrations of the components and low pH favored the growth of clusters.

The TEM measurements of the  $\gamma$ -PGA/ $Pb^{2+}$  mixtures in the dry state have shown that individual nanoparticles were formed together with some clusters of particles when the levels of added  $\gamma$ -PGA and  $Pb^{2+}$  ions were low. At high concentrations of the components, intermolecular  $\gamma$ -PGA/ $Pb^{2+}$  complexes (with a broad distribution of sizes) were formed and they appeared to be interconnected.

The DLS experiments also have shown the effects of pH and composition on the size and size distribution of the clusters. The general picture that emerged was that at high concentration of the components in the mixture, low pH values produced association complexes because of poorer solvent power, whereas at high pH, where the polymer is charged, intermolecular  $\gamma$ -PGA/ $Pb^{2+}$  complexes (with a broad distribution of sizes) were formed. An interesting feature was observed when there was a deficiency of  $Pb^{2+}$  ions in the sample. In this case, large clusters with a wide size distribution were formed at high pH values. The strong complexation capacity of  $\gamma$ -PGA nanoparticles for lead ions indicates a promising sorbent for removal of heavy metals in polluted water.

#### Acknowledgement

This work was supported by RET (Grant of Regional University Knowledge Center) contract number (RET-06/432/2004) and by ElizaNor Polymer LLC, USA.

#### References

- [1] R. Molinari, S. Gallo, P. Argurio, Metal ions removal from wastewater or washing water from contaminated soil by ultrafiltration-complexation, *Water Res.* 38 (2004) 593–600.
- [2] J. Llorens, M. Pujola, J. Sabate, Separation of cadmium from aqueous streams by polymer enhanced ultrafiltration: a two-phase model for complexation binding, *J. Membr. Sci.* 239 (2004) 173–181.
- [3] M. Taniguchi, K. Kato, A. Shimauchi, X. Ping, H. Nakayama, K.I. Fujita, T. Tanaka, Y. Tarui, E. Hirasawa, Proposals for wastewater treatment by applying flocculating activity of cross-linked poly- $\gamma$ -glutamic acid, *J. Biosci. Bioeng.* 99 (2005) 245–251.
- [4] U.S. Ramelow, C.N. Guidry, S.D. Fisk, A kinetic study of metal ion binding by biomass immobilized in polymers, *J. Hazard. Mater.* 46 (1996) 37–55.
- [5] D. Solpan, M. Torun, Investigation of complex formation between (sodium alginate/acrylamide) semi-interpenetrating polymer networks and lead, cadmium, nickel ions, *Colloid Surf. A* 268 (2005) 12–18.
- [6] L. Qi, Z. Xu, Lead sorption from aqueous solutions on chitosan nanoparticles, *Colloid Surf. A* 251 (2004) 183–190.
- [7] M. Taniguchi, K. Kato, A. Shimauchi, X. Ping, K.I. Fujita, T. Tanaka, Y. Tarui, E. Hirasawa, Physicochemical properties of cross-linked poly- $\gamma$ -glutamic acid and its flocculating activity against kaolin suspension, *J. Biosci. Bioeng.* 99 (2005) 130–135.
- [8] H. Yokoi, T. Arima, J. Hirose, S. Hayashi, Y. Takasaki, Flocculation properties of poly-( $\gamma$ -glutamic acid) produced by *Bacillus subtilis*, *J. Ferm. Bioeng.* 83 (1996) 84–87.
- [9] W.Y. Yang, J.W. Qian, Z.Q. Shen, A novel flocculant of Al(OH)<sub>3</sub>-polyacrylamide ionic hybrid, *J. Colloid Interf. Sci.* 273 (2004) 400–405.
- [10] A. Denizli, B. Garipcan, A. Karabacan, H. Senoz, Synthesis and characterization of poly(hydroxyethyl methacrylate-*N*-methacryloyl-(L)-glutamic acid) copolymer beads for removal of lead ions, *Mater. Sci. Eng. C* 25 (2005) 448–454.
- [11] B.L. Rivas, E.D. Pereira, I. Moreno-Villoslada, Water-soluble polymer–metal ion interactions, *Prog. Polym. Sci.* 28 (2003) 173–208.
- [12] I.L. Shih, Y.T. Van, L.C. Yeh, H.G. Lin, Y.N. Chang, Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties, *Bioresource Technol.* 78 (2001) 267–272.
- [13] M. Taniguchi, K. Kato, O. Matsui, X. Ping, H. Nakayama, Y. Usuki, A. Ichimura, K.I. Fugita, T. Tanaka, Y. Tarui, E. Hirasawa, Flocculating activity of cross-linked poly- $\gamma$ -glutamic acid against bentonite and *Escherichia coli* suspension pretreated with FeCl<sub>3</sub> and its interaction with Fe<sup>3+</sup>, *J. Biosci. Bioeng.* 100 (2005) 207–211.
- [14] K.L. Dearfield, J.F. Abernathy, Acrylamide—its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity, *Mutat. Res.* 195 (1988) 45–47.
- [15] C. Rattanakawin, R. Hogg, Aggregate size distributions in flocculation, *Colloid Surf. A* 177 (2001) 87–98.
- [16] W.C. Lin, D.G. Yu, M.C. Yang, Blood compatibility of novel poly-( $\gamma$ -glutamic acid)/polyvinyl alcohol hydrogels, *Colloid Surf. B* 47 (2006) 43–49.
- [17] C.Y. Hsieh, S.P. Tsai, D.M. Wang, Y.N. Chang, H.J. Hsieh, Preparation of  $\gamma$ -PGA/chitosan composite tissue engineering matrices, *Biomaterials* 26 (2005) 5617–5623.
- [18] I.L. Shih, Y.T. Van, The production of poly-( $\gamma$ -glutamic acid) from microorganisms and its various applications, *Bioresource Technol.* 79 (2001) 207–225.
- [19] S.S. Mark, T.C. Crusberg, C.M. DaCunha, A.A. Di Iorio, A heavy metal biotrap for wastewater remediation using poly- $\gamma$ -glutamic acid, *Biotechnol. Prog.* 22 (2006) 523–531.

- [20] H. Xu, M. Jiang, H. Li, D. Lu, P. Quyang, Efficient production of poly-( $\gamma$ -glutamic acid) by newly isolated *Bacillus subtilis* NX-2, *Process Biochem.* 40 (2005) 519–523.
- [21] A. Krecz, I. Pócsi, J. Borbely, Preparation and chemical modification of poly-gamma-L-glutamic acid, *Folia Microbiol.* 46 (2001) 183–186.
- [22] M. Borbely, Y. Nagasaki, J. Borbely, K. Fan, A. Bhogle, M. Sevoian, Biosynthesis and chemical modification of poly-(gamma-glutamic acid), *Polym. Bull.* 32 (1994) 127–132.
- [23] A.-L. Kjøniksen, B. Nyström, B. Lindman, Effect of temperature, surfactant concentration, and salinity on the dynamics of dilute solutions of a nonionic cellulose derivative, *Langmuir* 14 (1998) 5039–5045.
- [24] R.A. Lauten, B. Nyström, Time dependent association phenomena in dilute aqueous mixtures of a hydrophobically modified cellulose derivative and an anionic surfactant, *Colloid Surf. A* 219 (2003) 45–53.
- [25] A. Maleki, A.L. Kjøniksen, B. Nyström, Effect of shear on intramolecular and intermolecular association during cross-linking of hydroxyethylcellulose in dilute aqueous solutions, *J. Phys. Chem. B* 109 (2005) 12329–12336.