Preparation of new biosorbents based on poly-γ-glutamic acid and its adsorption of heavy metal ions

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Abstract. In this paper, adsorption effects and characteristics of heavy metal ions by poly-γ-glutamic acid (γ-PGA) and its two complexes (γ-PGA/Gelatin and γ-PGA/Fe₃O₄) were studied. The adsorption capacity of Cu²⁺, Pb²⁺ and Cd²⁺ with initial concentration of 100 mg/L by unmodified γ-PGA is 1158 mg/g, 1129 mg/g and 301 mg/g, respectively. However, γ-PGA is easy to dissolve in water, which is not conducive to the treatment of heavy metal wastewater under flow state, neither to its reuse. The γ-PGA/Gelatin was prepared by crosslinking γ-PGA with gelatin to enhance γ-PGA’s mechanical strength and adsorption stability. Moreover, Fe₃O₄ magnetic particles are also the ideal carrier of γ-PGA, which not only improves the strength performance of γ-PGA but also enhances the repetition rate of γ-PGA. The γ-PGA/Fe₃O₄ magnetic particles were prepared by co-precipitation. The scanning electron microscopy and infrared spectroscopy showed that γ-PGA and Fe₃O₄ were successfully synthesized, and the magnetic particles showed good paramagnetism. The data shows that the adsorption capacity of regenerated γ-PGA/Fe₃O₄ to Pb²⁺ and Cd²⁺ can reach more than 70% of the first one. The comparison of FTIR spectra shows that the amount of COO⁻ after reaction is greatly reduced, indicating that the -COOH in γ-PGA plays a major role in adsorption process. What’s more, the natural resolution of adsorbent γ-PGA, γ-PGA/gelatin and γ-PGA/Fe₃O₄ were 11.3%, 0.7% and 0 respectively 24 hours after adsorption for Pb²⁺.

1. Introduction

As a high-quality biosorbent, γ-poly-gamma-glutamic acid has received more and more attention. Gamma polyglutamic acid (Poly-gamma-glutamic acid) also known as poly glutamic acid or poly gamma glutamic acid, referred to as γ-PGA. Its natural source is the extracellular polymer (polyamino acid) produced by microbial fermentation in the environment, which is first discovered in the "Nadu" fermented bean. γ-PGA molecules contain a large number of -COOH and -NH₂ reactive groups, with strong water-soluble and strong adsorption. γ-PGA is a biodegradable substance that can be processed to the cosmetic and food grade levels, so it does not harm the human body and the environment and meets the requirements of environment-friendly adsorbents. From the perspective of water treatment, it is also a new type of biosorbent. The obvious advantages can be summarized as: fast adsorption of heavy metals, lower cost and no secondary pollution. γ-PGA’s molecular structure shown in figure 1.
As early as 1990, Mclean studied the binding force between γ-PGA and heavy metals. It was found that γ-PGA could bind to Cr$^{3+}$, Ni$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Al$^{3+}$ and other metals [1]. Although γ-PGA has good affinity to heavy metals, it is very soluble in water itself, which is not easy to control in actual industrial wastewater treatment. It is easily washed away by water current and is not conducive to the recovery of heavy metals and the regeneration of adsorbents. Therefore, some scientists have studied the modification of gamma polyglutamic acid. The modification of γ-PGA is mainly composed of γ-PGA and some functional materials or reagents. The composite methods include crosslinking, grafting and immobilization. The main purpose is to improve the adsorption and stability of γ-PGA, and to improve the mechanical strength and practicability of γ-PGA.

2. Materials and methods

2.1. Source materials

The experimental material is γ-PGA, which is provided by Guangzhou Yutai Chemical Technology Co., Ltd., with molecular weight above 800 kDa and purity above 98%. Gelatin, FeCl$_3$·6H$_2$O and FeCl$_2$·4H$_2$O are provided by Tianjin Da Mao chemical reagents factory.

2.2. γ-PGA/gelatin composite preparation

In this experiment, glutaraldehyde was selected as the crosslinking agent for γ-PGA and gelatin reaction, and glycerol was used as a cosolvent and lubricant for the reaction of γ-PGA and gelatin. Therefore, the main factors affecting the crosslinking modification of γ-PGA and gelatin include pH, temperature, amount of glycerol, amount of glutaraldehyde, and the ratio of γ-PGA and gelatin [2].

The 36 mL water was added to the 50 ml small beaker and placed in the magnetic stirrer at constant temperature. A certain amount of γ-PGA, gelatin as well as 0.5 ml glycerol are added. The pH value of the solution was adjusted to 6 by HNO$_3$ and NaOH of 0.1 mol/L. The temperature of the water bath was set at 323 K, and the speed of magnetic stirring was 400 r/min. For the complete dissolving of the mixture, 4 mL glutaraldehyde solution of 1% concentration is added to the small beaker. Then the reaction began and continued to reacted for 30 minutes. After the reaction is completed, the mixture in the small beaker was transferred to a petri dish, naturally cooled to room temperature, then the
crosslinked mixture can coagulate in a jelly-like form glue. The petri dish was then placed in a thermostatic oven and dried at a constant temperature of 333 K for 10 hours. By optimizing the experimental conditions, the film of γ-PGA/gelatin can be prepared and stripped from the culture dish for reserve. The SEM images of γ-PGA/gelatin complex are shown in figures 2 and 3.

It can be seen from the above two graphs that the surface of γ-PGA/gelatin has many tiny particles and a porous structure, which is beneficial to the affinity of heavy metal ions to some extent.

2.3. γ-PGA/Fe₃O₄ magnetic particle preparation
Preparation of γ-PGA/Fe₃O₄ magnetic particles by coprecipitation method [3], the preparation of Fe₃O₄ magnetic particles are the same. The ratio of Fe³⁺ to Fe²⁺ in the experiment is about 3:2, the reaction equation of Fe₃O₄ synthesis and the combination of γ-PGA/Fe₃O₄ are inferred as follows:

\[ Fe^{2+} + 2Fe^{3+} + BOH^- = Fe_3O_4 + 4H_2O \]  

(1)

A beaker with 200 mL deionized water heated at 353 K water bath. When the temperature reaches 351 K, add 12.8 g FeCl₃ • 6H₂O into the beaker, set the speed of the stirrer to 400 r/min, add 8.0 g FeCl₂ • 4H₂O and stir for about 1 min. When the temperature is raised to 353 K, take about 30 ml of 25% NH₃ • H₂O solution, add dropwise to the mixed solution of FeCl₃ and FeCl₂ and continue stirring for 5 minutes. Subsequently, about 100 ml of 30 g/L γ-PGA solution was added to the mixture and stirring was continued for 1.5 h to achieve the growth of Fe₃O₄ particles and the coating of γ-PGA on the particle surface.

The mixture was allowed to cool to room temperature, rinsed with deionized water and dried at 333 K for about 6 hours. Grind finely after drying, the magnetic particles of γ-PGA/Fe₃O₄ are obtained and kept in reserve. Scanning electron microscopy of two materials shown in the figures 4 and 5.

![Figure 4. SEM image of Fe₃O₄ (80,000 times).](image)

![Figure 5. SEM image of γ-PGA/Fe₃O₄ (80,000 times).](image)

Due to the γ-PGA film coated on the Fe₃O₄ core, steric hindrance and electrostatic repulsion are increased, which hinders the growth and aggregation of the Fe₃O₄ particles. Therefore, the γ-PGA/Fe₃O₄ particles are finer than the Fe₃O₄ particles.

As can be seen from figure 6, γ-PGA/Fe₃O₄ has characteristic absorption near 1600 cm⁻¹ like γ-PGA, corresponding to the asymmetric stretching vibration of COO⁻. γ-PGA/Fe₃O₄ also has obvious characteristic absorption at 1400.72 cm⁻¹, which corresponds to the symmetrical telescopic vibration of COO⁻. In addition, the γ-PGA/Fe₃O₄ has a very strong characteristic absorption at 582.94 cm⁻¹, which corresponds to the vibration of the Fe-O functional group [4]. It is concluded that γ-PGA has been successfully coated on the surface of Fe₃O₄ magnetic particles.

Material γ-PGA/Fe₃O₄ magnetic demonstration shown in figure 7.
Figure 6. Comparison of infrared spectra between γ-PGA and γ-PGA/Fe₃O₄.

Figure 7. γ-PGA/Fe₃O₄ in magnetic field.

As can be seen from figure 7, γ-PGA/Fe₃O₄ has good paramagnetic properties. With external magnetic field, it can be quickly and easily separated from the liquid.

2.4. Adsorption of metal ions by three adsorbents
The static adsorption experiments of Cu²⁺, Pb²⁺ and Cd²⁺ were carried out with γ-PGA, γ-PGA/gelatin and γ-PGA/Fe₃O₄ respectively to examine their ability to remove metal ions from the solution. The control conditions include pH (at 2, 3, 4, 5, 6 and 7), temperature (288 K, 293 K, 298 K, 303 K and 308 K) and adsorbent dosage (γ-PGA/Fe₃O₄ dose of 200 mg, 400 mg, 600 mg 800 mg, 1000 mg and 1200 mg). Through the experiments on the metal ions with an initial concentration of 100 mg/L, rotation speed of 150 r/min, the optimal reaction conditions were found. The removal characteristics of heavy metals by γ-PGA and two kinds of modified materials (γ-PGA/gelatin and γ-PGA/Fe₃O₄) were analyzed, and the three kinds of adsorbents were compared.

2.5. Kinetics and isotherm of adsorption for Pb²⁺
In order to determine the effect of Pb²⁺ concentration on the adsorption capacity of two adsorbents (γ-PGA/gelatin and γ-PGA/Fe₃O₄), two groups of different concentrations of Pb(NO₃)₂ solution were prepared. One group of concentration from 20 mg/L to 500 mg/L is added to 0.2 g γ-PGA/gelatin complex. The other set is 20 mg/L to 200 mg/L, adding 0.8 g γ-PGA/Fe₃O₄ magnetic particles. The pH of both solutions was adjusted to 4 with HNO₃ and NaOH and the lead concentration measured after shaking for 3 hours at 293 K. In this experiment, Langmuir and Freundlich adsorption isotherms were used to fit the adsorption process. The basic formulas for the Langmuir and Freundlich models are as
follows

\[ q_e = \frac{q_m b C_e}{1 + b C_e} \quad \text{or} \quad q_e = \frac{q_m C_e}{1/b + C_e} \]

(2)

\[ q_e = K_f C_e^{1/n} \]

(3)

Where \( q_e \) is the adsorption capacity at equilibrium, mg/g; \( q_m \) is the adsorption capacity, mg/g; \( b \) is the Langmuir adsorption equilibrium constant, L/mg; \( C_e \) is the concentration at equilibrium, mg/L; \( K_f \) and \( 1/n \) are Freundlich's adsorption constant.

To study the relationship between the adsorption process and time, 160 ml solution of Pb(NO₃)₂ (100 mg/L) was prepared, the pH was adjusted to 4, and 0.2 g of γ-PGA/gelatin complex was added. The mixture was continuously reacted with 150 r/min at 293 K. The concentration of Pb²⁺ in the supernatant was measured at intervals from 0 min to 120 min. The adsorption kinetics of γ-PGA/Fe₃O₄ magnetic particles is the same as that of γ-PGA/gelatin, but the dosage is 0.8 g. After the end of the two groups of experiments, the concentration was measured after standing in the dark for 24 hours and the natural desorption rate was calculated. The formula is as follows

\[ \eta = \frac{C' - C}{C_0} \times 100\% \]

(4)

Where \( \eta \) is the natural desorption rate; \( C' \) is the ion concentration after 24 h; \( C_0 \) is the initial concentration of metal ions; and \( C \) is the metal ion concentration after the adsorption is completed.

The pseudo-first-order and pseudo-second-order kinetic equations were used to fit the experimental process. The two kinetic equations are as follows

\[ q_t = q_e \left(1 - e^{-k_1t}\right) \]

(5)

\[ \frac{1}{q_t} = \frac{1}{k_2 q_e^2 t} + \frac{1}{q_e} \]

(6)

Where \( q_t \) is the adsorption capacity at time \( t \), mg/g; \( t \) is the time variable, min; \( q_e \) is the equilibrium adsorption capacity, mg/g; \( k_1, k_2 \) are the kinetic constants of the pseudo-first-order and pseudo-second-order equations, respectively.

2.6. Recovery and reuse of adsorbents

In order to improve the utilization of the adsorbent, the spent γ-PGA/gelatin and γ-PGA/Fe₃O₄ magnetic particles were eluted with 50 ml of a 0.5 mol/L HCl solution at 298 K and 100 r/min for 3 consecutive times 30 min. The eluted γ-PGA/gelatin film was dried at 333 K for 6 hours. After the elution, the γ-PGA/Fe₃O₄ magnetic particles were first regenerated by NaOH solution of pH 10, and then washed and dried at 333 K for 6 hours, to be reused.

3. Results and discussion

3.1. The effect of pH on the adsorption capacity

The pH has a great effect on the removal of Cu²⁺. At pH 2-6, the adsorption capacity of Cu²⁺ increased with the increase of pH. When the pH was in the range of 4-7, the adsorption amount was at a high level and the maximum adsorption amount was obtained at pH 6, which achieved 1157.5 mg/g. The adsorption amount of Pb²⁺ rapidly increased from 2 to 4, reached a peak at pH 4, and then decreased slightly with the increase of pH. When the pH reached 5, the amount of adsorption increased sharply again, and stabilized from 6 to 7, and the removal efficiency was the best. However, during the experiment, it was found that when the pH is above 5.5, the turbidity of Pb²⁺ solution appeared. It is judged that the concentration of OH⁻ in the solution is larger at this time, leading to the formation of Pb(OH)₂ precipitation. Therefore, high removal rates at pH 6 and pH 7 are believed to be contributed by the hydroxide precipitate, and from the point of view of adsorption, the optimum adsorption of Pb²⁺ by γ-PGA is achieved at pH 4. With the increase of pH from 2 to 6 and then to 7, the removal rate and
adsorption capacity of Cd\(^{2+}\) firstly increased steadily and then decreased slightly, that is to say, the removal rate and adsorption capacity reached the maximum at pH 6. The optimum adsorption pH was 6. Overall, the removal efficiency of γ-PGA for Cd\(^{2+}\) was much lower than that of Cu\(^{2+}\) and Pb\(^{2+}\). The removal rate of Cd\(^{2+}\) by γ-PGA was only about 24% under the same conditions, but the adsorption amount was still at a high level of 301 mg/g.

Based on the experimental results of the three metal ions, Cu\(^{2+}\), Pb\(^{2+}\) and Cd\(^{2+}\) have their best adsorption pH range. In general, adsorption reactions are poorly effective at low pH because γ-PGA is more prone to form intramolecular hydrogen bonds at low pH, making the α-helix structure more compact \([5]\), resulting in a decrease in the number of functional groups that can bind to the metal ion. When the solution pH is too high, the metal ions will form hydroxide micro precipitate, thus affecting the normal adsorption reaction.

At the same time, it is known from figures 8 and 9 that under 293 K and their respective optimum pH conditions, the adsorption capacity of Cu\(^{2+}\), Pb\(^{2+}\) and Cd\(^{2+}\) with initial concentration of 100 mg/L reached 1158 mg/g, 1129 mg/g and 301 mg/g respectively. Therefore, the affinity of γ-PGA to metal ions follows Cu\(^{2+}\) > Pb\(^{2+}\) > Cd\(^{2+}\).

### 3.2. The influence of temperature on the adsorption capacity

The figure showed the temperature effects for the adsorption of heavy metal ions Cu\(^{2+}\), Pb\(^{2+}\) and Cd\(^{2+}\). For Cu\(^{2+}\), as the temperature increased from 288 K to 298 K and then to 308 K, the amount of Cu\(^{2+}\) decreased gradually, which indicated that the low temperature was more favorable for copper ion adsorption. When the temperature rises from 288 to 293K, the adsorption amount of Pb\(^{2+}\) slightly increases. With the temperature increasing, the adsorption amount decreases slowly. The adsorption of Pb\(^{2+}\) is best at 293 K. However, the effect of temperature on the adsorption capacity of Cd\(^{2+}\) is not obvious. The amount of adsorption between 288 K and 298 K is higher than that between 303 K and 308 K. In general, for Cu\(^{2+}\), Pb\(^{2+}\) and Cd\(^{2+}\), 288 K to 293 K is ideal adsorption temperature. High temperature is not conducive to the adsorption of three kinds of metal ions by γ-PGA, and the higher temperature, the lower adsorption capacity.

### 3.3. Effect of adsorbent addition on adsorption

As can be seen from figure 10, the removal rates of Pb\(^{2+}\) and Cd\(^{2+}\) increase with the increase of γ-PGA/Fe\(_3\)O\(_4\) dosage, reaching 96.47% and 53.68% respectively in the experimental range. The removal rate of Cd\(^{2+}\) is higher than that of γ-PGA/gelatin, which may be related to the excellent structure of γ-PGA/Fe\(_3\)O\(_4\). Porous and large specific surface area contributes to the intermolecular forces between γ-PGA/Fe\(_3\)O\(_4\) and metal ions. The unit adsorption capacity decreases with the increase of adsorbents, which is due to the high adsorption saturation degree when the adsorption dose is low. When the amount of metal ions is fixed, the degree of adsorption saturation decreases with the increase of the
adsorption dose. In general, the unit adsorption amount of γ-PGA/Fe₃O₄ is much lower than that of γ-PGA and γ-PGA/gelatin because the specific gravity of Fe₃O₄ in γ-PGA/Fe₃O₄ magnetic particles is too large. However, the leading factor of adsorption is -COOH in γ-PGA.

3.4. Thermodynamics and dynamics

From figure 11, figure 12 and table 1, the correlation coefficient of Langmuir is greater than that of Freundlich, which indicates that the adsorption of Pb²⁺ by γ-PGA/gelatin and γ-PGA/Fe₃O₄ is more in accordance with the Langmuir equation. At 293 K, the adsorption capacity of Pb²⁺ by γ-PGA/gelatin was 199.80 mg/g and 27.90 mg/g for γ-PGA/Fe₃O₄ by Langmuir equation, which were much lower than that of γ-PGA for Pb²⁺ (1129 mg/g), suggesting that the predominant affinity for metal ions is γ-PGA.

| Adsorbent         | Langmuir model | Freundlich model |
|-------------------|----------------|------------------|
|                   |     |                  |
|                  | qₑ  | 1/b + Ce         | qₑ  = K_fCₑ¹/n  |
| γ-PGA/gelatin     | 199.80 | 6.30 | 0.949 | 59.47 | 0.24 | 0.804 |
| γ-PGA/Fe₃O₄       | 27.90  | 5.83 | 0.996 | 7.56  | 0.32 | 0.911 |

Table 1. Isotherm fitting parameters.
It is known from Table 2, the correlation coefficient $R^2$ of the pseudo-second-order kinetic equation is larger than the pseudo-one, that is, the adsorption process of $\gamma$-PGA/gelatin and $\gamma$-PGA/Fe$_3$O$_4$ to Pb$^{2+}$ are more consistent with pseudo-second-order kinetic equation. In other words, the adsorption rate is positively correlated with the amount of adsorbent and the amount of heavy metal ions. The adsorption capacity of Pb$^{2+}$ by $\gamma$-PGA/gelatin and $\gamma$-PGA/Fe$_3$O$_4$ fitted by pseudo-second-order kinetics were 81.97 mg/g and 17.86 mg/g, respectively, which were close to the experimental values. As you can see from Figure 13 and Figure 14, the equilibrium adsorption time of Pb$^{2+}$ by $\gamma$-PGA/gelatin and $\gamma$-PGA/Fe$_3$O$_4$ was about 60 min and 100 min, respectively.

Calculated, the natural resolution of adsorbent $\gamma$-PGA, $\gamma$-PGA/gelatin and $\gamma$-PGA/Fe$_3$O$_4$ adsorption of Pb$^{2+}$ after 24 hours were 11.3%, 0.7% and 0% respectively. This indicates that the adsorption of metal ions by $\gamma$-PGA/gelatin and $\gamma$-PGA/Fe$_3$O$_4$ is more stable than that of $\gamma$-PGA.

### Table 2. Kinetic fitting parameters.

| Adsorbent       | Pseudo-first-order kinetic $q_t = \frac{q_{e}}{1 - \exp(-K_1t)}$ | Pseudo second-order kinetic $q_t = \frac{1}{K_2q_e^2 t} + \frac{1}{q_e}$ |
|-----------------|-----------------------------------------------------------------|---------------------------------------------------------------------|
| $q_e$ | $K_1$ | $R^2$ | $\frac{1}{K_2q_e^2 t}$ | $\frac{1}{q_e}$ | $R^2$ |
| $\gamma$-PGA/gelatin | 74.65 | 0.123 | 0.937 | 0.0698 | 0.0122 | 0.993 |
| $\gamma$-PGA/Fe$_3$O$_4$ | 16.807 | 0.073 | 0.905 | 0.427 | 0.056 | 0.987 |

3.5. Adsorption mechanism

As you can see from Figure 15, $\gamma$-PGA/gelatin characteristic absorption consistent with $\gamma$-PGA.
During the reaction, forming a complex with free heavy metal ions. This research is supported by Key Research and Development Planning of Shandong Province (2016GSF117014) and Shandong Province Water Conservancy Program (2018LIBD0020).

References
[1] Mclean R J, Beauchemin D, Clapham L, et al 1990 J. Applied & Environmental Microbiology 56(12) 3671-7
[2] Chen H 2010 Research of γ-PGA composites preparation and the metal ions adsorption capability (Jiangsu University of Science and Technology) 13-35
[3] Chang J, Zhong Z, Xu H, Yao Z and Chen Z 2013 J. Chinese Journal of Chemical Engineering 21(11) 1244-50
[4] Inbaraj B S and Chen B H 2011 J. Bioresource Technology 102(19) 8868-76
[5] Ho G H, Ho T I, Hsieh K H, et al 2006 J. Chinese Chemical Society 53(6) 1363-84