Preparation of pH-sensitive polyacrylic acid hollow microspheres and their release properties

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The pH-sensitive polyacrylic acid (PAA) microspheres with a hollow structure are prepared via Pickering polymerization of acrylonitrile using diethanolamine-adipic acid modified silica nanoparticles as a stabilizer followed by hydrolysis in the presence of sodium hydroxide. The morphology and chemical structure of PAA hollow microspheres are investigated by scanning electron microscopy (SEM) and Fourier-transform infrared (FTIR) spectroscopy. The swelling of polymer hollow particles is observed by confocal microscopy. As a kind of functional material, the pH-sensitive PAA hollow microspheres can be used to load and release the substances. The release rates of the dye from PAA hollow microspheres are greatly dependent on pH value with sunset yellow (SSY) as a model molecule. It is proven that the release properties of PAA hollow microspheres can be well controlled via tuning the pH values.

Introduction

Hollow capsules of micro- and nano-size constitute an important class of materials, which exhibit interesting and unique properties such as optical scattering properties, low density and excellent thermal insulation.1,2 Important applications of hollow capsules can be found for example encapsulation for controlled release of drugs, lightweight fillers, and catalysis.1,4,14 There have been a variety of synthetic routes developed to produce hollow spheres, including layer-by-layer (LBL),2,5 seed-emulsion polymerization,6 self-assembling/phase separation procedures7-10 and suspension polymerization.11,12

An emulsion stabilized by solid particles instead of surfactant molecules is called a Pickering emulsion.11 Pioneering work by Velev et al.13 described the synthesis of capsules with microstructure via the self-assembly of polystyrene latex particles by using emulsion droplets as templates.14 Later, the structures, fully covering emulsion droplets with colloid building blocks, were named colloidosomes by Dinsmore et al.15 According to this method, “hairy” colloidosomes with shells of epoxy polymeric rods, laponite armored polystyrene microspheres, organic–inorganic hollow spheres and raspberry-shaped interpenetrating polymer network reinforced capsules were prepared.16-21 Currently, Pickering stabilization as a tool is being developed in the fabrication of polymer particles.22,23 Layered clay particles with mixed polymer brushes were used as a stabilizer to produce polymer colloid particles.24 Wang et al. prepared the thermo-sensitive hybrid microcapsules with tunable supracolloidal structures,25 magnetic polymer microspheres26 and Janus particles with polymer brushes.27

PAA is a kind of pH-sensitive polymer, which exhibits an electrostatic repulsion due to the ionization of the carboxyl groups at high pH. It exhibits reversible pH-dependent swelling behavior in the aqueous solution.28-30 Recently, stimuli-sensitive polymers have been introduced into novel delivery systems in which the release of substances is performed through different stimuli such as pH, temperature, and magnetism.31-34 Acidic pH as a stimulus is fascinating in chemical reactions as well as in the intracellular environment and compartments of cells. A well-defined pH-responsive double hydrophilic graft copolymer was prepared via ATRP.35 A degradable and pH-sensitive polyurethane was synthesized for lysozyme delivery.36 Armes et al. reported pH-sensitive polymers for controlled release of doxorubicin hydrochloride (DOX)37 and for DNA delivery.38

In this paper, pH-sensitive PAA microspheres with a hollow structure are prepared via the hydrolysis of polyacrylonitrile (PAN) hollow particles. PAN hollow microspheres with the size of about 4 μm have been fabricated using divinylbenzene (DVB) as a cross-linker and modified silica nanoparticles as a solid stabilizer. The PAA hollow microspheres are pH-sensitive and can be used as carriers to load and release sunset yellow in aqueous solutions of various pH.

Experimental

Materials

Sunset yellow (SSY, ≥99.0%), sodium hydroxide (NaOH, ≥99.0%), hydrochloric acid (HCl, ≥99.0%), hydrofluoric acid (HF, ≥40.0%), sodium chloride (NaCl, ≥99.0%), cyclohexane
(≥99%), azodiisobutyronitrile (AIBN, ≥99%), adipic acid (≥99.0%), diethanolamine (≥99.0%), sodium phosphate monobasic anhydrous (NaH$_2$PO$_4$) and sodium phosphate dibasic anhydrous (Na$_3$HPO$_4$) were purchased from Sinopharm Chemical Reagent Co. (China). Divinylbenzene (DVB, ≥99.0%) was provided by Aldrich. Silica hydrogel (average size: 28 nm, solid content: 40%) was supplied by Fujian Sanbang Chemical Co. Ltd. Acrylonitrile (AN) was purchased from Shanghai Jinshan Chemical Co. Ltd. Deionized water was used throughout the experiment. All the reagents were used as received.

**Modification of silica hydrogel**

The condensation product of diethanolamine–adipic acid was prepared according to the literature. In the preparation, adipic acid was mixed with equimolar diethanolamine in a 500 ml four-neck flask. The polymerization was conducted through vacuumization at 110 °C for 2 h to eliminate water. The acid value of diethanolamine–adipic acid was 80 mg KOH per g according to GB 1668-2008. 1.5 g diethanolamine–adipic acid aqueous solution (10 wt%) and 60.0 g deionized water was added to 6.0 g silica hydrogel (40 wt%) and stirred magnetically at room temperature for 30 min. The pH value of aqueous solution was then adjusted to 3.0 by hydrochloric acid.

**Preparation of PAA hollow microspheres**

PAN hollow microspheres were fabricated via the Pickering polymerization using modified silica hydrogel and cyclohexane as a Pickering stabilizer and porogenic agent separately. In a typical preparation, modified silica hydrogel (67.5 g) with 18.0 g NaCl was mixed with an oil phase containing 20.0 g AN, 1.5 g DVB, 0.15 g AIBN and 12.0 g cyclohexane in a 200 ml glass beaker. The mixture was emulsified with an FJ200–S homogenizer at 20 000 rpm for 5 min. The polymerization was conducted at 55 °C for 1 h, 60 °C for 1 h, and 68 °C for 8 h. The whole process was under N$_2$ atmosphere with constant magnetic stirring. The product was filtered and washed with deionized water three times before drying at 60 °C for 24 h.

PAA hollow microspheres were prepared via the hydrolysis of PAN hollow particles. In the preparation, 10 g PAN microspheres were reacted with 15 g NaOH solution (5 mol l$^{-1}$) in a 100 ml glass flask at 80 °C for 5 h with constant magnetic stirring. In order to obtain the PAA microspheres, HCl was added in the mixture followed by the filtration. The PAA hollow microspheres were washed with deionized water and then dried for the further characterization.

**pH-response behavior of PAA hollow microspheres**

pH-response studies were performed by dispersing the PAA hollow microspheres in buffer solutions (pH 2, 5, 6 and 9) at room temperature for 24 h and measuring their size and distribution until equilibrium was attained.

**Loading and controlled release of SSY**

The PAA hollow microspheres were loaded with SSY which was selected as the model molecule. 1.2 g hollow microspheres were dispersed in 60 g buffer solution at pH 9 containing SSY (40 mg ml$^{-1}$) with constant magnetic stirring at room temperature for 24 h. After HCl was dropped into the solution to acidic pH (pH 2), the polymer hollow microspheres with SSY were obtained by the filtration. The solid product was washed with deionized water and then dried at 60 °C for 24 h. SSY was assayed in the solution by measuring the absorbance (Unico UV-2000 spectrophotometer) at 480 nm. The concentration of SSY was calculated by interpolation from a standard curve. SSY loading capacity (LC) was computed from eqn (1):

\[
LC = \frac{W_0 - W_t}{W}
\]

where $W_0$ and $W_t$ are initial and residual weight of SSY, respectively; $W$ is the weight of PAA microspheres.

For the study of the release of the dye, 0.2 g SSY-loaded PAA hollow microspheres were placed into dialysis bags and immersed in 25 g buffer solutions at pH 2, 5, 6 and 9 in a thermostatic bath at 37 °C. After specific intervals, the soaking liquid was collected and dried at 60 °C. The concentration of SSY was assayed by measuring the absorbance (Unico UV-2000 spectrophotometer) at 480 nm. The release process was run for 30 h. The concentration of SSY was calculated by interpolation from a standard curve.

**Characterization**

A digital microscope EV5680 and a canon IXUS 850IS digital camera were used to take the microscopy image of the emulsion droplets and the photograph of the Pickering emulsion. The morphologies of the PAN and PAA microspheres were observed using a TESCAN 5136 MM scanning electron microscope (SEM). Before the characterization, the polymer microspheres were diluted with deionized water and dried on a mica plate, followed by sputter coating with gold. The morphologies of the polymer hollow microspheres were also characterized using a Hitachi H-600 transmission electron microscope (TEM). They were dropped onto carbon-coated lacy substrates for observation. Fourier-transform infrared (FTIR) spectra were taken on the Nicolet Nexus-470 FTIR spectrometer. The PAN microspheres were washed with hydrofluoric acid to remove the silica nanoparticles on the surface. The polymer hollow microspheres were then dried in an oven and pressed into KBr pellets for the FTIR measurements. Microscopy images of PAA hollow microspheres at pH 2, 5, 6 and 9 were taken by using a LEICA TCN SP5 II. The confocal microscope images of SSY-loaded polymer hollow microspheres were also observed. The microspheres were diluted with deionized water and dropped onto a glass slide. A Unico UV-2000 spectrophotometer was used to measure the absorbance of SSY in the buffer solution at 480 nm.

**Results and discussion**

**Characterization of the Pickering emulsion**

During the Pickering emulsion polymerization, solid particles as a stabilizer play an important role. It has been proven that the particles with extreme hydrophilicity or hydrophobicity cannot stabilize the emulsion droplets effectively. The weak...
Flocculation of the highly hydrophilic silica nanoparticles was observed after the addition of organic additives and the effective stabilization for droplets was expected.\textsuperscript{15,39,41} The mixture of cyclohexane and modified silica hydrosol was homogenized and the emulsion was used to investigate the stabilization of modified silica nanoparticles. The emulsion after a week is shown in Fig. 1. Since the density of cyclohexane is much less than that of water, the emulsion cream is driven to the top of the vessel and a clear aqueous phase is separated below, indicating that the modified silica nanoparticles have stabilized the emulsion droplets effectively. It has been proven that solid particles can act as a mechanical barrier to prevent the coalescence of droplets.\textsuperscript{18,42,43} The morphology of the Pickering emulsion from the upper layer was observed by an optical microscope, as shown in Fig. 2. The size of the emulsion droplets is about 5 μm, which is similar to the size of the initial droplets. This indicates that the modified silica particles have good stabilization for the Pickering emulsion and the initial droplets have been well stabilized.

**Characterization of PAN and PAA microspheres**

Bon and Colver had well investigated and described the mechanism of the Pickering miniemulsion polymerization.\textsuperscript{18} A model was presented that the droplets stabilized by solid particles acted as the reaction vessels where the polymerization occurred. The size and distribution of the latex particles were similar to that of the initial droplets. The morphology of obtained PAN microspheres is shown in Fig. 3a. It is clearly seen that the polymer microspheres are sunken with a size of about 3–6 μm from the SEM image. The hollow structure of PAN microspheres is induced by the addition of cyclohexane because it is not a good solvent for the polymer and the phase separation between cyclohexane and PAN occurs during the polymerization. As a result, the hollow PAN microspheres are formed and after the evaporation of the solvent before SEM examination, sunken polymer particles are observed. The size of hollow PAN microspheres is basically in agreement with that of the initial droplets, indicating that the emulsion droplets are stabilized effectively during the polymerization. The rough surface of these hollow PAN microspheres due to the deposition of the Pickering stabilizer also reveals the good stabilization of modified silica nanoparticles.

The hydrolysis of PAN hollow microspheres is conducted in the presence of NaOH to obtain PAA microspheres. The size of polymer microspheres with a sunken surface is similar to original

![Fig. 1](image1.png)  
**Fig. 1** Photograph of the Pickering emulsion after a week at room temperature.

![Fig. 2](image2.png)  
**Fig. 2** Optical microscopy image of the Pickering emulsion.

![Fig. 3](image3.png)  
**Fig. 3** SEM images of PAN hollow microspheres (a) before and (b) after hydrolysis. The insets show the TEM images for each corresponding sample.

![Fig. 4](image4.png)  
**Fig. 4** FT-IR spectra of PAN hollow microspheres before and after hydrolysis.
PAN microspheres as shown in Fig. 3b, indicating that hydrolysis does not destroy these PAN hollow microspheres. From the SEM image, it is found that the surface of the polymer microspheres becomes smooth after the hydrolysis due to the removal of silica particles. Fig. 4 shows the chemical structure of the polymer hollow microspheres after the hydrolysis. The typical absorption of nitrile groups at 2242 cm\(^{-1}\) for PAN microspheres almost disappears and new absorption bands at 1700 and 3000–3350 cm\(^{-1}\) are clearly seen. These two peaks can be assigned to the typical absorption of the carbonyl unit and the stretching vibration of the hydroxyl unit in the carboxyl group, respectively. These results indicate that the nitrile groups of PAN have been hydrolyzed to form carboxyl groups via our method and the PAA hollow microspheres are successfully prepared.

**pH response of PAA hollow microspheres**

As a kind of functional polymers, the pH-sensitive polymers have been studied extensively.\(^{28,44–46}\) The morphologies of PAA hollow microspheres at pH 2, 5, 6 and 9 were investigated by confocal microscopy, as shown in Fig. 5. It is clearly found that the dispersion of polymer hollow particles in the solution becomes better with the increasing pH value. At high pH values, the carboxyl groups of the PAA hollow microspheres are deprotonated and the charge repulsion between the negative ions improves the dispersion of polymer particles in aqueous solution obviously. These carboxyl groups are of low ionization at low pH values and insufficient charge repulsion leads to the aggregation of polymer particles.

The swelling of PAA hollow microspheres is studied for the further understanding of the pH-response behavior of the polymer particles. The size distribution of PAA particles in the solutions with different pH values is shown in Fig. 6 and their average diameters are listed in Table 1. It is found that the size of PAA hollow microspheres increases with the increasing pH value and an obvious increase in diameter of about 43.5% is obtained by just tuning the pH value from 2 to 9. The enhancement of the size of PAA hollow microspheres is also induced by the
deprotonation of carboxyl groups and the polymer network in the particles may become looser at higher pH values.

**Loading and controlled release of SSY**

The PAA hollow microspheres were incubated with SSY solution (40 mg ml\(^{-1}\)) for 1 day. The SSY-loaded polymer hollow microspheres were characterized by confocal laser scanning microscopy (CLSM). From Fig. 7a, the obvious fluorescence signals are observed, and the microspheres show negligible background fluorescence signals. The brightfield image of PAA hollow microspheres with SSY is shown in Fig. 7b. As shown in Fig. 7c, the overlap of confocal fluorescence and brightfield images reveals that the fluorescence signals are localized in every segment of the whole polymer microsphere, indicating that the shell and the cavity of hollow microsphere both contain SSY dye. These results prove the successful loading of the dye in PAA hollow microspheres.

The release profiles of SSY-loaded PAA hollow microspheres obtained in different pH buffer solutions (pH 2, 5, 6 and 9) at 37 °C are described in Fig. 8 with the same initial loading capacity of 0.618 mg mg\(^{-1}\). From Fig. 8, it is clearly found that the release rates of the dye are greatly dependent on the pH values. A high pH value leads to the rapid release of SSY from PAA hollow microspheres and the release rate at pH 2 is almost zero. The release rate of SSY is consistent with the swelling behavior of PAA hollow microspheres (Fig. 6). With the increase of the pH value, the microspheres become highly ionized, subsequently causing an increase of charge repulsion. At different pH levels, there are different mesh sizes of polymer microspheres induced by the charge repulsion interaction. The large mesh size of the microsphere matrix network allows an easy diffusion of the dye out of the matrix.\(^{39,47}\) During the first 5 hours, about 12, 25 and 85% of loaded SSY have been released at pH 5, 6 and 9, respectively. After 10 h, the release of the dye at pH 9 is almost completed and the release amount is about 18 and 40% at pH 5 and 6. The loaded SSY is further released at pH 5 and 6, whereas the release at pH 2 cannot be observed in our experiments. These behaviors demonstrate that the release rate of the dye can be well controlled by regulating the pH value of the solution.

In order to understand the nature of the SSY release from PAA hollow microspheres, we interpret the results by fitting the data to rate equations based on diffusion considerations. Higuchi’s model is used to analyze the release data, which has been applied for the diffusion controlled release of drug from a homogeneous planar matrix or from a porous matrix:\(^ {48}\)

\[
Q = \sqrt{D \frac{t}{\tau}} (2A - \varepsilon C)Ct
\]

where \(Q\) is the amount of drug release after time \(t\), \(D\) is the diffusivity of drug in the permeating fluid, \(\tau\) is the tortuosity factor of the capillary system, \(A\) is the total amount of the drug in the matrix, \(C\) is the solubility of the drug in the fluid and \(\varepsilon\) is the porosity of matrix.\(^ {49,50}\)

The release data are presented as the percentage of the drug released (\(C_t/C_w\), where \(C_t\) is the concentration of the drug release at time \(t\) and plotted against the square root of the time. The results are shown in Fig. 9. Two stages occur in the release of SSY from PAA hollow microspheres at pH 9, while only one stage can be found at pH 2, 5 and 6. The transition between two stages occurs at about 9 h at pH 9 and the amount of SSY released before the transition is 99%, which means no more SSY was released from polymer particles after 9 h. At pH 2, the release

<table>
<thead>
<tr>
<th>pH 2</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 9</th>
</tr>
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<tbody>
<tr>
<td>Average diameter/μm</td>
<td>4.78</td>
<td>5.45</td>
<td>6.29</td>
</tr>
</tbody>
</table>

**Fig. 7** Confocal fluorescence and brightfield images of SSY-loaded PAA hollow microspheres: (a) fluorescence image (\(\lambda_{ex} = 405\) nm, \(\lambda_{em} = 570\) nm); (b) brightfield image of PAA hollow microspheres shown in panel a; and (c) the overlap image of panels a and b.

**Fig. 8** Release of SSY loaded by the pH-sensitive PAA hollow microspheres at 37 °C.
of SSY can hardly be observed during our experimental process. The correlation coefficient $R_C$ of SSY release at pH 5, 6 and 9 (first stage) is given in Table 2.

All correlation coefficients are almost equal to 1, indicating that Higuchi’s model is well fit for SSY release from PAA hollow microspheres. Eqn (2) can be simply expressed as

$$Q = kt^{1/2}$$

(3)

and

$$k = \sqrt{\frac{Dc}{2}} (A - \varepsilon C) C$$

(4)

The parameters $D$, $A$, $C$ and $\tau$ can hardly change only by tuning the pH values of the solution. It is deduced that only the porosity of matrix, $\varepsilon$, should be responsible for the change of the slope of straight lines ($k$) because the increase of the particle size is clearly observed with the increasing pH value. The polymer network in PAA hollow microspheres may become looser with the increasing pH value due to the charge repulsion between the deprotonated carboxyl groups. Therefore, the release rate of SSY from PAA particles becomes larger accordingly.

In addition to Higuchi’s model, the release data of SSY are also analyzed using the Baker–Lonsdale model which is applicable for the drug release from diffusion-controlled matrices of spherical shape: \cite{49, 50}

$$\frac{3}{2} \left[ 1 - \left( 1 - \frac{M_t}{M_0} \right)^{4} \right] - \frac{M_t}{M_0} = kt$$

(5)

When applying the Baker–Lonsdale model, the results are similar to that of Higuchi’s model. There are two stages with the transition at about 9 h at pH 9, while only one stage is attained at pH 2, 5, and 6. We just compare the analyst data of pH 5, 6 and 9 (first stage). The correlation coefficients, $R_C$, are listed in Table 3, which show excellent correlation with the Baker–Lonsdale model. Thus, the release of SSY from PAA hollow microspheres appeared to be diffusion controlled.

Table 2  Correlation coefficient $R_C$ and slope of straight line $k$ of SSY release at pH 5, 6 and 9 (first stage) for Higuchi’s model

<table>
<thead>
<tr>
<th>Composition</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 9 (first stage)</th>
</tr>
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<tbody>
<tr>
<td>$R_C$</td>
<td>0.989</td>
<td>0.991</td>
<td>0.967</td>
</tr>
<tr>
<td>$k$</td>
<td>4.61</td>
<td>13.62</td>
<td>27.67</td>
</tr>
</tbody>
</table>

Table 3  Correlation coefficient $R_C$ of SSY release at pH 5, 6 and 9 (first stage) for the Baker–Lonsdale model

<table>
<thead>
<tr>
<th>Composition</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 9 (first stage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_C$</td>
<td>0.984</td>
<td>0.992</td>
<td>0.991</td>
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Conclusions

In summary, we showed an efficient way to synthesis PAA hollow microspheres which are fabricated via the hydrolysis of PAN hollow microspheres prepared by Pickering polymerization. The size of polymer microspheres is about 3–6 μm which is in good agreement with that of initial droplets. It is demonstrated that the emulsion droplets are stabilized effectively during the polymerization. PAA hollow microspheres disperse well in aqueous solution due to high deprotonation and strong charge repulsion between negative ions at high pH values, while PAA microspheres aggregate obviously at low pH due to their low ionization and weak charge repulsion. The size of PAA microspheres is dependent on the pH values and the release rate of SSY is consistent with the swelling behavior of polymer particles. The loaded substance released from PAA hollow microspheres is diffusion controlled. This kind of pH-sensitive materials may have applications in the controlled release of pigments and drugs.

References

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