

Analysis of Spherical Polyelectrolyte Brushes by Small Angle X-ray Scattering*

Xuan-ji Yu, Wei-hua Wang, Li Li**, Xu-hong Guo, Zhi-min Zhou and Fu-chen Wang
School of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, China

Abstract Spherical polyelectrolyte brushes (SPBs) with PS core and poly(acrylic acid) (PAA) brushes were prepared and analyzed by SAXS in this article. A radial electron density profile of SPB was brought up, which fits well with the SAXS result and shows a core-shell structure. The effect of pH on SPB form was represented by SAXS and it proves that the chains of SPB will stretch in response to increased pH owing to the increased electrostatic repulsion. SPBs immobilized with magnetic nanoparticles or bovine serum albumin (BSA) were prepared and analyzed by SAXS as well. SAXS could characterize the changes of electron density inside brushes of SPBs due to the immobilization of magnetic nanoparticles or BSA. This provides significant supports for further application of immobilized metal nanoparticles or proteins.

Keywords: SPB; SAXS; Nanoparticles.

INTRODUCTION

Spherical polyelectrolyte brush (SPB) is a polymer core densely grafted with linear polyelectrolyte chains, the linear dimensions of which are much larger than the average distance between two neighboring chains on the surface. Recently, there has been an intense research on these systems. This is due to the fact that the colloidal dimensions of the SPB may be used to create a large well-defined surface in an aqueous environment, which can be used for the immobilization of nanoparticles^[1–4] or proteins^[5–7].

When the polyelectrolyte chains such as PAA are charged, the form of SPB is dependent on the outer conditions *e.g.* pH and ionic strength. The situation would be much more complicated as nanoparticles or proteins are introduced into the brush layers. Thus an ideal tool was needed to investigate the radial structure of the SPB particles *in situ* and the distribution of nanoparticles or proteins in SPB, both of which may serve as catalysts and their distribution is of great significance when it comes to application.

As the X-rays pass through the material with uneven electronic area of nano-size, X-rays will interact with electrons and be scattered within a small angular range (3° – 5°). This phenomenon is called small angle X-ray scattering (SAXS). Thus SAXS is particularly sensitive to the unevenness of the electron density. This feature makes it a powerful tool of materials science and colloidal physics. By SAXS, the properties of polymer colloids, *e.g.* size, shape and internal structure, can be obtained, with the size ranging from 1 nm to several hundred nanometers. The analysis result is statistical and can reflect the overall information. What's more, SAXS is a non-destructive analysis method which can be used to detect a wide range of samples, despite of

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** Corresponding author: Li Li (李莉), E-mail: lili76131@ecust.edu.cn

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being wet or dry, with pores open or closed.

The SPBs with PS core and PAA brushes were studied by SAXS in this article. Different kinds of electron density profiles for describing spherical polymer colloidal particles have been brought up, including the step function profile^[8, 9], the parabolic profile^[10, 11] and the exponential profile^[10–12]. When the shell of SPB is modeled in terms of five subsequent concentric shell of homogeneous electron density, the step function profile could be obtained. However, in our opinion, this model seems relatively rough, and contains too many parameters. According to the mathematic principles, the more parameters the equations have, the more solutions there are, which makes the model inaccurate. Both parabolic profile and exponential profile can successfully describe a shell which decreases upon increasing the radial distance r from the center of the SPB. But they fail to provide the specific information of the polymer chains. Thus a new radical electron profile with parameters related with polyelectrolyte chains was brought up.

The analyzing result revealed a core-shell structure and the pH dependence of SPB, which were both in a good agreement with what was shown by dynamic light scattering (DLS). The SAXS results of SPBs immobilized with magnetic nanoparticles or BSA were also presented, which characterized the radial distribution and interaction between SPB and its inner particles or proteins. This may provide support for catalysis research of nanoparticles and optimization of industrial application in the future.

EXPERIMENTAL

Materials and Measurements

All solvents were analytical grade and used as received. Styrene (BASF) and acrylic acid (Guoyao Co. Ltd) were distilled under a reduced pressure to remove the inhibitor and stored at 4 °C before use. Bovine serum albumin (BSA), sodium chloride (NaCl), hydrochloric acid (HCl), standard phosphate solutions, potassium persulfate (KPS) and 2-(*N*-morpholino)ethanesulfonic acid (MES) were purchased from J & K Chemical and used as received. Standard sodium hydroxide (NaOH) solution (0.1 mol/L) were purchased from Sigma-Aldrich and used without further purification. Photoinitiator (2-[p-(2-hydroxy-2-methylpropiophenone)]-ethylene glycol-methacrylate) (HMEM) was synthesized following the previous publication^[13]. Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), ammonium hydroxide (25 wt%–28 wt%) were purchased from Sinopharm Chemical Reagent Co., Ltd. The water used in the reactions was purified by reverse osmosis and subsequent ion exchange (Millipore Milli-Q).

The size of the particles and their size distribution were determined by DLS (PSS, NICOMP 380 ZLS). The SAXS test was conducted on beamline BL16B1 of SSRF (Shanghai Synchrotron Radiation Facility). The focus beam size was 0.48 mm $\times$ 0.39 mm. The wavelength of the X-ray used was 0.124 nm. The detectors were Mar 165 CCD (Marresearch GmbH) and Gabriel 1D gas detectors.

Synthesis of Spherical PAA Brushes

The polystyrene (PS) core latex was synthesized by the redox emulsion polymerization. 10 g styrene was polymerized in 250 mL H_2O with 0.1 g/0.2 g SDS as surfactant and 0.6 g KPS as initiator. A thin shell of photoinitiator was generated by adding 1 g HMEM dissolved in 9 mL acetone. To achieve a well-defined initiator layer, HMEM was added under starved conditions. The prepared core latex was cooled to room temperature and purified by extensive serum replacement against pure water.

The latex of PS core and acrylic acid were added into a photoreactor, and then the reactor was degassed by repeat evacuation and subsequent addition of nitrogen. The polymerization started under UV radiation. Vigorous stirring ensured homogenous condition during the polymerization. After polymerization the latex was purified by serum replacement against pure water. The whole reaction process and further SPB application are represented in Fig. 1.

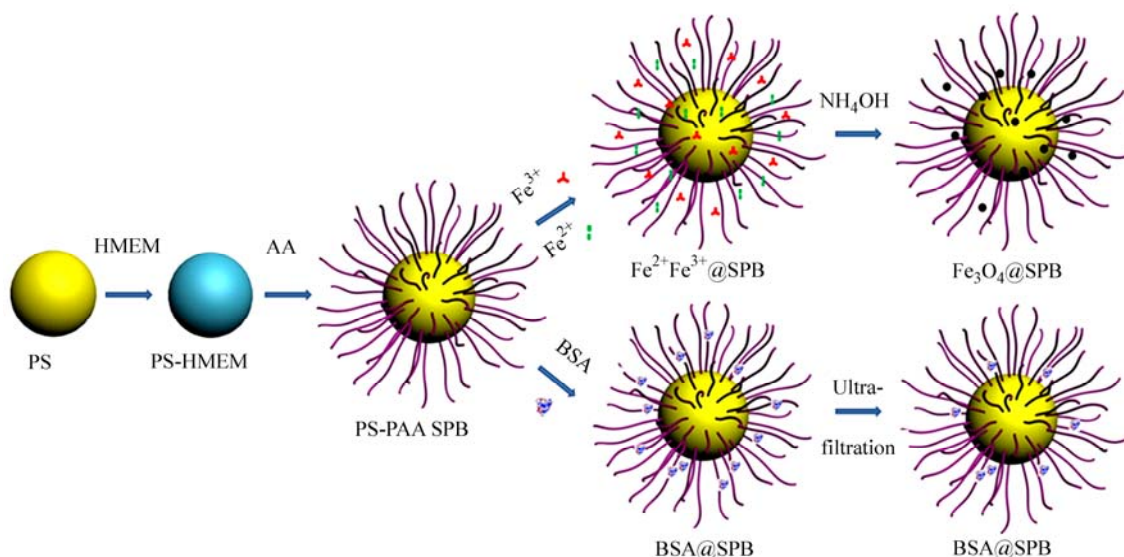


Fig. 1 Schematic representation of the synthesis of spherical PAA brushes and SPB immobilized with magnetic nanoparticles or BSA

Synthesis of Magnetic Nanoparticles in SPB^[4]

The solution of Fe^{2+} and Fe^{3+} (total iron concentration 25 mmol/L) with a molar ratio of 2:3 was freshly prepared, in the typical run, 10 mL of which was slowly added into 100 mL SPB latex (solid content 1.2 wt%) at a feeding rate of 1 mL/min with vigorous stirring in nitrogen atmosphere. Then 10 mL of ammonia (1 wt%) was added dropwise into the system at 80 °C. After 2 h reaction, the products were obtained, which were then purified by serum replacement against pure water.

BSA Adsorption

BSA solutions were prepared by adding BSA into 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer solutions. After mixing of BSA and SPB, the immobilization of protein by SPB was stirred for 24 h at 4 °C for equilibration. All samples were centrifuged (Microfuge 16, Centrifuge, Brinkmann) with 10000 r/min for 60 min at room temperature. And the supernatants were filtered by a 0.20 μm RC membrane (Corning Incorporated).

Analyzing SPBs by SAXS

The ionic strength of all aqueous solutions was tuned through the addition of NaCl. The pH of solutions was adjusted with NaOH or HCl standard aqueous solutions. It should be kept in mind that the pH was adjusted while keeping the ionic strength constant. Then the emulsion was put in the sample cell through syringe, which was placed on the sample stage. The sample cell was covered by 2 pieces of polyimide membrane front and back. The X-ray would go through the brush solution and the scattering signals were collected by a CCD detector.

THEORY AND FITTING

SAXS Theory on SPB

First the scattering intensity was normalized to the exposure time and the primary beam intensity. The intensity of the pure water is independent of the scattering angle and will remain constant as temperature is fixed. Thus all the scattering intensity was corrected to absolute intensity in agreement with pure water.

The scattering intensity of the SPB particles then was subtracted from background (sample cell, air and water), which was obtained by

$$I_{\text{SPB}}(q) = I_{\text{sample+cell}}(q) - (1 - \phi)I_{\text{water+cell}}(q) - \phi I_{\text{air+cell}}(q) \quad (1)$$

where ϕ denotes the volume fraction of the particles. $I_{\text{sample+cell}}(q)$, $I_{\text{water+cell}}(q)$ and $I_{\text{air+cell}}(q)$ represent the scattering intensities of the sample solution, water and air along with cell, respectively. All the intensities then were divided by the SPB concentration to 1 wt%.

The scattering intensity $I_{\text{SPB}}(q)$ of a single SPB can be split into three terms^[11].

$$I(q) = I_{\text{cs}}(q) + I_{\text{fluct}}(q) + I_{\text{ps}}(q) \quad (2)$$

The first term $I_{\text{cs}}(q)$ is due to the overall structure of the SPB that consists of a core with low contrast to water on electron density and a shell with high contrast.

$$I_{\text{cs}}(q) = B^2(q) \quad (3)$$

The shell composed of the polyelectrolyte chains and their counter ions is described by an average radial profile directly related to the excess electron density represented as $\rho(r) - \rho(m)$, where r is the radial distance, $\rho(r)$ is the radial electron density of the spherical polyelectrolyte brush and $\rho(m)$ is the electron density of the surrounding medium water. $B(q)$ is the scattering amplitude given by

$$B(q) = 4\pi \int_0^R [\rho(r) - \rho(m)] r^2 \frac{\sin(qr)}{qr} dr \quad (4)$$

The term $I_{\text{fluct}}(q)$ takes into account the fluctuations of the polyelectrolyte layer on the surface.

$$I_{\text{fluct}}(q) = \frac{I_{\text{fluct}}(0)}{1 + \xi^2 q^2} \quad (5)$$

where ξ characterizes the spatial extent of the fluctuations. In this context, both ξ and $I_{\text{fluct}}(0)$ are treated as adjustable parameters.

Finally, $I_{\text{ps}}(q)$ denotes the scattering intensity which is caused by the density fluctuations of the solid PS core of the particle, which could be neglected.

In summary, an appropriate radial electron density profile $\rho(r)$ is essential to analyze and simulate the SAXS curves of SPB. The electron density of water, PS and PAA is presented in Table 1, respectively. Thus when r is less than the radius of the PS core, $\rho(r) - \rho(m)$ is the difference between ρ_{PS}^e and ρ_{Water}^e , remaining constant. Owing to the fact that PAA chains are grafted on the PS core, $\rho(r)$ would change with r . A $\rho(r)$ was hereinafter brought up.

Table 1. Electron density

Material	Water	PS	PAA
$\rho^e (\text{e}^- \cdot \text{nm}^{-3})$	333	327	429

Lognormal Distribution Profile

Lognormal distribution theory is remarkably applied in analyzing small particle system by SAXS. Likewise, it can be used in SPB model. For the lengths of the PAA chains in one particle are different, we assume that the number of the PAA chains whose length equals L are submitted to lognormal distribution.

$$N(L) \sim \text{Log-} \mathcal{N}(\ln L_m, \ln \sigma) \quad (6)$$

Most of the chains are L_m in length and σ indicates the distribution of chains' length. It should be noted that one end of each chain is attached with the polymer core and there are less and less chains as the radial distance to the PS sphere center increases. Therefore the distribution of electron density can be described as below.

$$\begin{cases} \rho(r) = \rho_{\text{PS}} & (r < R_{\text{core}}) \\ \rho(r) = \frac{P_0 \cdot R_{\text{core}}^2}{2r^2} - \frac{P_0 \cdot R_{\text{core}}^2}{2r^2} \text{erf}\left[\frac{\ln(r - R_{\text{core}}) - \ln(R_{\text{SPB}} - R_{\text{core}})}{\sigma_{\text{shell}} \sqrt{2}}\right] & (R_{\text{core}} < r < R_{\text{shell}}) \end{cases} \quad (7)$$

where P_0 is the electron density of the shell part which is closest to the core and $\frac{R_{\text{core}}^2}{r^2}$ is introduced due to the spherical shape of SPBs. R_{SPB} characterizes the average radius of SPBs. σ_{shell} is the standard variance of the lognormal distribution and indicates the uneven distribution of PAA chains' length. The erf is the Gauss error function and an increasing function, which makes $\rho(r-R_{\text{core}})$ a decreasing function and obeys the fact that the electron density decreases as r increases.

Figure 2 shows the SAXS experimental intensities and fitting result by the usage of Lognormal Distribution Profile. The SAXS analysis exhibited that the radius of PS core was 54 nm and the radius of SPB was 77 nm, which were coincided with the result of DLS analysis. It exhibited a clear core-shell structure. The pH and ionic strength of the solution were both low. Herein the PAA chains were only weakly charged, and the entire particle may be treated as a neutral spherical brush. It should be noted that there existed a discrepancy of the fitting curve with respect to the experimental data at small q range, which could be caused by the interaction between SPB particles due to the finite concentration. But overall the SAXS simulation curve fitted well with the experimental result. The electron density of PS core was constant while the electron density of the shell decreased as r increased.

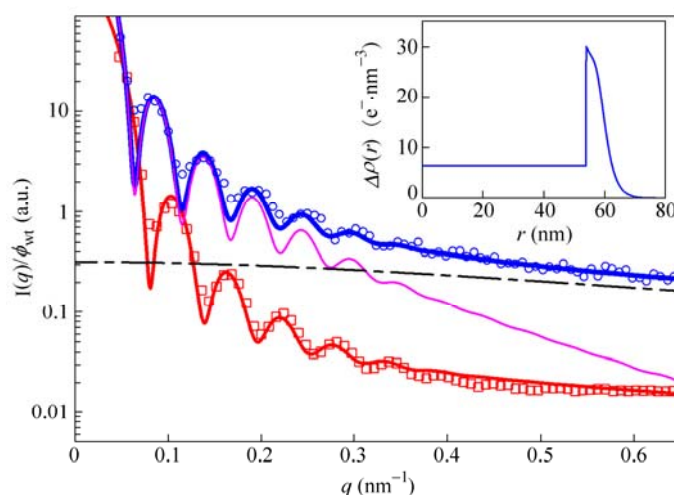


Fig. 2 The measured SAXS intensities of PS ($\square$), SPB ($\circ$), and their simulation results (bold lines) (pH 5 in 10 mmol/L NaCl solution)

The fine line represents $I_{\text{cs}}(q)$. The dashed line indicates $I_{\text{fluct}}(q)$. The inset presents the excess electron density $\rho(r)-\rho(m)$ as the function of the radial distance r which has been used for this fit.

RESULTS AND DISCUSSION

Effect of pH

Another batch of SPB was synthesized to investigate the effect of pH value on the structure of SPBs. After SAXS test, the same data treatment as above was conducted. The SPB samples of different pH were analyzed with Lognormal Distribution Profile. The experimental data and simulation results are shown in Fig. 3. The peak positions of SPBs shifted to a smaller scattering factor q as pH increased, indicating an increased gyration radius. The electron profile in the inset also confirmed that the radius of SPB increased as pH increased. Fitting results showed that the radius of PS core was 83 nm, and the radii of SPB were 93, 97 and 99 nm in 10 mmol/L NaCl solution at pH 6, 8 and 9, respectively, which were coincided with the DLS result.

The behavior of the SPB shell consisting of PAA are under the influence of the pH-value of solution, which can affect the dissociation degree of the carboxylic groups of the PAA chains. At very low pH the PAA-chains are hardly dissociated, thus the chains would clasp on the PS core. Upon increasing pH, there will be a marked stretching of the chains with increasing electrostatic repulsion.

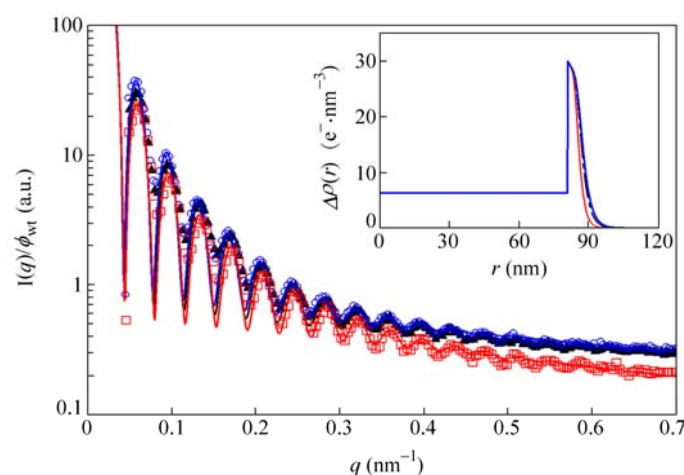


Fig. 3 The measured SAXS intensities of SPB in 10 mmol/L NaCl solutions of different pH values ($\square$) pH 6, ($\blacktriangle$) pH 8 and ($\circ$) pH 9. The solid lines are fitted results. The inset gives the radial distribution of the excess electron density of SPB at pH 6 (solid thin line), pH 8 (dashed line) and pH 9 (solid thick line).

Both SAXS and DLS shows that there were less difference in SPB formation at pH 8 and pH 9, which shows that the polyelectrolyte chains would stretch fully and remain constant in length when pH reaches a certain value.

Preparation of Magnetic Nanoparticles in SPB

The feature that SPBs can absorb counter-ions into their brush layer enables SPBs to serve as nano-reactors for preparation of metal or metal oxide nanoparticles. SPBs immobilized with magnetic nanoparticles can maintain good stability owing to the bi-dentate chelating interaction between iron and carboxyl group. Thus the pH and ionic strength can be manipulated by adding different amount of HCl, NaOH and NaCl as magnetic particles would not fall off. To achieve a minimized influence on samples' stability, pH 7.5 were adopted. This is due to the fact that after the coprecipitation reaction and dialysis, the pH ranged from 7 to 8. Then the SAXS test was conducted, and Fig. 4 shows the scattering intensities of blank SPB and SPB loaded with magnetic nanoparticles.

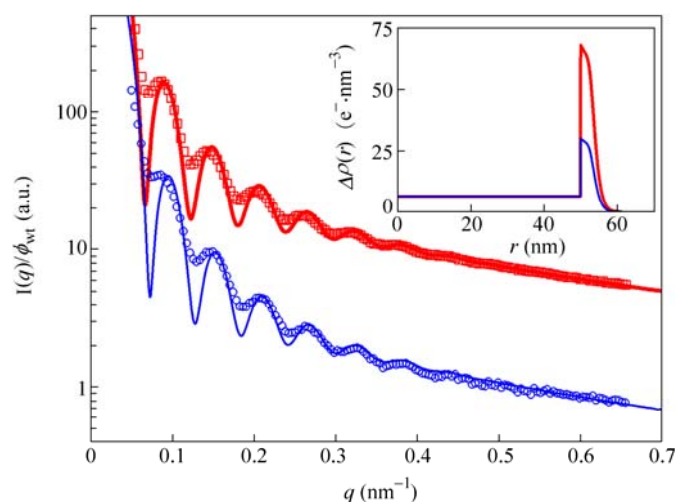


Fig. 4 SAXS spectra of SPB and SPB loaded with magnetic nanoparticles at pH 7.5 in 10 mmol/L NaCl solution: ($\circ$) blank SPB and ($\square$) SPB loaded with magnetic nanoparticles. The solid lines are fitted results. The inset gives the radial distribution of the excess electron density of blank SPB (thin line) and of SPB loaded with magnetic particles (thick line).

It can be observed from Fig. 4 that the intensity of SPB loaded with magnetic particles significantly increases after the coprecipitation reaction. Also the position of the peaks changes to smaller q , indicating a bigger gyration radius. The phenomena above both resulted from the dramatically increased amount of magnetic nanoparticles immobilized within the brush layers. The maximum amount of magnetic nanoparticles is distributed in the brush layer near the polystyrene core, where the majority of counter-ions initially lay owing to the spherical structure of SPB with polyelectrolyte chains.

BSA Adsorption in SPB

SPBs have the ability to adsorb proteins into their brush layer and thus can be carriers for proteins or enzymes. By a combination of turbidimetric titration, DLS and zeta potential measurements, different behaviors, architectures and phase states of pH dependent protein-SPB interactions have been investigated in our former publications^[14, 15] and the results claimed that SPB would adsorb BSA at pH 7–4.5. In this case, SPB and BSA were mixed at pH 5. The adsorption of BSA in SPB is caused mainly by electrostatic interaction, which leads to the change of electron density profile in SPB. This kind of change can be detected very well by SAXS. Figure 5 shows the SAXS spectra of SPB and its complexes with BSA at pH 5 in 10 mmol/L NaCl solution.

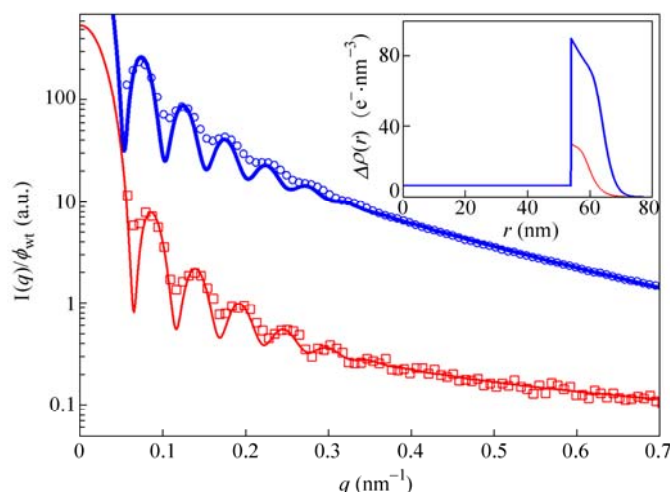


Fig. 5 SAXS spectra of SPB and its complexes with BSA at pH 5 in 10 mmol/L NaCl solution ($\circ$) BSA and SPB complexes and ($\square$) blank SPB. The solid lines are fitted results. The inset gives the radial distribution of the excess electron density of SPB loaded with BSA (thick line) and of blank SPB (thin line).

As presented in Fig. 5, the maxima of scattering intensity $I(q)$ increased and moved to smaller scattering factor q after adsorption of BSA. This indicates an increase of the electron density in the subsequent shell upon adsorption of BSA and a rise of the gyration radius. The profile of electron density demonstrates that the proteins are entering the brush layer and distributed inside the shell. This is another clear indication for the importance of electrostatic interaction for protein adsorption.

CONCLUSIONS

The SPBs were characterized *in situ* by SAXS. Lognormal Distribution Profile of SPB shell was brought up for the first time, which can be applied well in SPB SAXS analysis. The DLS characterizes the outline of SPB, while SAXS is related to the electron density and can delicately characterize the radial structure of polymer particles. The dependence of SPB on pH was reflected in SAXS, agreeing with the DLS result. Furthermore, SAXS could characterize the changes of electron density inside brushes of SPBs when they were immobilized with magnetic nanoparticles or BSA. These convincing results lay the theoretical support for further SAXS study

on SPBs immobilized with metal nanoparticles and proteins, both of which are of significant potential application in catalysis and biological field.

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