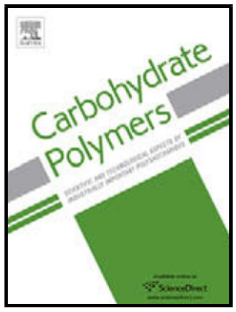

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Electroactive polyaniline/silica hybrid gels: controllable sol-gel transition adjusted by chitosan derivatives

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Highlights

- 1. The sol-gel transition of electroactive gels can be controlled by polysaccharide derivatives.
- 2. There is no organic solvent in the process of prepared hybrid gels.
- 3. Good biocompatibility and low toxic for the encapsulated enzyme or protein.
- 4. Good redox electroactivity. The polyaniline could be well compatible with the silica matrix.

Abstract: In this study, the electroactive hybrid gels with controllable sol-gel process were fabricated based on the water soluble polyaniline complex and water soluble silica precursor. β-cyclodextrin grafted on chitosan (CSCD) acted as a template, a new route for the synthesis of water soluble polyaniline complex (PA@CSCD) was designed by in-situ polymerization. Then, the hybrid silica gels without severe shrinkage were prepared by mixing PA@CSCD complex with water soluble precursor (tetrakis(2-hydroxyethyl)orthosilicates, THEOS). By dynamic rheological measurements, it was found that PA@CSCD complex could trigger and accelerate the sol-gel transition of the silica precursor. The gelation time could be largely shortened with the increase of PA@CSCD complex amount. By SEM observation, the PA@CSCD complex could be well compatible with the silica matrix. Moreover, the hybrid gels showed the good redox electroactivity, which could be successfully applied in a HRP-based biosensor.

Keywords: polyaniline; chitosan derivatives; hybrid gels; controllable sol-gel transition; electroactivity.

1. Introduction

Polyaniline has attracted particular interest because of its good environmental, special chemical and electrochemical properties as a conductive polymer(Han, Bai, Yang, Lee & Lin, 2008; Zhang, Gang, He, Fu, Gu & Sheng, 2017). Polyaniline can be doped into hydrogels by the three-dimensional network of hydrogels, which can exploit the application of polyaniline, such as drug controlled release, biosensor, artificial muscle, and so on(Fei, Chen, Bao, Wang & Wang, 2015; Roosz, Euvard, Lakard, Buron, Martin & Viau, 2017). However, due to the poor hydrophilicity, the polyaniline can not be effectively combined with hydrogel matrix and resulted to phase separation behavior. In recent years, the water soluble electroactive polyaniline has been prepared by self-doped or using polymeric templates(Jang, Han & Im, 2000; Li, Zhao, Zhuang, Wang & Gu, 2007; Marmisollé, Maza, Moya & Azzaroni, 2016) Water soluble polyaniline can be incorporated with inorganic components and formed hybrid composites via the sol-gel technology(Facio, Carrascosa & Mosquera, 2017; Wang et al., 2001). It is reported that the polyaniline/inorganic hybrid composites exhibited special conductivity, and good mechanical, electronic, electrochemical, and optical properties(Pei et al., 2014; Takei, Dong, Yonesaki, Kumada & Kinomura, 2011; Wan & Azarian, 2016). These hybrid composites also have been widely applied in electrode materials, electrochromic devices, biosensor, electrostatic discharge protection and drug delivery(Bai, Wang, Song, Wang & Wang, 2015; Mi, Zhang, Yang, Ye & Luo, 2008; Neiracarrillo et al., 2016; Tahir, Alocilja & Grooms, 2005). However, there are three main problems in the fabricated organic/inorganic composites: 1. organic co-solvents were added to prepare composites owing to the water insolubility and poor hydrophilicity of precursor, which could lead to phase separation between water soluble polyaniline and matrix (Wang & Zhang, 2007). 2. The gelation time of general precursors is too long and uncontrollable, such as TMOS, TEOS. The acid or base as catalyst needs to add in the gelation process (Ferrer, Levy, Gomez-Lor & Iglesias, 2004; Wang, Li, Yang, Shen & Wang, 2000). 3. The severe shrinkage was accompanied during the condensation process. It is reported that the dimension shrinkage is more than 70%. (Danielle C. Schnitzler, Michelle S. Meruvia, Ivo A. Hümmelgen & Aldo J. G. Zarbin, 2003). It is difficult to obtain stable and intact hybrid films. In our previous work, water-soluble silica precursor (THEOS) was developed, which can be accelerated the sol-gel process by polysaccharides and sharply decreased

the gelation time in aqueous solutions. Moreover, the intact thin films or monolithic hybrid gels can be obtained via the sol-gel process between chitosan and THEOS(Wang & Zhang, 2009; Zhang, 2006).

In this study, a new strategy for fabricating the electroactive polyaniline/silica hybrid gels was developed. The hybrid gels were prepared based on water soluble polyaniline and water soluble silica precursor. The sol-gel transition can be modulated by polyaniline complex. Firstly, a new route for the synthesis of water soluble polyaniline was developed by in-situ polymerization. *β*-cyclodextrin grafted chitosan (CSCD) was synthesized and acted as a template for the formation of the water soluble polyaniline complex (PA@CSCD) via the host-guest interaction. Secondly, THEOS was mixed with the PA@CSCD solution and a controllable sol-gel transition was induced under mild conditions. At last, horseradish peroxidase (HRP) was added to mixture solution and an amperometric biosensor was fabricated based on the PA@CSCD/silica hybrid films. The characteristics of polyaniline hybrid gels and the resulting hydrogen peroxide biosensor have been investigated.

2. Experimental section

2.1. Materials

β-cyclodextrin (*β*-CD), Tetraethoxysilane (TEOS), Chitosan (Mw=10 kDa, deacetylation degree=85.3%)), *β*-alanine, p-toluenesulfonyl chloride, n-hydroxysuccinimide (NHS) and 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), N,N-Dimethylformamide (DMF) were purchased from Energy Chemical (Shanghai, China). ***β***-CD was recrystallized twice from water before used. Horseradish peroxidase (HRP) (250U/mg) was purchased from Boao Biotechnology Corporation (Shanghai, China). The other reagents were analytical grade and were used as received.

2.2. Preparation of *β*-cyclodextrin-g-chitosan derivatives (CSCD)

6-Mono-(p-toluenesulfonyl)-***β***-CD (CD-Ts) was synthesized according to the previous reported(Kojima, Toi, Harada & Kono, 2008; Yi, Duan, Cai, Dong & Wei, 2016). CD-Ts (1.02 g, 0.79 mmol) was dissolved in 20 mL of anhydrous DMF solution and added dropwise to *β*-alanine solution (4.45 g/30 mL of DMF). The mixed solution was reacted at 60 °C under nitrogen for 3 days. Then 100 mL of acetone was added to stop the reaction. The precipitation was centrifuged and washed with acetone three times. The resulting precipitation (CD-COOH) was dried in vacuum. Yield: 79%. ^{1}HNMR (400 MHz, D_2O): d4.96 (s, H(1) of CD), 3.76-3.84 (m, H(3), H(6) and H(5) of CD), 3.54 (m, H(2) and

H(4) of CD), 2.71-2.84 (m, methylene of -$NHCH_2CH_2COOH$) (Fig. S1). IR (KBr, cm^{-1}) 3394, 2928, 1633, 1412, 1337, 1036, 938, 854,760,705 (Fig. S2).

0.20 g of chitosan was dissolved in 20 mL of water and adjusted the pH value to 5.0 by 0.1M HCl solution. EDC (0.20 g) and NHS (0.11 g) were added. CD-COOH (1.20g) was dissolved in 20.0 mL water and added to chitosan solution and reacted for 24 h at room temperature. Then 100 mL of ethanol was added and the precipitation was centrifuged. The precipitation was redissolved in water and dialyzed in a dialysis bag (MWCO=3KDa). The resulting products (CSCD) were dried in vacuum freeze-drying. ^{1}H NMR (400 MHz, D_2O) δ 4.94 (s, C-1 H of ***β***-CD and protons of chitosan), 3.30-4.00 (m, C-2, C-3, C-4, C-5, C-6 H of ***β***-CD and protons of chitosan), 1.98 (27 H, s -$NHCOCH_3$ of chitosan) (Fig. S1). IR (KBr, cm^{-1}) 3250, 2924, 1623, 1415, 1301, 1151, 1091, 1028, 815 (Fig. S2).

2.3. preparation of water soluble polyaniline(PA@CSCD)

0.20 g CSCD dissolved in 20 mL of water. 1 M HCl was added to the solution and adjusted the pH value to 3.0. Aniline monomer (0.20 g) was added dropwise with stirring at room temperature. After 2 h, $(NH_4)_2S_2O_8$ (0.60 g) aqueous solution (pH 3.0, adjusted by HCl) was added dropwise in an ice bath and reacted at 0 °C overnight. The precipitation was removed by centrifuged at 2000 rmp. The resultant solution was dialyzed to remove unreacted monomer and any oligomeric products in a dialysis bag (MWCO = 3 kDa). The PA@CSCD colloid solution was obtained. The obtained solution was vacuum freeze-dried.

2.4. Preparation of Hybrid Material and Hydrogen Peroxide Biosensor

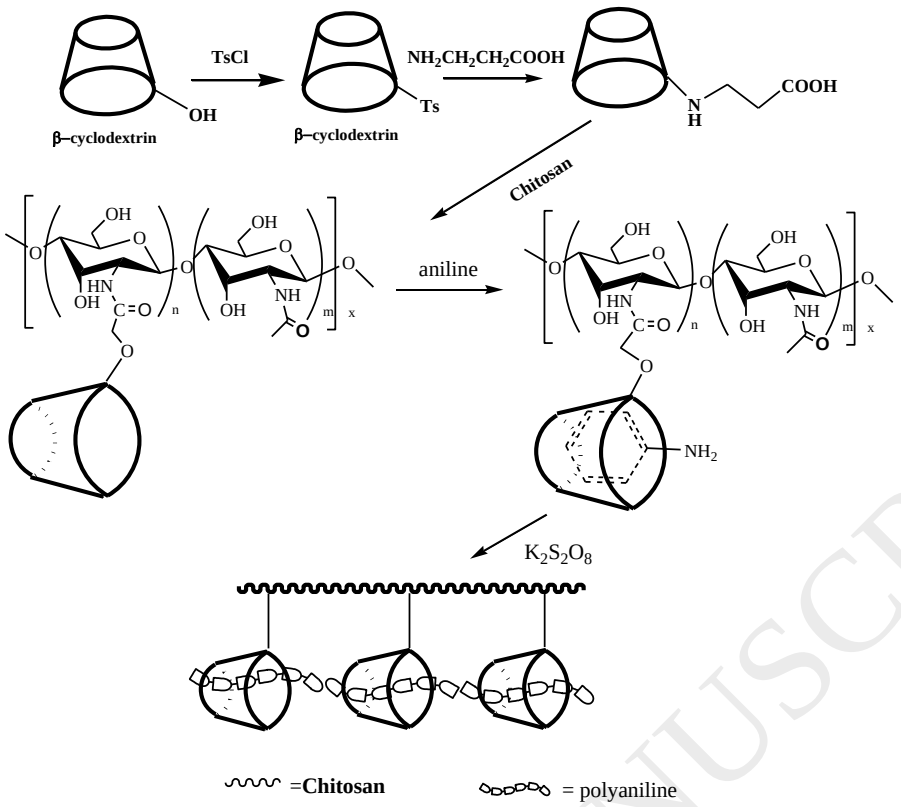
The fixed amount of PA@CSCD solution was directly mixed with precursor THEOS at room temperature. The homogeneous hybrid gels can be formed standing for a while. The biosensor construction was prepared as our previous reported. The enzyme electrode was prepared based on hybrid gels containing HRP as the working electrode. 1.0 wt % PA@CSCD solution blended with HRP to form a homogeneous solution (5 mg/mL HRP). Then, 10wt% THEOS was added and mixed uniformly. The mixture was rapidly coated on the purified glassy carbon electrode (GCE) surface, and then dried for 10 min at room temperature. The property of the enzyme electrode was measured according to our previous reported(Wang & Zhang, 2009).

2.5. Characterizations

The morphology of hybrid gels was observed by scanning electron microscope (SEM, JSM-6330F field emission). UV-vis spectra were obtained using a spectrophotometer (TU-1900). Thermal gravimetric analyses (TGA) were performed on a NETZSCH TG209 thermogravimetric analyzer at a scan rate of 10 °C/min in nitrogen atmosphere. The sol-gel process and the viscoelastic properties of hybrid gels were performed by an advanced rheometric extended system (ARES, TA Co.) with a cone-and-plate geometry (cone diameter, 25 mm; angle, 1°). The property of the hydrogen peroxide biosensor was performed with a CHI 630 electrochemical workstation (Shanghai Chenghua, China).

3. Results and discussion

β-CD is a cyclic oligosaccharide with internal hydrophobic cavities. The hydrophobic molecules can be included into the cavities and formed inclusion complex. It was reported that polymer chains were threaded through cyclodextrin molecules and formed complex like a "molecular necklace"(Yoshida, Shimomura, Kohzo Ito & Hayakawa, 1999). In this study, ***β***-CD grafted on chitosan derivatives (CSCD) were synthesized and acted as a template to prepare water soluble polyaniline complex in situ via the host-guest interaction (as shown in Scheme 1). The degree of substitution (D.S.) of CSCD was calculated from digital integration of the anomeric protons singles of s-Dglucopyranosyl residues (δ 5.0 ppm) in the β-CD moiety and the glucosamine proton single (δ 3.4-4.0 ppm) in the chitosan skeleton. From the area ratio of the signals of these anomeric protons, D.S. of the product was estimated to be about 29.2%.



Scheme 1. Synthesis process of PA@CSCD complex by in-situ polymerization.

Chitosan is well soluble macromolecules in water(Gomez, Chambat, Heyraud, Villar & Auzély-Velty, 2006), which act as a dispersant for polyaniline. The PA@CSCD complex can be stably dispersed in water and formed stable colloids solution without any other dispersants. The dispersed PA@CSCD complex in water was displayed at pH value of 3.0 in Fig. S3. No precipitation or degradation was observed over 10 days. Dynamic light scattering (DLS) was performed to investigate the particle size of PA@CSCD complex. As seen in the Fig. 1, PA@CSCD complex showed a green transparent colloidal solution in acidic solutions. The average diameter of complex was about 144.1 nm in the pH value of 3.0. Adjusted the pH value from 3.0 to 12.0, the color of PA@CSCD complex was changed from green to blue to purple. Moreover, the complex size was increase to 263 nm in the pH value of 7.0 (Fig. S 4A). At last, a purple precipitation was formed in the pH value of 12.0 (Fig. S 4B).

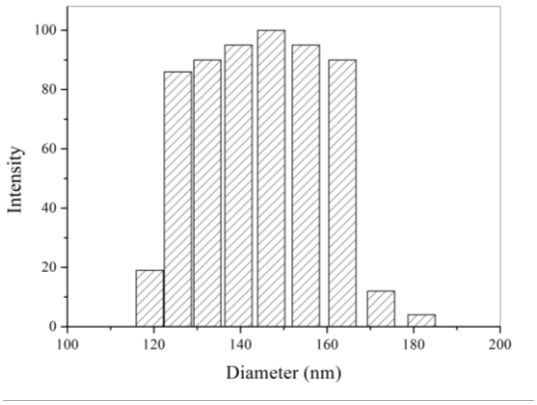


Fig. 1. The size distributions of PA@CSCD in pH value of 3.0.

Fig. 2 demonstrated the morphology and structure of freeze-dried PA@CSCD complex by SEM images. As seen in Fig. 2, PA@CSCD complex exhibited nanofiber morphology with the average diameter of ~80 nm. It is reported that *β*-cyclodextrin acted as a template for the formation of the polyaniline via the host-guest interaction(Hasegawa et al., 2012; Prasannan, Truong, Hong, Somanathan, Shown & Imae, 2011). The 1,4 coupling polymerization will be reacted owing to the oxidative coupling reaction, which can reduce the side chains of polyaniline(And & Jayakannan, 2006; Sen, Mishra & Shimpi, 2017). In this study, the aniline monomer can self-assemble into cyclodextrin of CSCD, which acted as templates during polymerization. The nanofibrous polyaniline complex will be fabricated via its intrinsic self-assembly aided during in situ polymerization.

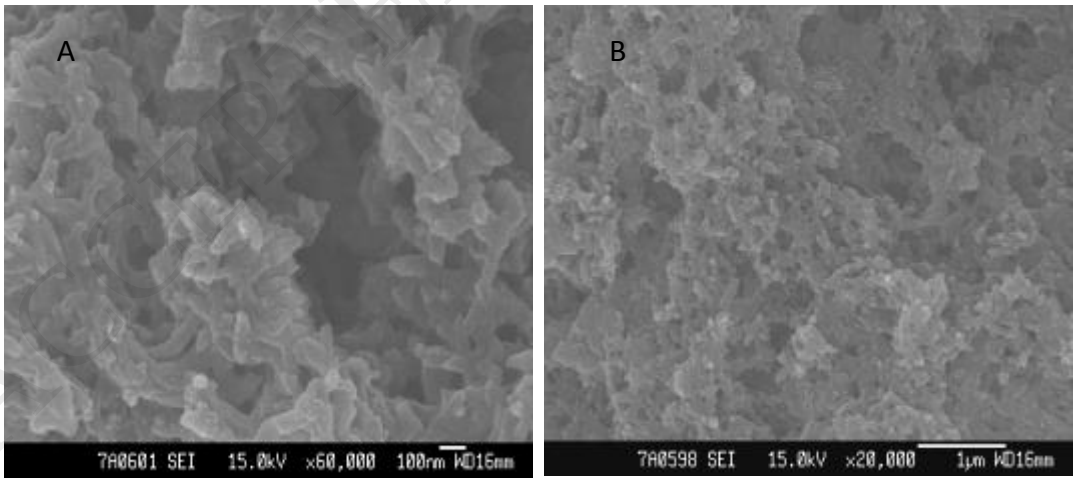


Fig. 2. The SEM images for PA@CSCD complexes with various magnifications:

(A) ×60000, (B) ×20000.

TGA was performed to investigate the amount of polyaniline in complex. As shown in Fig. 3A, three major weight loss zones can be observed in the TGA curves of pure polyaniline and polyaniline complex. The pure polyaniline exhibited an initial weight loss in the range of 70–100 °C, which correspond to a loss of adsorbed water. The second step around 220 °C associated with loss of dopant molecules. The final step exhibited a lost weight in the range of 500-600 °C, which corresponds to the decomposition of polyaniline backbone with different degradation temperatures(Planes, Rodríguez, Miras, García, Pastor & Barbero, 2010; Trchová, Šeděnková, Tobolková & Stejskal, 2004). In comparison with pure polyaniline, the TGA curve of polyaniline complex demonstrated a similar tendency and good thermal stability. The amount of polyaniline in the complex can be calculated about 18.7%.

The structure of PA@CSCD and pure polyaniline was determined by wide-angle XRD. As shown in Fig. 3B, three reflection peaks of 14.9、 20.9 and 25.1° were observed in pure polyaniline(Jasim, Ullah, Shi, Lin & Yang, 2017). XRD confirmed a crystallization of pure polyaniline. By comparison, the diffraction patterns of PA@CSCD were different to pure polyaniline. The degree of crystallinity of PA@CSCD was obviously decreased. No well-resolved reflection peaks was observed in the XRD diffraction patterns, which suggest the inclusion complex was formed between polyaniline and CSCD. Moreover, each glucosamine unit on chitosan chains does not match a β-CD moiety according to the degree substitution. And chitosan macromolecules chains is coiled and overlapped. In combination with the results of DLS, aniline monomers were polymerized with CSCD as template, then formed colloidal particle among a few CSCD macromolecules chains. Therefore, the complex could be exhibited different colors in different pH value.

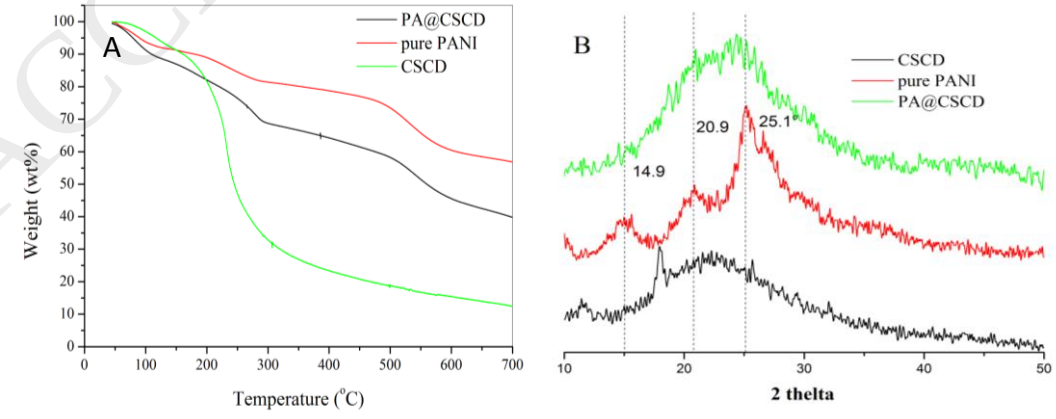


Fig. 3. TGA curves for the thermal degradation (A) and X-ray diffraction patterns (B) of pure PANI, CSCD and PA@CSCD

The reversible redox behavior of the PA@CSCD complex was investigated by adjusted the pH value. As shown in Fig. 4A, the UV-vis spectra of PA@CSCD complex are similar to characteristic spectrum of polyaniline. The polaron bands will be transferred from 780-800nm to t 550-600 nm owning to the polaron transitions while adjusted pH value from 2 to 12 by 0.1 mol/L NaOH(Sucharita Roy et al., 2002). Meanwhile, the absorption intensity of π-π conjugated system of benzene at 260 nm and 320 nm were increased obviously with the increase of the pH value(Hong et al., 2016; Li, Zhao, Zhuang, Wang & Gu, 2007). The color of complex was changed from green to blue to purple. By comparison, the color of complex also was changed from purple to blue to green and the UV-vis spectra also returned to origin with the decrease of the pH value from 12 to 2. This result indicated that PA@CSCD complex have good reversible redox electroactivity from polyaniline in the complex.

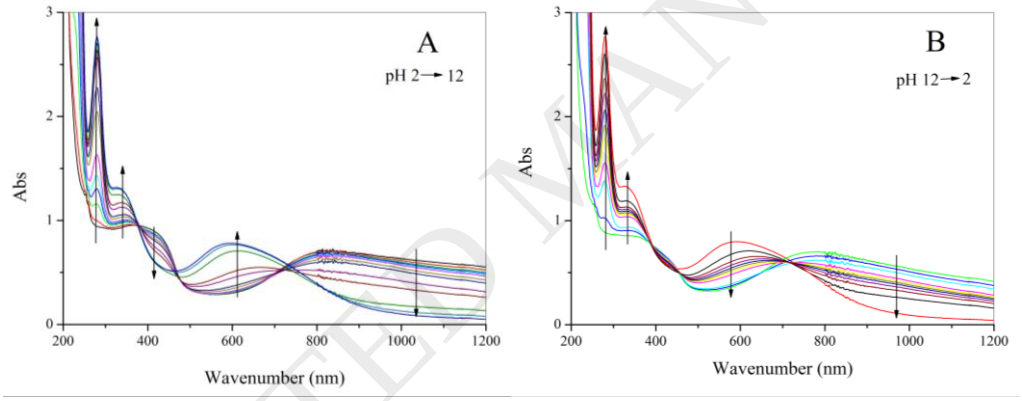
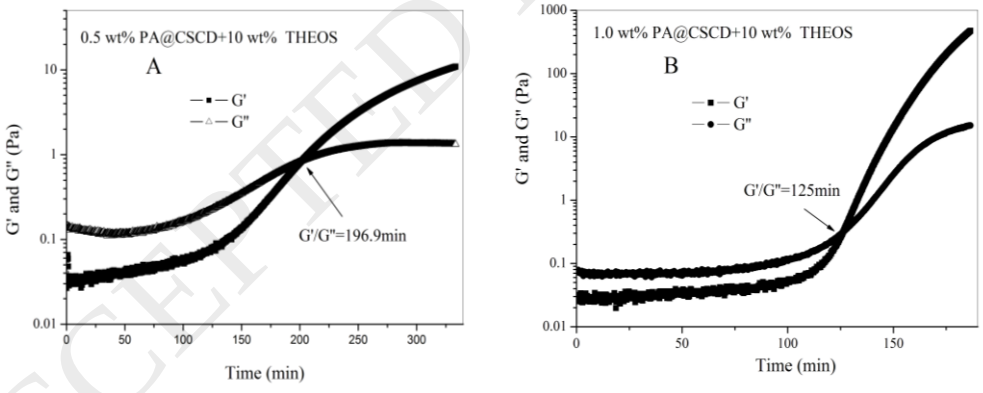


Fig. 4. UV-vis absorption spectra change of PA@CSCD complexes in different pH value. The pH changed from (A) 2.0 to 12 and (B) 12 to 2.0. pH value adjusted by 0.1 mol/L NaOH and 0.1 mol/L HCl.

In our previous research, we found polysaccharide was able to accelerate the sol-gel transition of THEOS precursor without organic solution, such as chitosan, guar gum, dextran. The polysaccharides acted as a catalyst in the sol-gel process due to changing the cluster aggregation kinetics of silica sol particles. Moreover, the homogeneous silica gels without severe shrinkage can be obtained (Wang & Zhang, 2009; Zhang, 2006). In this study, a homogeneous solution also can be formed while PA@CSCD colloid solution mixed with THEOS precursor. To monitor the sol-gel transition process affected by PA@CSCD, time sweep rheological was performed. The viscoelastic properties with time (storage modulus and loss modulus) of sol-gel transition process were measured at ambient temperature. As

shown in Fig. 5, there is a sharply increase of G' and G'' with the increase of time, then form a crossing point of G' and G'' curves. The corresponding time of the crossing point is regarded as the gelation time(Aoki & Watanabe, 2004). Beyond the crossing point, G' value is lager than G'' value, which indicates a gel in formative stage. It is interesting to observe a significant decline in the gelation time when the PA@CSCD concentration varied from 0.5 to 2.0 wt%. In PA@CSCD composites, CSCD also can change the cluster aggregation kinetics of silica sol particles and obviously accelerate the sol-gel transition (Sui, Cruzaguado, Chen, Zhang, And & Brennan, 2005). The viscoelastic property of hybrid gels also was investigated by the complex viscosity (η^*) and storage modulus (G'). As shown in Fig. S5, the hybrid PANI gels exhibited shear-thinning behavior and the storage modulus was increased with the increase of PA@CSCD concentration, which indicates PA@CSCD amount can help to enhance the strength of hybrid gels. These results indicate that PA@CSCD complex were strongly interacted with the silanols from precursor hydrolysis. The semi-interpenetrating networks were formed between PA@CSCD macromolecules and silica network. Moreover, the sol-gel transition process could occur without any catalyst or organic solvent at room temperature, which is a great advantage in application of the novel composite platforms for electronic devices, protein encapsulation, drug carrier(Kortesuo, Ahola, Karlsson, Kangasniemi, Yliurpo & Kiesvaara, 2000).



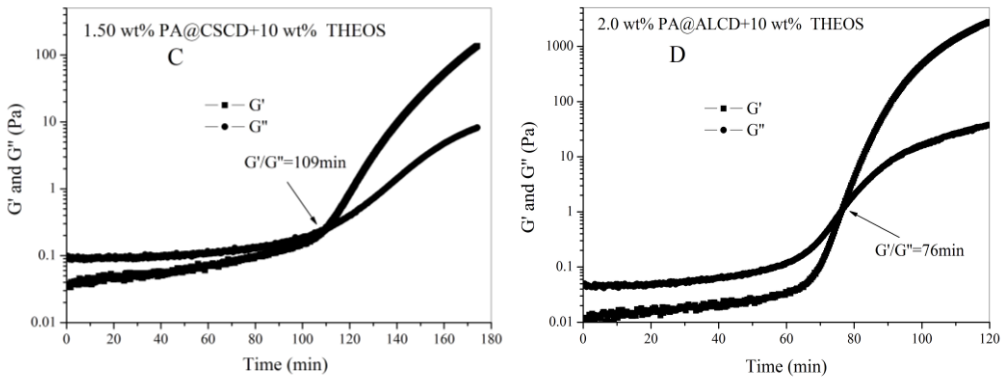


Fig. 5. Effects of complexes content on storage modulus (*G*') and loss modulus (*G*'') for PA@CSCD /THEOS systems (25 °C).

The morphology of hybrid gels was observed by SEM. As seen in Fig. 6, the hybrid gels showed a uniformly phase. Heterogeneous hybrid aggregates or the shape of polyaniline colloids was not found in the hybrid gels. Apparently, the PA@CSCD could be well compatible with the silica matrix in the hybrid gel. Owning to the water soluble precursor THEOS, PA@CSCD colloid particles can be dispersed in sol particles and form homogeneous solution. During the hydrolysis-condensation reaction, PA@CSCD colloid particles were compatible with gel matrix and form semi interpenetrating network structure, which are beneficial to improve the mechanical performance and electroactivity of the hybrids materials.

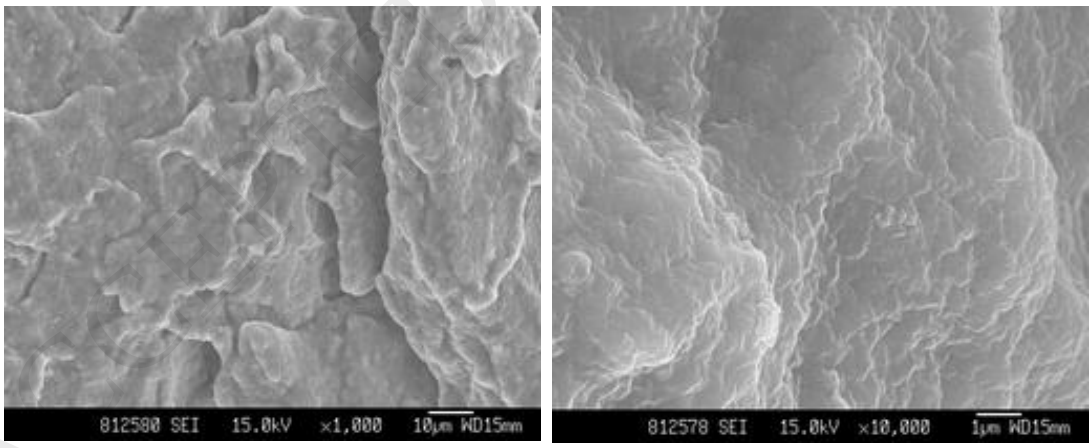


Fig. 6. The interior structure of SEM images for PA@CSCD/silica hybrid gels. A. ×1000, B. ×10000

The electrochemical characterization of the PA@CSCD hybrid gels was measured by cyclic voltammetry. Three groups of redox peak were observed in the Fig. 7, which showed the electroactivity and electron transfer in the macromolecule chains of polyaniline. The first couple

peaks appeared at 0.22 V and corresponding peaks at 0.10 V. The redox potential difference was 120 mV. The third couple redox peaks appeared in (0.80 and 0.76 V), which correspond to polyaniline change from intermediate oxidation to high oxidation(Li, Zhao, Zhuang, Wang & Gu, 2007). In addition, a couple of small redox peaks appeared in 0.55V, which may be caused by redox of quinone structure. These results indicate good electrochemical reversibility of polyaniline in the hybrid gels(Huang & Kaner, 2004).

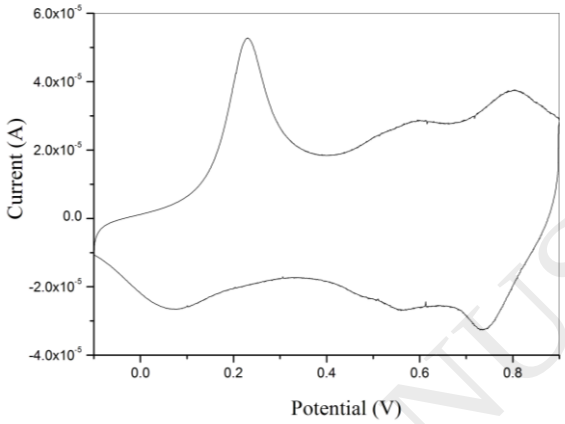


Fig. 7. Cyclic voltammograms of PA@CSCD/silica hybrid gels in 1mol/L HCl (10 mV/s).

Fig. 8 showed the effect of polyaniline for steady-state current was responsive to the hydrogen peroxide. As seen in Fig. 8A, the HRP biosensors have rapid and sensitive response to the detection of hydrogen peroxide. The responsive time of steady-state current is less than 4s, which indicates the catalytic activity of HRP can be kept in hybrid membrane. The polyaniline in hybrid membrane also did not affect on the activity of HRP. Fig. 8B showed the steady-state current depended on the concentration of hydrogen peroxide (C_{H2O2}). Compared to biosensor without polyaniline, biosensor with polyaniline leaded to a higher current intensity to hydrogen peroxide under the same conditions. The conductive polyaniline molecules as a non diffusion media, built a bridge of electron transfer between the enzyme and electrode(Garjonyte & Malinauskas, 2000; Kuwahara, Ogawa, Sumita, Kondo & Shimomura, 2018). The current intensity was linear increased with increase of the concentration of hydrogen peroxide. However, the results showed that the redox current intensity is not directly proportional to the content of polyaniline complex (as shown Fig.S5). The low content of polyaniline may have little effect to transfer electrons in hybrid films. On the contrary, the high content may affect the sensitivity. The biosensor based on the PA@CSCD/silica hybrid gels

has a linear range of C_{H2O2} (mol/L) from 1.0×10^{-6} to 2.6×10^{-4} with slope of 1.59×10^{-2}. The detection limit is 8.7×10^{-7} mol/L. In comparison, the biosensor without polyaniline has a linear range of C_{H2O2} (mol/L) from 2.1×10^{-6} to 2.8×10^{-4} with slope of 1.01×10^{-2}. The detection limit is 4.0×10^{-7} mol/L. The Michaelis-Menten constant K_m^{app} could be calculated by the Lineweaver-Burk equation:(Xu, Guo, Lu & Wang, 2010)

$$1/I_{ss} = 1/I_{max} + K_m^{app}/(I_{max}C)$$

Where I_{ss} is the steady-state current intensity, C is the concentration of the substrate, and I_{max} is measured the maximum current intensity. The K_m^{app} constant indicates the affinity between the enzyme and substrate. A smaller K_m^{app} value implies a higher affinity. In contrast, a higher K_m^{app} value implies a smaller affinity. The K_m^{app} was calculated to be 0.996 mmol/l of biosensor with polyaniline and 3.2 mmol/l of biosensor without polyaniline, respectively. A smaller K_m^{app} value of biosensor with polyaniline illustrated a higher affinity of the enzyme electrode to substrate(Yu, ‡ & §, 2003). In addition, we found the biosensor fabricated by polyaniline hybrid gels has good storage stability. The current intensity was not obviously changed to hydrogen peroxide after 30 days of storage at 4 °C.

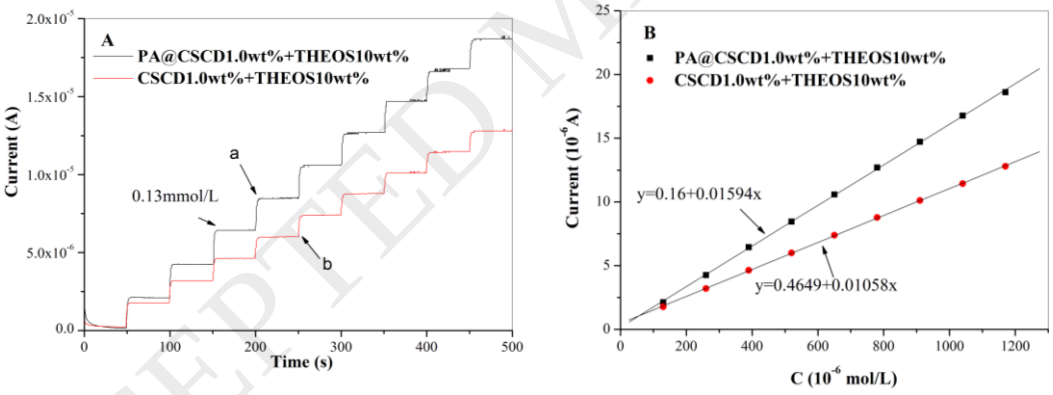


Fig. 8 (A) Amperometric response of the fabricated biosensor to successive addition of different concentration of H_2O_2 in 0.02 mol/l PBS solution (pH 7.0)

(B) Calibration plots between the current and the concentration of H_2O_2.

4. Conclusion

A new strategy for preparing the homogeneous polyaniline/silica hybrid gels was developed. *β*-cyclodextrin derivatives acted as a template, the aqueous polyaniline complex (PA@CSCD) was developed by in-situ polymerization. PA@CSCD complex showed good stability in water and

electroactivity, which can form homogeneous solution with water soluble precursor. Moreover, PA@CSCD complex could trigger and accelerate the sol-gel transition of precursor. The gelation time also could be largely shortened with the increase of PA@CSCD amount. By SEM observation, the PA@CSCD could be well compatible with the silica matrix in the hybrid gel. The hybrid gels have good redox electroactivity, which could be successfully applied in a HRP-based biosensor.

Acknowledgment

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