



# Multiple pH-responsive graphene composites by non-covalent modification with chitosan

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## ABSTRACT

Multiple pH-sensitive composites have been prepared through non-covalently functionalizing chemically converted graphene (CCG) with chitosan. Chitosan exhibits as polybases and CCG shows characteristics of polyacids. Owing to the synergistic effects of chitosan and CCG, chitosan decorated graphene (CS-G) presents a multiple pH-responsive behavior that it can be dispersed well whether in acidic or in basic solution but aggregated in near-neutral solution. After CS-G was modified through a controlled deposition and cross-linking process of chitosan, the resultant cross-linked chitosan decorated graphene (CLCS-G) can be converted to a different pH-sensitive material that disperses only in acidic solution. Both CS-G and CLCS-G present a reversible switching between dispersed and aggregated states with pH as a stimulus. The unique pH response mechanisms of CS-G and CLCS-G have been further investigated by zeta potential analysis. Based on the unique pH-responsive property of CS-G, a stable and repeatable pH-driven switch was developed for monitoring pH change.

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## 1. Introduction

Graphene exhibits unique structural, optical, mechanical, and electrical properties and has been intensively studied in recent years in materials science and biotechnology [1–5]. Similar with other carbon nanomaterials such as fullerene and carbon nanotubes, graphene generally presents poor water solubility and tends to form irreversible agglomerate in aqueous solution due to the strong  $\pi$ - $\pi$  stacking interaction [6]. However, chemically converted graphene (CCG) prepared through chemical reduction of graphene oxide (GO) by hydrazine can readily form stable aqueous colloids in basic solution through electrostatic stabilization, owing to the large amount of remaining carboxylic acid groups on CCG [7]. As well known, pH-sensitive materials can be classified into two categories, polyacids and polybases. CCG could be considered as a specific polyacids macromolecule. Thus, using CCG as a building block, exploiting novel pH-sensitive material has attracted increasing interest in recent years. In most previous reports, graphene-based pH-responsive materials generally present a single pH-response resulting from either modified functional molecules or CCG [8–10]. However, little has been done to exploit graphene-based multiple pH-sensitive materials by integrating pH-responsive properties of both CCG and functional molecule, which might provide a new pH-sensitive material for

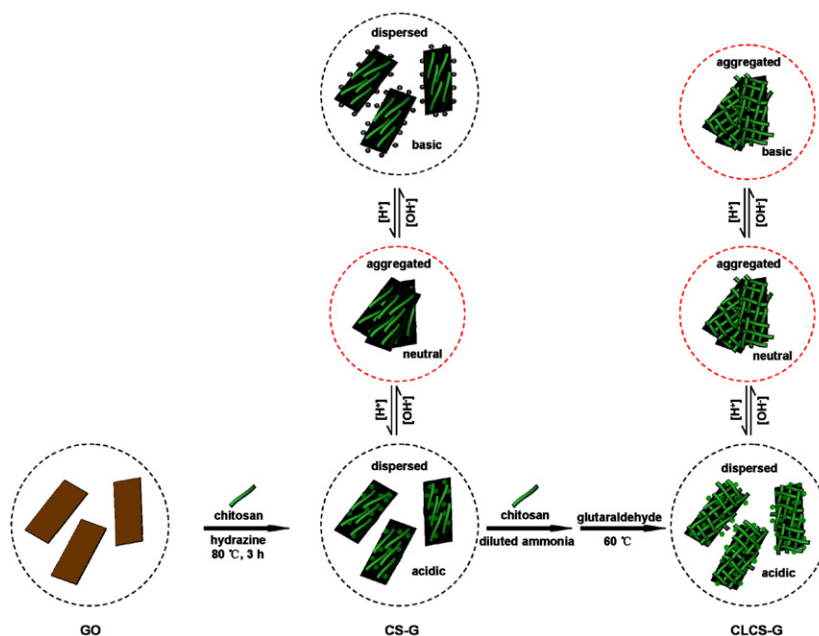
potential applications in switching devices, biosensor, novel gel, drug and gene delivery systems.

Chitosan is a natural polysaccharide, normally produced by extensive deacetylation of chitin and is a pH-responsive polymer with a unique solubility that can be reversibly alerted by pH changes [11]. Since the amino groups of chitosan have a  $pK_a$  value of  $\sim 6.5$ , chitosan is only soluble in acidic solution where the amino groups of chitosan get protonated [9,12]. Different from CCG, chitosan is a typical polybases material. Combined with its bioactivity, nontoxicity and biocompatibility, chitosan has found widespread applications in biosensor and pharmaceutical products [13–15]. It is expected that the graphene functionalized with chitosan could present excellent pH-responsive properties.

In this contribution, multiple pH-responsive graphene composites have been prepared for the first time by non-covalent modification with chitosan (Scheme 1). Owing to the perfect combination of chitosan and CCG, the as-prepared chitosan functionalized graphene (CS-G) presents a multiple pH-responsive behavior that it can disperse well either in acidic or in basic solution whereas aggregate in near-neutral solution. Furthermore, through a controlled surface deposition and cross-linking process of chitosan on CS-G, the resultant cross-linked chitosan decorated graphene CLCS-G can be converted to a different pH-sensitive material that disperses well only in acidic solution. Additionally, the dispersed and aggregated states of both CS-G and CLCS-G can be reversibly transferred with pH as a stimulus. On the basis of the unique pH-responsive property of CS-G, we have developed a stable and repeatable pH-driven

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**Scheme 1.** The synthesis process of CS-G and CLCS-G and corresponding pH response behaviors.

switch. These controllable multiple pH-responsive properties may provide new insights into application of graphene nanomaterial in pH sensor, controlled drug delivery systems, biomimetics and responsive plasmonics.

## 2. Experimental

### 2.1. Materials

Graphite was purchased from Alfa Aesar. Chitosan with low molecular weight (MW=9000 Da) was obtained from Aldrich. Hydrazine solution (50 wt%) was obtained from Beijing Chemical Reagent factory (Beijing, China). Unless otherwise stated, other reagents were of analytical grade and used as received. All aqueous solutions were prepared with ultrapure water ( $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ) from a Milli-Q Plus system (Millipore).

### 2.2. Apparatus

AFM was conducted with a SPI3800N micro-scope (Seiko Instruments, Inc.). UV-vis spectra were recorded on a Cary 50 UV-vis spectrophotometer (Varian, USA). Raman spectra were recorded on a J-Y T64000 Raman spectrometer with 514.5 nm wavelength incident laser light. X-ray photoelectron spectroscopy (XPS) analysis was carried out on an ESCALAB MK II X-ray photoelectron spectrometer. Zeta potential measurements were performed on a Malvern Zetasizer Nano-ZS particle analyzer.

### 2.3. Synthesis of CS-G and CLCS-G

Graphene oxide (GO) was prepared from natural graphite according to a modified Hummers' method [16]. CS-G composite was prepared as follows: GO dispersion (4 mL, 1 mg/mL) was mixed with an aqueous solution of chitosan (36 mL, 0.25%, pH 3.0). After being vigorously shaken, the mixture was ultrasonicated for 30 min to form a uniform dispersion. Hydrazine solution (50 wt%, 20  $\mu\text{l}$ ) was added into the resulting homogeneous dispersion under strong stirring and kept at 80 °C in a water bath for 3 h. Then, the resultant CS-G was collected by

centrifugation (15000 rpm) and washed with 0.1 mM of HCl solution for three times to remove the excess chitosan and hydrazine.

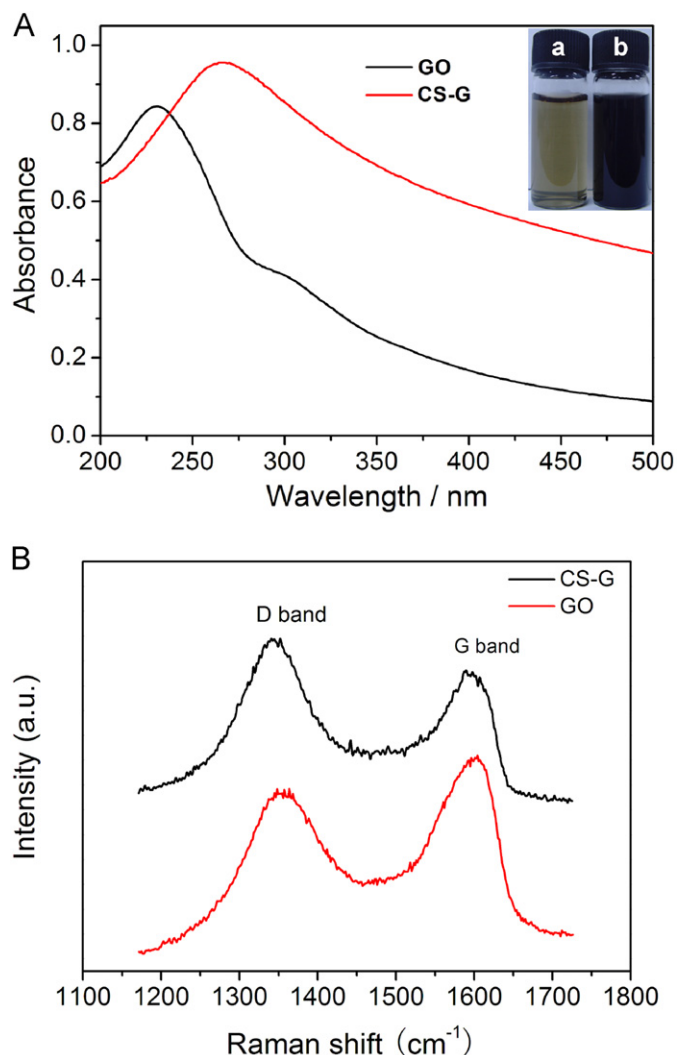
CLCS-G composite was prepared via a controlled surface-deposition and cross-linking process, which was a related approach reported previously for preparing cross-linked chitosan modified carbon nanotubes [12]. Briefly, the CS-G (0.2 mg/mL, 10 mL) solution was mixed with chitosan solution (0.2 mg/mL, 10 mL) and then ultrasonicated for 15 min. Diluted ammonia solution was added dropwise into the mixture until a weak aggregation occurred in the solution. Then 0.5 mg glutaraldehyde was added to the system under strong stirring and kept at 60 °C for 0.5 h. Finally, the CLCS-G was collected by centrifugation and then washed with diluted HCl solution for three times to remove the excess chitosan.

### 2.4. pH-response experiments of CS-G and CLCS-G

The pH-response experiments of CS-G and CLCS-G were conducted as follows: for CS-G, eleven equal copies of CS-G dispersions (2 mL) were adjusted to different pHs (from 2 to 12) by adding 1 M HCl or NaOH solution and followed by ultrasonication for 20 s. Then all the suspensions were sedimentated for 1 min after being vigorously shaken and used for further zeta potential analysis. For CLCS-G, three equal copies of CLCS-G suspensions (2 mL) with pH 3.0, 7.0 and 11.0 were prepared with the same procedures.

### 2.5. pH switch experiments of CS-G

The pH switch experiments of CS-G suspension between pH 4.0 and pH 7.0 were conducted as follows: CS-G suspension (0.2 mg/mL, 1 mL) was circularly modulated to different pHs (4.0 and 7.0) by adding 1.0 M HCl or NaOH solutions. After 30 min of incubation, the transmittance (at a wavelength of 500 nm) of the supernatant at each pH was collected by a UV-vis spectrophotometer. The pH switch experiments of CS-G suspension between pH 7.0 and pH 10.0 were conducted following the same procedures.



**Fig. 1.** (A) UV-vis spectra of GO and CS-G. The insets show the photographs of (a) GO dispersions, and (b) CS-G dispersions. (B) Raman spectra of GO and CS-G.

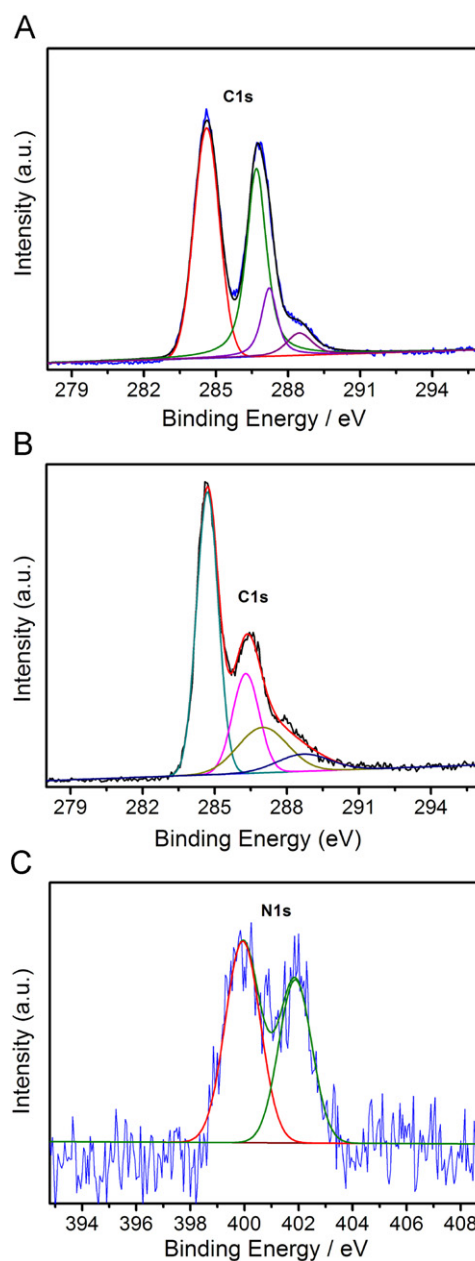
### 3. Results and discussion

#### 3.1. Characterizations of CS-G and CLCS-G

The formation of CS-G was first evidenced by UV-vis absorption spectroscopy. As shown in Fig. 1A, two peaks centered at 230 and 300 nm are observed for the GO dispersion, corresponding to the  $\pi$ - $\pi^*$  transition of aromatic C=C bonds and the  $n$ - $\pi^*$  transition of the C=O bond, respectively [17]. After the reaction, the color of the solution changes from light brown to dark (inset of Fig. 1A). The absorption peak of the GO dispersion at 230 nm red-shifts to 268 nm and the other absorption peak at 300 nm disappears, indicating that the electronic conjugation within the graphene sheets is restored upon hydrazine reduction [7].

Raman spectroscopy is one of the most widely used techniques to characterize the structural and electronic properties of graphene. As shown in Fig. 1B, a G band and D band are observed in both spectra of GO and CS-G, corresponding to the  $E_{2g}$  phonon of C  $sp^2$  atoms and breathing mode of  $\kappa$ -point phonons of  $A_{1g}$  symmetry, respectively [18]. The Raman D/G band intensity ratio is proportional to the average size of the  $sp^2$  domains [4], which increases significantly from 0.81 (GO) to 1.17 (CS-G), validating that CS-G was successfully synthesized by effective reduction of GO.

Detailed structural and compositional analyses of GO and CS-G were conducted by X-ray photoelectron spectroscopy (XPS). Learned from the C1s spectrum of GO (Fig. 2A), four different peaks centered at 284.6, 286.7, 287.2, and 288.5 eV indicate the presence of four types of carbon bonds: C=C/C-C in aromatic rings, C-O (epoxy and alkoxy), C=O, and COOH groups, respectively [7]. For the CS-G (Fig. 2B), the peaks associated with C=C/C-C (284.6 eV) become predominant, while the peaks related to the carbon binding to oxygen especially the peak of C-O (epoxy and alkoxy) decrease dramatically, suggesting that most of the oxygen-containing functional groups are removed after the reduction. Meanwhile, a new peak corresponding to C-N bond appears at 286.0 eV in the spectrum of CS-G [19], which is attributed to the coating of chitosan. Nitrogen 1s XPS data also proves that the chitosan molecules are adsorbed on the graphene sheets (Fig. 2C).



**Fig. 2.** Carbon 1s XPS profiles of GO (A) and CS-G (B). Nitrogen 1s XPS profile of CS-G (C).

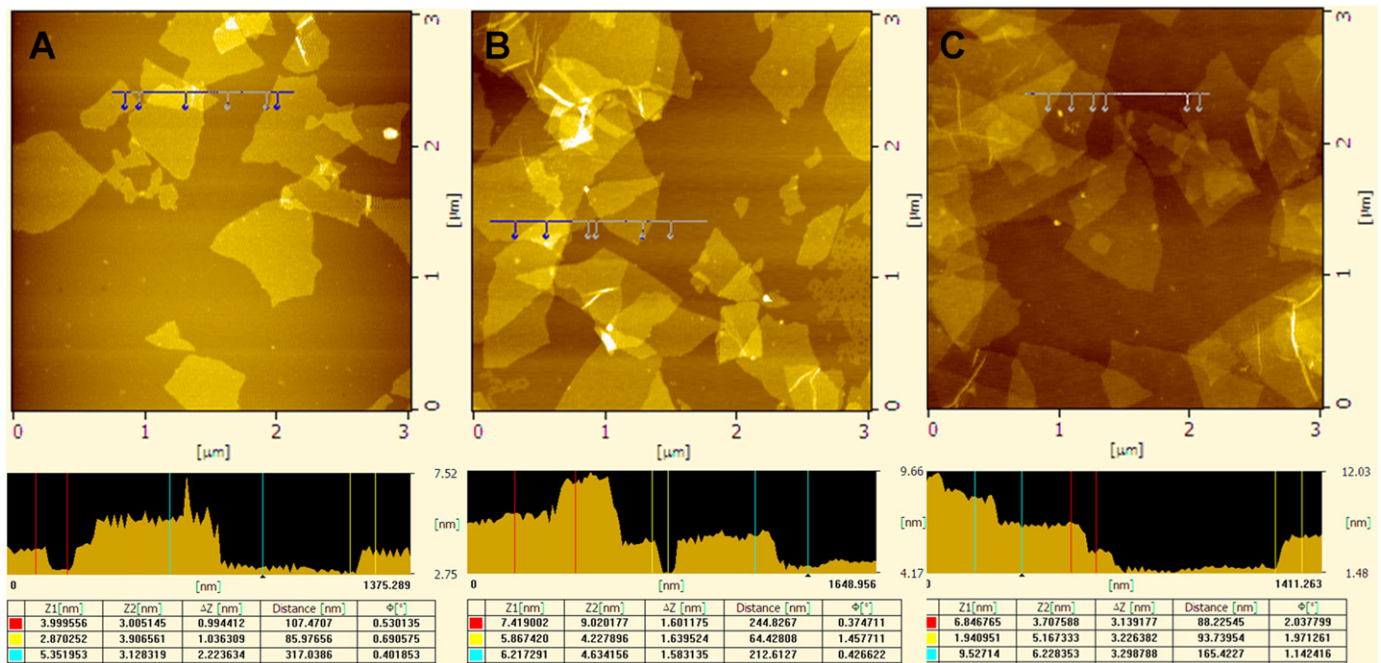


Fig. 3. Tapping mode AFM images of (A) GO, (B) CS-G and (C) CLCS-G on freshly cleaved mica.

The morphology and thickness of GO, CS-G and CLCS-G were characterized by AFM to provide direct information. Measured from the height profile of the AFM image (Fig. 3B), the average thickness of CS-G is  $\sim 1.6$  nm which is significantly greater than that of GO ( $\sim 1.0$  nm) (Fig. 3A), though most of the oxygen-containing functional groups are removed after a reduction. This result is mainly ascribed to the chitosan molecules grafted onto both sides of graphene through van der Waals force and hydrophobic interaction between the hydrocarbon main chain of chitosan and the basal plane of graphene, similar with other polymers interacted with graphene as reported previously [10,20]. For the CLCS-G, an average thickness of  $\sim 3.2$  nm is measured from the corresponding cross-sectional view of the AFM image (Fig. 3C). There is a 1.6 nm increment compared with that of CS-G, indicating that the loading amount of chitosan on CLCS-G is much more than that on CS-G after the process of surface deposition and cross-linking of chitosan.

### 3.2. pH response behaviors of CS-G and CLCS-G

CS-G was prepared through a non-covalent modification method for functionalizing graphene with chitosan in order to retain the intrinsic pH-responsive properties of CCG and chitosan. Photographs in Fig. 4A show the pH-responsive behavior of CS-G over a pH range from 2.0 to 12.0 in aqueous solutions. It is obviously seen that CS-G could readily form homogeneous aqueous colloid solutions with black color at pH below 5.0. When the pH increases to 6.0, 7.0 and 8.0, respectively, flocky precipitate appears in the solutions. However, the CS-G re-disperses well in aqueous solutions as the pH is over 9.0 and up to 12.0. The multiple pH-responsive behavior of CS-G is very much different from that of previously reported graphene-based pH-sensitive materials which have only a single pH-responsive property [8–10].

The novel pH-response of CS-G stimulates us to further explore its pH-responsive mechanism. pH sensing system is usually related to the surface charge of the materials changed with pH. Here, the surface charge of CS-G at different pHs was investigated by measuring its zeta potential, which is widely used

for investigating the stability of colloid. As is well known from colloidal science, the absolute value of zeta potential larger than 30 mV is generally considered to represent sufficient mutual repulsion to ensure the stability of a colloid aqueous solution [7]. Fig. 4B shows the variation of zeta potential along with the change of pH. As depicted by the curve, the zeta potential of CS-G is pH dependent and divides into three representative regions. At lower pH range from 5.0 to 2.0, the zeta potential is above +30 mV and reaches maximum +47.5 mV at pH 3.0. Positive zeta potential of CS-G at lower pH suggests that the well dispersion of CS-G in acidic solution is mainly ascribed to electrostatic repulsion resulting from the strong protonation of chitosan, since the  $pK_a$  of chitosan is 6.5. Under the circumstances, the solubility of CS-G is mainly related to the property of chitosan. When the CS-G dispersion is adjusted to nearby neutral pH range from 6.0 to 8.0, the zeta potential abruptly falls to +9.1 mV at pH 6.0 and is converted to negative values of  $-3.8$  mV and  $-15.3$  mV at pH 7.0 and pH 8.0, respectively. This result is mainly contributed to the cooperative effect of chitosan and CCG. With a gradual increase of pH, the protonation degree of chitosan gradually decreases. When the pH is over its  $pK_a$ , chitosan becomes deprotonated in aqueous solution. Meanwhile, the ionization of carboxylic acid groups on CCG is gradually enhanced along with increasing pH. Thus, the charge density of CS-G greatly decreases at nearby neutral pH range so that the resulting electrostatic repulsion force is too weak to make CS-G disperse in this pH range. As the pH further increases to 9.0 and up to 12.0, the zeta potential is below  $-30$  mV and reaches  $-41$  mV at pH 11.0, indicating that the ionization of carboxylic acid groups on CCG is greatly enhanced in basic solution and becomes dominant effects on dispersion of CS-G. Furthermore, whether the protonation and deprotonation of chitosan or the ionization and deionization of CCG are reversible, the behaviors of CS-G can therefore be reversibly manipulated with pH as a stimulus. According to the above discussion, the dispersion of the CS-G in acidic solution is mainly ascribed to the protonation of chitosan. In basic solution, however, the ionization of graphene mainly dominates the surface charge of the CS-G, leading to a stable dispersion. The aggregation of the CS-G in near-neutral solution is mainly due to a low surface charge density, which is caused by the cooperation of chitosan and graphene. Therefore, it is



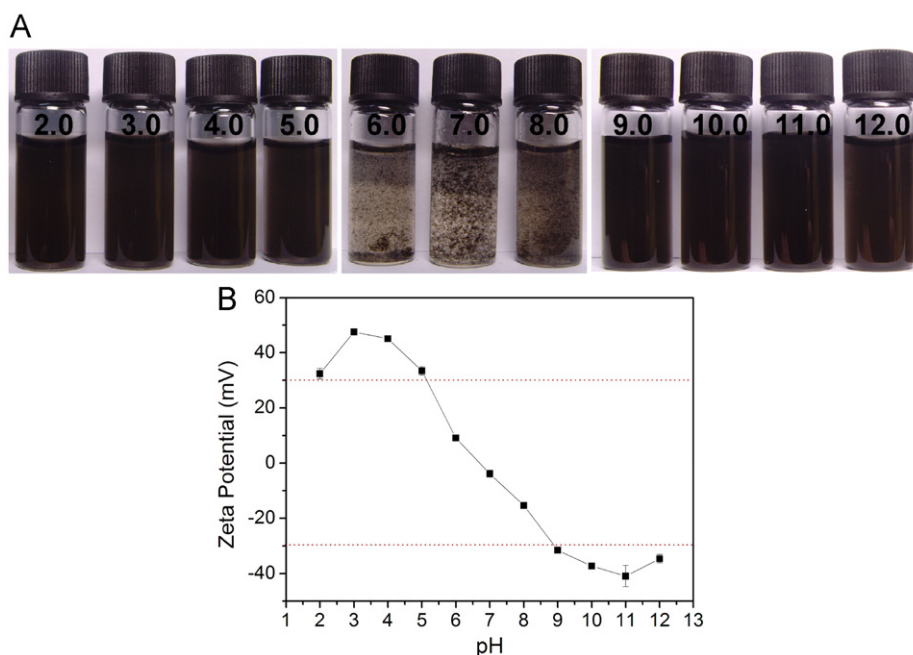


Fig. 4. (A) Photographs of CS-G aqueous solutions with different pH from 2.0 to 12.0. (B) Zeta potential of CS-G as a function of pH in aqueous dispersions.

reasonable to draw a conclusion that the charming pH responsive properties of the CS-G can be ascribed to the synergistic effects of chitosan and graphene.

To further validate the speculation, CLCS-G was synthesized via a controlled chitosan surface-deposition and cross-linking process to CS-G (Scheme 1). Compared with CS-G, the as-prepared CLCS-G presents a different pH-response. As shown in Fig. 5A, CLCS-G can disperse well in acidic solution but aggregate in neutral and basic solutions. We supposed that the increasing loading amount and cross-linking of chitosan might greatly affect the pH-responsive property of CCG. Zeta potential of CLCS-G at pHs 3.0, 7.0 and 11.0 were measured as shown in Fig. 5B. CLCS-G has a zeta potential of 43.1 mV (Fig. 5Ba) at pH 3.0 and  $-2.7$  mV (Fig. 5Bb) at 7.0, consisting that of CS-G. However, when the pH increases to 11.0, CLCS-G has a zeta potential of  $-9.3$  mV (Fig. 5Bc). The magnitude of the zeta potential is much lower than that of CS-G at pH 11.0 ( $-41$  mV). The results indicate that the solubility property of CLCS-G was mainly dominated by the large amount of chitosan which makes a strong electrostatic shielding effect on CCG in basic solution.

### 3.3. pH dependent switch of the CS-G

Based on the unique pH-responsive property of the CS-G, a repeatable pH-driven switch was developed for monitoring pH change. Learned from Fig. 6A, the dispersed and aggregated state of the CS-G suspension could be reversibly switched by alternating pH between 4.0 and 7.0. UV-vis spectrophotometer was used to measure the transmittances (at a wavelength of 500 nm) of the supernatants of the CS-G suspension at different states after 30 min of incubation. Fig. 6B shows the cyclical changes of the transmittance of the CS-G suspension resulting from solution pH oscillating between 4.0 and 7.0. The CS-G suspension at pH 4.0 was homogeneous with a transmittance lower than 5%. As the pH was increased to 7.0, the suspension became aggregated within several seconds by hand-shake, and the transmittance was larger than 90% after sedimentation. Considering the reversible pH response of the CS-G, the aggregated state of the CS-G with a high transmittance and the dispersed state of the CS-

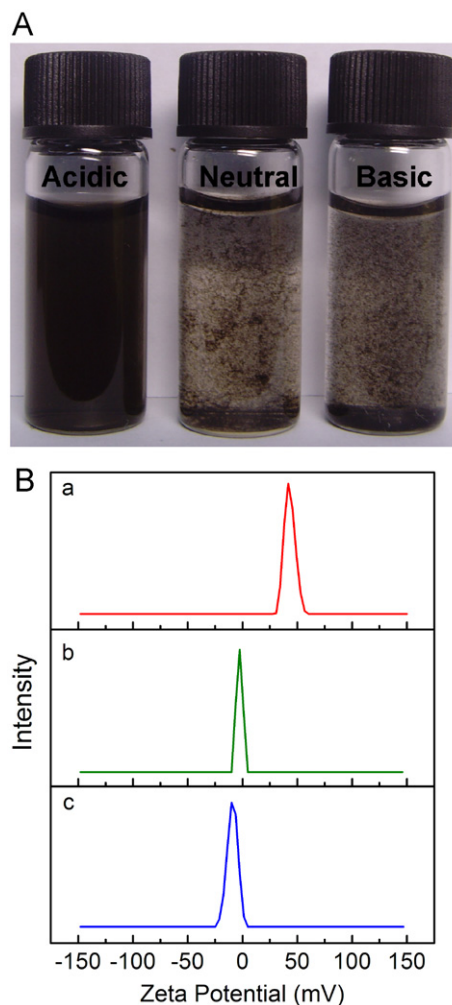
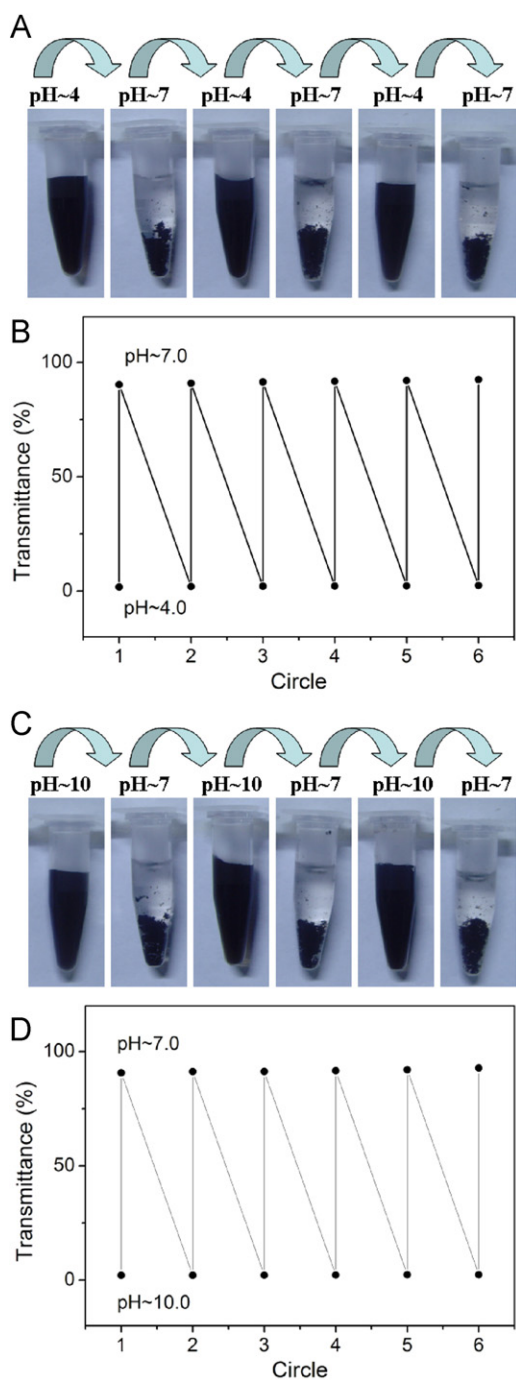


Fig. 5. (A) Photographs of CLCS-G in acidic, neutral and basic solutions. (B) Zeta potential of CLCS-G aqueous solutions at pH 3.0 (a), 7.0 (b) and 11.0 (c).



**Fig. 6.** The pictures show the reversible pH-response of CS-G suspension at pH between 7.0 and 4.0 (A) and also between 7.0 and 10.0 (C). Reversible switch of CS-G between the ON and OFF states through alternating pH between 7.0 and 4.0 (B) and also between 7.0 and 10.0 (D).

G with a low transmittance could be defined as ON and OFF states, respectively. Accordingly, a reversible pH-driven switch was developed for monitoring solution pH change between acidic and neutral. Meanwhile, the switch between ON and OFF states could also be obtained by modulating solution pH between 7.0 and 11.0 due to the unique multiple pH-responsive property

of CS-G, as shown in Fig. 6C and Fig. 6D. The results confirm that CS-G has potential application in the development of stable and multiple reversible pH-switch [9,10].

#### 4. Summary

In summary, we have successfully prepared graphene-based multiple pH-responsive composites through non-covalently functionalizing CCG with chitosan. Due to the perfect synergetic effect of chitosan and CCG, the as-prepared CS-G presents a multiple pH-responsive behavior that it can disperse well not only in acidic but also in basic solution whereas aggregate in near-neutral solution. By increasing the coverage and cross-linking of chitosan, the resultant CLCS-G can disperse only in acidic solution whereas aggregate in neutral and basic solutions owing to the strong electrostatic shielding effect of cross-linked chitosan on CCG. Zeta potential analysis demonstrates that electrostatic stabilization plays an important role in the forming of stable CS-G dispersions either in acidic or in basic solution. Both CS-G and CLCS-G can be reversibly transferred between dispersed and aggregated states by modulating pH value of the solutions. According to the unique pH-responsive property of the CS-G, a repeatable pH-driven switch was developed for monitoring pH change, which can work as a pH sensor. Combining the unique properties of graphene and the versatility of chitosan, the CS-G and CLCS-G biocomposites may provide new insights into the applications in controlled drug delivery systems, biosensor, biomimetics and responsive plasmonics.

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