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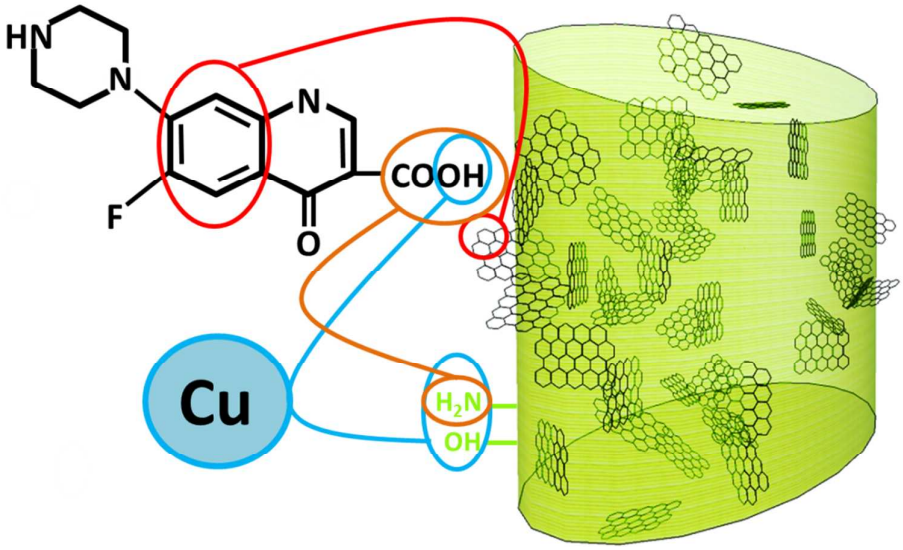
Graphical abstract



A new kind of biocomposite, porous graphene-soy protein (GS) aerogel, was prepared by a simple hydrothermal method and the mechanism of ciprofloxacin adsorption on GS was investigated.

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LETTER

Adsorption of Ciprofloxacin on Graphene-Soy Protein Biocomposite

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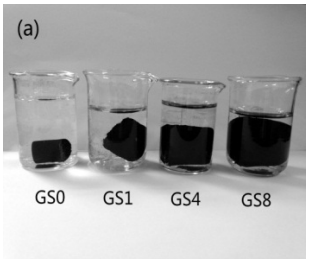
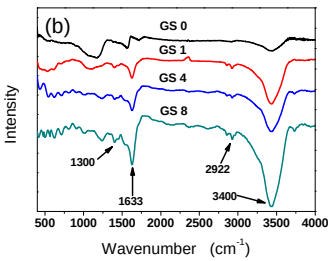
A new kind of biocomposite, porous graphene-soy protein (GS) aerogel, was prepared. Influence of protein content on GS and ciprofloxacin adsorption on GS were studied. The GS aerogel demonstrated good hydrophilicity and abundant functional groups. Cu could improve ciprofloxacin adsorption through coordination with GS8.

Antibiotics have received comparatively little attention as pollutants in the aquatic environment surprisingly because antibiotics have a direct biological action on microbes, unlike many other pollutants. Many of these antibiotics are not completely metabolized or eliminated in the body and between 30% and 90% are excreted unchanged into the waste system. Degradation productions of these antibiotics can also be considered as contaminants contributing to these complex mixtures. There is even some evidence that these degradation products can be as active and/or toxic as their parent. The presence of broad spectrum antibiotics like these in aquatic environments, albeit at low concentrations, may pose serious threats to the ecosystem and human health by inducing proliferation of bacterial drug resistance [1]. The global annual application of antibiotics has been estimated between 100,000 and 200,000 tons [2]. Ciprofloxacin is a second generation fluoroquinolone antibiotic of high use that has a high aqueous solubility under various pH conditions and a higher stability in soil and wastewater systems [3, 4]. Chemically, ciprofloxacin is a 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-quinolone-3-carboxylic acid. Since it has an extended aromatic part and functional groups suitable for hydrogen bonding, it can be expected that this phenolic-type molecule is able to interact strongly with biomacromolecules and that these noncovalent interactions may play a decisive role in its mechanism of action [5].

Carbon materials such as active carbon and carbon nanotubes present unique advantages in adsorption due to their low cost, high adsorption capacity and easy disposal [6, 7]. In general, it has been found that increasing the oxygen content of samples (i.e., turning the carbon surface more acidic), has detrimental effects on adsorption [8]. A dispersive interaction has been reported between the free electron of ciprofloxacin and the delocalized electron in carbon basal planes [9]. Electrostatic attraction and hydrogen bonding via the protonated amine and carboxylic groups of ciprofloxacin, respectively, seem to play important roles for adsorption on the surface of carbonaceous adsorbents [8]. Cation exchange was also reported to be an important interaction for ciprofloxacin disposition [10, 11]. Moreover, active site competition and/or multilayer adsorption between ciprofloxacin and coexisting metals rarely have been reported in the literature.

In this research, graphene-soy protein (GS) aerogels with different protein content were prepared by a simple thermal reduction method and then used as an adsorbent to remove antibiotics from aqueous solutions. The influence of protein content on material properties was investigated to study the combination of graphene and protein. Moreover, the adsorption of ciprofloxacin on GS and the influence of Cu(II) ions on adsorption were studied and the adsorption mechanism was discussed based on the tests of FTIR, BET and XAFS.





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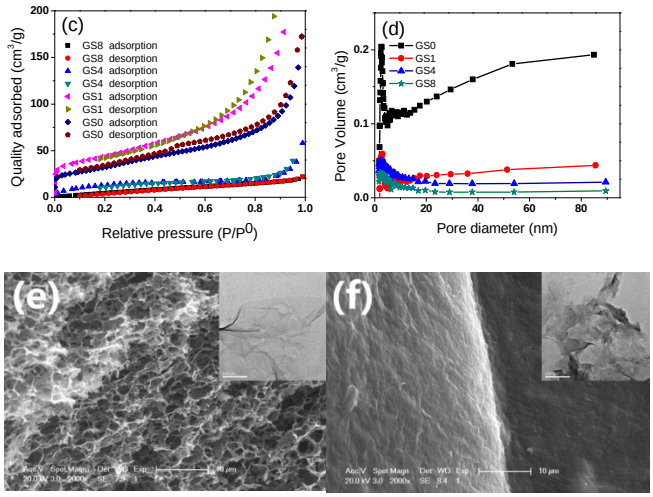


Fig. 1 Optical photograph (a) FTIR (b) N_2 adsorption and desorption curves (c) and pore size distribution (d) of GS0, GS1, GS4 and GS8 (e) SEM (insert TEM) of GS0 (f) SEM (insert TEM) of GS8

Digital images of GS0, GS1, GS4 and GS8 are shown in Fig. 1a. It can be observed that all the hydrogels have uniform structures; however, the hydrogels with a higher protein content have a much looser structure because the protein in hydrogels can help to retain more water. As a result, with higher water content, the hydrogels with a higher protein content have a more porous structure. Fig. 1a also reveals that the protein and graphene combine well in this biocomposite, under which condition the protein restrains the graphene from agglomeration, while the graphene combines with the protein to form a more ordered and larger structure. Fig. 1b shows the FTIR spectra of GS0, GS1, GS4 and GS8. It can be seen that many peaks become stronger while the protein content is higher, indicating that new bonds formed between the graphene and protein. The band at 2922 cm^{-1} is associated with the stretching vibration of the ring methane hydrogen atoms. The broad band around 3400 cm^{-1} was attributed to the O–H stretching vibrations, the strong band at 1227 cm^{-1} was assigned to the C–OH, and the band at 1633 cm^{-1} was carbonyl stretching vibrations (amide I) of hydrogen bonded, which indicated that hydrogen bonds are formed between the protein and graphene [16, 17]. Moreover, it can be seen that with a higher protein content, hydrogels had more functional groups.

Fig. 1c shows the N_2 adsorption-desorption isotherms of GS0, GS1, GS4 and GS8 and their pore distributions are shown in Fig. 1d. The isotherms of GS0 exhibit a typical type-I curve and a hysteresis loop at a relative pressure of 0.4, indicating the presence of slit-shaped pores between parallel layers of graphene [18]. Although the GS primarily contains protein, the GS has an obvious increase in N_2 adsorption. The specific surface areas of GS0, GS1, GS4 and GS8 were 119.17 m^2/g, 155.77 m^2/g, 40.51 m^2/g and 30.07 m^2/g, respectively, as shown in Table.1. As GS1 had a larger specific area than GS0, it indicated that the protein separated the graphene from agglomeration. As the protein itself was not porous, GS4 and GS8 had a smaller specific area than GS1. The pore size distribution of GS is also shown in Fig. 1d. It can be seen that GS0, GS1, GS4 and GS8 have the same tendency in the curves of pore volume and have peaks in the same position of around 2 nm, indicating the graphene maintains its nanopore in GS. Moreover, because the protein did not close the pores of graphene, it can be deduced that the combination force between the graphene and the protein may be a hydrogen bond. Fig1e and 1f show the SEM (insert TEM) of GS0 and GS8. It can be seen that GS0 contains more pores than GS8.

Table 1. Specific surface area, average pore diameters (BJH) and total pore volume of GS0, GS1, GS4 and GS8.

Samples	Specific surface area (m^2/g)	Average pore size (nm)	Total pore volume (cc/g)
GS0	119.17	2.86	0.26
GS1	155.77	3.05	0.56
GS4	40.51	3.91	0.02
GS8	30.07	4.96	0.03

We then investigated the adsorption capacity of ciprofloxacin on GS0, GS1, GS4 and GS8, as shown in Fig. 2. As can be seen, the protein content did not have an evident effect on adsorption, it was because that protein contributes to adsorption through chemical bonding while graphene contributes to adsorption mainly relating on its abundant pores, thus, whether protein or graphene hold dominant position, the material still have considerable adsorption capacity. However, as the cost of the adsorbent is an important factor for adsorbent evaluation and protein is a biopolymer that is much cheaper than graphene, we calculated the adsorption capacity based on the mass of graphene in GS. It can be seen that GS8, which has an adsorption capacity of 160 mg/g, is the best choice. So we chose GS8 for the following batch experiments.

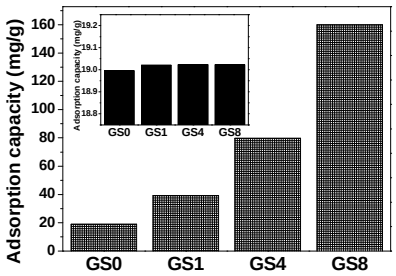
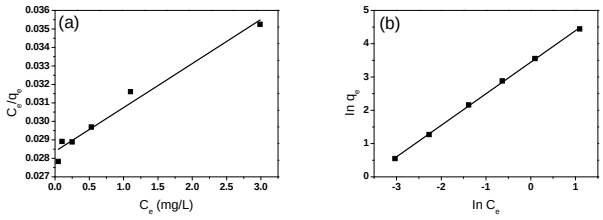


Fig. 2 Adsorption capacity of ciprofloxacin on GS with different protein content (25℃, ciprofloxacin initial concentration 20mg/L, solid-liquid ratio 0.5 mg/mL, 24h)

The adsorption isotherm of GS8 was calculated by Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich (D-R) isotherm as shown in Fig. 3a, 3b. 3c and 3d and their parameters were shown in Table.2. The good regression coefficients of the Langmuir isotherm and the Temkin model present a good affinity between the ciprofloxacin and GS8. Influence of pH and time on ciprofloxacin adsorption on GS8 was shown in Fig.3e and 3f. The GS8 showed better adsorption capacity under an acid solution as shown in Fig. 3e. This result was attributed to the charges difference of ciprofloxacin under different pH. Under aicd solution, the ciprofloxacin is positively charged which has electrostatic attraction toward graphene while in alkaline solution the action is repulsion. It can be seen from Fig.3f that the adsorption capacity reaches equilibrium around 24 h.



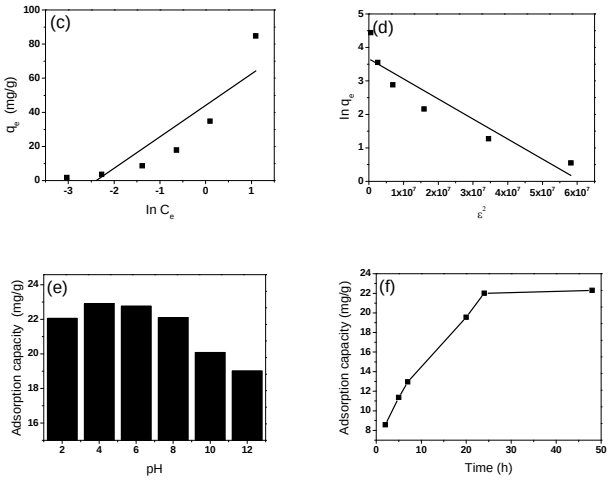


Fig. 3 Adsorption isotherm of GS8. (a) Langmuir, (b) Frendlich, (c) Tempkin, and (d) D-R; influence of (e) pH and (f) time on ciprofloxacin adsorption on GS8.

Table 2 The parameters derived from the Langmuir, Freundlich, Temkin, and D-R models

Isotherm	Characteristic	GS6
Langmuir	q_m(mg/g)	500
	R^2	0.967
Freundlich	R^2	0.999
Temkin	R^2	0.740
D-R	R^2	0.83

XRD and BET of GS8 before and after adsorption were shown in Fig.4. In Fig.4a, it can be seen that a peak around 6° appeared after adsorption, which indicated that crystal structure of GS8 changed after adsorption. Thus, a chemical reaction may have occurred during adsorption. During ciprofloxacin adsorption on GS8, both π–π and a hydrogen bond played an important role. Electron donor–acceptor interaction is one of the driving forces for the sorption of organic chemicals with benzene rings on graphene. The benzene rings with fluorine group a π-electron-acceptor due to the strong electron withdrawing ability of N and F. The -OH groups on the graphene surface can make the graphene act as electron donors. Thus, significant enhanced sorption was expected by the formation of a π–π bond. Moreover, -COOH in ciprofloxacin and –NH_2 in GS could form hydrogen bond between the adsorbents and the adsorbate [19]. It can be seen from Fig.4b that after adsorption the specific area of GS8 was nearly zero, which further proved that pores play an important role in adsorption. It was then believed that the adsorption followed a site to site behavior.

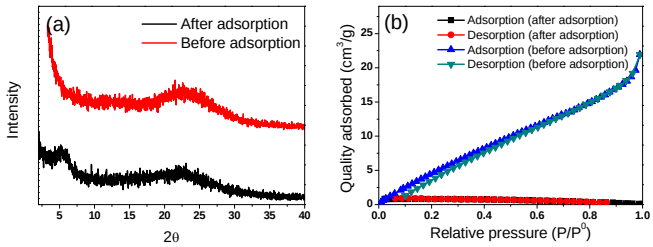


Fig. 4 Characterization of GS8 before and after adsorption (a) XRD, (b) N_2 adsorption and desorption curves.

Heavy metals cause potential risks for their impact on environmental quality and human health. Due to the use of copper as a growth promoter in animal feed, copper and antibiotics can coexist in the environment. Hence, we studied the influence of copper on ciprofloxacin adsorption. As shown in Fig. 5a, the ciprofloxacin adsorption capacity increased in certain concentration ranges of Cu because GS8 contains a deprotonated hydroxyl group and an amide group that enables Cu^{2+} coordination. Cu^{2+} can also strongly bind with the hydroxyl groups on the surface of ciprofloxacin through ligand-exchange reactions. Therefore, ternary complexes are expected to form among the Cu^{2+}, ciprofloxacin, and GS8 functional groups, resulting in Cu^{2+}-enhanced ciprofloxacin adsorption to GS8 through cation bridging between the metal ion and ciprofloxacin and the adsorbent ligand groups [20]. However, after the Cu concentration exceeded 10 mg/L, competitive adsorption strengthened and the adsorption of ciprofloxacin began to decrease. For further investigation of the influence of Cu on ciprofloxacin adsorption, we tested XAFS of Cu, Cu adsorbed on GS8 and Cu adsorbed on GS8 with ciprofloxacin, as shown in Fig.5b. Pure Cu has two characteristic peaks at 929.7 cm^{-1} and 949.8 cm^{-1}. After the adsorption on GS8, the peak at 949.8 cm^{-1} disappeared whether ciprofloxacin co-existed or not, further proving that that Cu^{2+} coordinated with GS8.

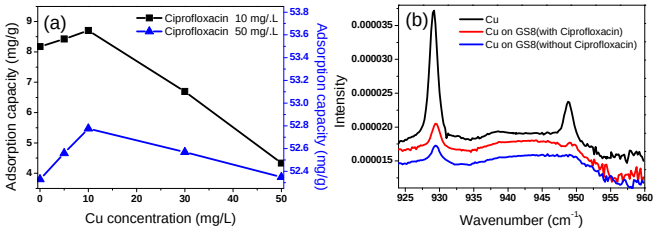


Fig. 5 Influence of Cu^{2+} on ciprofloxacin adsorption (a) adsorption capacity of ciprofloxacin on GS8 under different ciprofloxacin concentration and different Cu concentration, (b) XAFS characterization of Cu, Cu on GS with ciprofloxacin and Cu on GS without ciprofloxacin.

Biocomposite porous graphene-soy protein (GS) aerogels with different protein content were prepared by a simple hydrothermal method and then used as an adsorbent to remove ciprofloxacin. Batch adsorption experiments were carried out to remove ciprofloxacin from an aqueous solution using GS as an adsorbent. The GS aerogel demonstrated good hydrophilicity and abundant functional groups and the protein was able to to separate graphene from agglomeration. In ciprofloxacin on GS8, both the pores and the functional groups played an important role. Under a certain concentration, Cu^{2+} could improve ciprofloxacin adsorption.

Experimental

All chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) in analytical purity and used in the experiments directly without any further purification. All solutions were prepared using deionized water. Graphite oxide was obtained using the modified Hummers' method [12-15], dispersed in deionized water, and sonicated in an ultrasound bath for 12 h. Soy protein and ascorbic acid were added to the GO dispersion and placed into an ultrasound bath for 5 h to

form a uniform solution. The mass ratios of graphene to soy protein were 1:0, 1:1, 1:4, 1:8; the resulting products were denoted as GS0, GS1, GS4, GS8. The mixture solutions were heated in a water bath under 90 ℃ for 12 h to form hydrogels. The aerogels were synthesized after the hydrogels were washed with distilled water several times and then freeze-dried for 24 h. The surface morphologies of GN and GS were visualized using a field-emission SEM (Hitachi, S-4800) and TEM (JEOL, JEM-2010). The surface functional groups were observed by FTIR (NEXUS, 670). Measurements of micro-Raman spectra were carried out using a Raman Scope system (LabRam, 1B). XRD was collected on a Bragg-Brentano diffractometer (Rigaku, D/Max-2200). The BET isotherms were measured by an Accelerated Surface Area and Porosimetry system (Micromeritics, ASAP 2020). Fine local structure of Cu was studied by XAFS (Shanghai Synchrotron Radiation Facility, BL08U).

Batch experiments were conducted to evaluate the adsorption performance of ciprofloxacin on the adsorbents. GS0, GS1, GS4 and GS8 were used as adsorbents for ciprofloxacin adsorption in an aqueous solution. Then, 100 mg/L stock ciprofloxacin solution was prepared by dissolving 100 mg ciprofloxacin in 1 L deionized water. Working solutions of the required concentrations were obtained by diluting the stock solution with deionized water. All the sorption tests were conducted in well-capped 100 mL flasks containing 20 mL tetracycline solutions with the required concentration. After adding 10 mg GS8 aerogel, the flasks were shaken in a thermostatic shaker at 150 rpm at 298K for 24 h. All the adsorption experiments were conducted in duplicate, and only the mean values were reported. The maximum deviation for the duplicates was usually less than 5 %. Blank experiments without the addition of an adsorbent were conducted to ensure that the decrease in the concentration was actually due to the adsorbent rather than by the adsorption on the glass bottle wall. After adsorption, the adsorbent was separated using a 0.45 μm membrane. The residual concentrations in the solution were determined by an ultraviolet spectrophotometer (Tianmei UV-2310(II)) at 270 nm.

Acknowledgements

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