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# A facile approach for the synthesis of highly luminescent carbon dots using vitamin-based small organic molecules with benzene ring structure as precursors

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In this study, vitamin-based small organic molecules were used as precursors to synthesize carbon dots by means of a hydrothermal approach. The obtained carbon dots presented high luminescence and good cellar-imaging properties when folic acid was used as a precursor andthe quantum yield of the carbon dots obtained using the present method was more than 30%. Moreover, the green and yellow-green photoluminescence was also successfully achieved by adding the reagents of $H_3PO_4$ and $H_2C_2O_4$, respectively. The related formation mechanism and photoluminescence emission mechanism of carbon quantum dots were discussed. The present study will open one of new possible routes for the synthesis of carbon dots with high quality using small organic molecules as precursors.

## Introduction

Photoluminescent carbon-based materials, including carbon dots[1-10], carbon nanotubes[11], fullerene[12], and photoluminescent graphene[13-15], have attracted increasing attention in recent years owing to their excellent properties such as high optical absorptivity, chemical stability, biocompatibility, and low toxicity[16]. These superior properties provide a promise for the applications in bioimaging[2], biosensing[17], medical diagnosis[10], drug delivery[18], catalysis[9], and photovoltaic devices [19-21]. A variety of methods have been used to synthesize carbon dots, generally including arc-discharge[22], laser-ablation[23], electrochemical synthesis[24], plasma treatment[25] and combustion routes[26] and so on. Although these synthetic approaches are able to generate carbon dots, they require a complex and time-consuming process, which severely limits their application. Hydrothermal treatment method, as a traditional soft chemical preparation route, is considered to be one of the simplest and most cost-effective methods to synthesize nano materials due to its cheap apparatus, simple manipulation, good selectivity and low energy consumption. Recently, this method has been successfully used to prepare carbon dots with high quality. Basically, the used carbon sources include two general categories. One is carbon structural materials and carbon-based materials, such as graphene[27], carbon nanotubes[28], candle soot[29] and carbon powders[30], which can be effectively applied to

prepare carbon dots. However, the synthesis of some of these precursors generally requires complicated procedures, and some also need expensive equipment, which is not beneficial for large-scale production of carbon dots and hence limits their application. Another carbon source is from natural bio-resources like orange juice[31], peel[32], egg[33], soy milk[34], grass[35] and so on. However, these materials contain complex compositions, often causing unpredictable results due to the individual difference. Therefore, carbon source is a very important factor for the preparation of carbon dots with high quality. The optimal precursors for the hydrothermal synthesis of carbon dots might be the hydrosoluble aromatic compounds with rich amino and carboxyl groups. This is because benzene ring structure is conductive to the formation of carbon core structure by means of π-π stacking self-assembly mechanism while amino and carboxyl groups will contribute to the formation of surface state. The surface state will become the photoluminescence (PL) center created by a synergetic hybridization structure of carbon core and its surface chemical groups. In this study, folic acid, a small organic molecule with benzene ring structure, would be presented as an example to synthesize nano carbon dots by using a hydrothermal synthesis strategy. So far, there has a little report on the synthesis of nano carbon dots using single small organic molecules as a precursor. The present study will be expected to provide a reference for the hydrothermal synthesis of carbon dots with high quality in the selection of small organic molecule precursors. Furthermore, in order to achieve evidence for our proposed mechanism, some other vitamins with or without benzene ring structure would also be studied to investigate the role of benzene ring structure in the formation of nano carbon dots during

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hydrothermal synthesis. The obtained results from them could make the right conclusion for our proposed mechanism. Moreover, in order to make sure whether or not the surface state plays a crucial role in photoluminescence mechanism as reported before, some as-previously-reported metal ions, acid and alkali would also be introduced to modify the surface state of nano carbon dots to see whether or not the luminescence emission can be changed. If it is true, the obtained results would be able to provide further support for that. Meanwhile, vitamins with benzene ring structure but with different radical groups were also used to investigate the effect of radical groups of precursors on the surface state and luminescence emission so as to further explore the formation mechanisms of the surface state and luminescence emission. The related work will be described and discussed in the later section.

## Experimental

### Materials

All the chemicals were purchased from Kelong Co, Chengdu, China and used without further processing or purification: vitamin B9:folic acid (B.R.), vitamin B1: thiamine (B.R.), vitamin B5: pantothenic acid (B.R.), vitamin A: retinoid (B.R.), vitamin C: L-ascorbic acid (B.R.), vitamin E (B.R.), phosphate buffer solution (PBS, VB.R.), phosphate (A.R.), oxalic acid (A.R.), quinine sulfate (A.R.), and the other (A.R.)

### Synthesis of carbon dots

The typical experimental procedure is described as follows: first, 0.1g folic acid was added to 35mL distilled water and dissolved at 90℃ under agitation. After 20 minutes, the obtained solution was transformed into a stainless steel autoclave with a Teflon liner with a capacity of 50 mL and heated in an oven at 180℃ for 3h, followed by a natural cooling process to room temperature. The obtained light yellow solution was centrifuged at 12,000 rpm for 20 min to remove the agglomerated particles. After being lyophilized at -80°C temperature, the carbon dot powder was obtained. The obtained carbon dot powder was dispersed in water for further characterization. The experimental procedure was the same when other vitamins were used as precursors to synthesize nano carbon dots.

### Effect of metal ions on the PL of carbon dots

The detection of effect of metal ions on the PL of carbon dots was performed at room temperature in PBS buffer solution. In a typical run, 200μg carbon dot powder was added into PBS buffer (1mL, 0.2M), followed by the addition of a calculated amount of metal ions (1mL, 100μM). The PL spectra were recorded after reaction for 10 min at room temperature.

### Preparation of acid-modified photoluminescent carbon dots

The typical experimental procedure is described as follows: firstly, 0.1g folic acid (Vitamin B9) was added to 35mL distilled water and was dissolved at 90℃ under agitation. After 20 minutes, 1ml phosphate or 0.2g oxalate was added to the above mixture and the mixture was stirred for 10 minutes and then it was transformed them into a stainless steel autoclave with a Teflon liner with a capacity of 50mL and heated in an oven at 180℃ for 3h, followed by a natural cooling process to room temperature. The obtained light yellow solution was centrifuged at 12,000 rpm for 20 min to remove the agglomerated particles. The carbon dots thus obtained were dispersed in water for further characterization.

### Live cell imaging experiments

SCC-7 cells seeded on 24-well plates with D-minimum essential medium (DMEM) that contained 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotics was carefully cultured in a humidified incubator with 5% $CO_2$ at 37℃. After an incubation of 24 h, compared with the control culture, the cells did not show noticeable abnormality. Subsequently, the incubation media was removed and then the cells were rinsed with phosphate buffer solution (PBS) for three times. The cells were further incubated in 1.0 mL fresh DMEM containing 0.3 mL aqueous solution of carbon dots (200μg mL$^{-1}$) at 37℃ for 6 hours. Finally, the cells were washed with PBS for three times and then they were imaged under an Olympus BX51TRF microscope.

### Characterizations

The phase analysis of the sintered samples was carried out using an X-ray diffractometer (XRD) (X´Pert Pro MPD, Philips, Netherlands) with Cu Kα radiation at 45kV accelerating voltage and 35mA emission current. Data were collected in the 2θ range of 20° to 80°. Fourier transform-infrared spectroscopy (Spectrum One, Perkin Elmer, USA) was used for the analysis of material composition. Small amount of carbon dots were ground together with KBr and then pressed into discs for the IR measurements. The spectra were recorded from 500 to 4000cm$^{-1}$ wavenumber. In order to deeply observe the lattice and morphology of carbon dots, Transmission electron microscope (TEM) images were taken at an accelerating voltage of 200 kV(HR-TEM, JEOL 2100F, JEOL Ltd., Japan), Prior to TEM analysis, the sample was dropped on a Cu grid coated with an ultrathin amorphous carbon film, and then dried under ambient condition. UV-vis absorption was carried out using a UV-2550 ultraviolet–visible–near infrared (UV-vis-NIR) spectrophotometer (SHIMADZU, Japan).

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Photoluminescence spectroscopy was performed using a photoluminescence spectrophotometer （F-7000, HITACHI, Japan ）equipped with a 150W xenon lamp as the excitation source. Zeta potential was measured using a Zetasizer (Zen3690, Malvern, UK). The photoluminescence microscopy images were taken with an Olympus BX51TRF microscope. The light source for photoluminescence microscopy observation was a mercury lamp with a photoluminescent filter.

### The quantum yield of the carbon dots

The quantum yield (Φ) of the carbon dots was estimated by comparing the integrated PL intensities (excited at 350 nm) and the absorbance values (at 350 nm) of the carbon dots with that of the quinine sulfate in 0.1 M $H_2SO_4$ (literature quantum yield 0.54 at 350 nm). Then, the integrated photoluminescence intensity against the absorbance was plotted and a linear regression was attained for each curve with intercept at zero. Absolute values are calculated using the standard reference sample that has a fixed and to know photoluminescence quantum yield value, according the following equation:

$$\Phi_X = \Phi_{std}(Grad_X / Grad_{std})(\eta_X / \eta_{std})^2$$

Where Φ is the quantum yield, Grad is the gradient from the linear regression, and η the refractive index of the solvent (both are 1.33). The subscript "std" refers to the reference fluorophore of known quantum yield. In order to minimize re-absorption effects absorbencies in the 10 mm photoluminescence cuvette were kept under 0.1 at the excitation wavelength (350 nm).

## Results and discussion

### Characterization of structure and morphology

The X-ray diffraction (XRD) pattern showed a very broad peak at the 2θ of 23° (Figure 1A), generally indicative of a characteristic of weak crystalline. The average size of the obtained carbon particles was estimated by full width at half maximum (FWHM) of the peak at the 2θ of 23° using the Scherrer's formula as follows[36-38]:

$$\tau = K\lambda / \beta \cos\theta$$

where K is a dimensionless shape factor, which is 0.94; λ is the wavelength of X-ray, which is 1.5406 Å for Cukα1; β is a dimensionless shape factor, which is the FWHM of the relevant peaks; θ is the Bragg's angle.

The calculated result showed that the average size of the obtained carbon particles was about 2.5 nm, indicative of a structure of a cluster. To clearly investigate the real microstructure of the obtained carbon particles, an examination on a transmission electron microscope (TEM) was conducted. Figure 1B shows the low magnification TEM image of the products, revealing that they consisted of nanodots separated from each other, which is further evidenced by the high-resolution transmission electron microscope (HRTEM) images of the carbon dots, as shown in Figure 1C，indicating that these nanodots were in a size range of 2 to 5 nm. The calculated average crystal size was around 2.8 nm, which was in good agreement with the result of XRD analysis. From Figure 1D, it is more intuitive to be observed that the carbon particles were composed of carbon dots with weak crystallinity, presenting the same characteristic with that the XRD pattern showed. It can be seen that most of the carbon atoms were arranged in a close-packed hexagonal structure. A measured lattice spacing was about 0.25nm, corresponding to the (100) planes of graphite, displaying that the obtained carbon might be a kind of graphite. The SAED pattern presented a diffraction ring structure which was composed of a broad ring. These TEM results were consistent with the XRD results.

To investigate the surface functional groups of the carbon dots, FTIR analysis was performed. The result was shown in Figure 1F. The broad absorption bands at 3100–3500 $cm^{-1}$ were assigned to ν(O-H) and n(N-H). These functional groups could improve the hydrophilicity and stability of the carbon dots in aqueous system. The absorption bands at 1650–1740 $cm^{-1}$ were related to ν(C=O), and the absorption band at about 1300 $cm^{-1}$ corresponded to ν(C–O), indicating the presence of carboxylic acid and other oxygen-containing functional groups39. Furthermore, The absorption bands from 1640–1550 $cm^{-1}$ were attributed to δ(N–H), suggesting the existence of amino-containing functional groups40. Moreover, the weak vibration absorption bands at 2850–2710 $cm^{-1}$ corresponded to ν(C–H). The absorption band at about 1400 $cm^{-1}$ was associated with δ(O–H). Compared with the IR result of folic acid, it can be seen that the obtained carbon dot powder still contained amino, hydroxyl and carboxyl groups, indicating that these functional groups on the precursors might be the main source of functional groups of carbon dots or surface state of carbon dots.
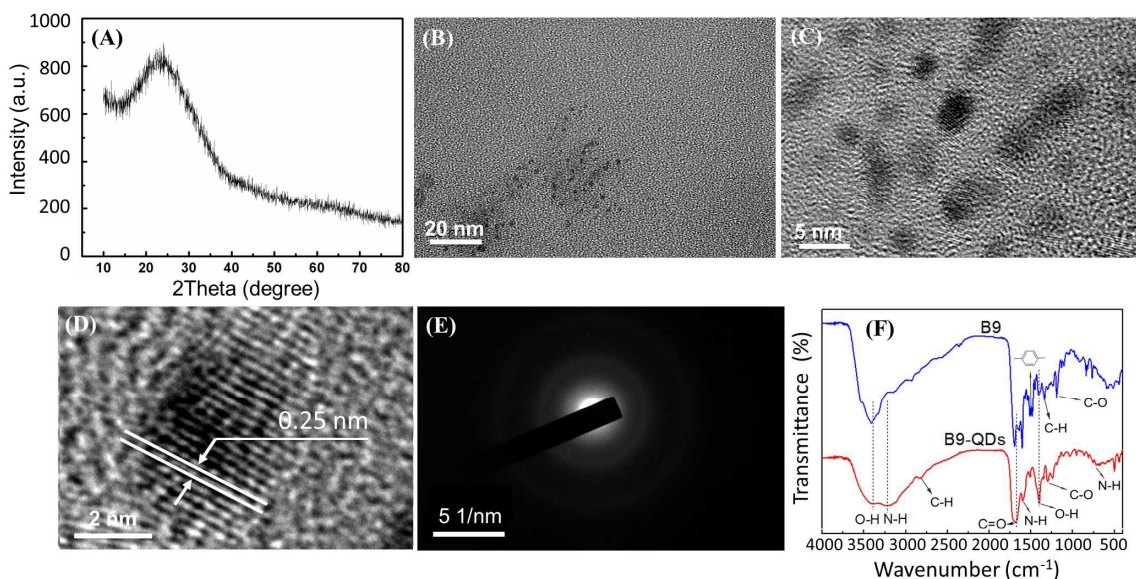
Fig.1 (A) XRD pattern of carbon dots, (B) Low- and (C) high resolution TEM images of the products thus formed and (D) an individual carbon dot at higher magnification, (E) SAED patterns of the CDs, (F) FTIR spectra of the precursor B9 and the carbon dots.
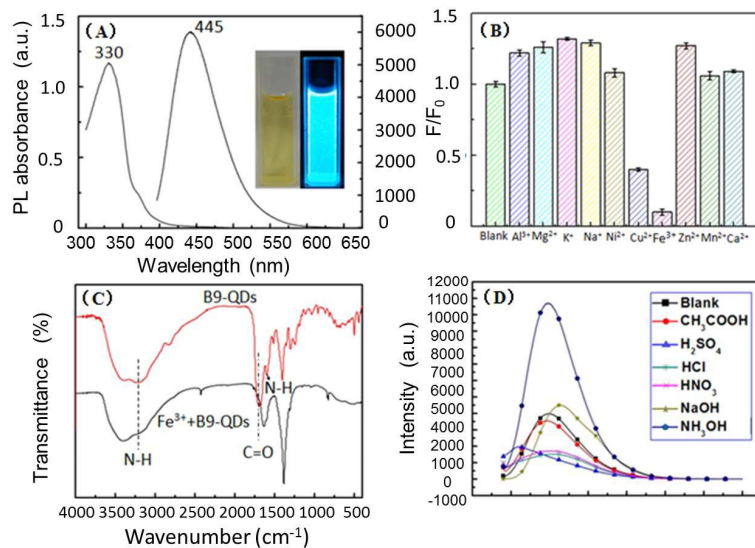


Fig. 2 (A) UV absorption and PL emission spectra (ex = 380 nm) of carbon dots, inset: optical images under daylight (left) and UV light (right) (B) The difference in PL intensity of carbon dots dispersion between the blank and solutions containing different metal ions (excitation at 380 nm; F0 and F are photoluminescence intensities of carbon dots at 445 nm in the absence and presence of ions, respectively) (C) FTIR spectra of the carbon dots before and after treatment with $Fe^{3+}$ (D) PL intensity of carbon dot solutions containing different Acid and alkali.

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### The photoluminescent properties of carbon dots and the effect of various ions on their photoluminescence

Figure 2A shows the UV-vis absorption and photoluminescent (PL) emission spectra of the aqueous dispersion liquid of the carbon dots. It can be seen that the UV-vis spectrum presented a strong peak at 330 nm, which was assigned to the K absorption bands caused by $\pi \rightarrow \pi^*$ transition, revealing that there might be benzene rings in a carbon dot. It can be noted that the dispersion liquid showed a very strong PL emission peak with an intensity of 6000 at 445 nm when it was excited at 380 nm, indicative of a very good photoluminescent property of the obtained carbon dots in an aqueous system. The dispersion liquid under a UV light (365 nm) also exhibited blue colour (inset).

We examined the PL intensity in the presence of representative metal ions, including $Ag^+$, $Zn^{2+}$, $Ca^{2+}$, $Al^{3+}$, $K^+$, $Fe^{3+}$, $Na^+$, $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$ and $Cu^{2+}$, as shown in Figure 2B. It can be seen that most metal ions could lead to a stronger PL peak at 445 nm compared with the blank. However, the presence of $Cu^{2+}$ ions and $Fe^{2+}$ ions caused an obvious decrease of photoluminescence in intensity, indicating that $Cu^{2+}$ ions and $Fe^{2+}$ ions could effectively quench the photoluminescence of carbon dots. These results would be beneficial for the explanation of photoluminescence mechanism of the obtained carbon dots. For that, we further analyzed the FTIR changes in the presence of $Fe^{2+}$ ions, as shown in Figure 2C, and found that the broad absorption band at 3210 cm$^{-1}$ which was assigned to $\nu$(N-H) and absorption band at 1590 cm$^{-1}$ which was related to $\delta$(N–H) disappeared, and that the absorption bands at 1630–1700 cm$^{-1}$ associated with $\nu$(C=O) became weak and were accompanied with a certain offset. In addition, all the absorption bands at 1400–500 cm$^{-1}$ also disappeared. A possible explanation is that $Cu^{2+}$ ions and $Fe^{2+}$ ions reacted with amino and carboxyl groups and formed coordination bond, and that as a result, the photoluminescence of carbon dots was quenched via electron or energy transfer from functional groups to $Cu^{2+}$ ions or $Fe^{2+}$ ions[41-43]. Therefore, from these results, it can be inferred that functional groups such as amino and carboxyl groups play a crucial role on emitting the photoluminescence of carbon dots. However, the enhanced effect of other metal ions are probably due to that metal ions formed some new surface states on the surface of the carbon dots and then formed new energy levels, thus leading to more radiative recombination between holes and electrons. Consequently, the luminous intensity for carbon dots was enhanced.

We also examined the change of PL intensity in the

presence of representative acid and alkali, including $CH_3COOH$, $H_2SO_4$, HCl, $HNO_3$, NaOH, $NH_4OH$, as shown in Figure 2D. It can be seen that the presence of $CH_3COOH$ led to a slight decrease of photoluminescence in intensity. It might be due to the increase of non-radiative recombination on the surface of carbon dots induced by carboxylate radical[40,44]. It can also be seen that the presence of $H_2SO_4$, HCl and $HNO_3$ caused an obvious decrease of photoluminescence in intensity, indicating that some inorganic acid can effectively quench the photoluminescence of carbon dots. In contrast, some increase was observed by addition of alkali into the carbon dots dispersion. The most obvious increase was detected when $NH_4OH$ was added into the carbon dots dispersion. According to the literatures[40,44,45], the presence of hydroxyl radical and ammonia radical has a promoting effect on luminescence. Ammonium radical ions didn't change the level of energy of surface state, which dominated in the photoluminescence emission of carbon dots. In order to realize it, a possible approach is to modify the surface state on the carbon dots by introducing new or more functional groups with a low level of energy onto the surface of carbon dots. For that, we adopted phosphoric acid and oxalic acid to modify the surface state on the carbon dots. The results showed that the carbon dots modified with phosphoric acid and oxalic acid exhibited green photoluminescence and yellow-green photoluminescence under UV light (365 nm), as shown in Figure 3A. Strong PL peaks at 515 nm and 550 nm were observed, respectively, for the carbon dots modified with phosphoric acid and oxalic acid. Compared with the untreated carbon dots with a PL peaks at 445 nm, there was a red shift of 70 nm or 105 nm for the treated carbon dots, respectively.

To analyze the change of surface state and band-gap energy of the carbon dots, a further IR examination and the diffuse reflectance were conducted on the carbon dots before and after modification. Figure 3B shows the FTIR spectra of the products modified without and with oxalic acid. For the products with green photoluminescence, the absorption band at 3100–3500 cm$^{-1}$ assigned to $\nu$(O-H) and $\nu$(N-H) become broader. These functional groups would play an important role in improving the hydrophilicity and stability of the carbon dots in aqueous system. The absorption band at 2369 cm$^{-1}$ was assigned to the antisymmetric stretching vibration of $CO_2$. It might be caused by the absorbed carbon dioxide during the preparation of samples. The absorption band at 1650–1740 cm$^{-1}$ assigned to $\nu$(C=O) had a certain offset compared with carbon dots with blue photoluminescence and meanwhile, a strong absorption band appeared at 1000–1125cm$^{-1}$ which
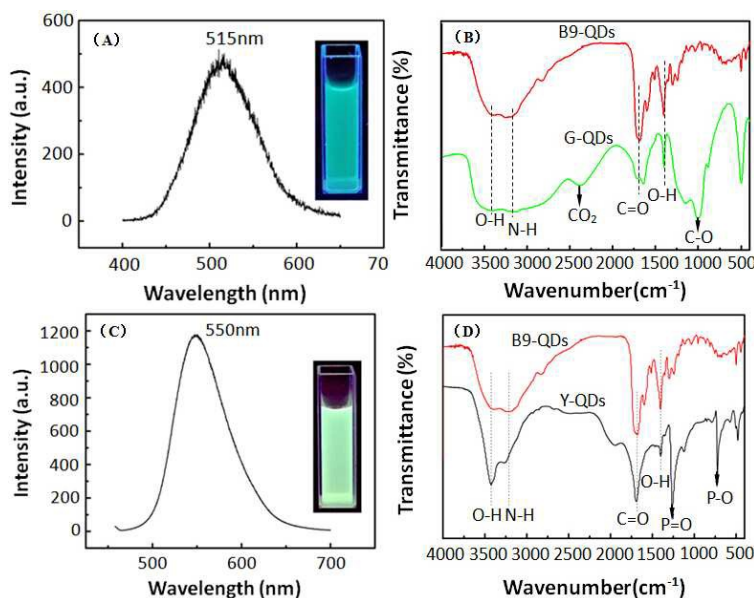
Fig.3 (A) PL emission spectrum (ex = 385 nm) of carbon dots with green photoluminescence (G-QDs), inset: optical image under UV light. (B) FTIR spectra of the carbon dots modified (B9-QDs) without and (G-QDs) with oxalic acid. (C) PL emission spectra (ex = 400 nm) of carbon dots with yellow-green photoluminescence, inset: optical image under UV light. (D) FTIR spectra of the carbon dots modified (B9-QDs) without and (Y-QDs) with phosphoric acid.

was assigned to $\nu(C-O)$, indicative of the formation of more carboxyl groups, which might be the reason for the red-shift of photoluminescence emission.

Figure 3D shows the FTIR spectra of the products modified without and with phosphoric acid. For the products with yellow-green photoluminescence, it can be seen that the absorption bands at 3300–3500 $cm^{-1}$ assigned to $\nu(O-H)$ turned sharper and stronger. However, two new absorption bands at 1300 $cm^{-1}$ and 738 $cm^{-1}$, which were assigned to $\nu$ (P=O) and $\nu(P-O)$[46], respectively, were observed, indicative of the presence of phosphate radical in carbon dots, which led to the further red-shift of photoluminescence emission. This can be explained that phosphoric acid is a strong acid and it can react with amino group of folic acid via a salt or hydrogen bond way to form a composite.

Additionally, measurement of diffuse reflectance with a UV-visible spectrophotometer has been used as a standard technique to determine the light absorption properties of materials whilst the diffuse reflectance has also been used a standard technique based on Kubelka-Munk remission function to estimate the band-gap energy[47]. The results show that the band-gap energy of carbon quantum dots before and after treatment with oxalic acid was 2.35 eV and 1.75 eV, respectively, as shown in Figure 4. Obviously, the use of oxalic acid led to the reduction of the band-gap energy. One possible explanation is that in the

presence of oxalic acid more surface states such as carboxyl groups were introduced onto the surface of carbon dots, thus leading to the decrease of band-gap energy. A more detailed discussion will be described in the later section. Because the products modified
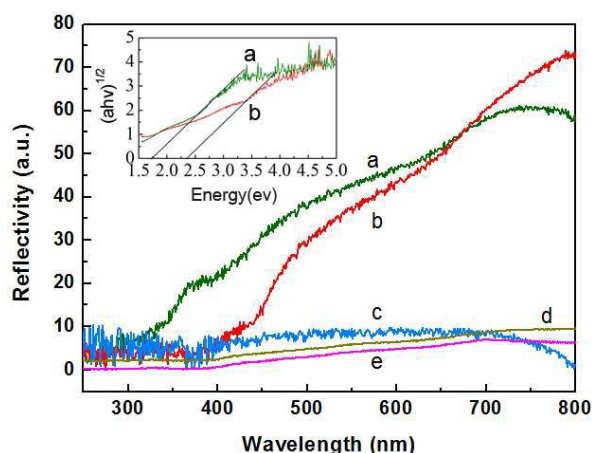


Fig.4 Diffuse reflection spectrum of carbon dots (a) without modification, modified with (b) oxalic acid,(c) phosphoric acid, (d) $Cu^{2+}$ ions and (e) $Fe^{2+}$ ions. The band-gap energy of oxalic acid-modified carbon dots and pure folic acid carbon dots is 1.75eV and is 2.35eV, respectively.

with phosphoric acid were black, reflectivity was very low. This method is not suitable for the black samples. It can be seen that the obtained samples using metal ions to modify had low reflectivity.

**The formation mechanism of carbon dots and their photoluminescence mechanism and their further evidences**

The formation of carbon dots can be explained as follows: due to the presence of the benzene ring structure of folic acid, carbon core structure can be formed via π-π stacking self-assembly mechanism while some amino and carboxyl groups in folic acid can be remained after the formation of carbon cores, thus leading to the formation of surface states on the surface of the carbon dots as shown in Figure 5.

It is worth noting that the precursor vitamin B9 was non-emissive under UV light, therefore, the luminescence should arise from the formed carbon dots after hydrothermal processing. As has been seen, the photoluminescence intensity of carbon dots could be changed with adding various ions, suggesting that the PL emission of carbon dots might be mainly due to the surface state rather than the carbon dot cores. Different functional groups have different energy levels, thus forming different emissive traps. Hsu et al. [40] found that compounds containing amino and carboxyl groups are beneficial for synthesizing carbon dots with high PL quantum yield. The presence of amino can enhance the luminous intensity of carbon dots. Liu et al.[44] found that the hydroxyl has a promoting effect on luminescence. Their results are in good agreement with our present ones. In addition, our experimental results also reveal that a high degree of surface oxidation can create more surface defects with a lower level of energy, which can lead to the red-shifted emission. These photoluminescence properties allow these dots typically valuable for in vitro and in vivo bioimaging.

Although there are still many debates about the origin of the photoluminescence of carbon dots, a widely accepted mechanism for luminescence emission is that the emission arises from both the intrinsic bandgap resulting from confined sp[2] conjugation in the core of carbon dots and the surface state of carbon dots, which is lower than the π* state but higher than the π state and can be excited or indirectly excited by energy transfer from intrinsic band, as shown in Figure 6. The energy level of functional groups might be associated with their ability to donor electrons. The stronger ability to donor electrons the functional groups have, the higher energy level they create. The ability of functional groups to donor electrons increases in the order phosphate radical < carboxyl group < hydroxyl group < amino group. Clearly, the regulation of photoluminescence emission

wavelength can be achieved by the modification of the chemical groups on the surface of carbon dots. Before this, Zhu et al. also found that modification and redox both can tune surface chemistry groups of carbon dots and tailor the photoluminescence colour from green to blue or from blue to green[45]. For example, the green PL can be translated to blue one when -COOH changes into -OH during chemical reduction [48,49]. In this present study, we employed phosphoric acid and oxalic acid to modify the surface state of carbon dots and successfully introduced more carboxyl groups and phosphate radical onto the surface of carbon dots and realized the red-shift of photoluminescence emission of carbon dots.
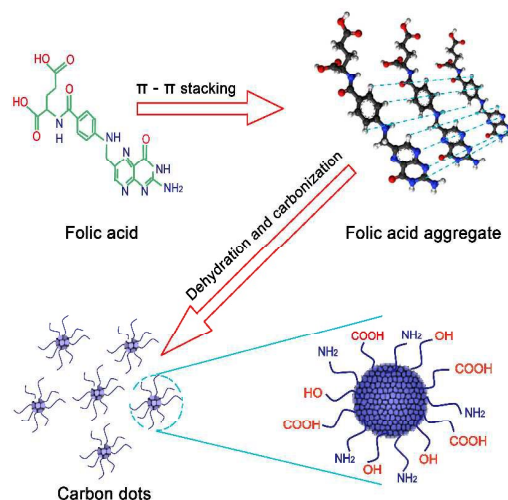


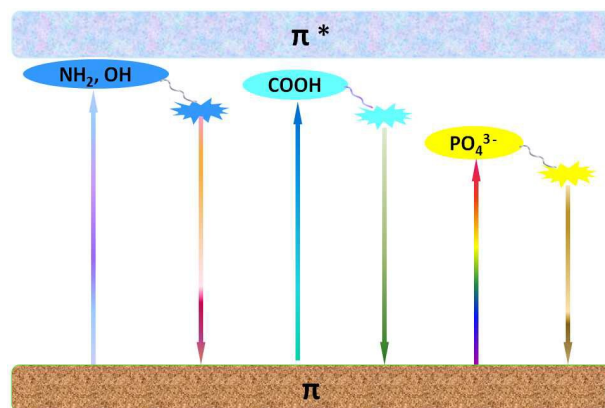Fig.5 Schematic diagram for the formation of carbon dots



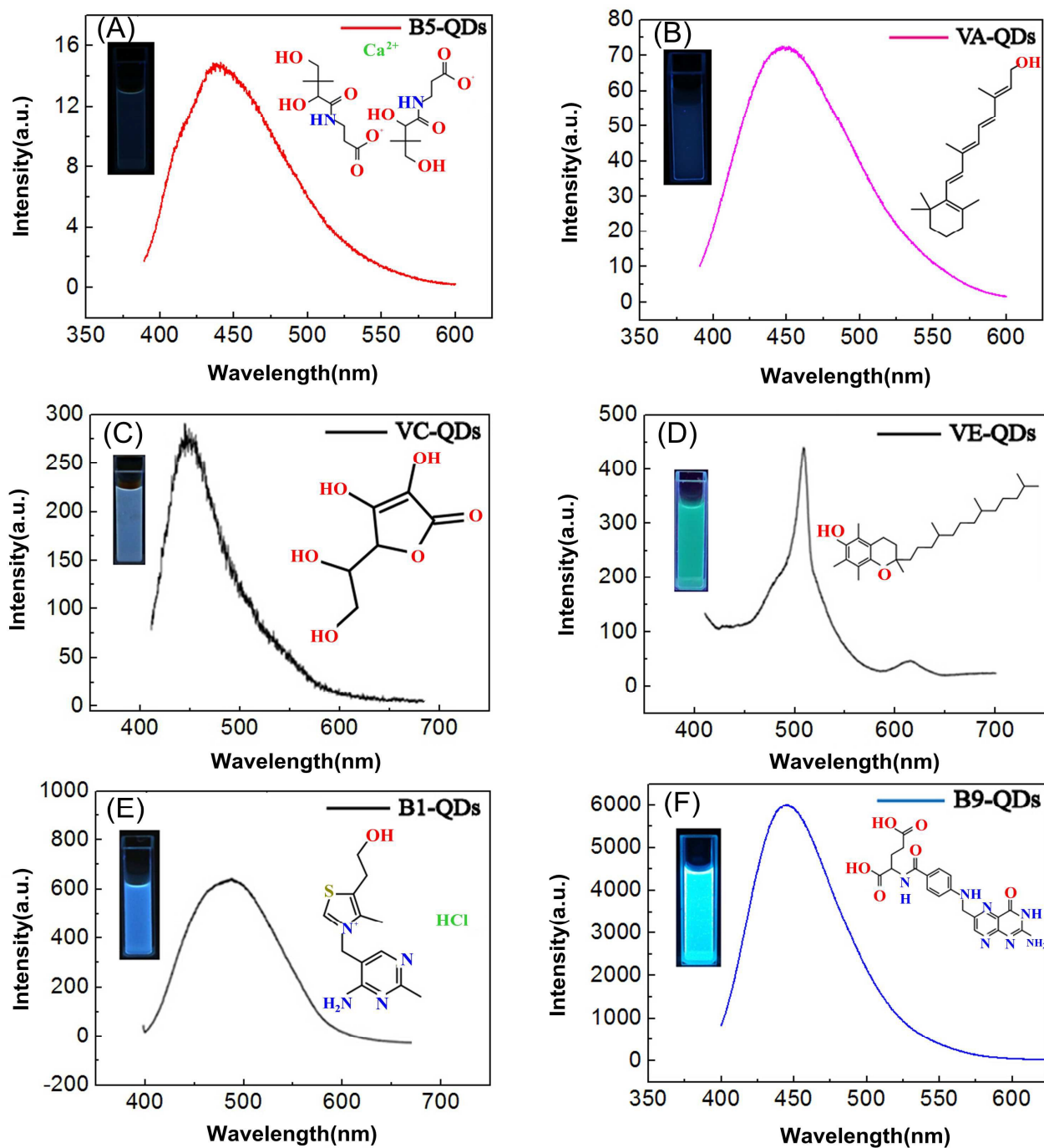Fig. 6 Proposed PL emission mechanisms.

Fig. 7 The chemical structures of the precursors used to synthesize carbon dots and the PL emission spectra of their carbon dots and their optical images under UV light: (A) vitamin B5, (B) vitamin A, (C) vitamin C, (D) vitamin E, (E) vitamin B1, (F) vitamin B9
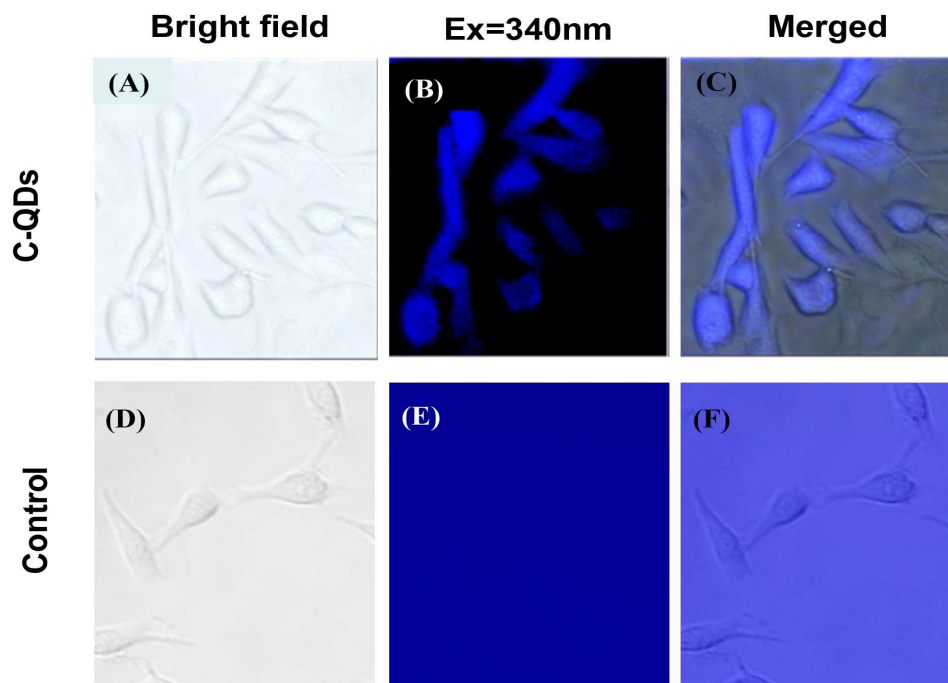
Fig. 8 (A) Bright field and (B) photoluminescence images of SCC-7 cells incubated in the presence of carbon dots for 6 h obtained through a band-pass filter of 420 nm under irradiation of UV light (340 nm), (C) the merged image of (A) and (B) ; (D) Bright field and (E) photoluminescence images of SCC-7 cells incubated in the absence of carbon dots for 6 h obtained through a band-pass filter of 420 nm under irradiation of UV light (340 nm), (F) the merged image of (D) and (E). The objective used was a 50 * 0.75 NA (300X magnification). The exposure time is 150 ms.

In order to get deep insight into our proposed formation mechanism of carbon dots for this present study and to provide further direct evidences, some other vitamins with or without benzene ring structure would also be studied to investigate the role of benzene ring structure in the formation of nano carbon dots during hydrothermal synthesis; and additionally, these vitamins with benzene ring structure but with different radical groups were used to investigate the effect of radical groups of precursors on the surface state and luminescence emission so as to further explore the formation mechanisms of the surface state and luminescence emission. Figure 7 shows the chemical structures of the precursors used to synthesize carbon dots and the PL emission spectra of their carbon dots and their optical images under UV light. It can be clearly seen that vitamin B5 without benzene ring structure, although it has rich radical groups, didn't present any photoluminescence property after hydrothermal processing whilst all the vitamins A, C, E, B1 and B9 with benzene ring structures presented photoluminescence property after hydrothermal processing, although their intensity was different. There is no doubt that this result has provided a vital evidence and strong support for our proposed formation mechanism of carbon dots. Additionally, it can also be seen that the precursors that contain phenylbenzene and rich radical groups are more beneficial for the formation of photoluminescent quantum dots compared with those that don't contain phenylbenzene and have poor radical groups. These results might offer us important clues for the selection of precursors when using small organic molecules to synthesize carbon dots with high luminescence.

### Cellular imaging

We chose SCC-7 cells as the labeled cells and cultivated them in the presence of the as-prepared carbon dots. As depicted in Figure 8, strong blue photoluminescence of the SCC-7 cells could be seen after incubation with carbon dots for 6 h, indicating good cellar imaging properties. It is worth noting that

there is no auto photoluminescence emitting from the cells (Figure 8(E)). Only after carbon dots being internalized into the cells, a large number of luminescent spots started to appear in the SCC-7 cells. Most of the available cellar imaging studies of carbon dots generally suggests a possible endocytosis mechanism to account for the inhomogeneous distribution of carbon dots inside the cell. In addition, after 24h, there were more than 90% of the SCC-7 cells remaining alive, indicating carbon dots' good biocompatibility.

## Conclusions

The carbon dots with high luminescence were successfully synthesized by using folic acid as precursor and the quantum yield of the obtained carbon dots was about 31.59%. The luminescence of the obtained carbon dots could be quenched by $Cu^{2+}$ ions and $Fe^{2+}$ ions and inorganic acid but enhanced by other metal ions and alkali. The results showed that the luminescence emission of carbon dots was mainly due to the surface state. The green and yellow-green photoluminescence was successfully achieved by adding the reagents ($H_3PO_4$ or $H_2C_2O_4$). The carbon dots presented good property in cell imaging. The present method to synthesize carbon dots with high photoluminescence by using small organic molecules as precursors was proved to be very effective, which opens a new avenue for the synthesis of carbon dots with high luminescence and might offer us one of some clues for the selection of precursors when using small organic molecules to synthesize carbon dots with high luminescence.

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## Acknowledgements

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