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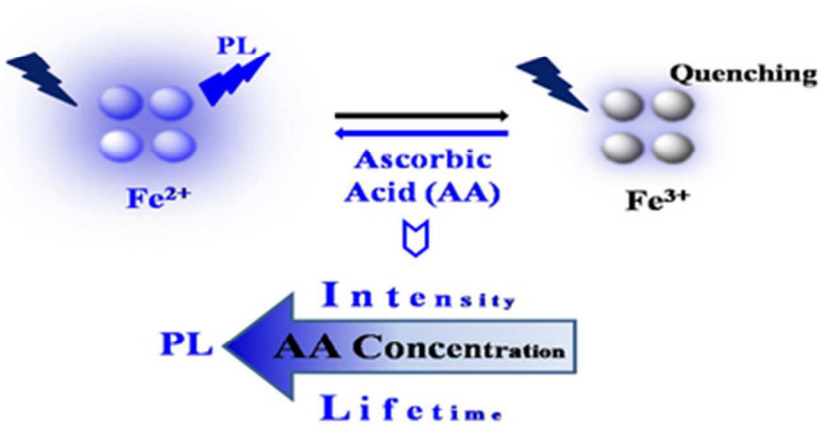

A fluorescent C-dots/Fe3+ nanosensor with off-on switch was established for ascorbic acid detection
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ARTICLE TYPE

Carbon dots as fluorescent off-on nanosensors for ascorbic acid detection

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An "off-on" approach for detection of ascorbic acid using carbon dots as fluorescent probe was presented, which based on the fluorescence recovery of the quenched C-dots/Fe^{3+} complex when ascorbic acid was introduced. And this sensor was employed to determine the encapsulation efficiency of ascorbic acid in a liposome formulation.

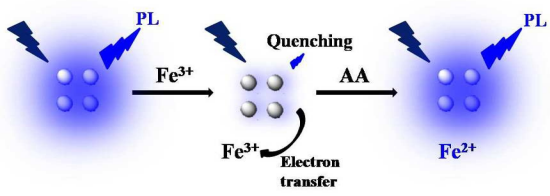
Ascorbic acid (AA, also known as vitamin C), is a strong antioxidant and a cofactor in at least eight essential enzymatic reactions. Because AA can efficiently scavenge toxic free radicals and other reactive oxygen species (ROS) that are associated with several forms of tissue damage, disease and the process of aging formed in cell metabolism, it has been used for the prevention and treatment of scurvy, cardiovascular disease, cancer, age-related macular degeneration, gout, heavy metal toxicity, diabetes and AIDS[1]. Additionally, AA is also a vital vitamin in the diet of human. On the other hand, AA is sensitive to various environmental factors such as temperature, oxygen concentration, metallic ions and UV exposure. This vulnerable feature makes AA suffering from low efficient absorbing or utilizing by user in food products that are fortified with AA in order to supply the recommended daily allowance. In order to reduce degradation and improve the absorbance of AA, some nanocarriers are used to protect AA from unwanted interactions, such as microemulsion[2], micelle[3] and liposome[4, 5]. Liposomes are spherical vesicles made of phosphatidylcholine-enriched phospholipids for cosmetic/drug active materials delivery, and they have been used to encapsulate AA in food industry. To evaluate the concentration of AA in liposome, an assay is needed to determine the entrapment efficiency of AA in the liposome.

In the past ten years, various approaches have been developed for the quantitative determination of AA, such as titration[6], colorimetry[7], electrochemical method[8] and fluorescence assay[9-12]. Among the above methods, the strategy based on changes of fluorescence intensity or anisotropy[13] possesses the advantages of operational simplicity, high sensitivity and real-time detection. In the fluorescent assay, fluorescent nanomaterials such as semiconductor quantum dots[10], novel metal nanoparticles[12] and rare earth doped luminescent nanomaterials[11] have been employed for AA detection. However, these assays suffer from the usage of hazardous materials[6, 9, 10] or precious materials,[12] complicated process[7], complex modified electrode[8] and limited sensor range[9, 11, 12]. So better methods for detection of AA are still highly need.

As a new-member of the nanomaterials family, fluorescent carbon dots (C-dots) are small carbon nanoparticles with sizes below 10 nm and have many intriguing merits[14, 15]. As an alternative to semiconductor quantum dots, C-dots have shown versatile applications in bio-imaging, sensors, photo-catalysts as well as optronics based on their exciting fluorescent, nontoxic, biocompatible and electronic properties[16, 17]. The functionalized C-dots have great potential in analytical applications and have been used to sense pH, metal ions and molecules[18-20]. Among C-dots based sensor, "off-on" sensor has appeared as a novel type of sensor with flexible functional platform[21]. In this sensing system, the PL signal of the carbon dots is initially quenched by one substance and then recovered by the analytes. An "off-on" fluorescent sensor has been reported based on C-dots/Cr(VI) system for AA determination, but which suffered from hazardous Cr(VI) as well as limited sensor range.[9] Therefore, it is still highly desired to explore better off-on sensor for AA determination.

In this communication, a novel fluorescent nanosensor based on C-dots for AA detection with the merit of wide detection range, low detection limit and high selectivity was developed. In this sensing method, the PL of C-dots sensor that was quenched by Fe^{3+} was recovered after the adding of AA, as shown in Scheme 1. The detection range, limit of detection and the selectively of this off-on sensor were studied and the PL recovery mechanism was explored. This off-on method was employed to determine the encapsulation efficiency of AA in a liposome formulation.

C-dots were prepared by M-H method using AA as carbon source[22]. The detail of synthetic process was showed in ESI. The morphology analysis, XRD and FTIR spectrum of C-dots were presented in Fig. S1. The diameters of C-dots obtained from TEM and dynamic light scattering (DLS) were about 3 nm and 6 nm (Fig. S1(b) and (d)), respectively. The hydrodynamic diameter of



Scheme 1 Schematic illustration of AA sensor based on the off-on fluorescent probe of C-dots adjusted by Fe^{3+}

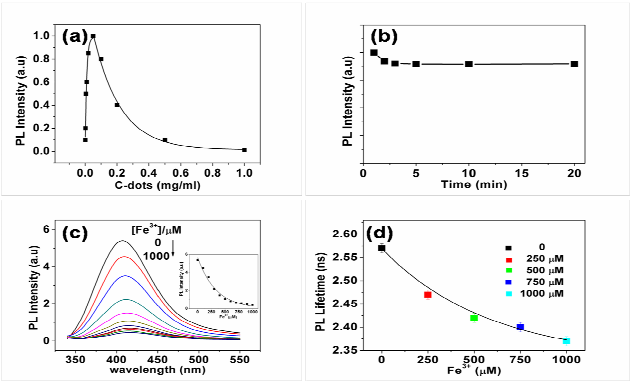


Fig. 1 (a) Concentration dependence of the PL behaviour of C-dots in aqueous solution. (b) Kinetic behaviour of the PL intensity of C-dots/Fe^{3+} system. (c) PL spectra of C-dots aqueous solution with different concentration of Fe^{3+}, inset is the PL intensity of C-dots aqueous solution against the concentration of Fe^{3+}. (d) PL lifetime values of C-dots with different concentration of Fe^{3+}. The excitation and emission wavelength was 330nm and 410nm, respectively in (a)-(d), the concentration of C-dots was 0.05mg/ml in (b)-(d), the concentration of Fe^{3+} was 1mM in (b).

6 nm (DLS) was larger than the diameter of 3 nm (TEM) because the hydrodynamic diameter includes all solvent molecules attracted to the surface of C-dots. It can be found that the non-crystalline hydrophilic carbon dots are spherical and well dispersed. The C-dots showed highly luminescent fluorescent and excellent optical stability, and the intensity was independent of pH value in aqueous solution as shown in Fig. S2. The AA detection can proceed in aqueous solution without buffer. The superior fluorescent properties of C-dots favour their sensor applications.

In the "off" step of the "off-on" process, ferric chloride ($FeCl_3$) aqueous solution was employed to quench the PL intensity of C-dots. In order to optimize the concentration of C-dots for AA detection, the concentration dependent PL behaviour of C-dots in aqueous solution was researched. As shown in Fig. 1(a), the PL intensity of C-dots increased with the concentration when it below 0.05mg/ml, but it decreased with the concentration when it greater than 0.05mg/ml. The decrease of PL intensity at high concentration was ascribed to self-absorption quenching[23]. As a result, the concentration of C-dots used in the following experiments was chosen as 0.05 mg/ml. The effect of incubation time on the PL intensity of C-dots/Fe^{3+} was investigated to assure the accuracy of this analytical procedure (Fig. 1(b)). C-dots (0.05mg/ml) and Fe^{3+} solution (1 mM) was mixed and the PL intensity of the mixture was stable within 3 min. Therefore, the optimal incubation time was confirmed as 3 min. The effect of Fe^{3+} on the PL spectra of C-dots was studied to optimize the concentration of Fe^{3+} for further AA detection. The PL intensity of C-dots decreased sharply with increase of concentration of Fe^{3+} when the concentration of Fe^{3+} below 0.6 mM, and the decrease slope was tended to flat when the concentration of Fe^{3+} approaching to 1mM, which indicated a quenching saturation at 1 mM. So the optimal Fe^{3+} concentration was chosen as 1 mM for quenching PL of C-dots. This fluorescent quenching may be attributed to non-radiative electron transfer from the excited state of the C-dots to the *d* orbital of Fe^{3+} ions[20].

Time correlated single photon counting (TCSPC) was used to reveal PL quenching mechanism of C-dots in the "off-on" process. PL lifetimes of C-dots with different concentration of Fe^{3+} were shown in Fig. 1 (d). The fluorescent lifetime of C-dots was 2.57 ns by fitting with mono-exponential function, which reflects a fast electron-hole recombination. Additionally, the lifetime of C-dots aqueous solution was decreased from 2.57 ns to 2.38 ns with the enhancement of Fe^{3+} concentration from 0 to 1mM. This phenomenon supported the dynamic quenching mechanism because the decrease of PL lifetime with the addition of quencher is a unique phenomenon for dynamic quenching process[24].

In the "on" step of the "off-on" process, AA was added to the PL quenched C-dots/Fe^{3+} system, and the PL intensity was increased with the AA concentration as shown in Scheme 1. The PL intensity of C-dots/Ferric ions with 1 mM AA was studied to obtain optimal incubation time for AA detection (Fig. 2(a)). It was found that a stable PL intensity signal was obtained within a reaction time of 2 min. So the incubation time of 2 min is enough for the PL intensity measurement. The quenched PL of C-dots/Fe^{3+} was recovered gradually with the increase of AA concentration (Fig. 2(b)). In this process, Fe^{3+} was reduced by AA into Fe^{2+} that had no quench effect on the PL of C-dots and the PL intensity of C-dots/ferric ions recovered.[25]. As indicated in Fig 2(c), some sigmoidal fitting is suitable for the range of 0-1000μM. And a good linear relationship of PL intensity and the concentration of AA from 0.2 to 284 μM was obtained, with a linear equation of $I = 3.414 + 0.02024\,[AA]$ (μM) ($R^2 = 0.993$) and a limit of detection (LOD) of 0.05 μM.

TCSPC experiment of C-dots/Ferric ions with different concentrations of AA was used to further explore the mechanism of the PL intensity recovery. When the concentration of AA increased from 0 to 284 μM, the lifetime of C-dots/ferric ions complex increased from 2.38 ns to 2.42 ns as shown in Fig. 2(d). The increase of PL lifetime indicated that dynamic quenching process between C-dots and Fe^{3+} was decreased by the addition of AA because the dynamic quenching process decreased the PL lifetime.[23] So the PL intensity recovery could be ascribed to the suppression of dynamic quenching process that origin from the reduction of Fe^{3+}. Additionally, the changes of PL lifetime with

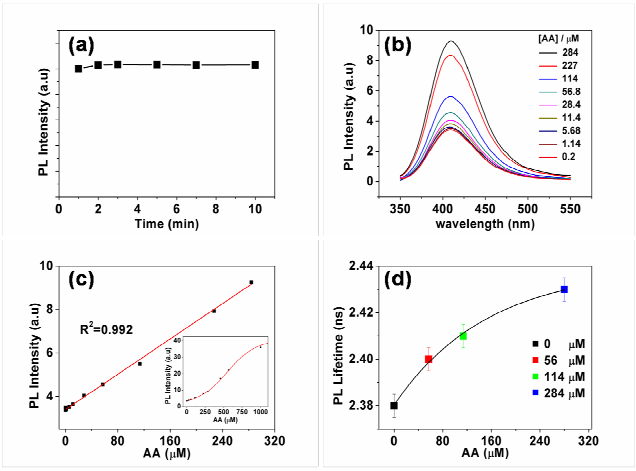


Fig. 2 (a) The kinetic behaviour of the PL intensity of C-dots/Fe^{3+} system with 1mM AA. (b) The PL spectra of C-dots/Fe^{3+} mixture with different concentration of AA. (c) The plot of the fluorescence intensity against the AA concentration within 0.2-280μM according to (b), inset is the whole vision of the relationship between PL intensity and AA from 0-1000μM. (d) PL lifetime values of C-dots/Fe^{3+} mixture with different concentration of AA. The excitation wavelength is 330nm in (a)-(d).

Table 1 Comparison of previously reported fluorescent methods with present method for AA detection

Sensor	Linear Range (μM)	LOD (μM)	Reference
C-dots/Cr(VI)	30-100	-	9
$CuInS_2$ quantum dots	0.25-200	0.05	10
LaF_3:Ce, Tb nanoparticle	8-100	2.4	11
Au nanoparticle	1.5-10	0.2	12
C-dots/Fe^{3+}	0.2-284	0.05	This work

Table 2 Effect of co-existing substances on the PL intensity of C-dots/Fe^{3+} with 0.1 mM AA

Coexisting Substances	Concentration / mM	$\Delta F^a/F_0$ (%)
Citric acid	5.0	+3.0
Glucose	5.0	+2.7
Glycine	5.0	+2.0
Lysine	5.0	+2.8
BSA	5.0	+2.0
Na^+	5.0	+1.4
K^+	5.0	-0.9
Mg^{2+}	5.0	-0.5
Ca^{2+}	5.0	-0.8

[a] $\Delta F=F-F_0$, where F and F_0 are the PL intensity of AA-C-dots/Fe^{3+} in the presence and absence of co-existing substance.

the addition of analyte provide a new idea for sensing based on PL lifetime changes if this change correlates linearly with the variation of analyte's concentration.

Table 1 showed the performance comparison between this method and other methods in the aspect of detection range and LOD for AA detection. The results show that C-dots/Fe^{3+} system for AA detection possess the widest linear range (0.2-284 μM) and almost lowest detection limit.

In order to investigated the selectivity of this C-dots/Fe^{3+} sensor for detection of AA, interference of usual physiological molecules and ions, such as citric acid (Cit), glucose (Glu), glycine (Gly), lysine (Lys), bovine serum albumin (BSA), Na^+, K^+, Mg^{2+}, Ca^{2+}, was studied under the optimum experimental conditions, and the results were shown in Table 2. The results reveal that most molecules/ions could be allowed to coexist at a concentration of >50 fold of AA under the relative error within 3.0% for AA detection. Therefore, those molecules/ions had little interference on this sensor for AA detection. In summary, this method had a high selectivity and it could be applied for AA detection in liposome.

To further explore the potential utility of this sensor, the C-dots/Fe^{3+} system was studied as a probe to detect AA in liposome. The AA encapsultated liposome was prepared by membrane evaporation method with the details presented in ESI. To measure the entrapment efficiency (EE) of AA in the liposome solution, the dissociative AA was separated by dialysis process and the dialysate was collected for AA determination (see ESI for detailed process). The fluorescent off-on nanosensor was used to determine the AA concentration in the dialysate solution. And then the EE% of AA in liposome was calculated using the following equation: EE %= $(1-C*V_{dialysate}/m_{AA})*100\%$. The C, V and m_{AA} in the equation represent the concentration of AA in dialysate, the volume of dialysate and the total mass of AA, respectively. To verify the accuracy of result obtained by this off-on assay, a traditional HPLC analysis assay was presented to determine the EE of AA as a control. The results of the two methods for EE detection of AA in the liposome were listed in Table S1 for comparison. The EE obtained by the traditional HPLC assay was in accordance with that obtained by off-on assay. Comparing with the HPLC analysis assay, the off-on fluorescence assay is more suitable for determining the EE of AA in the liposome because it possessed the advantage of wider linear detection range (0.2-284μM *vs*.5-70μM) and lower detection limit.

In conclusion, an "off-on" approach for detection of AA using C-dots as fluorescent probe was presented based on the AA's PL recovering ability of the quenched carbon dots/Fe^{3+}complex. The quenching and recovery mechanism were investigated. It found that the quenching of fluorescence by Fe^{3+} belongs to dynamic quenching and the fluorescence was recovered after adding of AA by reducing Fe^{3+} into Fe^{2+} that had no quench effect with C-dots. Besides wide detection range, low detection limit and high selectivity, this C-dots/Fe^{3+} sensor also has advantage of low cost and facile fabrication. So it could be concluded that this sensor is excellent for AA detection. This off-on sensor system was successfully used to determine the encapsulation efficiency of ascorbic acid in a liposome formulation.

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Notes and references

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† Electronic Supplementary Information (ESI) available ncluding details of synthetic of C-dots and sensening of AA. See DOI: 10.1039/b000000x/

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