Fluorescent sensing platform based on green luminescence carbon dots and AuNPs for clenbuterol detection in pork liver

Ying Guo[a*], Min Zheng[a], Wei Zhang[a], Daodao Hu[b]

[a] *College of Chemistry & Chemical Engineering, Xianyang Normal University, Xianyang712000, P. R. China.*

[b] *School of Materials Science and Engineering, Shaanxi Normal University, Xi'an 710062, P. R. China.*

** Corresponding author: Ying Guo*

Tel.: +86-(0)29-33720704

E. mail: guoying207@126.com

Materials

GSH, p-phenylenediamine, clenbuterol and rhodamine B were purchased from Aladdin Reagent Co., Ltd.. Chloroauric acid and trisodium citrate were obtained from Sinopharm Reagent Co., Ltd.. All chemicals were analytical reagent grade and used without further purification. Ultrapure water was used in all experiments.

Apparatus and characterizations of G-CDs

Transmission electron microscopy (TEM) images of the G-CDs and AuNPs were taken on JEM-2100 (Japan) at 200 kV accelerating voltage. A FL-7000 spectrofluorometer (Hitachi, Japan) was used to obtained fluorescence spectra and emission lifetimes of G-CDs. All absorption spectra were obtained using a Lambda 35 UV-vis spectrophotometer (Perkin-Elmer, Japan). Fourier transform infrared spectra (FTIR) analysis of the prepared G-CDs was performed using on a FT-IR spectrophotometer (Perkin-Elmer, Japan).

Synthesis of G-CDs

The G-CDs was synthesized via hydrothermal method using p-phenylenediamine and GSH as precursor. In brief, 0.2600 g of GSH and 0.0173 g of p-phenylenediamine were added into 30 mL of ultrapure water and stirred to completely dissolve. The obtained solution was transferred to a polytetrafluoroethylene autoclave, heated at a constant

temperature of 160 °C in an oven for 11 h, and cooled naturally to room temperature after the reaction was completed. The obtained solution was centrifuged at a high speed of 16000 rpm for 15min and then filtered through a 0.22 μm membrane filter. Finally, dichloromethane was added to the filtrate, and the upper yellow aqueous solution was collected for dialysis and freeze-drying to obtain G-CDs powder.

Preparation of AuNPs

AuNPs were synthesized in the reference to the classic Frens method.[31] Glassware ware was immersed with the freshly prepared aqua regia for 24 h, then thoroughly washed with ultrapure water, and dried for later use. During preparation, 100 mL of 1 mM $HAuCl_4$ solution was heated under stirring to boiling, 10 mL of 38.8 mM sodium citrate solution was then quickly added, and the mixture was stirred vigorously. The color of the solution changed from light yellow to light gray and finally to wine red. Heating was terminated when the reaction lasted for 30 min, and stirring was continued for 10 min. After cooling to room temperature, the solution was filtered with a 0.45 μm microporous membrane and stored at 4°C in the dark.

Detection of CLB

In brief, 100 μL of AuNPs and CLB stock solutions at different

concentrations were added into a 1.5 mL centrifuge tube and shaken thoroughly. After incubation for 10 min at room temperature, 30 μL of G-CDs dispersion solution and 200 μL of pH 6.0 B-R buffer solution were added. The final volume of the solution is 500 μL. the fluorescence intensity of the system at 522 nm was recorded by a fluorescence spectrophotometer with the excitation wavelength of 410 nm.

Real Sample Pretreatment

Briefly, 5 g of mashed fresh pork liver sample was transferred into a 50 mL centrifuge tube, added with 10 mL of 10% sodium carbonate solution, and mixed for 2 min by vortex. The protein was precipitated in a water bath at 80 °C. After cooling, 15 mL of ethyl acetate was added for extraction for 10 min, and the mixture was centrifuged for 10 min (8000 rpm). The residue was extracted with 15 mL of ethyl acetate. The extracts were mixed, added with 5 mL HCl (0.1 mol/L), sonicated for 15 min, and centrifuged at 8000 rpm/g for 10 min. The lower layer of water was collected. These extraction steps were repeated twice with HCl to obtain a pork liver sample solution.

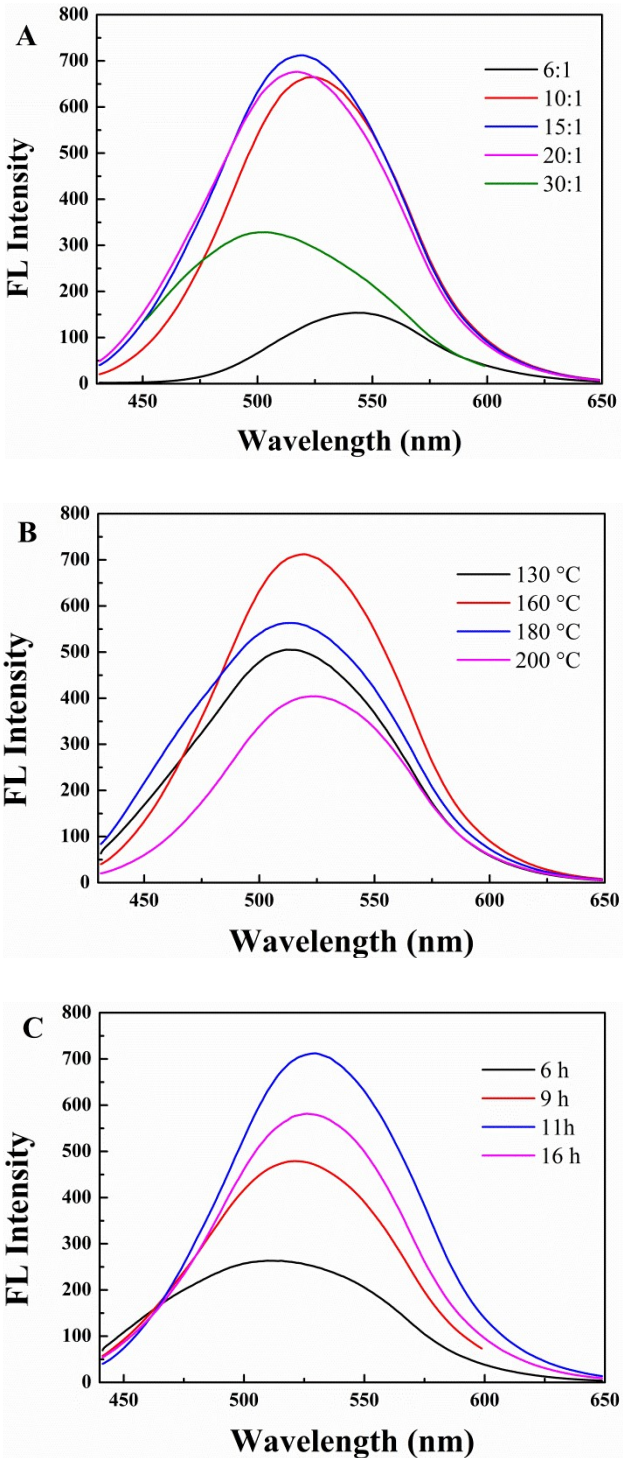

Fig. S1. Fluorescence emission spectra of the G-CDs at different starting material mass ratio (A), different temperature (B), different reaction time (C).

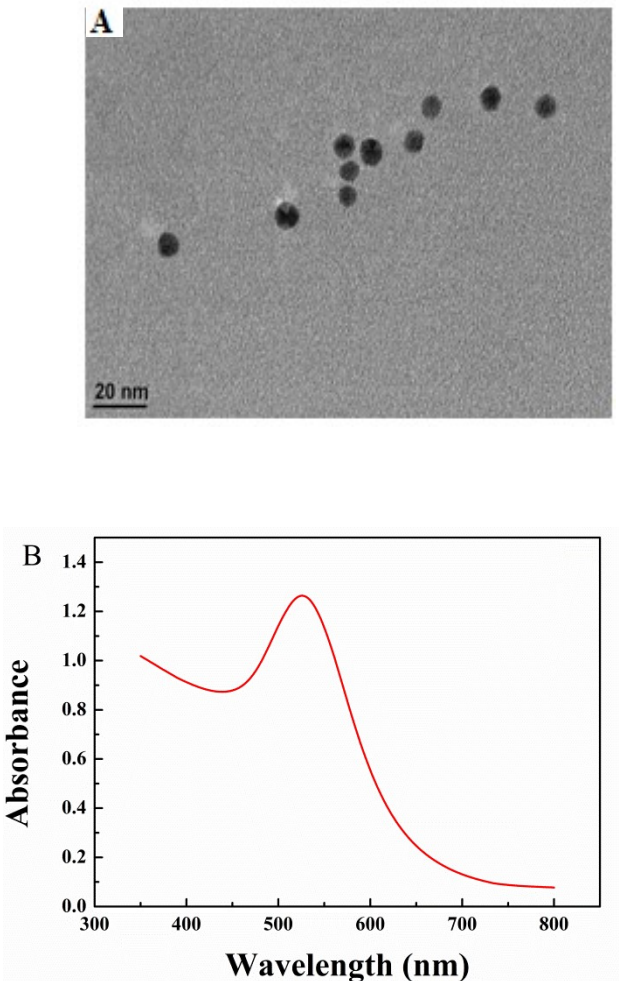

Fig. S2. TEM image (A) and absorption spectrum (B) of the AuNPs.

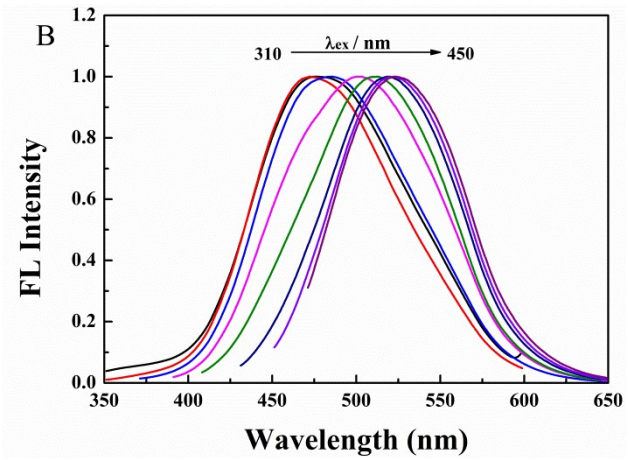

Fig. S3. Normalized fluorescence spectra of the G-CDs.

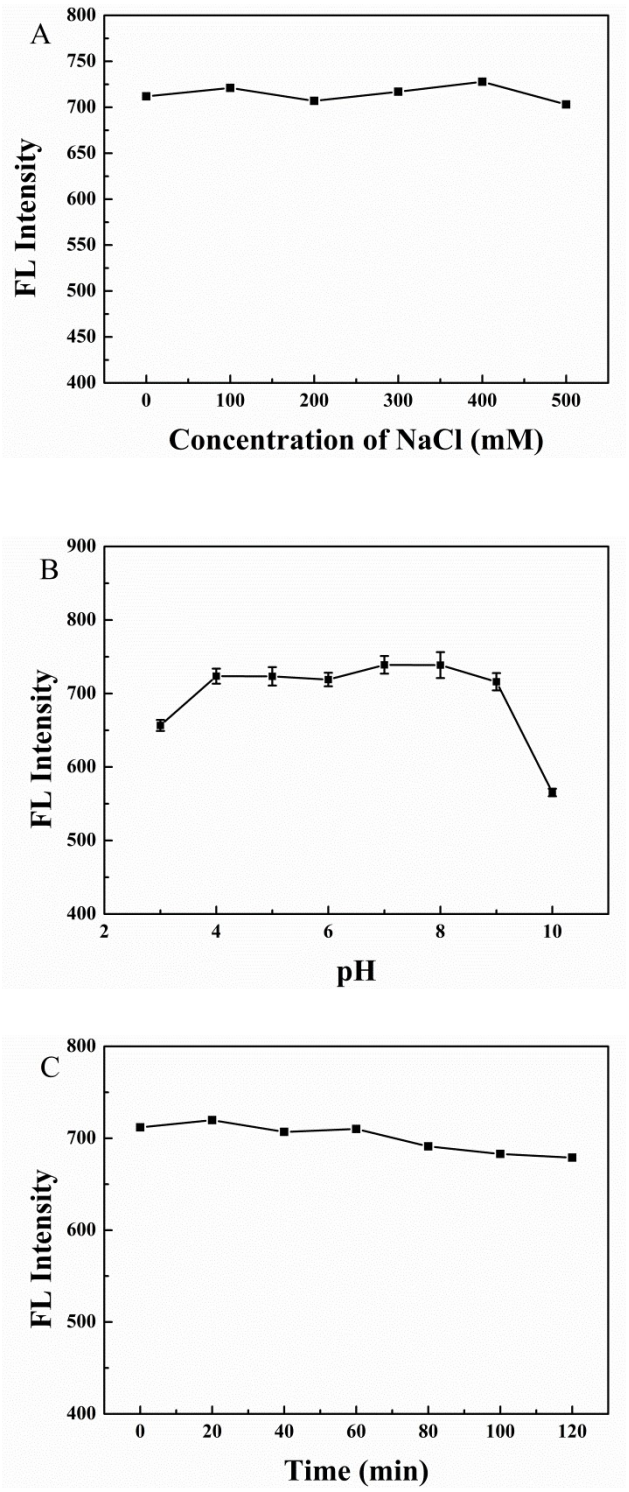

Fig. S4. (A) Fluorescence emission spectra of G-CDs in the different NaCl solution.

(B) Effect of pH on G-CDs fluorescence. (C) Photo stability of G-CDs.

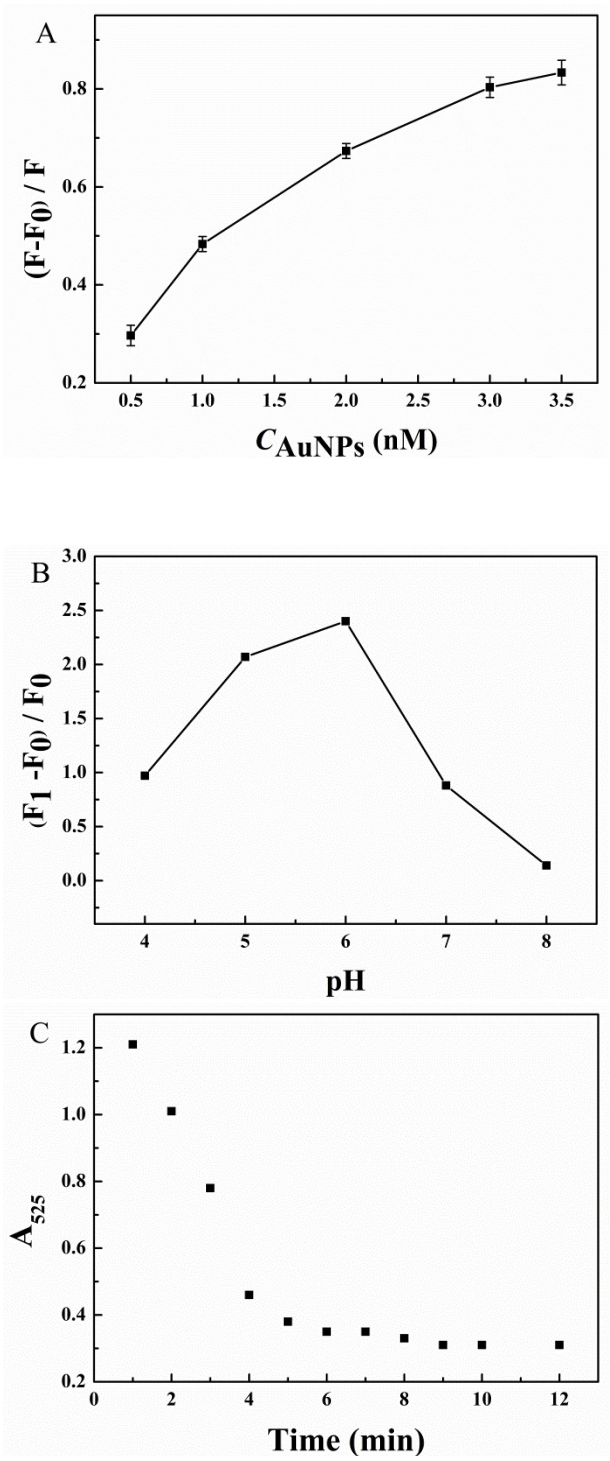

Fig. S5. (A) Influence of AuNPs concentration on the fluorescence quenching efficiency (F - F_0)/F of G-CDs. (B) Influence of pH on the fluorescence recovering efficiency (F_1- F_0)/F_0 of the G-CDs–AuNPs-CLB system. (C) Influence of CLB and AuNPs-incubation time on the A_{525} value of AuNPs.