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Fluorescent carbon nanodots for sensitive and selective detection of tannic acid in wines

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ABSTRACT

Herein we describe an easy one step synthesis of carbon nanodots (C-dots) by thermal carbonization of 6-bromohexylboronic acid using two different amine compounds, polyethyleneglycol bis(3-aminopropyl (PEGA) and 1,2-aminopropane (DPA), at 180 °C in atmospheric oxygen. The as-synthesized C-dots were characterized by FTIR, HRTEM, NMR and fluorescence. The C-dots prepared using PEGA showed a strong emission at 440 nm with excitation at 362 nm. These C-dots exhibited analytical potential as sensing probes for tannic acid (TA) determination. pH effect, interferences, and analytical performance of the method were investigated. The method was found effective in the linear concentration range from 0.1 to 10 mg L^{-1} TA achieving a limit of detection equal 0.018 mg L^{-1} TA. The applicability of the method was demonstrated by direct measurements of TA in red and white wine samples. Validation of the method was achieved by spiking the wine samples with different standard TA concentrations obtaining recoveries in the range (90–112.5%). A probable mechanism by which TA quenched the C-dots fluorescence was proposed.

1. Introduction

Tannic acid (TA) is a natural hydrolysable polyphenol compound present in fruits and different kinds of vegetables and, along with other condensed polyphenols, can be found in several beverages including wine, beer, coffee, black tea and white tea. TA is composed of a polyol residue derived from D-glucose, which hydroxyl groups may be partially or fully substituted with galloyl units (gallotannins) [1]. It is used as a food additive (code number E-181) as clarifying agent, flavor adjunct and flavoring agent [2] as well as additive in medicinal products due to its astringent, diuretics and anti-inflammatory activities [3,4]. Moreover, TA has also applications in the tannery industry to transform animal skins to leather and for re-tanning with Cr(III) to prevent leather putrefaction [5]. As an organic pollutant associated with the tanning industry, TA has been found to be toxic to aquatic microorganisms and may form metal complexes that alter the aquatic ecosystem [6,7]. Due to its wide range of applications, analysis of tannic acid is of importance not only in food but also in the medical and environmental fields. Many analytical methods are based on the overall oxidation properties of polyphenols and, consequently, devoted to the determination of total phenolic content rather than specific determination of each component [8–12]. However, many efforts were attempted to measure TA in several kinds of food and beverage samples, as well as in industrial waters. So, a number of methods are available to quantitatively determine tannic acid content in waters, pharmaceuticals and foods, including spectrophotometry [13], electrochemical methods [14–16], luminescence [17–19] and chromatography [20,21]. Each method has its advantages and drawbacks. For example, the determination of tannic acid in wines by the traditional spectrophotometric Folin–Ciocalteu method, based on the formation of a blue phosphotungstic phosphomolybdenum complex, is simple but lacks selectivity as many other compounds in wine interfere. Chromatographic methods allow the determination of tannic acid along other polyphenols but are time consuming and expensive. Electroanalytical methods with different types of electrodes were used for TA determination, but the presence of ascorbic acid limits the use of some of these methods, or laborious sample pretreatments are needed to remove ascorbic acid before analysis [15,16]. These examples demonstrate that sensitive, selective and rapid TA detection is still a challenge.

Recent developments in analytical nanotechnology open the opportunity to develop new sensitive and selective methods for tannic acid determination. Carbon nano dots (C-dots) were found recently to be promising materials in analytical and bioanalytical

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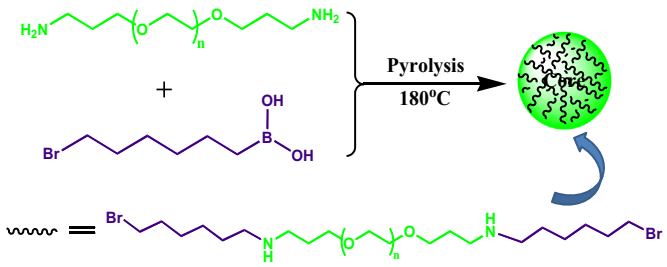

Scheme 1. Schematic representation of PEGA-C-dots synthesis .

applications, due to their unique optical properties, such as broad excitation spectra, tunable emission wavelength and stable photoluminescence [22]. Exploiting C-dots in analytical chemistry is relatively recent and most methods depend on the C-dots surface functional groups and/or their surface passivation effects. The number of analytical assays using C-dots has been increasing, but in the best of our knowledge, no work has been described for TA determination in real samples using carbon nanodots.

Herein we report a straightforward synthesis of passivated C-dots in one step via thermal carbonization method (Scheme 1), using two different amino precursors, polyethyleneglycol bis(3-aminopropyl) (PEGA) and 1,2-aminopropane (DPA). Those C-dots prepared with PEGA were found sensitive and selective fluorescent nanosensors for TA and were successfully applied to direct TA detection in real red and white wine without sample pretreatment. The synthesis reaction process as well as the mechanism for the selective sensing are also proposed.

2. Experimental

2.1. Materials

All the reagents used were highly pure analytical grade chemicals and used without further purification. The following reagents were used in this study: polyethylene glycol bis(3-aminopropyl) (PEGA), 6-bromohexylboronic acid (BrHBA), glucose, fructose, sucrose, gallic acid, citric acid, calcium chloride, and disodium hydrogen phosphate, all purchased from Sigma-Aldrich. Ascorbic acid, sodium fluoride, potassium chloride, and sodium sulfite were purchased from Merck. 1,2-diaminopropane, tartaric acid and caffeine were purchased from Fluka. NaOH, and HCl were purchased from Prolabo. TA was purchased from Hopkin & Williams chemicals (England).

2.2. Synthesis of C-dots

PEGA-C-dots and DAP-C-dots were synthesized by a thermal carbonization method, using PEGA and DAP, respectively. Typically, 1 mmol of PEGA was dissolved in about 25 mL of milli-Q water. To this solution, 0.25 mmol of BrHBA was added. The solution was then stirred and heated at 150 °C. The heating was continued until near dryness, after which 1 mL of milli-Q water was added. The process was repeated 5 times. Finally the temperature was raised to 180 °C. A yellow solution was formed and heating continued until obtaining a reddish-brown color solution to ensure the formation of the C-dots. The obtained PEGA-C-dots solution was then completed to 25 mL of milli-Q water filtered by nylon filters (0.45 μm) and purified through dialyzer tube (MWCO, 3.5 kDa) for 3 days. The purified solution was divided into two aliquots, the first one was dried completely for characterization analysis while the second was used for the analysis experiments of tannic acid. The pH of the aqueous PEGA-C-dots solution resulted to be 6.43. The same procedure was carried out to prepare DAP-C-dots using DAP instead of PEGA.

2.3. Spectrofluorimetric measurements

In a typical pH effect determination procedure, 100 μL of TA (so that the final concentration is 5 mg L^{-1}) were diluted by about 4 mL of universal buffer (in the range 3–11.5) and then 100 μL of C-dots solution was added. Finally, the solution was completed by the same buffer until a final volume of 5 mL. The fluorescence was measured immediately after the preparation in a 1-cm quartz cuvette 3 times at 440 nm with excitation at 362 nm and slit widths of excitation and emission as 20 and 10 nm, respectively. The average fluorescence data were calculated and presented as a graph. Similarly, for interference measurements, 100 μL of TA (final concentration is 5 mg L^{-1}) was mixed with the interference material (final concentration is 10 mg L^{-1}) and diluted by about 4 mL of universal buffer solution pH=9. Then, 100 μL of C-dots solution was added and finally the solution was completed to 5 mL using the same buffer solution. The subsequent fluorescence was measured as mentioned above with the same instrumental conditions.

2.4. Fluorescence quantum yield measurement

The fluorescence quantum yield was calculated through the well-established comparative method using quinine sulfate as a reference. The following equations were used in the quantum yield measurement:

$$\Phi_C = \Phi_{st}\frac{F_C}{F_{st}}\frac{A_{st}}{A_C}\frac{{n_C}^2}{{n_{st}}^2} \quad (1)$$

$$\Phi_C = \Phi_{st}\frac{G_C}{G_{st}}\frac{{n_C}^2}{{n_{st}}^2} \quad (2)$$

where ϕ is the quantum yield, F is the calculated integrated fluorescence intensity, n is the refractive index, A is the optical density (measured with a UV–Vis spectrophotometer, Perkin Elmer, Lambda 900), and G is the gradient of F vs A linear plot. The subscripts C and st refer to C-dots and the reference fluorophore, respectively. Quinine sulfate dissolved in 0.1 M H_2SO_4 (n=1.33) of quantum yield equal 0.54 at λ_{ex}=350 nm was used as a reference. C-dots were dissolved in milli-Q water (n=1.33).

2.5. Analysis of wine samples

The white wine sample (Soldepeñas, www.felixsolis.com) and red wine samples (Don Mendo, www.sanvelro.com) were used in the application experiment. The pHs of the wines were found 3.31 and 3.43, respectively. TA standards were prepared in 10% ethanol solution to avoid the effect of alcohol and sample pretreatment. Wine samples were diluted so that the alcoholic content was reduced to 10%. In a typical procedure, 100 μL of sample were spiked by 100 μL standard TA (0, 1, and 3 mg L^{-1}) followed by 4 mL buffer solution pH=9 (Na_2HPO_4 and NaOH and/or HCl) and 100 μL of the PEGA-C-dots solution. Finally, the solution was diluted to 5 mL by the same buffer. Fluorescence was measured at 440 nm with excitation at 362 nm. TA was quantified by running a calibration curve using standard solutions. All determinations were carried out in triplicate.

2.6. Instrumentation

HRTEM (JEOL JEM-2100F, 200 kV) was used to determine the size and morphology of the synthesized C-dots. A Varian 620-IR instrument was used to analyze FTIR spectra in the range 600 to 4000 cm^{-1}. ^{1}HNMR and ^{13}CNMR (NAV400 with 9.0 T magnet shielded, 600 MHz) were used for structural analysis of the synthesized C-dots in D_2O

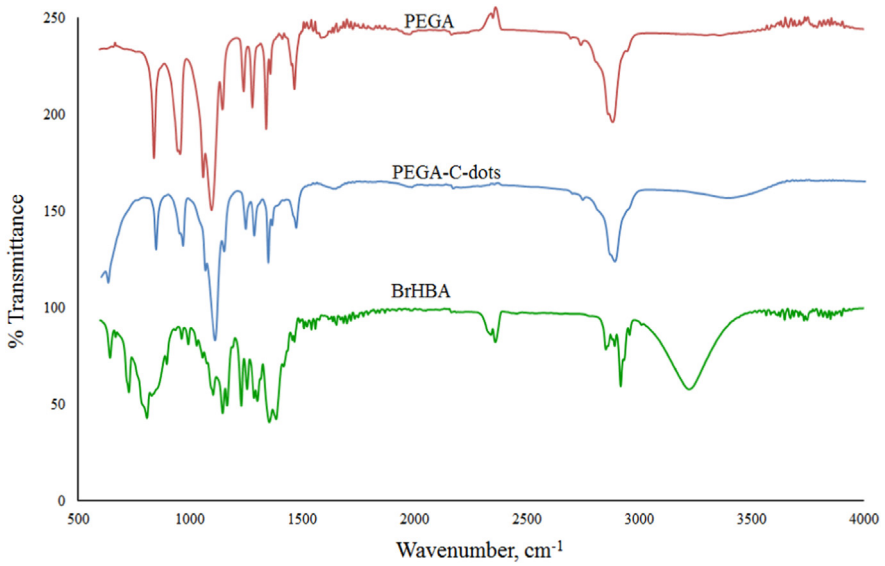

Fig. 1. FTIR spectra of PEGA-C-dots and their precursors.

solvent. Fluorescence spectra were measured using a Cary Eclipse Varian spectrofluorimeter.

3. Results and discussion

3.1. Synthesis and characterization of C-dots

The up-down strategy was used to synthesize C-dots via thermal carbonization method. It is well known that the reaction between the amine group and the alkyl halides proceeds easily and does not need extreme conditions. Higher temperatures than 180 °C were avoided in order to prevent product decomposition. According to the Scheme 1, it was expected that upon heating the long chain product formed between PEGA and BrHBA adhered to the C-dots surface. To confirm this reaction route, the same strategy of synthesis was performed using DAP, a small molecular weight amine, as precursor (Fig. S1).

3.2. FTIR analysis

Fig. 1 shows the spectra of the synthesized PEGA-C-dots and that of reaction precursors for comparison. Two characteristic peaks were identified in the synthesized PEGA-C-dots, the first at 1290 cm^{-1} ascribed to C–H wag (observed only in terminal alkyl halides) and a second at 650 cm^{-1} ascribed to C–Br stretching vibration. Similarly, the same two characteristic peaks were observed in DPA-C-dots (Fig. S2). The absence of characteristic peaks of B–O deformation at 500–550 cm^{-1}, B–O rocking at 725 cm^{-1}, B–OH deformation (at 1210 and 1305 cm^{-1}) and B–N stretching at 780 cm^{-1} in both types of C-dots suggested a pyrolytic deboronation mechanism according reaction route depicted in Scheme 1.

3.3. HRTEM and NMR analysis

The size and the morphology of the as-synthesized PEGA-C-dots and DPA-C-dots are spherical with size range 5 ± 3 nm as demonstrated by the HRTEM images (Fig. S3). Typical ^{13}C NMR spectra of PEGA-C-dots (Fig. S4) showed no signals in the range of 165 to 180 ppm corresponding to sp^2 carbons; however, signals in the range of 40 to 80 ppm revealed the presence of aliphatic sp^3 carbons [23]. On the other hand, ^{13}C NMR spectra of DAP-C-dots (Fig. S5) showed signals in the range of 165 to 170 ppm due to sp^2 carbons that could be ascribed to graphite carbons/planes and signals in the range of 40 to 80 ppm corresponding to aliphatic sp^3 carbons. The ^{1}HNMR spectra of PEGA-C-dots and DPA-C-dots are displayed in Figs. S6 and S7, respectively. Peaks in the range 3.4–3.5 ppm for RCH_2-Br and 0.5–5.0 ppm for R_2NH [23] for both types of C-dots confirmed the information obtained by FTIR.

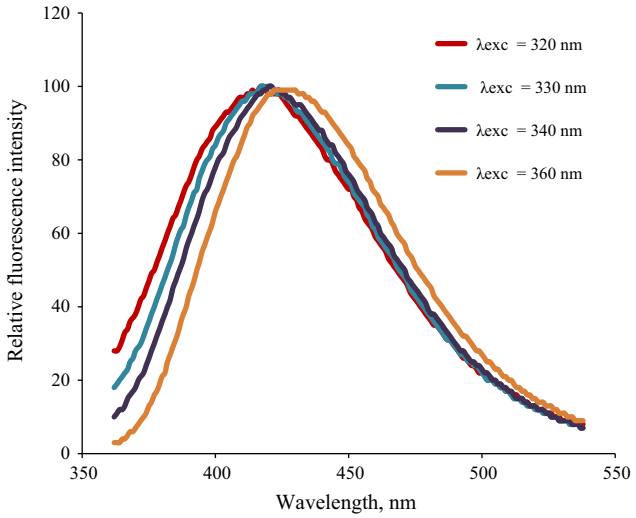

Fig. 2. PEGA-C-dots emission fluorescence as a function of excitation wavelength. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Fluorescence features of the as-synthesized carbon nanodots

The fluorescence emission peak of PEGA-C-dots at pH 3 (0.2 M Na_2HPO_4/0.1 M citric acid) was blue shifted with the change of excitation wavelength (Fig. 2), a phenomenon frequently observed in C-dots which origin still remains unclear. Notwithstanding, it is frequently ascribed to different functional groups that create surface defects with the result of different energy levels [24]. In further experiments, excitation and emission wavelengths of 362 and 440 nm, respectively, were used. The full width at a half maximum at the different excitation wavelengths demonstrated a narrow size distribution of as-prepared PEGA-C-dots (90 ± 5 nm), thus confirming the absence of fluorescence color-dot size relationship.

On the other hand, the fluorescence quantum yield of the C-dots excited at 362 nm in milli-Q water was calculated to be 0.3%, a value similar to that found for other C-dots obtained from combustion soot of candles [25] or by using a plasma-induced method [26].

3.5. pH effect

The effect of pH is very important in this investigation, as the fluorescence of PEGA-C-dots in the absence and presence of TA is pH dependent. The influence of pH in the range 3–11.5 was investigated for TA interaction with PEGA-C-dots and results shown in Fig. 3. In the acidic pH range 3–6 a white precipitate was formed in presence of TA, particularly at concentrations of TA higher than 5 mg L^{-1}. Maximum quenching effect took place in basic media and a pH=9 was taken as the optimum value for further experimental measurements.

3.6. Analytical figures

Under the optimum experimental conditions, the sensitivity, the linear response range and the limit of detection of TA by PEGA-C-dots fluorescence quenching were determined. Calibration curve, obtained from a Stern–Volmer semilog plot, was linear within the range 0.1 to 10 mg L^{-1} TA being the calibration equation $\log(F^{\circ}/F)=0.0597\ C\ \{mg\ L^{-1}\}$, ($R^2=0.994$), where F° and F account for the fluorescence intensities of PEGA-C-dots in the absence and presence of TA, respectively and C represents the concentration of TA in mg L^{-1}. A detection limit of 0.018 mg L^{-1} TA was calculated (%RSD=0.2). As far as we know, no fluorescence methods for TA based on the use of C-dots have been described to date. From Table 1, it is apparent that the present method, exhibits high sensitivity and low detection limit when compared with some electrochemical and optical methods. Among them, that based on the TA oxidation by chitosan capped silver nanoparticles has been recently published for TA determination [27].

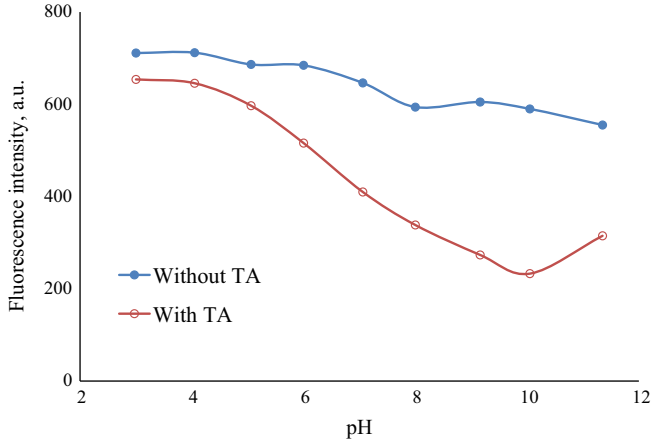

Fig. 3. pH influence on PEGA-C-dots fluorescence in absence and presence of TA (5 mg L^{-1}) at $\lambda_{ex}=362$ nm.

The effect of coexisting compounds on the PEGA-C-dots fluorescence quenching detection of 5 mg L^{-1} TA at pH 9 was investigated. The concentration of all compounds used was 10 mg L^{-1}. The tolerance limit was estimated with a ±5% relative error in fluoresence intensity. The major interference compounds chosen were ascorbic acid, sulfite, caffeine, Mg^{+2} and Ca^{+2} in addition to some abundant components in wine such as gallic acid, sucrose, glucose, fructose, tartaric acid and Na^{+}. It was found that these compounds have no significant effect on the fluorescence emission of PEGA-C-dots by TA. These analytical figures offer a high potential of sensitivity and selectivity for TA in wine samples with no need of sample treatment and it could be comparable to other analytical techniques used for TA determination in such samples.

3.7. Interaction of PEGA-C-dots with TA

The above experimental results showed that the fluorescence quenching observed on adding TA to a C-dots solution at pH=9 remained stable over a long time, indicating that a stable method could be optimized for TA. On the other hand, it was observed that gallic acid did not quench the PEGA-C-dots fluorescence. Carbon dots are known to be excellent electron acceptors and donors [28]; so, a possible mechanism for TA quenching may be attributed to an electron transfer process from the photo excited C-dots to the aromatic groups in TA. The PEGA-C-dots may be wrapped by the TA mimicking dendrimers, so allowing an effective non-radiative energy transfer process (Fig. 4).

On the contrary, in presence of gallic acid such interaction with PEGA-C-dots was not possible and fluorescence quenching did not take place.

3.8. Real sample analysis

Under the optimum experimental conditions described, TA determination in red and white wine samples was performed to validate the method. No sample pre-treatments were made but dilution with buffer. The fluorescence spectra of PEGA-C-dots without and with wine samples spiked by different concentrations (0, 1, and 3 mg L^{-1}) are illustrated in Fig. S8. Table 2 shows the results of TA obtained in wine samples after their dilution. Each result was the average of three samples. It is obvious that satisfactory recoveries were achieved for the spiked samples and results demonstrated that the method offers potential for quantitative determination of TA in wine samples.

4. Conclusions

In summary, we have been successfully synthesized passivated C-dots in an easy one step in which a deboronation process took

Table 1
Figures of merit of different analytical methods for tannic acid determination.

Method	Detection limit (μM)	Linear range (μM)	Samples	Ref.
Inhibition of luminol electrochemiluminescence	0.02	0.05 to 100	Chinese gall, hop pellet	[19]
Chemiluminescence/FIA	0.06	0.06 to 17	Pharmaceuticals, human urine, surface waters	[17]
Quenching of the 3-aminophthalate fluorescence	0.58	3 to 180	Tea beverages	[18]
Anodic stripping voltammetry	0.01	0.01 to 1	–	[14]
Colorimetric: oxidation by chitosan capped Ag nanoparticles	1	1–100	Water	[27]
Spectrophotometric	0.08	1 to 10	Ayurvedic formulation	[21]
Carbon dots fluorescence quenching	0.01	0.05 to 0.6	Wines	This method

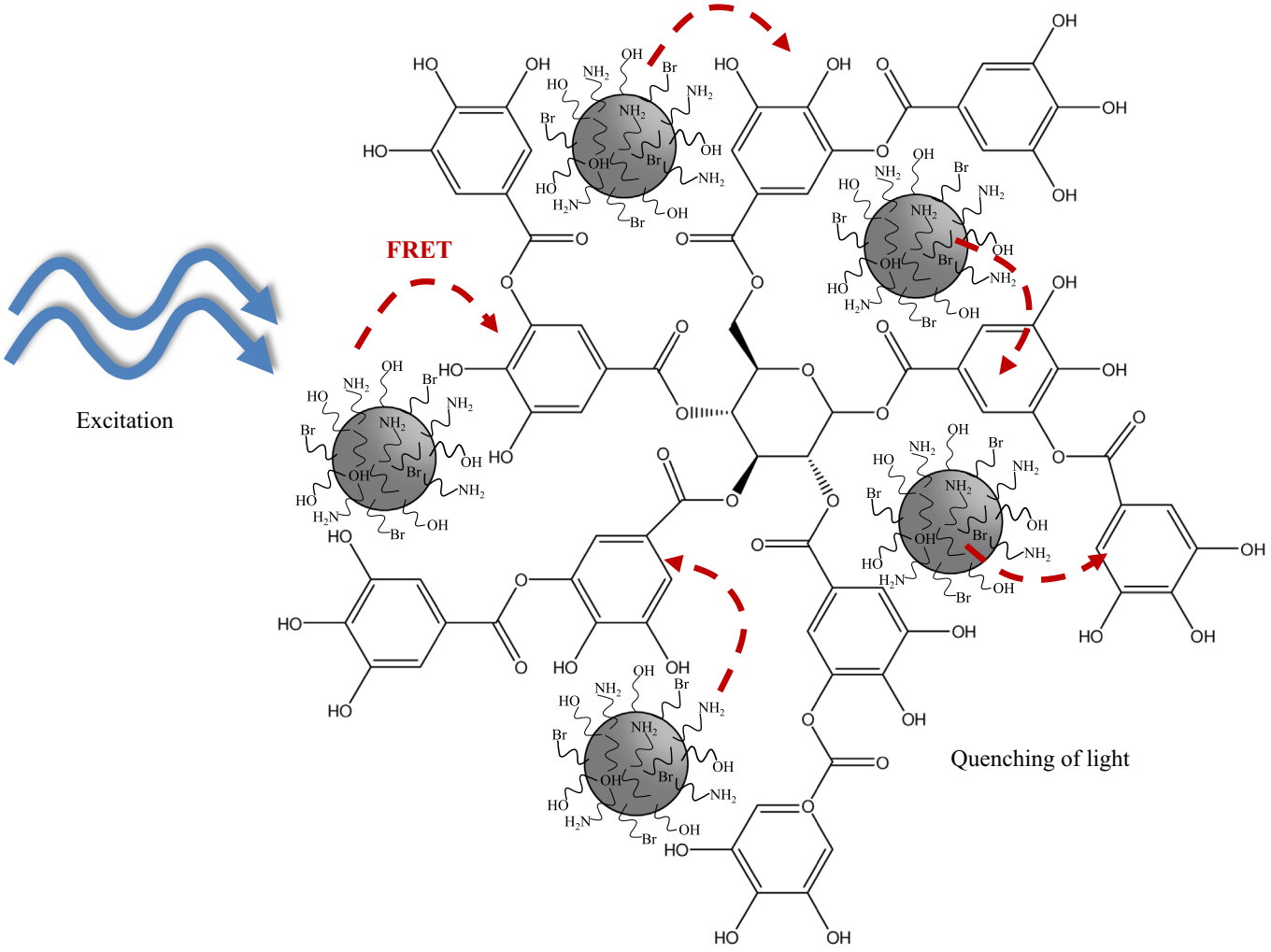

Fig. 4. PEGA-C-dots non-radiative energy transfer to tannic acid dendrimers under excitation wavelength λ_{ex}=362 nm, at pH=9.

Table 2
Results for TA determination in raw and spiked wines.

Spiked conc. (mg L^{-1})	Red wine			White wine		
	Found conc. (mg L^{-1})	% RSD	Recovery (%)	Found conc. (mg L^{-1})	% RSD	Recovery (%)
0	2.69	0.1	–	0.055	0.13	–
1	3.81	0.25	112.2	1.18	0.1	112.5
3	5.4	0.34	90.2	2.95	0.36	96.6

place. The as-synthesized PEGA-C-dots were found sensitive and selective towards TA, so that a promising fluorescence sensing system for TA detection in wines has been developed. The probe system of TA based on PEGA-C-dots fluorescence quenching showed analytical advantages such as rapid detection, high sensitivity and selectivity, wide linear response range, and low cost. A possible mechanism for TA sensing was attributed to a wrapping of the PEGA-C-dots by the TA mimicking dendrimers, so allowing an effective non-radiative energy transfer process.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.09.028.

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