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PAPER

Antioxidant activity assay based on the inhibition of oxidation and photobleaching of L-cysteine-capped CdTe quantum dots†

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Quantum dots (QDs) have recently been the focus of attention of many investigators for development of diagnostic tools in many research areas. In this work, we established a new QD-based assay to evaluate the antioxidant/polyphenolic activity. This assay is based on measurement of the inhibitory effect of the antioxidant/polyphenolic compounds on the UV-induced bleaching of CdTe QDs with L-cysteine capping. QDs exhibited excellent photostability without any UV exposure, while they bleached rapidly under UV irradiation. Generation of reactive oxygen species (ROS) under UV irradiation is probably the main cause of the photobleaching of QDs. By comparing the photostability of QDs in buffer solution in the absence and presence of sodium azide, as a known $^1O_2$ quencher, the involvement of $^1O_2$ in photobleaching of QDs was confirmed. The photobleaching effect induced by ROS could be reduced in the presence of antioxidant/polyphenolic compounds. We tested several antioxidant/polyphenolic compounds as well as known antioxidants such as trolox and 4 different types of tea. The results obtained by the QD-based assay revealed a very good correlation with the data acquired by Folin–Ciocalteu assay. Furthermore, a deeper understanding of the mechanism and the solution for photobleaching of QDs under UV irradiation might be very meaningful in promoting their clinical applications.

Introduction

Reactive oxygen species (ROS), such as the superoxide anion radical (O_2^{-}), hydroxyl radical (OH) and peroxyl radical (ROO), are constantly generated by aerobic metabolism as well as exogenous sources such as UV radiation and environmental pollution. The formation of ROS may cause oxidative modification of lipids, DNA, proteins and other biomolecules.[1,2] This plays an important role in ageing and also in the pathogenesis of diseases such as atherosclerosis and cancer.[3,4] Many epidemiological studies suggest that consumption of a fruit- and vegetable-rich diet inversely correlates with the risk of cardiovascular diseases and certain forms of cancer. These chemoprotective effects have been, at least in part, attributed to the antioxidant activities of polyphenolic compounds and antioxidant vitamins.[5,6]

Antioxidant assays have been widely used to evaluate the antioxidant properties of pure compounds or complex samples. Some methods for measuring antioxidant activity of materials have already been known and used in many fields of research.[7,8] For example electrochemical techniques, based on phenolic OH groups' behavior, offer direct determination of the total AO activity, *i.e.*, evaluation of the overall reducing power without the use of reactive species. Various electrochemical techniques, relying upon direct or indirect oxidation of phenolic compounds, such as cyclic voltammetry (CV),[9–12] flow-through column electrolysis and flow injection analysis,[13,14] coulometric detection and the combined high-performance liquid chromatography-electron capture detection[15] have been employed in such analyses. The main electrochemical approaches used are CV and flow injection analysis with amperometric detection (FIA-AD).[16] Also inhibition of substrate oxidation by antioxidants has been measured by steady-state fluorescence or the spin-trapping ESR method.[7,17] However, more sensitive methods which can be applicable in quantitative antioxidant assays are desirable because they could perform measurements of small amounts of samples, and even those for spatial distribution of antioxidant actions. Fluorescence detection is one of the solutions to these needs, and many fluorescence probes for antioxidant assays have been reported.[18,19] However, in biological systems, many synthesized probe molecules encounter obstacles because of their solubility and activity limitations. Semiconductor nanocrystal quantum dots (QDs) with size dependent optical properties are emerging as

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alternative or complementary tools to the organic fluorescent dyes. Compared with the traditional organic fluorescence probes, QDs have many advantages, including broadband excitation, narrow bandwidth and high intensity emission.[20] In order for synthesized QDs to become applicable for the study of the biological systems, their surfaces are usually modified with a ligand such as mercaptopropionic acid or cysteine.[21,22]

A typical feature of QDs is their response to illumination. While some researchers reported photobrightening properties for QDs, *i.e.* their increased emission intensity upon illumination,[21,23,24] others observed photoquenching or photobleaching property for QDs.[12] In addition, there are reports on excellent photostability of some types of QDs.[22] Detailed studies of Ma *et al.*[20,25] on the photochemical stability of thiol-coated CdTe QDs in living cells and aqueous solutions revealed their photochemical instability. They attributed the bleaching of photoluminescence of QDs under laser irradiation (named photoquenching or photobleaching) to the enhanced non-radiative transfer in exited QDs caused by increased surface defects due to losing of thiol ligands. They proposed that, among different ROS that may be produced, singlet oxygen ($^1O_2$) is mainly involved in the photobleaching of QDs, as revealed by the inhibiting effects of $^1O_2$ quenchers such as histidine and sodium azide on the photobleaching of QD solutions.[26]

QDs have many applications in biology, especially in cell imaging. In an imaging process, production of ROS species due to instability of QDs causes quenching of QD fluorescence and serious restriction in the application of QDs in cell imaging. The reaction of cells with ROS causes oxidative damage of DNA[27–32] and inhibits the peroxidation of low-density lipoprotein.[33–35]

While different investigators are now attempting to improve the photochemical stability of the photosensitive QDs,[36–39] we used the interesting photochemical instability of a cysteine-coated QD to discover a novel method for assessment of antioxidant activity. The antioxidant materials by scavenging the generated ROS under UV irradiation of QDs can inhibit the photobleaching effect. Polyphenols/flavonoids found in plants have begun to receive much attention among researchers as a new natural antioxidant.[40] Several studies have shown that phenolic compounds play a role as antioxidants through different mechanisms of action, including scavenging of free radicals,[41,42] quenching of reactive oxygen species,[43] and chelating of transition metals.[42,44]

In this work, we will demonstrate the use of synthesized L-cysteine-capped CdTe QDs to measure the antioxidant activity of a series of polyphenolic compounds including, quercetin, tannic acid, caffeic acid, gallic acid, naringin, trolox, and some different tea samples. The proposed method was compared with the method described by the International Organization for Standardization (ISO) 14502-1.[45] The use of antioxidant materials along with QDs has two advantages: protection of QD from bleaching and preservation of cells from reaction with ROS.

Experimental

Chemicals

$CdCl_2$, L-cysteine, $NaBH_4$, quercetin, and Te powder were purchased from Sigma-Aldrich (St. Louis, MO, http://www.sigmaaldrich.com). Caffeic acid, Folin–Ciocalteu's reagent, gallic acid, H_3PO_4, methanol, Na_2CO_3, NaOH, naringin, tannic acid and trolox were obtained from Merck Chemicals (Darmstadt, Germany, http://www.merck.de). All reagents were of analytical grade and used as supplied without further purification. Black, green, white, and red tea samples (*Camellia sinensis*) were provided by local sources. Phosphate buffer (pH = 7.4) was prepared by the addition of appropriate amounts of concentrated sodium hydroxide to phosphoric acid solution.

Instrumentation

All fluorescence spectra were recorded on a Perkin-Elmer spectrofluorimeter (model LS50B) equipped with 1.0 cm quartz cell. UV-visible spectra were recorded using a HP diode array spectrophotometer (Hewlett-Packard model 8452A) employing a quartz cuvette with 10 mm path length. A Metrohm pH meter (model 780) with a combined glass pH electrode, calibrated against standard buffer solutions at pH 4.0 and 7.0, was used for pH measurements. A previously homemade UV exposure chamber equipped with a UV light source (254 nm) was used.[46] All measurements were made at room temperature and atmospheric pressure.

Preparation of CdTe nanoparticles

The CdTe colloidal solution was prepared using the reaction between Cd^{2+} ($CdCl_2$, Sigma-Aldrich) and a NaHTe (Sigma-Aldrich) solution.[21] Tellurium powder was chosen as a starting material to prepare the NaHTe aqueous solution. It was reduced by excessive sodium borohydride in water under stirring and Ar bubbling. After Te was completely reduced, a certain volume of the NaHTe solution was injected into a $CdCl_2$ solution containing L-cysteine (Cys, Sigma-Aldrich) which was deaerated by Ar for 20 min. The molar ratio of Cd^{2+}/Te^{2-}/Cys was set to 1 : 0.5 : 2.4. Then, it was heated until boiling. Under refluxing, fluorescence of the solution appeared and could be tuned in color by prolonging the refluxing time (see Fig. S1, in ESI†). The colloidal solution can be kept for more than 3 months without any visible aggregation.

Extraction of polyphenols

The method described by the International Organization for Standardization (ISO) 14502-1 was used.[45] Black, green, white, and red tea samples (*Camellia sinensis*) were purchased from a commercial grocery store. Briefly, 0.200 g of each sample was weighed in an extraction tube, and 5.0 mL of 70% methanol (Merck) at 70 °C was added. The extract was mixed and heated at 70 °C on a vortex for 10 min. After cooling at room temperature, the extract was centrifuged at 200*g* for 10 min. The supernatant was decanted and placed in a graduated tube. The extraction step was repeated twice. Both extracts were pooled and the volume was adjusted to 10 mL with cold 70% methanol.

Determination of total polyphenol content (TPC)

The total polyphenol content (TPC) was determined spectrophotometrically, using gallic acid (Merck) as a standard, according to the method described by the International

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Organization for Standardization (ISO) 14502-1 with minor changes.[45] Briefly, 1.0 mL of the diluted sample extract was transferred in duplicate to separate tubes containing 1.0 mL of a 1/10 dilution of Folin–Ciocalteu's reagent (Merck) in water. After 5 min, 4.0 mL of a sodium carbonate solution (7.5% w/v, Merck) was added. The tubes were then allowed to stand at room temperature for 90 min. Absorbance at 764 nm was measured against a solution with the same composition of samples without Folin–Ciocalteu's reagent, as blank. Experiments were performed in duplicate.

Assay of the antioxidant/polyphenolic activity

The fluorescence spectra of the QD solution at 30 nM concentration before and after irradiation with UV light were recorded and F_0/F was calculated. A previously homemade UV exposure chamber equipped with a UV light source (254 nm) was used.[46] F_0 and F are the fluorescence signals of QD in the absence and the presence of UV light at 610 nm, respectively. This procedure was repeated three times and $(F_0/F)_{\text{uninhibited}}$ was calculated from the average of all measurements. $(F_0/F)_{\text{inhibited}}$ was computed from the fluorescence signal of the QD solution at 30 nM and different concentrations of each antioxidant before (F_0) and after (F) irradiation with UV light. The percent of inhibition was calculated from the following equation:

$$\%\text{Inhibition} = \left(1 - \left(\frac{(F_0/F)_{\text{Inhibited}} - 1}{(F_0/F)_{\text{Uninhibited}} - 1}\right)\right) \times 100 \quad (1)$$

Results and discussion

The L-cysteine-capped CdTe colloidal solution was prepared using the reaction between Cd^{2+} and a NaHTe solution.[21] Observation of the color of the synthesized quantum dots (QDs) of different sizes, under ambient and UV lights (see Fig. S1†), revealed that as the size of QDs increased the color changed from yellow to red (red shift). The same phenomenon was observed for the UV-vis absorption and fluorescence emission spectra (Fig. S2†). The size and concentration of the synthesized CdTe QDs in aqueous solutions were determined from the optical densities of the first excitonic absorption peak and extinction coefficients.[47] The properties of synthesized QDs observed in this work were highly consistent with those reported by Ma *et al.*[20,25] The QDs of variable sizes were stable for a long time (more than 3 months). However, the degree of stability was found to be directly correlated with the particle size. Thus, in order to gain the highest stability, we used the largest synthesized particles (with a diameter of about 3.5 nm) for our experiments.

The change in fluorescence emission spectra of an aqueous solution of L-cysteine-capped CdTe QDs at the concentration of 30.0 nM irradiated by a 254 nm UV light is shown in Fig. 1. As is obvious, in the presence of UV light, a rapid decay in the fluorescence signal with time is observed, so that after 150 s, the emission intensity decreases to 50% of the original intensity before exposure to the light (Fig. 1B). However, no significant changes in the fluorescence signal are observed when the solution is kept in the dark (Fig. 1A). The observed photobleaching effect is reproducible with a relative standard deviation (RSD) of 1.4% for 6 repeated experiments. Similar trends were reported by Ma

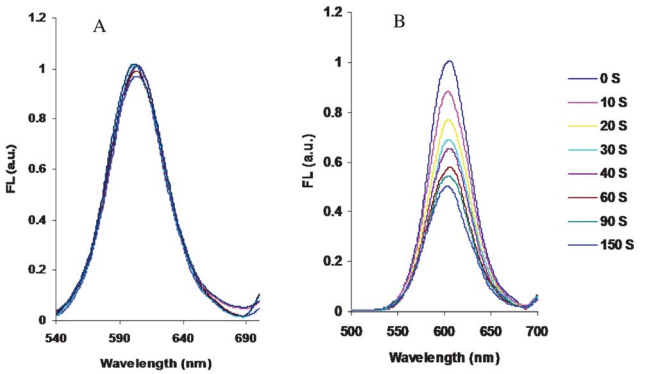

Fig. 1 Effect of UV light on the stability of the fluorescence signal of QDs. Fluorescence signal of QDs in the absence (A) and presence (B) of UV light illuminated at times of 0, 10, 20, 30, 40, 60, 90, and 150 s. *Conditions*: 0.04 M PBS of pH = 7.4; [QD] = 30 nM; λ_{exi} = 360 nm. All fluorescence spectra are normalized to 1.

et al.,[25] when the authors illuminated the aqueous solution of mercaptoacetic acid-capped CdTe QDs with a 532 nm laser beam. Photobleaching of the CdTe emission was attributed to the production of reactive oxygen species (ROS), catalyzed by CdTe QDs. Once produced in this manner, the ROS cause the photobleaching of the fluorescence signal. Photobleaching of QDs by ROS has been previously reported by other authors.[20,25] The production of ROS in living cells and culture media by exposure of QDs to UV irradiation has also been reported.[12] However, to the best of our knowledge, there is no report on the photobleaching of aqueous solutions of thiol-capped CdTe QDs illuminated with UV irradiation.

As seen in Fig. 2, after a 5 min bubbling of the aqueous solution of QDs buffered at pH 7.4 with Ar and subsequent irradiation by a UV lamp for 30 s, no photobleaching was observed. Instead, an increase in photoluminescence

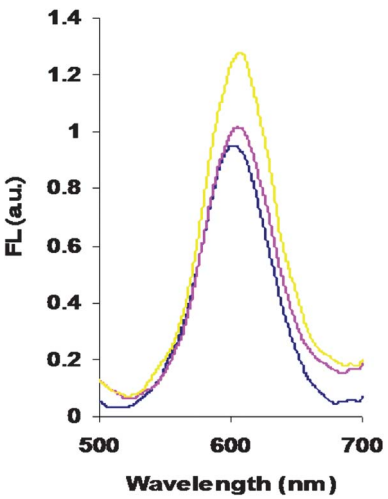

Fig. 2 Effect of Ar bubbling on the fluorescence signal of QDs. The fluorescence signal of QD solution without Ar bubbling before UV irradiation (blue), after 5 min of Ar bubbling before UV irradiation (pink) and after 5 min of Ar bubbling and 30 s UV irradiation (yellow). *Conditions*: 0.04 M PBS of pH 7.4; [QD] = 30 nM; λ_{exi} = 360 nm. All fluorescence spectra are normalized to 1.

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(photobrightening) with respect to the UV illuminated air-saturated solution was observed. This observation confirms the contribution of ROS to the photobleaching of the L-cysteine-capped CdTe QDs upon irradiation at 254 nm. The photobrightening of the QDs in the absence of oxygen has already been reported for some types of QDs,[21,23,24] which have been attributed to the reduction of defects in the QD surface under UV irradiation.

Singlet oxygen ($^1O_2$) is one of the ROS that can be considered as the main species responsible for photobleaching. To investigate the role of singlet oxygen in photobleaching of QDs, the change in fluorescence spectra of the QDs under UV irradiation before and after sodium azide (NaN_3) treatment, as a physical quencher of singlet oxygen, in the concentration range of 1.0×10^{-8} to 8.0×10^{-2} M was studied. It was found that NaN_3 can effectively inhibit the QD photobleaching in buffer solution. With increasing concentrations of NaN_3, photobleaching of QDs was decreased (Fig. S3†). This phenomenon can be attributed to the quenching effect of NaN_3 on $^1O_2$. The percentage inhibition was calculated according to eqn (1). There is a linear relationship between inhibition percentage and logarithmic concentration of NaN_3 (Fig. S3†).

Our results clearly demonstrate the role of oxygen in the photobleaching of L-cysteine-capped CdTe QDs. Treatment with sodium azide (NaN_3) as a physical quencher of singlet oxygen showed that the photobleaching of QDs is decreased with increasing concentrations of NaN_3. This phenomenon can be attributed to the quenching effect of NaN_3 on $^1O_2$. Therefore, it is observed that QDs can catalyze the photoproduction of $^1O_2$ (and perhaps other ROS as well), which consequently photobleach the QDs.

Polyphenols/flavonoids found in plants have begun to receive much attention among researchers as a new natural antioxidant.[40] It is well known that, because of scavenging of the ROS, antioxidant/polyphenolic compounds play a fundamental role in balancing the oxidative stress of the human body.[48] Thus, in this section, we studied the effect of six different antioxidant/polyphenolic compounds, namely, quercetin (QCN), tannic acid (TA), caffeic acid (CA), gallic acid (GA), naringin (NGN) and trolox (TLX), on the photobleaching of the CdTe QDs upon irradiation with a UV source at 254 nm. In all experiments, a 30 nM concentration of QD was used. At higher QD concentrations, overloading of the fluorescence signal was observed, and at lower ones, the assay sensitivity was decreased. The irradiation time was set at 30 s. At higher irradiation times, more concentrated antioxidant/polyphenolic solutions were needed to inhibit the photobleaching which, in turn, resulted in lower sensitivities. While, at lower irradiation times, a small fluorescence quenching effect was achieved that was not suitable for further studies.

As an example, the changes in the fluorescence spectra of QDs, incubated with different concentrations of GA, after 30 seconds of UV irradiation are shown in Fig. 3. A similar effect was observed for other antioxidant/polyphenolic compounds. This figure clearly demonstrates the role of antioxidant/polyphenolic compounds in preventing the photobleaching of QDs. GA prevents the photobleaching of QDs, most probably by scavenging the ROS. This behavior is dependent on the concentration of GA, so that by augmenting the concentration

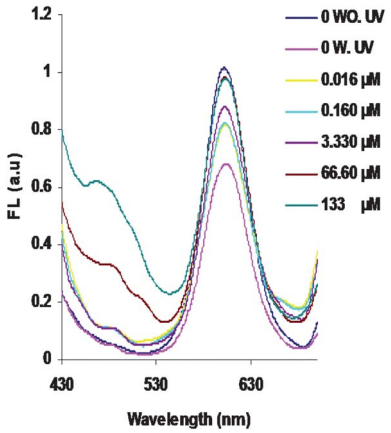

Fig. 3 Effect of gallic acid on photobleaching of QDs. The fluorescence signal of QDs in the absence of UV irradiation (WO. UV) and in the presence of 30 s UV irradiation with 0 (W. UV), 0.16 μM, 1.6 μM, 33 μM, 0.66 mM and 1.3 mM gallic acid. 30 s UV irradiation. *Conditions*: 0.04 M PBS of pH 7.4; [QD] = 30 nM; λ_{exi} = 360 nm. All fluorescence spectra are normalized to 1.

of GA, the prevention of photobleaching of QDs is increased. At a 1.30 mM concentration of GA, the photobleaching is completely prevented and the fluorescence spectrum is matched

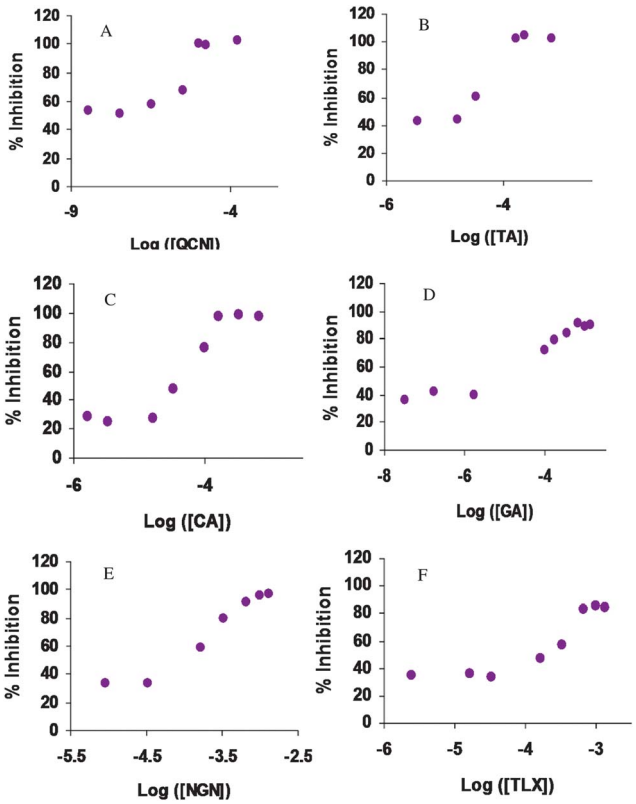

Fig. 4 Effect of different antioxidant/polyphenolic compounds on the photobleaching of QDs. The percent inhibition of photobleaching, calculated according to the formula described in eqn (1), against the concentration of different antioxidant/polyphenolic compounds after 30 s UV irradiation of QDs in 0.04 M PBS of pH 7.4. [QD] = 30 nM; λ_{exi} = 360 nm.

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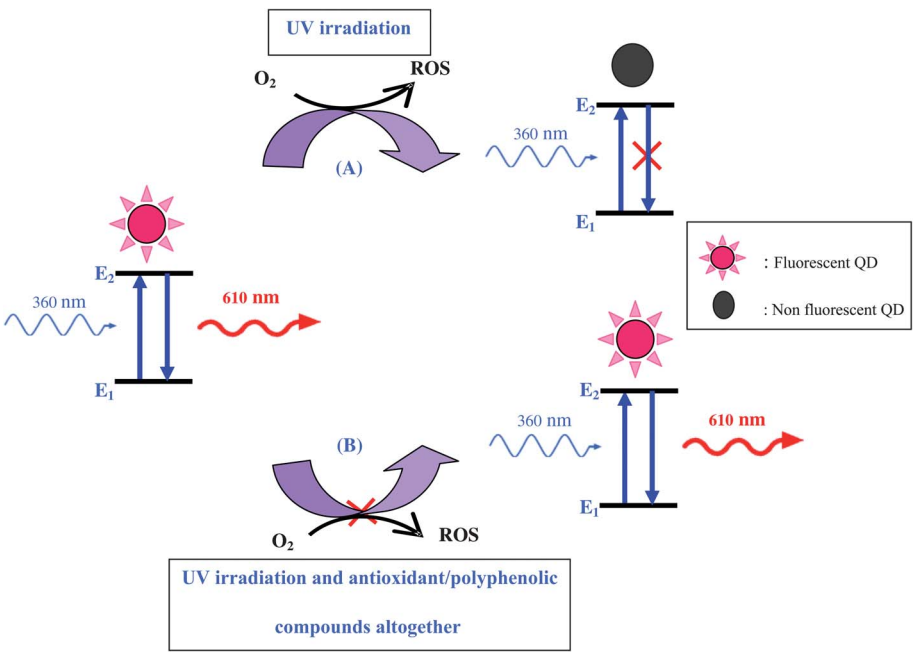
with that obtained in the absence of UV irradiation. The data of percent inhibition of photobleaching by different concentrations of antioxidant/polyphenolic compounds are shown in Fig. 4. All experiments were performed three times. An S-shape (sigmoid) relation between the logarithmic concentration of antioxidant/polyphenolic compounds and the percent inhibition was observed. The concentration of antioxidant/polyphenolic compounds, at which the inhibition of photobleaching is started, was not the same for the studied molecules and varied by about three orders of magnitude. Similarly, the concentration required for maximum inhibition also varied significantly. Among the studied compounds, QCN, TA, CA, and NGN could completely return the fluorescence intensity of the QDs to the original values before irradiation, upon 30 s UV irradiation. They could do this function at the concentrations of 0.016, 0.160, 0.330, and 4.00 mM, respectively. However, GA and TLX were not able to inhibit the photobleaching of CdTe completely and maximum percent of inhibition obtained by these compounds was 90% and 85% at concentrations of 4.00 and 1.30 mM, respectively.

The above results further elucidate the mechanism of photochemical instability of thiol-capped QD solutions: The QDs could transfer energy to nearby oxygen molecules and lead to ROS formation.[22] UV irradiation has the potential to generate a relatively high level of ROS and photobleaching of QDs.[49] Therefore, antioxidant/polyphenolic compounds, as ROS scavengers, could affect the fluorescence intensity of QDs. The general mechanism of this process is represented in Scheme 1. We used these observations to develop a quantum dot-based assay of antioxidant/polyphenolic activity.

The middle part of the sigmoidal curve obtained by plotting the percent inhibition of photobleaching against the logarithmic concentration of antioxidant/polyphenolic compounds represents a linear pattern (Fig. 4). The concentration ranges and slope of the linear regression line (Table S1†) are different among the studied compounds. All experiments were performed three times.

For measuring the ROS scavenging activity, we calculated percent inhibition at the same concentration for all compounds. It should be noted that the choice of such a unique concentration for the evaluation of ROS scavenging activity of the antioxidant/polyphenolic compounds tested is a critical task. In fact, the selected concentration should not be set too low so that the compounds are not able to inhibit the photobleaching. On the other hand, if the concentration selected is too high, most compounds will completely inhibit the photobleaching and, thus, ranking of the compounds is not possible. It should be noted that, although this observation could be a drawback of this new method, the presented work is the first step in starting the application of quantum dots to the antioxidant/polyphenolic assay and can be improved in the future.

Thus, according to the plots of percent inhibition against concentration (Fig. 4), a concentration of 0.33 mM was selected for this purpose. The inhibitory effect in the 0.33 mM concentration of antioxidant/polyphenolic compounds, evaluated from three repetitive analyses, was found to decrease in the order QCN > TA > CA > GA > NGN > TLX (Fig. 5). The standard deviation of the antioxidant activity measurements has been shown as an error bar in Fig. 5; the standard deviations are in the range of 2.4–4.5 units of percent of inhibition. In Table 1 are compared the observed trend in inhibitory effects of different antioxidant/polyphenolic compounds obtained by the proposed method with those previously reported in the literature by a variety of established testing methods.[50–63]



Scheme 1 General mechanism for the QD-based antioxidant/polyphenolic assay. In the presence of UV irradiation ROS was produced and fluorescent QD converted to nonfluorescent QD (A). In the presence of both UV irradiation and antioxidant/polyphenolic compounds quenching of QD fluorescence reduced depending on antioxidant/polyphenolic concentration (B).

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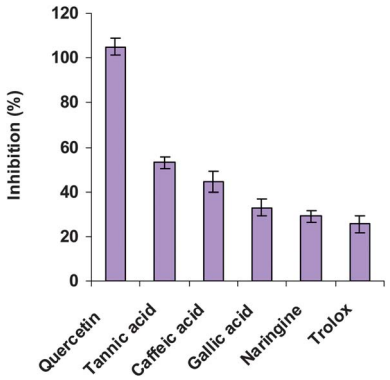

Fig. 5 Percent inhibition of different antioxidant/polyphenolic compounds at a concentration of 0.33 mM on the photobleaching of QDs after 30 s UV irradiation. *Conditions*: 0.04 M PBS of pH = 7.4; [QD] = 30 nM; λ_{exi} = 360 nm.

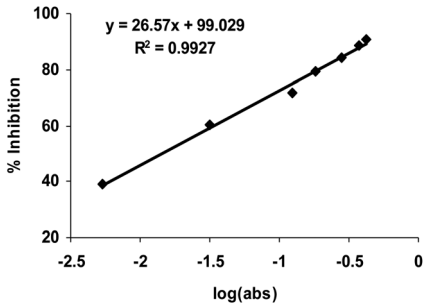

Fig. 6 Correlation graph between the inhibition percentages which are calculated with the QD assay and the Folin–Ciocalteu reference method. Experimental condition for fluorescence measurement is as in Fig. 3, and for absorbance measurements as is mentioned in the text in the section: Determination of total polyphenol content.

Measurement of antioxidant/polyphenolic content is one of widely used approaches for determining the antioxidant activity of plants. The color development using a Folin–Ciocalteu reagent (Folin–Ciocalteu assay) is the generally preferred approach for measuring antioxidant/polyphenolic content because most plant derived antioxidants contain large amounts of antioxidant/polyphenolic compounds.[64] The results of the QD-based assay were also compared with the corresponding values obtained by the use of the standard Folin–Ciocalteu spectrophotometric method,[22] commonly used for the determination of total antioxidant/polyphenolic content. The results obtained by the proposed method and the Folin–Ciocalteu (FC) method for all tested antioxidant/polyphenolic compounds were found to be very similar, and a good correlation was observed between the two sets of results. The results for GA are shown in Fig. 6. As can be seen, there is a good correlation between the proposed method and the FC method, in the concentration range of 0.1–1.0 mM (R^2 = 0.9927). In our suggested method, %inhibition is a criterion for comparison of the antioxidant/polyphenolic content. Because of the good correlation between the two methods, it could be concluded that the new method is comparable with a standard method and its results are reliable.

Research on the putative health effects of tea has demonstrated the contribution of phytochemicals, particularly phenolic acids and flavanoids.[65] Tea (*Camellia sinesis*) is the most widely consumed beverage worldwide and has become an important agricultural product.[66] The type and quantity of tea taken varies in different countries and races.[67–69] Black ("fermented") tea is popular in the West; "semifermented" Oolong-type tea is commonly consumed in Taiwan and parts of China; green (nonfermented) tea is favored in the rest of China, Northern Africa, and Japan.[68] Tea contains large amounts of polyphenolic compounds with antioxidant properties. In this work, the proposed method was used to estimate the antioxidant/polyphenolic capacity of different tea samples (*i.e.*, white, green, red and black tea samples). Addition of 20 μL of these tea samples to 3.0 mL of 32 nM of QD in buffer solution before UV irradiation led to a significant inhibition of photobleaching. White tea was able to approximately return the fluorescence of QDs to their original values (Fig. S4†). All experiments were done three times. The antioxidant power of the tea samples obtained by the proposed method was found to follow the order: white

Table 1 Comparison of trend in inhibitory effects of different antioxidant/polyphenolic compounds obtained by the proposed method with those previously reported in the literature

Testing method	Reference method	The order of antioxidant activity	Ref.
Modified TEAC	TEAC	QCN > NGN > TLX	50
Modified FRAP	DPPH	QCN > TA > CA > GA > TLX	51
FRAP-CV	—	QCN > TLX	52
CUPRAC	ABTS/persulfate FC	QCN > CA > GA	53
HPS-CUPRAC	GSH-Px-DTNB (HPLC-ECD)	GA > QCN > CA > TLX	54
MTT	LPO	QCN > CA	55
Modified CUPRAC	TBARS	—	56
ORAC-PGR	—	QCN > GA > TLX > CA	57
ORAC-PGR_{MIC}		GA > QCN > TLX > CA	
ORAC-PGR/ORAC-PGR_{MIC}		QCN > GA > TLX = CA	
HPLC-CUPRAC	CUPRAC	QCN > CA > GA > TLX	58
Ferric–ferrozine	CUPRAC-FRAP-FC	QCN > GA > CA > TLX	59
CERAC	ABTS-CUPRAC	QCN > GA > CA > NGN	60
SIA-CL	DPPH-FC	—	61
FRAP	—	QCN > CA > TLX	62
CV	DPPH-FC-FRAP-TEAC	—	63
QDA	FC	QCN > TA > CA > GA > NGN > TLX	This work

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Table 2 The amount of antioxidant/polyphenolic content of tea samples according to all studied polyphenolic/antioxidant compounds in this work[a]

Type of tea	QCN (M)	TA (M)	CA (M)	GA (M)	NGN (M)	TLX (M)
White	$>1.66 \times 10^{-5}$	1.51×10^{-4}	$>1.66 \times 10^{-4}$	$>6.66 \times 10^{-4}$	$>1.00 \times 10^{-3}$	$>3.33 \times 10^{-4}$
Green	$>1.66 \times 10^{-5}$	1.21×10^{-4}	$>1.66 \times 10^{-4}$	$>6.66 \times 10^{-4}$	8.60×10^{-4}	$>3.33 \times 10^{-4}$
Red	3.72×10^{-7}	2.54×10^{-5}	4.31×10^{-5}	1.02×10^{-5}	1.01×10^{-4}	3.02×10^{-4}
Black	3.8×10^{-7}	2.62×10^{-5}	4.42×10^{-5}	1.11×10^{-5}	1.10×10^{-4}	3.13×10^{-4}

[a] All experiments were repeated three times and RSD for all measurements were lower than 5%.

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tea > green tea > red tea ≈ black tea, which is similar to that previously reported in the literature.[70] The amount of the antioxidant/polyphenolic content of tea samples has been calculated from the equations reported in Table S1†. These quantities were reported relative to all studied polyphenolic/antioxidant compounds (see Table 2). The amount of antioxidant/polyphenolic content of the diluted tea samples was not in the reported linear ranges of antioxidant/polyphenolic compounds, and are presented in Table S1†. It is worth mentioning that the concentrations out of linear ranges of antioxidant/polyphenolic compounds could be measured by appropriate dilution of tea samples.

Similar to other antioxidant methods reported in the literature,[71,72] the proposed method cannot differentiate between antioxidant/polyphenolic compounds and other reducing agents that contribute to the total antioxidant/polyphenolic capacity. Indeed, due to the complexity of the composition of food and biological samples, and taking into account the possible synergistic interactions among the antioxidant compounds in the samples, separation and study of each individual antioxidant/polyphenolic compound is a costly and inefficient process.

Conclusion

In summary, we developed a quantum dot-based assay for the evaluation of antioxidant/polyphenolic activity. The method is based on the capacity of some antioxidant/polyphenolic compounds to inhibit the UV-induced bleaching of fluorescence of chiral CdTe QDs with L-cysteine capping. In the presence of UV irradiation, the excited QDs, by absorbing photons, could react with the surrounding oxygen molecules to produce reactive oxygen species (ROS). Once ROS are produced, they can initiate the oxidation of QDs. The effect of $^1O_2$ on the photobleaching of QDs was confirmed by showing that NaN_3 treatment inhibits this process. The proposed method was applied to the assessment of the antioxidant/polyphenolic activity of individual antioxidant/polyphenolic compounds as well as total antioxidant/polyphenolic activity of four different tea samples. This report also constitutes a starting point for studies related to the application of antioxidant/polyphenolic compounds in fluorescence imaging with QDs. The photostability of QDs in cells could be improved by the use of a proper antioxidant/polyphenolic compound that reduces the UV-induced photobleaching of QDs.

References

1 K. Hensley and R. A. Floyd, *Biochem. Biophys.*, 2002, **397**, 377–383.
2 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, USA, 2007.
3 M. Valko, C. J. Rhodes, J. Moncol, M. Izakovic and M. Mazur, *Chem.-Biol. Interact.*, 2006, **160**, 1–40.
4 K. K. Griendling and G. A. FitzGerald, *Circulation*, 2003, **108**, 1912–1916.
5 G. G. Duthie, S. J. Duthie and J. A. M. Kyle, *Nutr. Res. Rev.*, 2000, **13**, 79–106.
6 P. C. H. Hollman and M. B. Katan, *Food Chem. Toxicol.*, 1999, **37**, 937–942.
7 D. Huang, B. Ou and R. L. Prior, *J. Agric. Food Chem.*, 2005, **53**, 1841–1856.
8 J.-K. Moon and T. Shibamoto, *J. Agric. Food Chem.*, 2009, **57**, 1655–1666.
9 P. A. Kilmartin, H. Zou and A. L. Waterhouse, *J. Agric. Food Chem.*, 2001, **49**, 1957–1965.
10 A. P. Kilmartin, H. Zou and L. A. Waterhouse, *Am. J. Enol. Vitic.*, 2002, **53**, 294–302.
11 J. Piljac, S. Martinez, T. Stipčević, Ž. Petrović and M. Metikoš-Huković, *Am. J. Enol. Vitic.*, 2004, **55**, 417–422.
12 C. S. Petrovic, *Am. J. Enol. Vitic.*, 2009, **60**, 373–378.
13 B. G. Osborn and J. F. Tyson, *Int. J. Food Sci. Technol.*, 2007, **23**, 541–554.
14 L. M. Magalhães, M. Santos, M. A. Segundo, S. Reis and J. L. F. C. Lima, *Talanta*, 2009, **77**, 1559–1566.
15 H. A. G. Niederländer, T. A. van Beek, A. Bartasiute and I. I. Koleva, *J. Chromatogr., A*, 2008, **1210**, 121–134.
16 A. J. Blasco, A. G. Crevillén, M. C. González and A. Escarpa, *Electroanalysis*, 2007, **19**, 2275–2286.
17 S. Rohn and L. W. Kroh, *Mol. Nutr. Food Res.*, 2005, **49**, 898–907.
18 A. Gomes, E. Fernandes and J. L. F. C. Lima, *J. Fluoresc.*, 2006, **16**, 119–139.
19 P. Wardman, *Free Radical Biol. Med.*, 2007, **43**, 995–1022.
20 J. Ma, Y. J. Chen, Y. Zhang, P. N. Wang, J. Guo, W. L. Yang and C. C. Wang, *J. Phys. Chem. B*, 2007, **111**, 12012–12016.
21 Y. Zhang, Y. Li and X.-P. Yan, *Anal. Chem.*, 2009, **81**, 5001–5007.
22 R. Bakalova, H. Ohba, Z. Zhelev, T. Nagase, R. Jose, M. Ishikawa and Y. Baba, *Nano Lett.*, 2004, **4**, 1567–1573.
23 Z. Zhelev, R. Jose, T. Nagase, H. Ohba, R. Bakalova, M. Ishikawa and Y. Baba, *J. Photochem. Photobiol., B*, 2004, **75**, 99–105.
24 Y. Wang, Z. Tang, M. A. Correa-Duarte, I. Pastoriza-Santos, M. Giersig, N. A. Kotov and L. M. Liz-Marzán, *J. Phys. Chem. B*, 2004, **108**, 15461–15469.
25 J. Ma, J.-Y. Chen, J. Guo, C. C. Wang, W. L. Yang, L. Xu and P. N. Wang, *Nanotechnology*, 2006, **17**, 2083–2089.
26 X. Peng, M. C. Schlamp, A. V. Kadavanich and A. P. Alivisatos, *J. Am. Chem. Soc.*, 1997, **119**, 7019–7029.
27 B. Zhao, X. Li, R. He, S. Cheng and X. Wenjuan, *Cell Biophys.*, 1989, **14**, 175–185.
28 B. C. Scott, J. Butler, B. Halliwell and O. I. Aruoma, *Free Radical Res. Commun.*, 1993, **19**, 241–253.
29 N. Cook and S. Samman, *J. Nutr. Biochem.*, 1996, **7**, 66–76.
30 C. A. Rice-Evans, N. J. Miller and G. Paganga, *Free Radical Biol. Med.*, 1996, **20**, 933–956.
31 S. A. Wiseman, D. A. Balentine and B. Frei, *Crit. Rev. Food Sci. Nutr.*, 1997, **37**, 705–718.
32 J. Zhang and X. Shen, *J. Nutr. Environ. Med.*, 1997, **7**, 79–89.
33 S. Miura, J. Watanabe, T. Tomita, M. Sano and I. Tomita, *Biol. Pharm. Bull.*, 1994, **17**, 1567–1572.
34 T. Ishikawa, M. Suzukawa, T. Ito, H. Yoshida, M. Ayaori, M. Nishiwaki, A. Yonemura, Y. Hara and H. Nakamura, *Am. J. Clin. Nutr.*, 1997, **66**, 261–266.

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35 M. Luo, K. Kannar, M. L. Wahlqvist and R. C. O'Brien, *Lancet*, 1997, **349**, 360–361.
36 B. O. Dabbousi, J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen and M. G. Bawendi, *J. Phys. Chem. B*, 1997, **101**, 9463–9475.
37 M. A. Hines and Ph. Guyot-Sionnest, *J. Phys. Chem.*, 1996, **100**, 468–471.
38 I. Stensvold, A. Tverdal, K. Solvoll and O. P. Foss, *Prev. Med.*, 1992, **21**, 546–553.
39 D. Gerion, F. Pinaud, S. C. Williams, W. J. Parak, D. Zanchet, S. Weiss and A. P. Alivisatos, *J. Phys. Chem. B*, 2001, **105**, 8861–8871.
40 J.-K. Moon and T. Shibamoto, *J. Agric. Food Chem.*, 2009, **57**, 1655–1666.
41 Q. Y. Zhu, R. M. Hackman, J. L. Ensunsa, R. R. Holt and C. L. Keen, *J. Agric. Food Chem.*, 2002, **50**, 6929–6934.
42 T. Wang, R. Jónsdóttir and G. Ólafsdóttir, *Food Chem.*, 2009, **116**, 240–248.
43 J. Lee, N. Koo and D. B. Min, *Compr. Rev. Food Sci. Food Saf.*, 2004, **3**, 21–33.
44 K. Liao and M. Yin, *J. Agric. Food Chem.*, 2000, **48**, 2266–2270.
45 ISO 14502-1:2005. Determination of substances characteristic of green and black tea. Part 1: Content of total polyphenols in tea. Colorimetric method using Folin-Ciocalteu reagent, 2005.
46 B. Hemmateenejad, N. A. Mobaraki, F. Shakerizadeh-Shirazi and R. Miri, *Analyst*, 2010, **135**, 1747–1758.
47 W. W. Yu, L. Qu, W. Guo and X. Peng, *Chem. Mater.*, 2003, **15**, 2854–2860.
48 C. Kaur and H. C. Kapoor, *Int.J. Food Sci. Technol.*, 2001, **36**, 703–725.
49 S.-q. Chang, Y.-d. Dai, B. Kang, W. Han, L. Mao and D. Chen, *Toxicol. Lett.*, 2009, **188**, 104–111.
50 R. Berg, G. R. M. M. Haenen, H. Berg and A. Bast, *Food Chem.*, 1999, **66**, 511–517.
51 R. Pulido, L. Bravo and F. Saura-Calixto, *J. Agric. Food Chem.*, 2000, **48**, 3396–3402.
52 O. Firuzi, A. Lacanna, R. Petrucci, G. Marrosu and L. Saso, *Biochim. Biophys. Acta*, 2005, **1721**, 174–184.
53 R. Apak, K. Güçlü, M. Özyürek and S. E. Çelik, *Microchim. Acta*, 2008, **160**, 413–419.
54 M. Özyürek, B. Bektaşoğlu, K. Güçlü, N. Güngör and R. Apak, *J. Food Compos. Anal.*, 2010, **23**, 689–698.
55 Y. Liu and M. G. Nair, *J. Nat. Prod.*, 2010, **73**, 1193–1195.
56 M. Özyürek, B. Bektaşoğlu, K. Güçlü and R. Apak, *Anal. Chim. Acta*, 2008, **616**, 196–206.
57 M. Romero, B. Rojano, J. Mella-Raipán, C. D. Pessoa-Mahana, E. Lissi and C. López-Alarcón, *Molecules*, 2010, **15**, 6152–6167.
58 S. E. Çelik, M. Özyürek, K. Güçlü and R. Apak, *Anal. Chim. Acta*, 2010, **674**, 79–88.
59 K. I. Berker, K. Güçlü, B. Demirata and R. Apak, *Anal. Methods*, 2010, **2**, 1770–1778.
60 D. Ozyurt, B. Demirata and R. Apak, *J. Fluoresc.*, 2011, **21**, 2069–2076.
61 E. Fassoula, A. Economou and A. Calokerinos, *Talanta*, 2011, **85**, 1412–1418.
62 B. L. Halvorsen and R. Blomhoff, *Food Chem.*, 2011, **127**, 761–768.
63 D. Zhang, L. Chu, Y. Liu, A. Wang, B. Ji, W. Wu, F. Zhou, Y. Wei, Q. Cheng, S. Cai, L. Xie and G. Jia, *J. Agric. Food Chem.*, 2011, **59**, 10277–10285.
64 I. Parejo, F. Viladomat, J. Bastida, A. Rosas-Romero, N. Flerlage, J. Burillo and C. Codina, *J. Agric. Food Chem.*, 2002, **50**, 6882–6890.
65 T. Bahorun, A. Luximon-Ramma, A. Crozier and O. I. Aruoma, *J. Sci. Food Agric.*, 2004, **84**, 1553–1561.
66 D. A. Balentine, H. C. Tang, Y. L. Chang and H. H. Tuan, Manufacturing and Chemistry of Tea, *Phenolics Compounds in Food and their Effects on Health*, American Chemical Society, 1992, vol. 1, Ch. 8.
67 J. H. Weisbuger, E. Cadenas and L. Packer, Tea Antioxidants and Health, *Handbook of Antioxidants*, Dekker, New York, 1996, Ch. 15.
68 L. Kohlmeier, *Br. J. Nutr.*, 1997, **78**, 1–3.
69 L. Kohlmeier, K. G. C. Weterings, S. Steck and F. J. Kok, *Nutr. Cancer*, 1997, **27**, 1–13.
70 M. P. Almajano, R. Carbo, J. A. L. Jiménez and M. H. Gordon, *Food Chem.*, 2008, **108**, 55–63.
71 F. Sari, N. Turkmen, G. Polat and Y. S. Velioglu, *Food Sci. Technol. Res.*, 2007, **13**, 265–269.
72 M. Scampicchio, J. Wang, A. J. Blasco, A. Sanchez Arribas, S. Mannino and A. Escarpa, *Anal. Chem.*, 2006, **78**, 2060–2063.

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