Ultrathin g-C3N4/TiO2 composites as photoelectrochemical elements for the real-time evaluation of global antioxidant capacity†

Weiguang Ma,ab Dongxue Han,ab⁎ Min Zhou,ab Hao Sun,c Lingnan Wang,ab Xiandui Dongab and Li Niu⁎ab

The antioxidants in biological organisms can scavenge excess free radicals and effectively reduce oxidative stress, which protects DNA, protein and lipids in the human body from damage, thus preventing diseases from being induced. Therefore, it is particularly significant to assay the antioxidant capacities of our habitual foods during dietary evaluation. Herein, ultrathin graphitic carbon nitride (utg-C3N4)/TiO2 composites have been introduced as sensing elements into a photoelectrochemical platform with a thin layer structured flow-cell, for the real-time assay of the global antioxidant capacity in practical samples. In this system, the two-dimensional utg-C3N4 nanosheet/TiO2 nanoparticle composite material provided a much better optoelectronic function than the individual materials. In comparison with previous reports, this photoelectrochemical strategy shows considerable advantages, including excellent anti-interference properties, a high level of stability and reproducibility, and it is also proved to be the most prompt, convenient and cost-effective method for antioxidant capacity detection up to now. Moreover, utilizing theoretical and experimental examinations, we revealed its photoelectrochemical sensing mechanism in depth. It is proposed that the developed method will pave the way for the development of excellent antioxidant assays with the advantages of photoelectrochemistry and fluidic cells. It is expected to be further applied in food quality inspections and health guides, as well as in other fields.

Introduction

High concentrations of free radicals will induce oxidative stress, which can damage DNA, protein and lipids in organisms, leading to serious diseases such as Alzheimer’s, Parkinson’s, cancer, etc.¹ Antioxidants, which exist in a wide range of foods, can effectively scavenge free radicals and protect organisms, helping them to maintain a healthy state.² Therefore, it is of significant importance to assay the antioxidant capacity in foods. At present, several methods have been developed for the evaluation of antioxidants including chromatography,³ spectrophotometry⁴ and electrochemical methods.⁵ Although great progress has been achieved using these methods, several problems still need to be resolved: (i) with the chromatographic method, it is the concentrations of individual antioxidants, but not the global antioxidant capacity, of the studied system that is determined; (ii) results are frequently interfered with by the inherent colour of foods during the detection process when spectral methods are applied; (iii) the working electrode is apt to be fouled by production generated at the electrode, which seriously affects detection via the electrochemical method. To solve these problems, therefore, a more suitable method, with the properties of rapid response time, high stability and excellent anti-interference, is in urgent need of development for the detection of the antioxidant capacities of foods.

Bearing these facts in mind, a photoelectrochemical platform has herein been designed, with ultrathin graphitic carbon nitride/titanium dioxide components (utg-C3N4/TiO2) serving as the photoelectrochemical elements for the assay of the antioxidant capacities of foods (scheme in Fig. 1). It is well known that use of a material with good optoelectronic properties plays a key role in the creation of an excellent photoelectrochemical platform. Although TiO2 has a lot of remarkable properties such as good photostability, a chemically/biologically inert nature, nontoxicity and a high electronic mobility, the performance of
pristine TiO₂ will not sufficiently meet the needs of an ideal photoelectrochemical platform. Inorganic semiconductors (such as CdS, ZnS, MoS₂) were used to functionalize TiO₂ to improve its properties, due to their narrow bandgap. However, toxicity and corrosion limit their further application. Owing to its outstanding properties, which include a metal-free nature, nontoxicity, a narrow bandgap, and easy processability into desired shapes, graphitic carbon nitride (g-C₃N₄) has been widely explored by many researchers. In g-C₃N₄/TiO₂ composites, due to the proper band level between the g-C₃N₄ and the TiO₂, the photo-generated electron and hole can be easily separated. This results in a great improvement of the photocatalytic activity. In previous reports, g-C₃N₄/TiO₂ composites have been synthesized for hydrogen evolution and environmental purification. Yet, bulk g-C₃N₄ materials, with a low specific surface area and poor solubility, were used in this research, which limited their photoelectrochemical properties. Luckily, g-C₃N₄ nanosheets with atomic-scale thickness can greatly promote the photoresponse in contrast to bulk materials due to their high specific surface area. The fully exposed surface of the ultrathin g-C₃N₄ (utg-C₃N₄) nanosheets makes it possible to utilize all of the surface active sites. Recent theoretical investigations also revealed that utg-C₃N₄ nanosheets exhibit unique electronic and optical properties. In this work, utg-C₃N₄/TiO₂ composites were synthesized via a simple method. Compared to g-C₃N₄/TiO₂, the photoelectrochemical properties of utg-C₃N₄/TiO₂ are greatly improved. By employing utg-C₃N₄/TiO₂ composites as photoelectrochemical elements, a thin layer flow cell has been designed and the resulting system demonstrated a series of advantageous properties, such as a rapid response, and anti-fouling and colour-interference-proof properties. These important features finally allowed the real-time assay of the antioxidant capacities of foods (e.g. tea, coffee, etc.).

Results and discussion

The chemical synthesis route for the utg-C₃N₄/TiO₂ nanocomposites is illustrated in Fig. 1. Typically, bulk g-C₃N₄ (bg-

C₃N₄) was first synthesized via thermal polycondensation of melamine at 550 °C, according to the previous literature. Thereafter, the obtained bg-C₃N₄ was ultrasonicated for 2 h in water and utg-C₃N₄ nanosheets with a few layers were obtained. Cross-sectional atomic force microscopy (AFM) was further conducted to investigate the structural features of the utg-C₃N₄ nanosheets (Fig. 2a). Randomly measured nanosheets demonstrated very close thicknesses of ~1.2 nm (Fig. 2b). This indicated that the exfoliated nanosheets were comprised of less than five C-N layers, which is much thinner than those reported previously. A TEM image of utg-C₃N₄ also showed similar results (Fig. 2c). Unlike bg-C₃N₄, the utg-C₃N₄ nanosheets can be very well dispersed in water, and the suspension can even be maintained for a few weeks without aggregation occurring (Fig. S2, ESIF). Finally, ca. 10 nm TiO₂ nanoparticles were uniformly decorated on to the as-prepared utg-C₃N₄ nanosheets (Fig. 2e) via a self-assembly process. High-resolution TEM (HRTEM) analysis revealed the highly crystalline features of the TiO₂ nanoparticles, with a lattice spacing of 0.350 nm (Fig. 2f), which can be assigned to the (101) plane of anatase TiO₂.

X-ray diffraction (XRD) patterns of bg-C₃N₄, utg-C₃N₄ nanosheets, and utg-C₃N₄/TiO₂ nanocomposites are shown in Fig. 3a. For bg-C₃N₄, the strong XRD Bragg peak at 27.7° is attributed to the characteristic interlayer stacking reflection of conjugated aromatic systems, with graphic materials assigned to the (002) peak. After exfoliation, the intensity of the (002) peak significantly decreased (Fig. 3a), which demonstrated that the layered g-C₃N₄ has been successfully exfoliated into the layer structures as expected. Bragg peaks corresponding to TiO₂ and the utg-C₃N₄ nanosheets can also be observed simultaneously in Fig. 3a, which indicated that consolidated structures of utg-C₃N₄/TiO₂ have been successfully obtained. X-ray photoelectron spectroscopy (XPS) measurements were taken to probe the chemical composition of utg-C₃N₄ and utg-C₃N₄/TiO₂. As shown in Fig. 3b, the utg-C₃N₄ nanosheet sample exhibited C1s and N1s signals with a C/N ratio of 1.31, which is very close to the chemical composition of utg-C₃N₄ and utg-C₃N₄/TiO₂. Inorganic semiconductors (such as CdS, ZnS, MoS₂) were used to functionalize TiO₂ to improve its properties, due to their narrow bandgap. However, toxicity and corrosion limit their further application. Owing to its outstanding properties, which include a metal-free nature, nontoxicity, a narrow bandgap, and easy processability into desired shapes, graphitic carbon nitride (g-C₃N₄) has been widely explored by many researchers. In g-C₃N₄/TiO₂ composites, due to the proper band level between the g-C₃N₄ and the TiO₂, the photo-generated electron and hole can be easily separated. This results in a great improvement of the photocatalytic activity. In previous reports, g-C₃N₄/TiO₂ composites have been synthesized for hydrogen evolution and environmental purification. Yet, bulk g-C₃N₄ materials, with a low specific surface area and poor solubility, were used in this research, which limited their photoelectrochemical properties. Luckily, g-C₃N₄ nanosheets with atomic-scale thickness can greatly promote the photoresponse in contrast to bulk materials due to their high specific surface area. The fully exposed surface of the ultrathin g-C₃N₄ (utg-C₃N₄) nanosheets makes it possible to utilize all of the surface active sites. Recent theoretical investigations also revealed that utg-C₃N₄ nanosheets exhibit unique electronic and optical properties. In this work, utg-C₃N₄/TiO₂ composites were synthesized via a simple method. Compared to g-C₃N₄/TiO₂, the photoelectrochemical properties of utg-C₃N₄/TiO₂ are greatly improved. By employing utg-C₃N₄/TiO₂ composites as photoelectrochemical elements, a thin layer flow cell has been designed and the resulting system demonstrated a series of advantageous properties, such as a rapid response, and anti-fouling and colour-interference-proof properties. These important features finally allowed the real-time assay of the antioxidant capacities of foods (e.g. tea, coffee, etc.).

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The introduction of bg-C3N4, the photocurrent greatly increased to could be attributed to the following reasons: (i) utg-C3N4 presents a better conductivity than that of bg-C3N4 (Fig. 4b); (ii) TiO2 nanoparticles can disperse more uniformly onto the utg-C3N4, thus efficiently reducing the recombination between electrons and holes. As shown in Fig. 4c, the charge-transfer resistance of utg-C3N4/TiO2 is 685.6 Ω cm², which is much smaller than that of bg-C3N4/TiO2 (1429.0 Ω cm²) under visible light radiation. This smaller arc radius implies a much higher efficiency of charge transfer. In a fluorescence quenching experiment, the fluorescence intensity of utg-C3N4 is observed to gradually decrease at 440 nm with an increase in the TiO2 concentration (Fig. 4d), as the recombination between electrons and holes is suppressed.

As shown in Fig. 4a, upon the addition of 50 μmol L⁻¹ catechin (CT, a typical example of an antioxidant), the photocurrents of all three modified electrodes increased as expected, which can be attributed to the oxidation of CT by the holes. Among these cases, utg-C3N4/TiO2 exhibited the largest photocurrent (284.28 nA), which was about 5.1 and 1.7 times greater than those of pristine TiO2 and bg-C3N4/TiO2, respectively. Other antioxidants also showed obvious signals on the utg-C3N4/TiO2-modified electrode. Therefore, the utg-C3N4/TiO2 nano-composite material seems to be an advisable candidate for use in antioxidant capacity assays.

For the development of an antioxidant capacity detection device, a kind of static cell was firstly introduced (Fig. S6a, ESIf). When using this device, although a photoelectrochemical assay could be realized and excellent responses were obtained towards various antioxidants, the utg-C3N4/TiO2-modified electrode was easily fouled during successive electrochemical measurements (Fig. S7a, ESIf). In order to improve the recyclability of the photocatalyst, a flow photoelectrochemical cell with a thin layer structure was then developed to replace the initial static cell (Fig. 5a, Fig. S6b and c, ESIf). Such a flow photoelectrochemical cell shows specific properties of high...
mass diffusion and depletion. With only a very small amount of sample needed, strong signals and a high detection sensitivity could be obtained. Just as expected, the photocurrent is quite stable even after a long period of activity in such a flow photoelectrochemical cell (Fig. S7b, ESI†). This has successfully confirmed the recyclability of the photocatalyst, and made this device an excellent candidate for the real-time evaluation of antioxidant capacity. As shown in Fig. S8 (ESI†), 0 V was selected for carrying out the photoelectrochemical assay, since simultaneous advisable sensitivity and anti-interference can be achieved at this potential. Then, the antioxidant capacities of nine typical antioxidants, including quercetin (QR), gallic acid (GA), caffeic acid (CA), catechin (CT), fisetin (FT), rutin (RT), trolox (TR), ascorbic acid (AA) and glutathione (GSH), were successfully detected. As shown in Table S1 (ESI†), all of these nine antioxidants exhibited good responses with a wide linear range. Among them, the relationships between the photoelectrocurrent and the concentrations of CA, CT and AA are shown in Fig. 5. Their linear concentration ranges are depicted as 15.00–193.31 μmol L⁻¹, 24.96–192.31 μmol L⁻¹ and 25.00–243.09 μmol L⁻¹, respectively, and their relative standard deviations (RSD, %) are 4.6%, 3.2% and 5.6%, respectively. It is noticeable that, even after continual use for two weeks, such photoelectrochemical sensors could still perceive at least 95% of the initial detection signals, which indicated that the fouling of the photocatalyst could be reduced to a great extent within such a thin layer photoelectrochemical flow cell system.

Theoretically, two main mechanisms for the sensor have been proposed, including hydrogen atom transfer and single electron transfer during the reaction process of the antioxidant species. As shown in Fig. 6, the valence band (VB) of utg-C₃N₄ is found to be −6.257 eV, as obtained from Mott–Schottky (Fig. S9, ESI†) and UV-vis diffuse reflectance spectra (Fig. S3b, ESI†). This is much higher than the oxidation of H₂O (−5.30 eV). However, in this system a fluorescence peak at 425 nm could not be observed in a solution containing terephthalic acid and utg-C₃N₄/TiO₂ under visible light irritation for various time periods (Fig. S10, ESI†). This indicated that no hydroxyl radical was produced in this case. Therefore, such a photoelectrochemical approach for antioxidant capacity assays, using utg-C₃N₄/TiO₂ as a photocatalyst, is ascribed to an electron transfer process. The detailed mechanism is proposed to be: when utg-C₃N₄ is excited by visible light irritation, an electron (e⁻) and hole (h⁺) are generated, and an immediate electron transfer to the conduction band (CB) of TiO₂ occurs. This is followed by the prompt arrival of the electron at the ITO substrate, leading to the generation of a photocurrent. Meanwhile, with the introduction of antioxidants, the h⁺ of the utg-C₃N₄ can be refilled by electrons from the antioxidants, and these occupied holes are then ready for the next excitation. This process could significantly enhance the photocurrent. Therefore, we believe that based on single electron transfer reactions, the regeneration ability of h⁺ and e⁻ transfer in the utg-C₃N₄/TiO₂ nanocomposite during antioxidant reactions should be defined as a comprehensive antioxidant capacity evaluation, rather than a simple detection of antioxidant concentration.

As shown in Fig. 6, CT, CA, FT, GA, QR, and RT showed amazing antioxidant capacity, in contrast to TR, AA and GSH. Since the mechanism of the present photoelectrochemical sensor is based on an electron transfer reaction, the stronger the antioxidant capacity is, the easier it is for it to lose an electron. In order to clarify the antioxidant capacity of different kinds of antioxidant, a detailed reaction process between the photocatalyst and antioxidants under visible light radiation has been thoroughly investigated. First, the ionization potential of each individual antioxidant was estimated using a B3LYP/6-311G (d,p) method, through the theoretical structural simulation of each antioxidant molecule, since the ionization potential is the most significant energetic factor for antioxidant capacity.
evaluation. It is observed from Fig. 6 that most of the phenol samples, for example, CT, CA, FT, GA, QR, and RT, have relatively low ionization potentials and show strong antioxidant capacities. This conclusion can also be proved by calculating the results for l-cysteine and glucose, which do not cause any interference with our detection of the antioxidant capacities of food (Fig. S12, ESIF) due to their high ionization potentials (187.95 kcal mol\(^{-1}\) and 191.17 kcal mol\(^{-1}\)). However, the results for GA and TR are disputable if this explanation is used. Other factors should therefore be considered here besides the ionization potential. The redox potentials of the antioxidants on a glassy carbon electrode were thus determined, which is also key for the evaluation of antioxidant capacity. As expected, GA showed a low redox potential and a high antioxidant capacity. Compared to GSH, other phenols also presented low redox potentials (Fig. S11, ESIF) and high antioxidant capacities. It is suggested that the effect of molecular structure also plays a key role in this photoelectrochemical reaction. Theoretical analysis indicated that the number and position of the hydroxyl groups, as well as the degree of conjugation of the entire molecule, can also affect the antioxidant capacity assay. Since CT, FT, QR, and RT present two ortho-hydroxyl groups, they showed a stronger antioxidant capacity in contrast to TR. The contact mode between the antioxidants and photocatalyst is another important factor during the reaction. The utg-C\(_3\)N\(_4\) has a \(\pi\)-conjugated structure, which can easily induce adsorption of the benzene-structured antioxidants through \(\pi\)–\(\pi\) interactions, promoting electron transfer. Considering all of the above factors, it is concluded that phenol antioxidants, such as CT, CA, FT, QR, GA and RT, should show higher antioxidant capacities, while TR, AA and especially GSH might present lower antioxidant capacities. This result is similar to that obtained with the optical method.

The anti-interference characteristics of this photoelectrochemical sensor were further investigated. A typical antioxidant system was studied as an example, which contains 25 \(\mu\)mol L\(^{-1}\) GA as well as some possible interfering species commonly found in food and drink (shown in Fig. S12, ESIF). Just as shown, 25 mmol L\(^{-1}\) L-proline, L-glycine, L-histidine, ethanol, methanol, 12.5 mmol L\(^{-1}\) of L-threonine, fructose, glucose, L-citric acid, L-malic acid and 0.5 mmol L\(^{-1}\) L-cysteine did not cause distinct interference with the results of the antioxidant capacity assays using our photoelectrochemical sensor.

These results further confirmed that the present photoelectrochemical sensor can be applied in practical complex conditions for antioxidant capacity evaluation.

Additionally, using this photoelectrochemical sensor, the antioxidant capacity of four brands of commercial tea and four brands of commercial coffee were examined. Compared to coffee, all of the tea samples presented higher antioxidant capacities (Table 1), and all these results were found to be consistent with the results of both the Folin–Ciocalteu (F–C) and DPPH methods. The detection data for coffee as obtained by our photoelectrochemical measurement is smaller than that of the F–C method, which should be assigned to the possible interference by the natural coffee and reducing sugar colours in the F–C method. Here, with our photoelectrochemical detection system, the utg-C\(_3\)N\(_4\)/TiO\(_2\) component-modified ITO electrode was irradiated from the back, which can completely avoid such colour interference during antioxidant capacity determination. Therefore, the results of such a novel photoelectrochemical sensor would be closer to the true values.

### Conclusions

In summary, as an excellent optoelectronic material, utg-C\(_3\)N\(_4\)/TiO\(_2\) composites have been designed and applied in a thin layer structured photoelectrochemical flow cell to detect the antioxidant capacities of food. Detailed studies have unambiguously revealed that, besides advantages including rapid response time, high sensitivity, long-lasting stability and minimal sample requirement, this photoelectrochemical platform can fully overcome three major problems of other antioxidant capacity assays (global antioxidant capacity determination, interference by sample colours and poisoning of the working electrode). Furthermore, the mechanism of this photoelectrochemical sensor was elaborately explored based on a single electron transfer reaction, and the results are comparable with the Folin–Ciocalteu and DPPH methods. By using such a thin layer photoelectrochemical flow cell, the actual samples could be rapidly detected in real time. This novel method paves the way to a high-performance antioxidant capacity assay combining the advantages of photoelectrochemistry and fluidic cells, which is expected to be further applied in developing analytical instruments for commercialization.

### Table 1

The antioxidant capacities of teas (T) and coffee (C) as found with our photoelectrochemical sensor, the Folin–Ciocalteu method and the DPPH method, respectively.

<table>
<thead>
<tr>
<th>Practical samples</th>
<th>Photoelectrochemical sensor (mg g(^{-1}) GA)</th>
<th>Folin–Ciocalteu method (mg g(^{-1}) GA)</th>
<th>DPPH method (mg g(^{-1}) trolox)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(_1)</td>
<td>72.212 ± 0.612</td>
<td>62.190 ± 0.426</td>
<td>163.610 ± 0.925</td>
</tr>
<tr>
<td>T(_2)</td>
<td>95.860 ± 0.536</td>
<td>88.610 ± 0.731</td>
<td>227.880 ± 0.834</td>
</tr>
<tr>
<td>T(_3)</td>
<td>21.010 ± 0.214</td>
<td>23.270 ± 0.198</td>
<td>113.600 ± 0.736</td>
</tr>
<tr>
<td>T(_4)</td>
<td>30.570 ± 0.165</td>
<td>34.960 ± 0.767</td>
<td>132.560 ± 0.961</td>
</tr>
<tr>
<td>C(_1)</td>
<td>3.334 ± 0.068</td>
<td>9.020 ± 0.213</td>
<td>14.740 ± 0.346</td>
</tr>
<tr>
<td>C(_2)</td>
<td>3.759 ± 0.086</td>
<td>9.645 ± 0.324</td>
<td>15.490 ± 0.425</td>
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<tr>
<td>C(_3)</td>
<td>1.937 ± 0.034</td>
<td>5.520 ± 0.228</td>
<td>6.890 ± 0.123</td>
</tr>
<tr>
<td>C(_4)</td>
<td>2.353 ± 0.056</td>
<td>6.830 ± 0.621</td>
<td>9.682 ± 0.456</td>
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</table>
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Notes and references


