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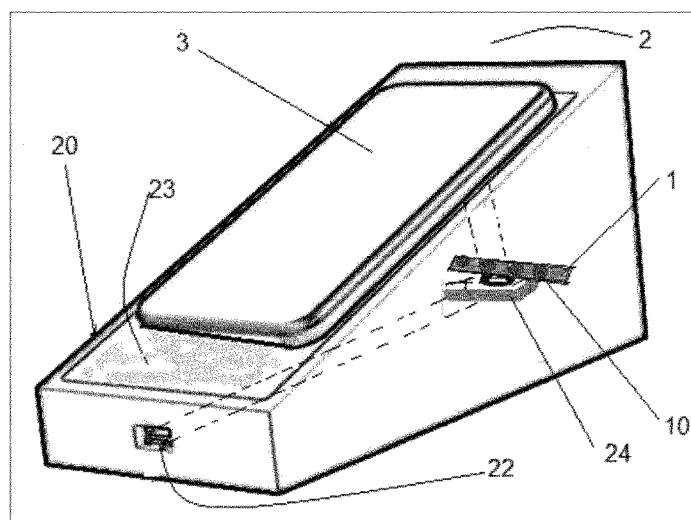
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(54) Title: AN ANALYTICAL TEST SUBSTRATE AS FLUORESCENT PROBE FOR PERFORMING A DETECTION OF AN ANALYTE, A PORTABLE DEVICE FOR PERFORMING SUCH DETECTION AND A SYSTEM THEREOF

FIG 1A



(57) Abstract: The application discloses an analytical test substrate (1) comprising PL-GQDs, as fluorescent probes, suitable for performing a detection of an analyte on the basis of the Förster resonance energy transfer (FRET) effect. The present application also discloses a portable device (2) having a body (20), light means (21), energy connection means (22) light-readout means (23) and electronic processor means (24), which is suitable for performing a detection of an analyte. The present application also discloses a system for performing a detection of an analyte comprising the analytical test substrate (1), the portable device (2) and being capable of detecting the analyte on the bases of the Förster resonance energy transfer (FRET) effect. The present application further discloses a kit comprising the portable device (2), the analytical test substrate (1) and instructions for carrying out such detection.



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AN ANALYTICAL TEST SUBSTRATE AS FLUORESCENT PROBE FOR PERFORMING A DETECTION OF AN ANALYTE, A PORTABLE DEVICE FOR PERFORMING SUCH DETECTION AND A SYSTEM THEREOF

Field of the invention

5 The present invention relates to the field of detection of an analyte from a sample. In particular, the invention relates to an analytical test substrate comprising PL-QDs, as fluorescent probes, suitable for performing a detection of an analyte on the basis of the Förster resonance energy transfer (FRET) effect.

The present invention also relates to a portable device suitable for performing such detection,
10 and to a system for performing such detection as well as to a kit comprising a portable device, an analytical test substrate and instructions for carrying out such detection.

Background of the invention

Recently, Petryayeva, E. et al. in "*Proteolytic assays on quantum-dot-modified paper substrates using simple optical readout platforms*", Analytical chemistry, 85 (18), 8817-8825
15 (2013) discloses paper-based assays for diagnostic enzymes and ratiometric transduction of nucleic acid hybridation by using paper fibers chemically modified with thiol ligands to immobilize CdSeS/ZnS QDs, so that the QDs have been self-assembled with dye-labeled peptides to generate efficient FRET, and steady-state and fluorescence lifetime imaging microscopy (FLIM) were used for characterization. This type of assay was used for detecting
20 colorimetric changes.

Also, Ahmed, G. et al. in "*Highly fluorescent carbon-dots as nano-probes for sensitive and selective determination of 4-nitrophenol in surface waters*", Microchimica Acta, 182 (1-2), 51-59 (2015) discloses synthesis of carbon dots (C-dots) by thermal carbonization of a mixture of ethylenglycol bis-(2-aminoethyl ether)-N, N,N',N'-tetraacetic acid (EGTA) and
25 tris(hydroxymethyl)aminomethane (Tris). These C-dots were highly fluorescent with an emission peak that shifts from 427 to 438 nm if the excitation wavelength is increased from 310 to 360-370nm. The fluorescence was quenched by 4-nitrophenol. This method has been used for detecting 4-nitrophenol in a test solution of spiked river and sea water.

By the other side, it has been disclosed the use of graphene quantum dots for detecting an
30 analyte in a test solution through cyclic voltammetry and a three electrode system via the graphene quantum dots, such as in CN106248769 or CN106290543.

Patent Application US2014/193839 describes the use of an image sensor of a mobile device as a spectrophotometer, with a light source to illuminate the detection platform. As a result, the

mobile captures the image and the detection is made by identifying the wavelength of the spectrum being analyzed. The use of a light source to excite graphene quantum dots in order to excite their own light is not described nor hinted, and neither their advantages.

Patent Application US2014/312247 describes the use of two filters, one for excitation at a specific wavelength of a type of fluorophore, and another to filter the emission light at a specific wavelength and this is captured by the mobile. The use of a filter only to block the excitation wavelengths from being captured by the mobile is not described nor hinted, and neither their advantages.

Rongbin Xie et al: "Graphene quantum dots as smart probes for biosensing", Analytical Methods, vol. 8, no. 20, 21 April 2016, pp 4001-4016, ISSN:1759-9660, despite being a review of the quantum dots of graphene and applications, throughout the document does not describes, nor hint an analytical test substrate of paper as a probe for sensing. Quenching has been disclosed related to the use of graphene oxide in order to turn off the quantum dots. All these articles do conceptual tests in solution without using any detection platform either in paper or another substrate. They use expensive laboratory equipment.

Therefore, up to day, aqueous solutions and spectroscopic equipment, fluorimeters and sophisticated fluorescence microscopes are required to detect analytes by similar procedures, which became an expensive sensing system that cannot be used for fast and in-field applications. Accordingly to the current technologies, firstly the user takes the sample, and later, on request, send it to a specialized laboratory to perform the corresponding analysis, thus time consuming, requiring too many preparation steps, the techniques used for analysis are complicated and in most cases the generated signals results difficult to interpret by a non-specialized user.

Therefore, there is still the need to provide a simplest, faster, and easier way for detecting an analyte in-situ, which further be reliable, inexpensive, with reproducible data and which can be carried out by a user without requiring specific knowledges.

There is also the need to provide a portable device that enables such detection with the above stated advantages.

Description of the invention

The present invention was made in view of the prior art described above, and the first object of the present invention is to provide an analytical test substrate comprising PL-GQDs, as fluorescent probes, suitable for performing a detection of an analyte on the basis of the Förster resonance energy transfer (FRET) effect in a solid state.

To solve the problem, the analytical test substrate comprising PL-GQDs is characterized in that it is of paper and comprises:

- i) PL-GQDs embedded in the paper, the PL-GQDs being deprotonated GQDs, and
 - ii) at least one area delimited by a hydrophobic barrier to confine the embedded PL-GQDs
- 5 within this area, thereby, in use, the area is suitable to receive the analyte to be detected and the PL-GQDs embedded therein emit fluorescence light when excited with UV light.

Advantageously, the analytical test substrate of the first aspect is suitable for performing a detection in solid state in a simplest, faster and easier way, which a user can be performed without requiring specific knowledges.

- 10 The analytical test substrate can comprise a plurality of delimited areas each one confining PL-GQDs therein.

In the present invention, "sensing areas" means the same as "delimited areas".

- The hydrophobic barrier can be made of a waxy material. The meaning of a "waxy material" is of general knowledge for a skill person in the art. It is known in the art that waxes are a diverse
- 15 class of organic compounds that are lipophilic, malleable solids near ambient temperatures. They include higher alkanes and lipids, typically with melting points above about 40 °C, melting to give low viscosity liquids. Waxes are insoluble in water but soluble in organic, nonpolar solvents. Therefore, a waxy material also encompasses a liquid wax including a wax dissolved in an organic, nonpolar solvent.

- 20 Although the scope of the present invention is not restricted to a determined analytical test paper, it is preferable that the analytical test paper be of nitrocellulose for properties reasons. Advantageously, the detection assays carried out in a nitrocellulose paper have shown higher capabilities of PL-GQDs confinement. In an embodiment, the PL-GQDs are embedded in the nitrocellulose paper.

- 25 The substrate or the paper, preferably, has a strip shape.

In a second aspect, the present invention refers to the use of the analytical test substrate comprising PL-GQDs of the first aspect for performing a detection of an analyte.

- Different types of analytes can be detected by using the analytical test substrate of the first aspect like flavonols such as quercetin, miricetin, kaemferol, morin; anthocyanidins such as
- 30 cyanidin, delphinidine and aurantadine, pollutants such as nitrophenols; or PL-GQDs.

In the present invention, the analytical test substrate works as a fluorescent probe, that is as

a sensor, due to the PL-GQDs in a particular arrangement and configuration in the paper. Thus, a fluorescence quenching, a fluorescence light emitted by PL-GQDs, an intensity of the fluorescence or even a brightness of an image can be sensed and quantified. As an example, the brightness values of an image can be calculated by converting RGB pixels to brightness values using the formula $V=(R+G+B)/3$, wherein R=RED, G=GREEN, B=BLUE, each one in the range from 0 to 255. Additional information can be revised in https://en.wikipedia.org/wiki/RGB_color_model.

In the invention, the term "sensor" means everything that has a property sensitive to a magnitude of the medium, and by varying this magnitude also varies with some intensity the property, that is, the presence of that magnitude, and also its measurement. A sensor in the industry is an object capable of varying a property before physical or chemical magnitudes, called instrumentation variables, and transforming them with a transducer into electrical variables.

In a third aspect, the invention provides a method for manufacturing the analytical test substrate of the first aspect. The method comprises the following steps:

- a. imprinting hydrophobic barrier patterns delimiting at least one area in the paper;
- b. preparing an aqueous solution including PL-GQDs, wherein the aqueous solution is buffered at a pH ranging from 8 to 13 by using a base, thereby the PL-GQDs in the basic solution result in deprotonated PL-GQDs, which show their fluorescence when they are irradiated with UV light; and
- c. wetting the delimited area in the paper with the deprotonated PL-GQDs solution and let it drying at room temperature, thereby the PL-GQDs being adsorbed in the paper.

In step a), a waxy material is employed to imprint the hydrophobic barrier pattern. Preferable, the barrier pattern is circular. Then, a thermal treatment is performed to melt the wax printed in the paper to form the hydrophobic barrier and, thus, to delimit a sensing area in which the PL-GQDs are adsorbed.

The PL-GQDs are deposited onto or into the analytical test substrate after a wax-printing method as defined in step a) above is performed.

In step b), the aqueous solution includes PL-GQDs in a concentration between 0.5 mg/ml and 500 mg/ml. More preferable, the aqueous solution includes PL-GQDs in a concentration higher than 12.5 mg/ml. The minimum concentration of PL-GQDs can vary depending on factors such as the analyte to detect, the type of paper. The solution including PL-GQDs are buffered with

an inorganic base, preferably at a concentration from 8 mg/ml to 10 mg/ml, in order the solution has a pH ranging from 8 to 13.

Once prepared the solution containing PL-GQDs, step c) is performed.

In step c), the prepared solution is deposited into the delimited area in order to wet the paper in such area. The solution can be pipetted on the paper substrate. The solution can be buffered to a pH ranging from 8 to 10, specially for detecting anthocyanidins, flavonols, as analyte. The solution can be buffered to a pH ranging from 10 to 13, specially for detecting nitrophenol pollutants, as analyte.

The amount of solution deposited on the paper sensing area can vary depending on the area size to be wetted. In an embodiment, the PL-GQDs are pipetted on the substrate at a concentration which ranges from 1 μ l of a solution at 6.25 mg/ml of PL-GQDs to 4 μ l of a solution at 50mg/ml of PL-GQDs.

A solution containing PL-GQDs with a concentration from 0.5 mg/ml to 500 mg/ml is equivalent to a concentration of PL-GQDs in the paper from 5 to 20 mg/mm³.

In a fourth aspect, the invention provides a portable device suitable for performing a detection of an analyte from a sample received in an analytical test substrate on the basis of the Förster resonance energy transfer (FRET) effect by means of an analytical test substrate.

In a first variant, the portable device comprises an analytical test substrate, a body, light means, energy connection means, light-readout means, and electronic processor means, and is characterized in that

- the analytical test substrate is as defined in the first aspect of the invention, and is configured to receive, in use, the sample containing the analyte to be detected, and to emit fluorescence light when excited with UV light, and

- the light means are UV means,

wherein

- the body is configured and adapted to house therein at least part of the analytical test substrate,
- the UV means operates on the basis of ultraviolet wavelength and is configured and arranged in the body
 - to irradiate UV light at the analytical test substrate for exciting the PL-GQDs comprised therein to make them emit fluorescence light in a wavelength range; and

- to generate a fluorescence-indicative signal (S211, S212) in the analytical test substrate which is indicative of the quenching of the fluorescence light emitted by the excited PL-GQDs and reveals the presence of an analyte by quenching the fluorescence light emitted of the excited PL-GQDs in case an analyte is received in the analytical test substrate and the analyte has an absorption wavelength range that at least in part overlaps the fluorescence light emitting wavelength range of the excited PL-GQDs;

- the energy connection means are configured and adapted to be coupled to an energy source;
- the electronic processor means are operatively connected to the energy connection means to supply energy to the UV means; and
- the light-readout means are associated to the analytical test substrate for taking of an optical readout of the fluorescence-indicative signal (S211, S212) generated in the analytical test substrate, and are adapted to provide the optical readout to a user of a quenching of the fluorescence light emitted by the PL-GQDs.

Advantageously, the portable device provides a fast and reliable data of the presence or absence of the analyte without requiring the use of expensive equipment, nor requiring the data resulting from the detection to be subsequently analyzed in a computer.

Advantageously, the portable device is capable to detect several chemical compounds as analytes, without requiring adaptations of the portable device to each specific analyte in each detection. It is preferable the use of this variant for performing a detection of an analyte selected from an anthocianidin, a flavonol, and a nitrophenol pollutant.

That is, the authors of the present invention have found that the portable device of this variant provides the optical readout of a fluorescence quenching of the fluorescence light emitted by the PL-GQDs to an user in a more versatile way, in situ-application, and whose operations of detection are easier to be practiced by a user.

It is relevant in the present invention that the PL-GQDs are switched off with an analyte directly. The fluorescence-indicative signal (S211, S212) indicates a signal Yes/Not (positive/negative).

In another aspect, the invention provides a second variant of the portable device suitable for performing a detection of an analyte from a sample received in an analytical test substrate on the basis of the Förster resonance energy transfer (FRET) effect by means of an analytical

test substrate.

Advantageously, the portable device of this variant is capable to detect directly the presence of PL-GQDs in a sample with a high level of sensibility. The analyte to be detected are the PL-GQDs directly by fluorescence.

- 5 In this second variant, the portable device is particularly suitable for copy detection of products in a solid state, that is to say a suitable device for the analysis counterfeiting of a solid product.

The second variant of the portable device is suitable for performing a detection of an analyte received in an analytical test substrate on the basis of the Förster resonance energy transfer (FRET) effect by means of an analytical test substrate, the portable device comprising an
10 analytical test substrate, a body, light means, energy connection means, light-readout means, and electronic processor means, and is characterized in that:

- the analytical test substrate is as defined in the first aspect of the invention, and is configured to receive, in use, the analyte from a sample to be detected, and to emit fluorescence light when excited with UV light, wherein the analyte from a sample to be detected are PL-GQDs
15 embedded in a sample, and

- the light means are UV means,

wherein

- the body is configured and adapted to house therein at least part of the analytical test substrate,
- 20 ○ the UV means operates on the basis of ultraviolet wavelength and is configured and arranged in the body
 - to irradiate UV light at the analytical test substrate for exciting the PL-GQDs comprised therein to make them emit fluorescence light in a wavelength range; and
 - 25 ▪ to generate a fluorescence-indicative signal (S211, S212) in the analytical test substrate, which is indicative of the fluorescence light emitted by the excited PL-GQDs and reveals the presence of PL-GQDs by fluorescence;
- the energy connection means are configured and adapted to be
30 coupled to an energy source;
- the electronic processor means are operatively connected to the energy connection means to supply energy to the UV means; and
- the light-readout means are associated to the analytical test

substrate for taking of an optical readout of the fluorescence-indicative signal (S211, S212) generated in the analytical test substrate, and are adapted to provide the optical readout to a user of a fluorescence of the fluorescent PL-GQDs;

5 and **in that**, the device further comprises

- a UV-blocking filter arranged between the light-readout means and the analytical test substrate and is configured to block, in use, the UV light of the UV means (21), thereby enhancing the detection level of PL-GQDs.

10 Advantageously, the enhancing of detection level can be two or three orders of magnitude.

In this aspect, the analyte to be detected are the PL-GQDs. The sample can be, for example, a picture. Thus, the portable device is suitable for performing a detection of PL-GQDs embedded in the picture, for example, for preventing a copy thereof.

The PL-GQDs can be detected in a concentration of 0.1 mg/mm^3 (0.5mg/ml) even in a
15 concentration as lower as 0.0001 mg/mm^3 (0.0005mg/ml).

The technical features of the portable device disclosed herein belong to the first or to the second variants indistinctly, whenever is not specified different.

Advantageously, the portable device provides an optical readout of the fluorescence-indicative signal generated in the analytical test substrate in a reliable, easy, inexpensive and secure
20 way for carrying out *in situ* by a user, who does not need technical acknowledgment.

Advantageously, the portable device can be coupled to a handheld computing device, that wears almost all the users in their pocket, in an easy way and low cost for a rapid detection of an analyte in situ, making this technology accessible to the whole public. The handheld computing device can be selected from the group consisting of a handheld digital camera
25 device, a cellular phone, a smart phone, and a tablet computer.

Particularly, the energy connection means can be adapted to be coupled to an energy source of a handheld computing device, so as to provide the energy source to the energy connection means from the handheld computing device. Preferable, in the portable device according to the first or second variants, the energy connection means is a USB connector.

30 The light-readout means can also independently be adapted to be coupled to an optical readout, i.e. a camera, of a handheld computing device, so as to provide the optical readout from the handheld computing device. Preferable, in the portable device according to the first or second variants, the light-readout means is the structural part of the body that allows take

a direct readout of the fluorescence-indicative signal generated in the analytical test substrate upon UV irradiation.

Preferable, the UV means is a LED.

5 In an embodiment, the electronic processor means is an electric circuit of the UV means: (365nm) UV LED, connected to the energy connection means at the male USB port.

Advantageously, the authors of the present invention has found that the intensity of the fluorescence of PL-GQDs is directly dependent upon the concentration of the analyte in the sample. Therefore, the concentration of the analyte in the sample can be quantified.

10 The portable device is suitable for determining the concentration of the analyte once detected their presence in the sample. To determine the concentration, the electronic processor means are configured to receive data relating to the fluorescence-indicative signal (S211, S212), at an intensity indicative of the concentration of the analyte in the sample, and are adapted to perform by means of processing means a calculation of said intensity, based on the received data, to generate a concentration-indicative output signal (S3).

15 Preferable, the portable device is sized to be suitable stored in a pocket of a user. The portable device can be sized to a size as small as a matchbox.

Preferable, the body is configured to isolate the analytical test substrate housed therein of the external light of the body, so to protect the analytical test substrate from external light fluctuations. Preferable, the body is a black chamber.

20 Advantageously, highly reproducible data is obtained in a portable device embodiment having this configuration of the body.

As stated above in the second variant, PL-GQDs can be detected in a very low concentration in the sample.

25 Advantageously, the presence of the UV-blocking filter permits to barr the ultraviolet wavelength through thereof. With the configuration and arrangement described above in this variant, the ultraviolet light emitted by UV means (LED) does not interference in the optical readout of the fluorescence indicative signal (S211 S212) generated in the analytical test substrate.

30 The UV-blocking filter can be also used in the first variant of the portable device in order to enhance the level of detection.

In the invention, the term "UV-blocking filter" means a filter that at least barr the pass of

ultraviolet wavelengths through thereof. A filter that exclusively prevent the pass of the UV wavelengths is also contemplated in the present invention. A "longpass filter" from which wavelengths higher than 400 nm can through thereof is also contemplated as a UV-blocking filter according to the invention.

5 In a further aspect, it is provided a system suitable for performing a detection of an analyte from a sample received in an analytical test substrate on the basis of the Förster resonance energy transfer (FRET) effect by means of an analytical test substrate, the system comprising an analytical test substrate, and a portable device having a body, light means, energy connection means, light-readout means, and electronic processor means, and is characterized
10 in that

- an analytical test substrate is as define in the first aspect, and is configured to receive, in use, the analyte from a sample to be detected, and to emit fluorescence light when excited with UV light, and

- a portable device comprises:

- 15 ○ the body is configured and adapted to house therein at least part of the analytical test substrate,
- the UV means operates on the basis of ultraviolet wavelength and is configured and arranged in the body
 - 20 ▪ to irradiate UV light at the analytical test substrate for exciting the PL-GQDs comprised therein to make them emit fluorescence light in a wavelength range; and
 - to generate a fluorescence-indicative signal (S211, S212) in the analytical test substrate which is indicative of the quenching of the fluorescence light emitted by the excited
25 PL-GQDs and reveals the presence of an analyte by quenching the fluorescence light emitted of the excited PL-GQDs in case an analyte is received in the analytical test substrate and the analyte has an absorption wavelength range that at least in part overlaps the fluorescence light
30 emitting wavelength range of the excited PL-GQDs; then
- the energy connection means are configured and adapted to be coupled to an energy source;
- the electronic processor means are operatively connected to the energy connection means to supply energy to the UV means; and
35 ○ the light-readout means are associated to the analytical test

substrate for taking of an optical readout of the fluorescence-indicative signal (S211, S212) generated in the analytical test substrate, and are adapted to provide the optical readout to a user of a quenching of the fluorescence light emitted by the PL-GQDs;

5 or alternatively,

- a portable device, which comprises:

- the body is configured and adapted to house therein at least part of the analytical test substrate,
- the UV means operates on the basis of ultraviolet wavelength and is
10 configured and arranged in the body
 - to irradiate UV light at the analytical test substrate for exciting the PL-GQDs comprised therein to make them emit fluorescence light in a wavelength range; and
 - to generate a fluorescence-indicative signal (S211, S212) in
15 the analytical test substrate, which is indicative of the fluorescence light emitted by the excited PL-GQDs and reveals the presence of PL-GQDs by fluorescence; then
- the light-readout means are associated to the analytical test substrate for taking of an optical readout of the fluorescence-indicative signal (S211, S212) generated in the analytical test
20 substrate, and are adapted to provide the optical readout to a user of a fluorescence of the fluorescent PL-GQDs;
- a UV-blocking filter arranged between the light-readout means and the analytical test substrate and is configured to block, in use, the
25 UV light of the UV means (21), thereby enhancing the detection level of PL-GQDs.

wherein the electronic processor means are connected to a handheld computing device, the handheld computing device being selected from the group consisting of a handheld digital camera device, a cellular phone, a smart phone, and a tablet computer.

30 In an embodiment, the electronic processor means are connected to the handheld computing device wirelessly.

In an embodiment, the energy connection means are coupled to an energy source of the handheld computing device, and/or the light-readout means are coupled to an optical readout of the handheld computing device, so as to supply the energy to the UV means and/or to
35 provide an optical readout by means of the handheld computing device.

In an embodiment, in the system, the portable device further comprises remote means associated to the light-readout means for wireless communication with the handheld computing device.

Advantageously, using the handheld computing device coupled to the portable device it is allowed to record data, process data, and display readings at any time after finalized the detection of the analyte, which makes the portable device more versatile, of simplified design, reduced size and low cost in the detection.

In an embodiment of the portable device, it further comprises a lens configured to couple to the optical readout, i.e. the camera, of the handheld computing device. The lens is arranged in the optical readout of a handheld computing device, making such lens possible that the size of the portable device be reduced to a smaller size.

In a still a further aspect, the invention provides a kit comprising a portable device, an analytical test substrate and instructions for carrying out such detection.

In this aspect, it is provided a kit for detecting an analyte from a sample, the kit comprising a portable device according any one of variants described above, an analytical test substrate of the first aspect of the invention and instructions for carrying out the detection by using a handheld computing device, the instructions comprising:

- depositing a sample of the analyte to be detected in the analytical test substrate;
- introducing the analytical test substrate including the sample into the portable device according to first or second variant at the body;
- coupling the energy source of the handheld computing device to the energy connection means and, optionally or alternatively, coupling the optical readout of the handheld computing device to the light-readout means of the portable device, and
- determining the presence or absence of the analyte in the sample by fluorescence quenching yes/not, respectively, by an optical readout of the analytical test substrate by means of the handheld computing device.

The skilled in the art is aware that when the user does not have a handheld computing device, the optical readout of the analytical test substrate can be read directly by the eyes of the user.

Brief Description of the Drawings

Figure 1A depicts a schematic view of an embodiment of the portable device 2 of this invention showing the different parts thereof, and showing the portable device coupled

with a smart phone and the analytical test substrate 1 housed therein.

Figure 1B depicts a schematic view of another embodiment of the portable device 2 of this invention showing the different parts thereof, without a smart phone 3 coupled thereon, and with the analytical test substrate 1 outside the portable device.

5 **Figure 1C** depicts a schematic view of another embodiment of the portable device 2 of this invention showing the different parts thereof, with a smart phone 3, the analytical test substrate 1 and the UV-blocking filter 4.

Figure 2 depicts a graph showing the absorption spectrums of two analytes (Quencher 1 and Quencher 2) and the emission spectrum of the PL-GQDs depending on the normalized absorbance and the normalized fluorescence.

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Figure 3A depicts how fluorescence decreased as the concentration of 4-nitrophenol (D) and paraoxon (E) increases.

Figure 3B depicts two graphs of the corresponding Stern–Volmer relationship for Paraoxon and 4-nitrophenol.

15 **Figure 4** depicts two bar diagram that show the fluorescence quenching of the portable device according to the second aspect of the invention by using the analytical test paper as a response to A) wine samples of different vintage and B) seawater samples spiked with 4-nitrophenol (NP) and Paraoxon (PO).

Figure 5A depicts both the UV-visible absorbance spectrum (left), and the Photoluminescence spectra (right) when PL-GQDs are excited at 365 nm.

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Figure 5B is a HRTEM image depicted showing the morphology of synthesized PL-GQDs.

Hereinafter, a mode for carrying out the present invention is described in detail (see Figs 1A and 1B).

25 The PL-GQDs were placed on top of the nitrocellulose substrate 1. The substrate resulted ideal for the assay where the PL-GQDs as fluorescent probes were bound to the paper interstices and reagents (whether needed) and samples placed on its surface. Some wax-printed hydrophobic circular patterns 10 were previously delineated on the nitrocellulose matrix 1 by a wax printer to confine the sensing probes, thus defining the sensing area. A hydrophobic barrier spread all around the hydrophilic nitrocellulose matrix was ready and rapidly delineated and the PL-GQDs were physically adsorbed into, while the barrier contained the fluorescent probes in the as-defined area. PL-GQDs pH value showed to be the most crucial parameter to

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be controlled. While at pH 4 and 6 protonated GQDs showed to dramatically quench the fluorescence, the deprotonated GQDs at pH 8 and 13 were absolutely fluorescent.

The portable device 2 has been used to detect two groups of phenolic compounds from a sample with no instrumentation required other than the analytical test paper 1 and a simple mobile phone 3. PL-GQDs as sensing probes were embedded into a nitrocellulose matrix 1. The sensing probes were synthesized from citric acid by a pyrolysis procedure, and with no modifications further physisorbed and confined into small wax-traced spots 10 at the nitrocellulose substrate 1. A UV LED 21 that excites the PL-GQDs was placed into a dark chamber 20 and fed by a mobile phone 3, used for both as the energy source 22 and digital color imaging capture 23. The dark chamber 20 was isolated the analytical test paper 1 comprising embedded PL-GQDs from external light fluctuations. The nitrocellulose-based device 2 was able to detect the antioxidant capacity related with flavonoid content in wine samples and 4-nitrophenol and paraoxon pollutants in seawater samples.

The sensing mechanism is based on fluorescence quenching of the sensing probes because of their interfacing with some phenolic compounds through the polar-polar resonance of acceptor-donor interaction. The portable device is able to detect the presence of a compound (Quencher 1 and Quencher 2, see Fig 2) which absorption spectrum is overlapped with the emission spectrum of the photoluminescent GQDs. The overlapped area, see Fig 2, is proportional to the percentage of quenching. According to the FRET mechanism, the quantum dots play a donor role transferring the light to the compound that absorbs it in the overlapped region (acceptor).

Thus, in the FRET phenomena, excited-state electrons from the PL-GQDs are transferred to the analyte when coming back to ground state, while this coupling transition results in the highly efficient quenching of the PL-GQDs. In contrast, when electrons can't be transferred, for example when conjugation from the aromatic ring is interrupted thus, the energy is not transferred and consequently the quenching not observed. Quenching of the PL-GQDs is the evidence of a positive sensing event, which can be pictured by a smartphone camera and quantified by a electronic processor unit.

The dark chamber 20 contains a strip hole where the paper strip 1 passes through (see Fig 1B). Each spot 10 is passed manually one by one to reach the UV LED area, where the confined PL-GQDs are excited by the LED 21 at 365 nm. The LED 21 is fed by a smartphone through a USB port 22, utilizing the simple circuit. The smartphone 3 captures the light that the PL-GQDs emit at 460 nm. White paper provides a proper background for optical readouts. The amount of captured fluorescent light is further related to the concentration of the analyte, which

can be quantified after processing by the image J program.

Hereinafter, a mode for carrying out the present invention is described in detail (see Fig 1C).

A UV-blocking filter 4 between the paper 1 that contains the GQDs and the camera 3 of the mobile phone. The UV-blocking filter 4 would prevent the light coming from the UV LED 21 from interfering with the light captured by the camera, thus increasing the exposure time. In other words, the camera cannot see the light coming from the LED 21 and only see the light of the excitation. An increase in the detection limit is obtained by increasing the exposure time to capture more light when the concentration of the GQDs is very low. It is also possible to detect a minimum difference of quenching.

As described above in detail, this invention has at least one of the following advantages:

The portable devices provide a simplest, faster, and easier way for detecting an analyte in-situ, which further is reliable, inexpensive, with reproducible data and which can be performed by a user without requiring specific knowledges.

High yield of the PL-GQDs along with outstanding features of paper platforms and the smartphone readout have been combined with the portable device, thereby providing rapid, inexpensive and simple paper-based analytical tools with versatile applications.

It is a matter of course that the features mentioned above and those explained below can be used in other combinations in addition to those described, on in isolation, without departing from the scope of the invention.

Examples

Hereinafter, the present invention is described in more detail and specifically with reference to the Examples and Figures, which however are not intended to limit the present invention.

PL-GQDs Synthesis

Photoluminescent Graphene oxide quantum dots can be synthesized according to the procedure reported by Dong, Y. et al. "Blue luminescent graphene quantum dots and graphene oxide prepared by tuning the carbonization degree of citric acid. Carbon 50, 4738-4743 (2012)". Briefly, 1g of citric acid was pyrolyzed at 200 °C until the color changed from transparent to orange in approximately 30 min, then the orange liquid was slowly transferred to a 50 ml 10 mg/ml NaOH solution, under vigorous stirring. The stock suspension of PL-GQDs was adjusted with 1M HCl to get 50 mg/ml PL-GQDs solution with a pH value down to 9 and kept in the fridge at 4 °C.

In order to expend less time in the quantum dots synthesis, another approach was used: the citric acid was pyrolyzed in a microwave oven for 2 min and then was added to the NaOH solution (10 mg/ml) under vigorous stirring. This methodology produces similar graphene quantum dots with the same emission wavelength range.

- 5 Once synthesized, the GQDs were further characterized by spectrophotometry, fluorimetry, Fourier transform infrared (FT-IR) spectroscopy and high-resolution transmission electron microscopy (HRTEM) imaging. Figure 5A shows both the UV-visible absorbance spectrum (left), characterized by a wide pick between 300 and 430 nm; and the Photoluminescence spectra with a strong fluorescent emission peak at 460 nm (right) when is excited at 365 nm.
- 10 The top inset depicts a picture of the PL-GQDs under UV (right) and visible (left) light, respectively. HRTEM image depicted in Figure 5B shows the morphology of fresh synthesized GQDs. The image depicts well-dispersed GQDs with a circular shape and an average size of 2.33 ± 0.12 nm.

Analytical test paper preparation and set up

- 15 Wax circumferences of 4 or 5 mm of diameter and 0.5 mm of width were printed on top of a nitrocellulose paper strip of (9 mm x 20 mm) with a Xerox ColorQube 8570 solid ink printer. Afterward, the strip was put on a hot plate at 84 °C for 2 minutes to melt the wax and create an internal structure confining spot. Then 4-8 µL of the as-prepared PL-GQDs was added to the spots; and let it dry at room temperature, for approximately 45 min. The paper strip was
- 20 placed either under the UV lamp or into the dark chamber. Either a 20 W UV lamp (HQ Power vdl20uv) or a 5 mm LED of 20 mA at 365 nm wavelength was used as an UV source (the LED introduced into the dark chamber). All the pictures were taken with a Samsung Galaxy S7, in manual mode, autofocus; at ISO 100 and shooter speed 1/180 s. Images were analyzed either with Image J, or directly from the camera using "IJ Mobile" App

Sensing experiments

- 4-8 µL of the analyte at different concentrations were added to the as-prepared fluorescent sensing area in the paper strip. After drying at room temperature, pictures of the different spots were taken with the smartphone adapted to the black chamber. Photoluminescence was estimated from the pictures. Wine samples were diluted 1/10 with PBS pH 7.4, which final
- 30 ethanolic content was 15%. Seawater samples were spiked with the desired concentration of 4-nitrophenol or paraoxon and the resultant solution, containing 5% ethanol tested without dilution. The seawater was collected from the beach of "La Barceloneta" (41°22'34.4"N 2°11'29.8"E), and tested without any further dilution.

Example 1

The nitrocellulose-embedded PL-GQDs strip was tested first with quercetin as a model phenolic compound of double bonds conjugated within multiple aromatic rings. Its molecular structure contains five –OH groups conjugated with the double bonds of its three aromatic rings.

Fluorescence quenching at different concentrations of quercetin was collected: 0 (0.01 M PBS buffer), 1.66×10^{-5} , 4.98×10^{-5} , 8.30×10^{-5} , 1.33×10^{-4} , 1.99×10^{-4} , 2.51×10^{-4} , 5.01×10^{-4} , 7.52×10^{-4} , 1.25×10^{-3} , 1.50×10^{-3} M.

The PL-GQDs-modified paper platform under UV light showed that the fluorescence quenching was higher as concentrations of quercetin buffered solutions increasing in a range of two orders of magnitude. Quenching of the fluorescence was negligible in the first spot with only PBS buffer.

Herein, at the pH of the assay, the deprotonated quercetin and PL-GQDs have shown to produce an efficient FRET with the concomitant quenching of the PL-GQDs. Stabilization of delocalized electrons resonating within the quercetin structure by electron transference may be accounting for such PL-GQDs intermolecular deactivation processes (quenching).

The quenching capabilities of a series of quercetin-analogous compounds in aqueous solutions were also tested. Indeed, quercetin structurally analogous compounds, with antioxidant properties (e.g. morin, myricetin, and kaempferol), also demonstrated to quench the fluorescence of QDs. Stability of the analytical test paper comprising embedded PL-GQDs was evaluated upon time based on the $\pm 3\sigma$ criterion. For this purpose, a control chart was plotted with the average intensity of the sensing area the first day of the study as central value. The estimated fluorescence was still within the control chart even after 15 days of storage at 4 °C, thus demonstrating the stability of the analytical test paper comprising embedded PL-GQDs.

The analytical information was extracted from pictures taken with a smartphone camera after illuminating the sensing areas with a simple UV light lamp.

Example 2

The sensing capabilities of the nitrocellulose-embedded PL-GQDs were tested for sensing another group of phenolic compounds of environmental interest. For example, 4-nitrophenol has shown to quench PL-GQDs in aqueous solutions. Nitrocellulose-embedded GQDs strip irradiated by a simple LED and coupled only to a smartphone camera was able to provide a

sensitive response to this kind of pollutants. At the proper pH, deprotonated 4-nitrophenolate molecules work as acceptors of electrons while PL-GQDs are the donors, being the FRET susceptible of taking place. At basic pH, given by a 1 M NaOH solution, hydrolyzes of the paraoxon pesticide produces 4-nitrophenol as bi-product. The as-developed nitrocellulose-embedded PL-GQDs thus demonstrated capabilities for sensing not only 4-nitrophenol but also hydrolyzed paraoxon. Figure 3A shows how fluorescence decreased as the concentration of 4-nitrophenol (D) and paraoxon (E) increased from 2.5×10^{-5} to 5×10^{-4} M (in the direction of the purple arrow in the figure). Figure 3B shows the corresponding paraoxon Stern–Volmer plot, which correlates well with the equation Fluorescence intensity_{UV LED} = -827.84 [paraoxon] + 0,9939, with a regression coefficient, $r^2 = 0.9972$. In the inset, the corresponding linear correlation for 4-nitrophenol that fits well with Fluorescence intensity_{UV LED} = -910.10 [4-nitrophenol] + 0,9959, with a regression coefficient, $r^2 = 0.9912$. LOD were estimated to be $4,36 \times 10^{-5}$ and $3,97 \times 10^{-5}$ M for paraoxon and 4-nitrophenol, respectively (3σ criteria). These results demonstrate the potential of the newly developed analytical test paper comprising embedded PL-GQDs for environmental monitoring in place.

Example 3

The versatile sensing portable device was used for testing in food and environmental samples, see Figure 4.

For this purpose, Tempranillo wine samples of different vintage were interrogated with the PL-GQDs-paper sensor. The fluorescence quenching extent resulted in being as higher as older the wine. For instance, the 'crianza' wine led to a 41.1 ± 1.3 % quenching while 'roble' and 'joven' wines only 23.4 ± 1.3 and 15.0 ± 2.4 %, respectively. These results are in agreement with the higher antioxidant phenolic compound content of vintage wines as compared to younger wines. Results suggest the sensor strip holds potential for a rapid characterization of wines based on their antioxidant capacity, which could be exploited in other beverages and foods.

Sensing capabilities of the analytical test paper strip were also tested in environmental samples. A seawater sample was spiked with 50 and 100 mM 4-nitrophenol (NP), which produced a quenching of the PL-GQDs of 17.1 ± 1.2 and 35.7 ± 2.6 %, respectively. 50 and 100 mM hydrolyzed paraoxon (PO) led to similar quenching extents (16.9 ± 1.5 and 35.3 ± 1.7 , respectively) as expected from the 4-nitrophenol, stoichiometrically produced as the by-product of the hydrolysis of PO. In agreement, unlike a mixture of 100 mM NP and 100 mM unhydrolyzed PO that produced only 36.5 ± 1.9 % of quenching, when the mixture was hydrolyzed while simultaneously tested the extent of quenching reached 62.3 ± 1.9 %. The

corresponding sensing areas (spots) are in the upper part Figure 4. These results demonstrate that the as-developed paper-based device can be used for in field monitoring of not only 4-nitrophenol (NP) and Paraoxon (PO) separately, but also when they coexist in the same environmental sample.

5 The response of the device appears to be associated with the antioxidant capacity, related to the flavonoid content, of wine samples of different vintage; but could be exploited in other beverages and foods. Quenching of fluorescence also correlated well with changes in the concentration of 4-nitrophenol and paraoxon pollutants separately or coexisting in seawater samples. Overall results are promising for monitoring in settings where sophisticated
10 instrumentation is not always available.

Example 4

Use of the UV blocking filter of Fig.1C

15 In certain situations where the concentration of GQDs in the paper is very low, it is necessary to use much longer exposure times, 1-10 seconds. Under these conditions, the camera's sensor captures the light coming from the ultraviolet light source, which can be more intense than the emission light of the GQDs. In order to avoid this, a filter is added that allows the light captured by the camera sensor to come only from the emission of the GQDs.

20 If it is made a (white) paper without GQDs, for long shutter times we can obtain a BLACK image that shows that there is no presence of GQDs. The way to achieve it is by adding the filter. Without the filter in these conditions a saturated image (white-violet) is observed that does not allow to identify the presence or not of GQDs. The same will happen at low concentrations of GQDs.

Quantification of the level of detection

25 When the filter is not used at concentrations of GQDs lower than 12.5mg/ml in the tested range of 0.5mg/ml to 12.5mg/ml, the shutter speed of the chamber should be increased to values between $\frac{1}{4}$ sec to 10 sec. This causes the camera to capture the LED's light signal, producing a saturation in the image. This translates to RGB values close to 255, 255, 255 respectively (white image) the GQDs are not different from anything because the image is completely white.

30 The use of the filter allows detection of GQDs even at concentrations lower than 0.5mg, even two or three orders of magnitude, that is about 0.0005 mg/ml. Because only the signal of the GQDs is seen and when increasing the shutter speed the light of GQDs accumulates in the time until completing the photo. The filter is placed exclusively between the camera and the paper.

CLAIMS

1. Analytical test substrate (1) comprising PL-GQDs, as fluorescent probes, suitable for performing a detection of an analyte on the basis of the Förster resonance energy transfer (FRET) effect, **characterized in that** the analytical test substrate (1) is of paper and comprises:
 - . PL-GQDs embedded in the paper, the PL-GQDs being deprotonated GQDs, and
 - i. at least one area (10) delimited by a hydrophobic barrier to confine the embedded PL-GQDs within this area (10),thereby, in use, the area (10) is suitable to receive the analyte to be detected and the PL-GQDs embedded therein emit fluorescence light when excited with UV light.
2. Analytical test substrate (1) of claim 1, wherein the hydrophobic barrier is of a waxy material.
3. Analytical test substrate (1) of claim 1, wherein the paper is of nitrocellulose.
4. Analytical test substrate (1) of claim 1, wherein the substrate (1) has a strip shape.
5. Use of the analytical test substrate (1) of any one of claims 1 to 4, for detecting an analyte selected from an anthocyanidin, a flavonol, a nitrophenol pollutant and PL-GQDs.
6. Method for manufacturing the analytical test substrate (1) defined in any one of claims 1 to 4, **characterized in that** the method comprises:
 - a. imprinting hydrophobic barrier patterns delimiting at least one area in the paper;
 - b. preparing an aqueous solution including PL-GQDs, wherein the aqueous solution is buffered at a pH ranging from 8 to 13 by using a base, thereby the PL-GQDs in the basic solution result in deprotonated PL-GQDs, which show their fluorescence when they are irradiated with UV light;
 - c. wetting the delimited area in the paper with the deprotonated PL-GQDs solution and let it drying at room temperature, thereby the PL-GQDs being adsorbed in the paper interstices.
7. Method of claim 6, wherein in step a) to imprint the hydrophobic barrier pattern, preferable a circular pattern, it is employed a waxy material and, then, a thermal treatment is performed to melt the wax printed, thus delimiting a sensing area by the hydrophobic barrier.
8. Method of claim 6, wherein in step b) the PL-GQDs in the aqueous solution are in a concentration between 0.5 mg/ml and 500 mg/ml, preferably a concentration higher than 12.5 mg/ml.
9. Method of claim 6, wherein in step c) the delimited area is wetted with the deprotonated

PL-GQDs solution by adding from 1 μ l of the solution at 6.25mg/ml of PL-GQDs to 4 μ l of the solution at 50mg/ml of PL-GQDs.

10. Portable device (2) suitable for performing a detection of an analyte from a sample received in an analytical test substrate (1) on the basis of the Förster resonance energy transfer (FRET) effect by means of an analytical test substrate (1), the portable device
5 (2) comprising an analytical test substrate (1), a body (20), light means, energy connection means (22), light-readout means (23), and electronic processor means (24), **characterized in that** the analytical test substrate (1) is as defined in any one of claims 1 to 4, and is configured to receive, in use, the sample containing the analyte
10 to be detected, and to emit fluorescence light when excited with UV light, and the light means are UV means (21), wherein

- the body (20) is configured and adapted to house therein at least part of the analytical test substrate (1),
- the UV means (21) operates on the basis of ultraviolet wavelength
15 and is configured and arranged in the body (20)
 - to irradiate UV light at the analytical test substrate (1) for exciting the PL-GQDs comprised therein to make them emit fluorescence light in a wavelength range; and
 - to generate a fluorescence-indicative signal (S211, S212) in
20 the analytical test substrate (1) which is indicative of the quenching of the fluorescence light emitted by the excited PL-GQDs and reveals the presence of an analyte by quenching the fluorescence light emitted of the excited PL-GQDs in case an analyte is received in the analytical test
25 substrate (1) and the analyte has an absorption wavelength range that at least in part overlaps the fluorescence light emitting wavelength range of the excited PL-GQDs;
- the energy connection means (22) are configured and adapted to be coupled to an energy source;
- the electronic processor means (24) are operatively connected to the
30 energy connection means (22) to supply energy to the UV means (21); and
- the light-readout means (23) are associated to the analytical test substrate (1) for taking of an optical readout of the fluorescence-indicative signal (S211, S212) generated in the analytical test
35 substrate (1), and are adapted to provide the optical readout to a

user of a quenching of the fluorescence light emitted by the PL-GQDs.

11. Portable device (2) suitable for performing a detection of an analyte received in an analytical test substrate (1) on the basis of the Förster resonance energy transfer (FRET) effect by means of an analytical test substrate (1), the portable device (2) comprising an analytical test substrate (1), a body (20), light means, energy connection means (22), light-readout means (23), and electronic processor means (24), **characterized in that** the analytical test substrate (1) is as defined in any one of claims 1 to 4, and is configured to receive, in use, the analyte from a sample to be detected, and to emit fluorescence light when excited with UV light, wherein the analyte from a sample to be detected are PL-GQDs embedded in a sample, and the light means are UV means (21), wherein

- the body (20) is configured and adapted to house therein at least part of the analytical test substrate (1),
- the UV means (21) operates on the basis of ultraviolet wavelength and is configured and arranged in the body (20)
 - to irradiate UV light at the analytical test substrate (1) for exciting the PL-GQDs comprised therein to make them emit fluorescence light in a wavelength range; and
 - to generate a fluorescence-indicative signal (S211, S212) in the analytical test substrate (1) which is indicative of the fluorescence light emitted by the excited PL-GQDs and reveals the presence of PL-GQDs by fluorescence;
- the energy connection means (22) are configured and adapted to be coupled to an energy source;
- the electronic processor means (24) are operatively connected to the energy connection means (22) to supply energy to the UV means (21); and
- the light-readout means (23) are associated to the analytical test substrate (1) for taking of an optical readout of the fluorescence-indicative signal (S211, S212) generated in the analytical test substrate (1), and are adapted to provide the optical readout to a user of a fluorescence of the light emitted by the PL-GQDs;

and **in that**, the device (2) further comprises a UV-blocking filter (4) arranged between the light-readout means (23) and the analytical test substrate (1) and is configured to block, in use, the UV light of the UV means (21), thereby enhancing the detection level

of PL-GQDs.

12. Portable device (2) according to claims 10 or 11, wherein the electronic processor means (24) is an electric circuit of the UV means (21), which is a LED, connected to the energy connection means (22), which is the male of the USB port.

5 13. Portable device (2) according to claims 10 or 11, wherein the device is sized to be suitable stored in a pocket of a user.

14. Portable device (2) according to claims 10 or 11, wherein the body (20) further is configured to light isolate the analytical test substrate (1) housed in the body (20) of the external light of the body (20).

10 15. Portable device (2) according to claims 10 or 11, wherein the fluorescence light emitted by the excited PL-GQDs is indicative furthermore of the intensity of fluorescence, which is directly dependent upon the concentration of the analyte in the sample, and thereby measurable.

15 16. Portable device according to claims 10 or 11, wherein the electronic processor means (24) are further configured to receive data relating to the intensity of the fluorescence-indicative signal (S211, S212), which is indicative of the concentration of the analyte in the sample, and adapted to perform by means of processing means a calculation of said intensity, based on the received data, to generate a concentration-indicative output signal (S3).

20 17. System suitable for performing a detection of an analyte from a sample received in an analytical test substrate on the basis of the Förster resonance energy transfer (FRET) effect by means of an analytical test substrate, the system comprising an analytical test substrate (1), and a portable device (2) having a body (20), light means (21), energy connection means (22), light-readout means (23), and electronic processor means (24), **characterized in that** the analytical test substrate (1) is as defined in any one of claims 1 to 4, and is configured to receive, in use, the analyte from a sample to be detected, and to emit fluorescence light when excited with UV light, and

- the portable device (2) comprises:

- the body (20) is configured and adapted to house therein at least part of the analytical test substrate (1),
- the UV means (21) operates on the basis of ultraviolet wavelength and is configured and arranged in the body (20)
 - to irradiate UV light at the analytical test substrate (1) for exciting the PL-GQDs comprised therein to make them emit fluorescence light in a wavelength range; and

- to generate a fluorescence-indicative signal (S211, S212) in the analytical test substrate (1) which is indicative of the quenching of the fluorescence light emitted by the excited PL-GQDs and reveals the presence of an analyte by quenching the fluorescence light emitted of the excited PL-GQDs in case an analyte is received in the analytical test substrate and the analyte has an absorption wavelength range that at least in part overlaps the fluorescence light emitting wavelength range of the excited PL-GQDs; then
 - the energy connection means (22) are configured and adapted to be coupled to an energy source;
 - the electronic processor means (24) are operatively connected to the energy connection means to supply energy to the UV means; and
 - the light-readout means (23) are associated to the analytical test substrate (1) for taking of an optical readout of the fluorescence-indicative signal (S211, S212) generated in the analytical test substrate (1), and are adapted to provide the optical readout to a user of a quenching of the fluorescence light emitted by the PL-GQDs;
- or alternatively
- the portable device (2) comprises:
- the body (20) configured and adapted to house therein at least part of the analytical test substrate (1),
 - the UV means (21) operates on the basis of ultraviolet wavelength and is configured and arranged in the body
 - to irradiate UV light at the analytical test substrate (1) for exciting the PL-GQDs comprised therein to make them emit fluorescence light in a wavelength range; and
 - to generate a fluorescence-indicative signal (S211, S212) in the analytical test substrate (1), which is indicative of the fluorescence light emitted by the excited PL-GQDs and reveals the presence of PL-GQDs by fluorescence; then
 - the energy connection means (22) are configured and adapted to be coupled to an energy source;
 - the electronic processor means (24) are operatively connected to the energy connection means to supply energy to the UV means; and

- the light-readout means (23) are associated to the analytical test substrate (1) for taking of an optical readout of the fluorescence-indicative signal (S211, S212) generated in the analytical test substrate (1), and are adapted to provide the optical readout to a user of a fluorescence of the fluorescent PL-GQDs; and
- a UV-blocking filter (4) arranged between the light-readout means and the analytical test substrate and is configured to block, in use, the UV light of the UV means (21), thereby enhancing the detection level of PL-GQDs.

wherein the electronic processor means (24) are connected to a handheld computing device (3), the handheld computing device (3) being selected from the group consisting of a handheld digital camera device, a cellular phone, a smart phone, and a tablet computer.

18. System according to claim 17, wherein the electronic processor means (24) are connected to the handheld computing device (3) wirelessly.
19. System according to claim 17, wherein the energy connection means (22) are adapted to be coupled to an energy source of the handheld computing device (3), and/or the light-readout means (23) are adapted to be coupled to an optical readout of the handheld computing device (3), so as to supply the energy to the UV means (21) and/or to provide an optical readout by means of the handheld computing device (3).
20. System according to claim 17, further comprising remote means associated to the light-readout means (23) for wireless communication with the handheld computing device readout (3).
21. A kit for detecting an analyte from a sample, the kit comprising a portable device (2), an analytical test substrate (1) and instructions for carrying out the detection by using the handheld computing device (3) of claim 17 or of any of claims 18 to 20 when depending on claim 17, the instructions comprising:
 - depositing a sample of the analyte to be detected in the analytical test substrate (1) of claim 1 or of any of claims 2 to 4 when depending on claim 1;
 - introducing the analytical test substrate (1) including the sample into the portable device (2) of claim 10 or of claim 11 or of any of claims 12 to 16 when depending on claim 10 or claim 11 at the body (20);
 - coupling the energy source of the handheld computing device (3) to the energy connection means (22) and, optionally or alternatively, coupling the optical readout of the handheld computing device (3) to the light-readout means (23) of the portable device (2), and

- determining the presence or absence of the analyte in the sample by fluorescence quenching or not, respectively, by an optical readout of the analytical test substrate (1) by means of the handheld computing device (3).

FIG 1A

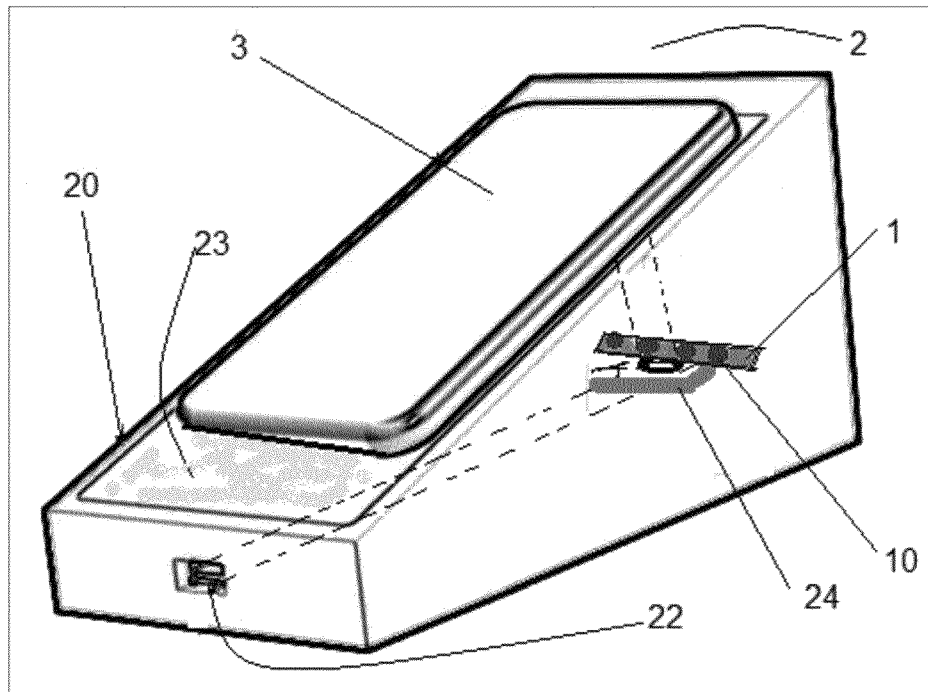
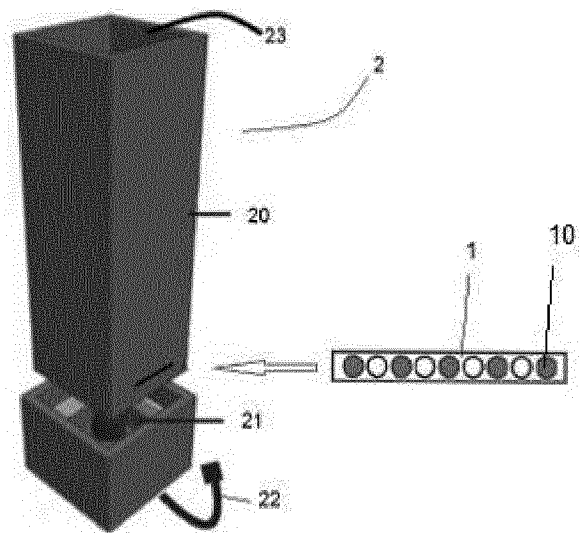


FIG 1B



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FIG 1C

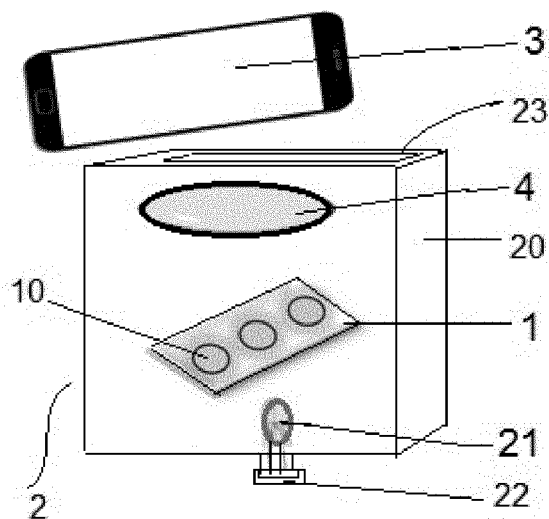
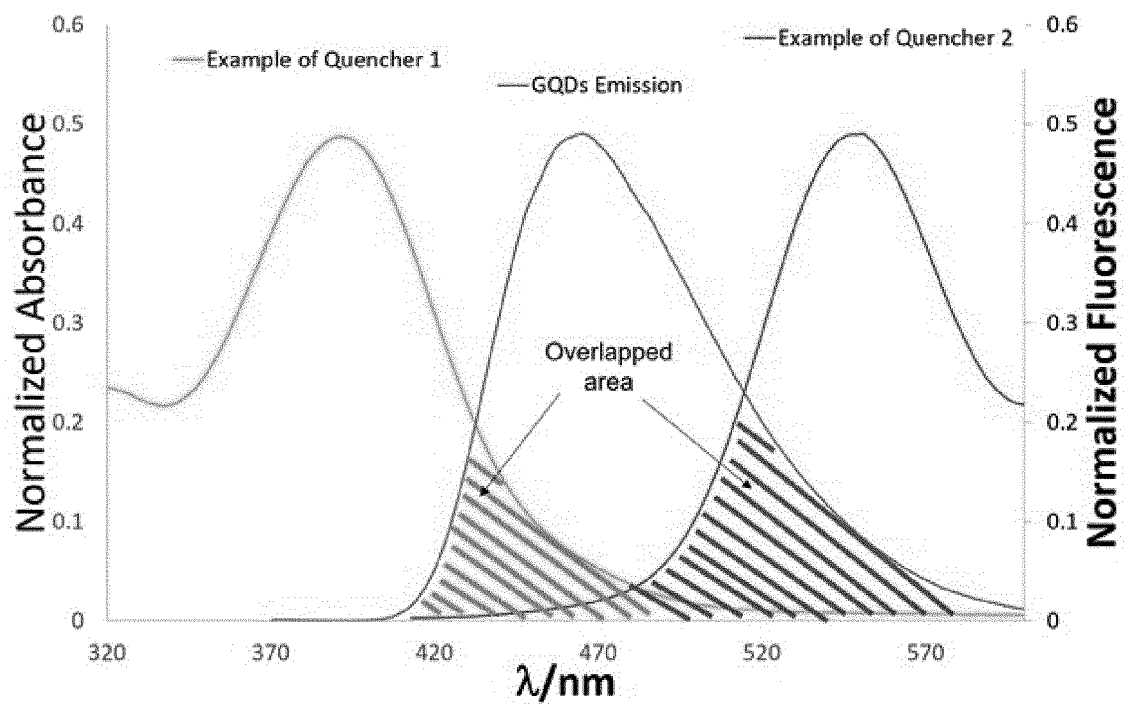


FIG 2



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FIG 3A

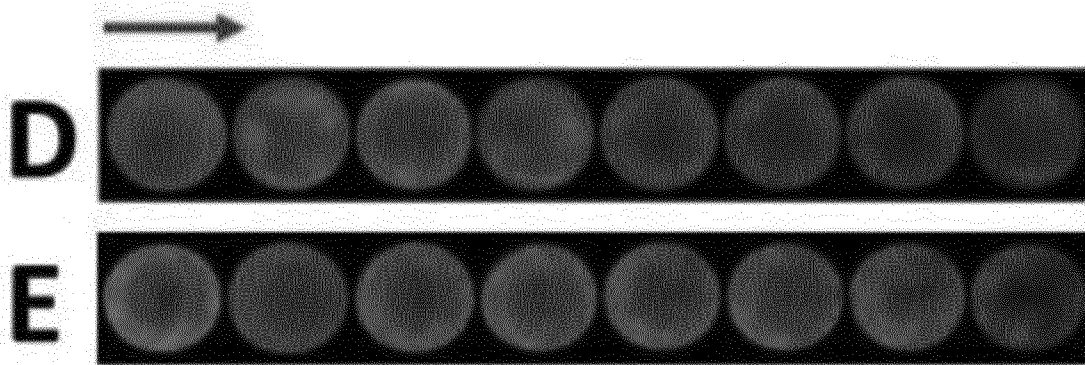


FIG 3B

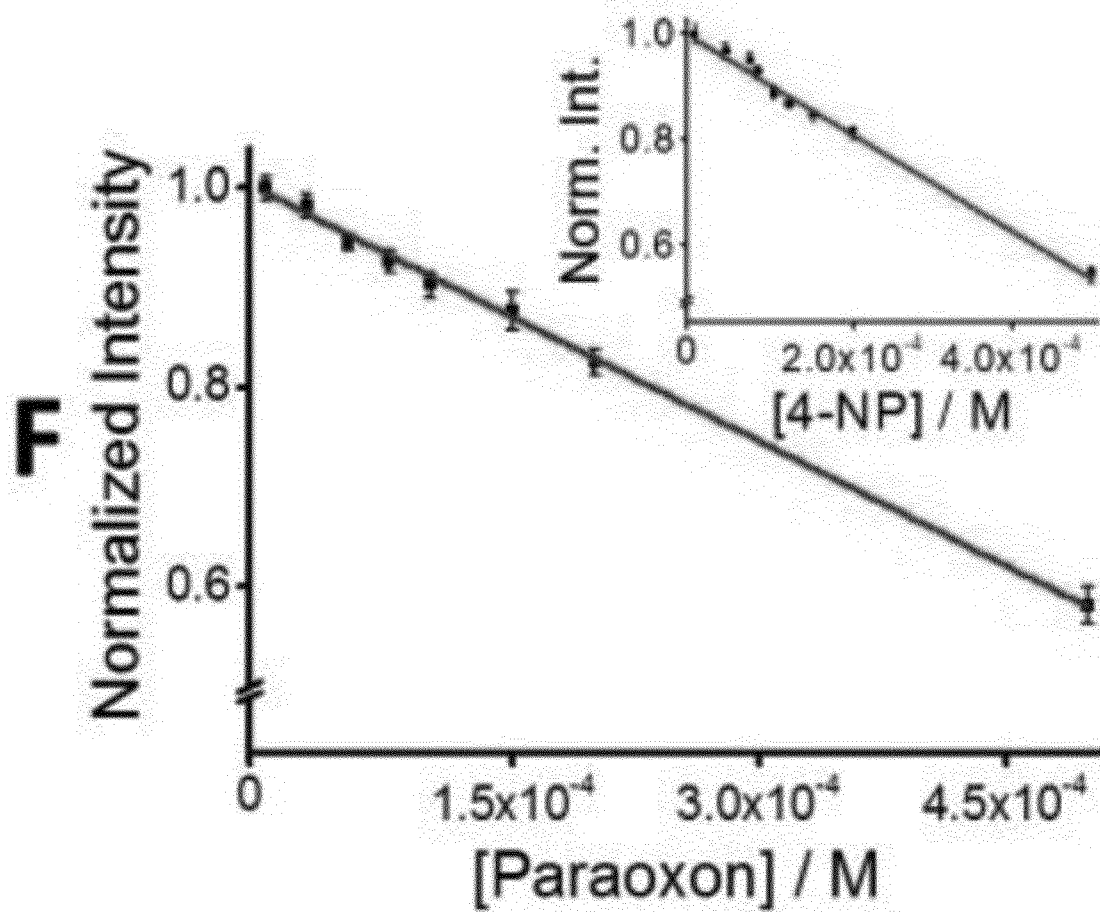


FIG 4

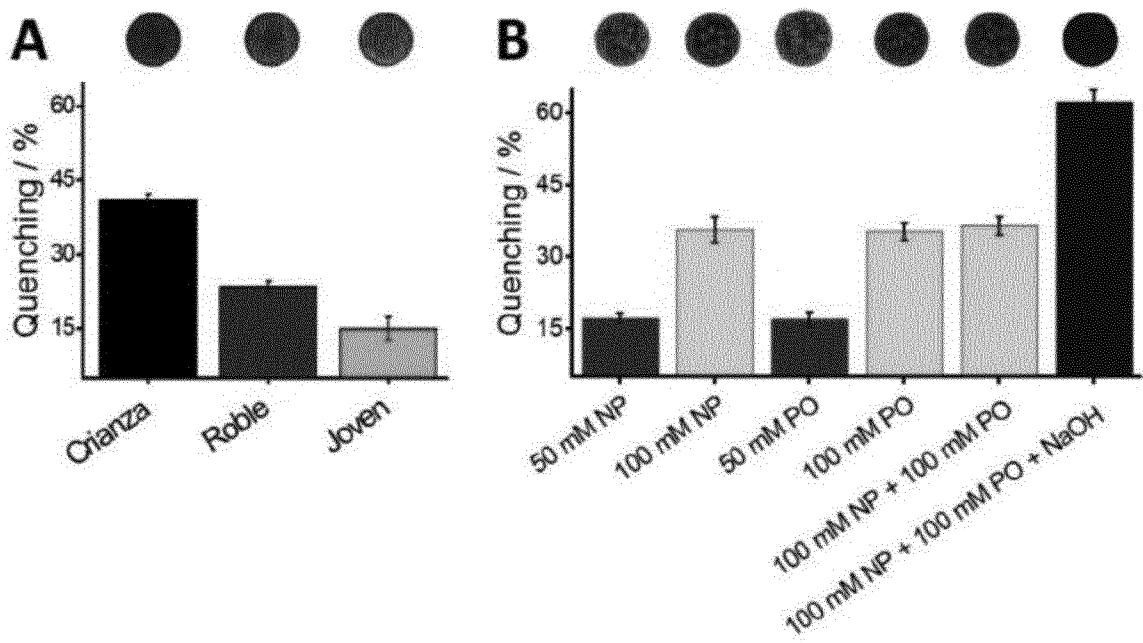
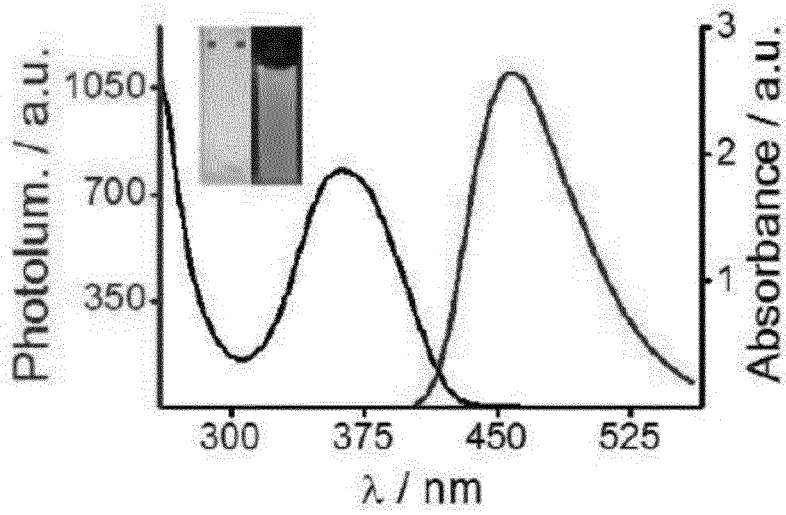
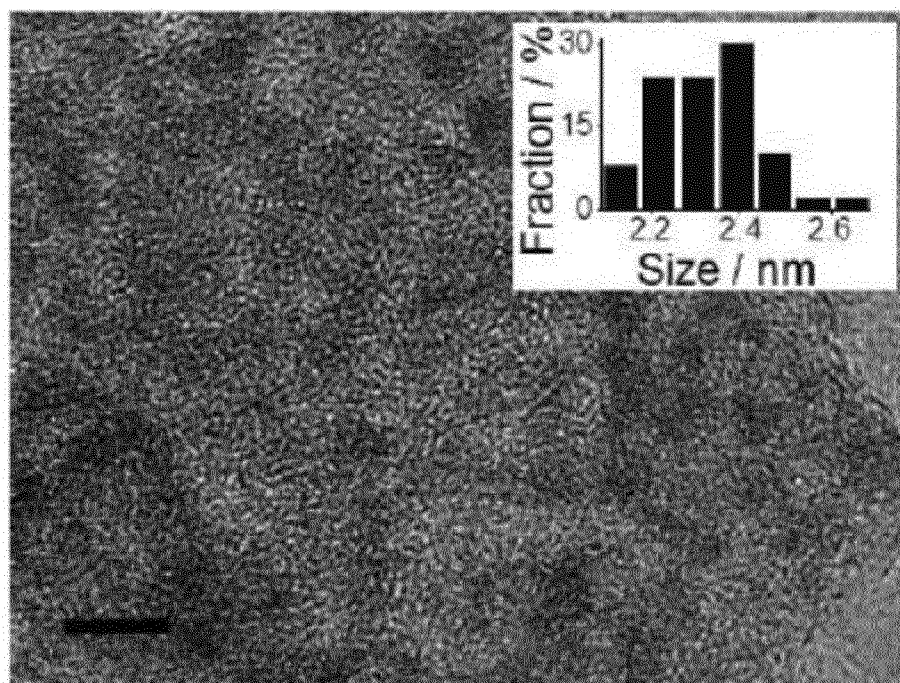


FIG 5A



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FIG 5B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/054583

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/64 C12Q1/68 G01N33/542
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12Q G01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, INSPEC, COMPENDEX, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. OMAIR NOOR ET AL: "Camera-Based Ratiometric Fluorescence Transduction of Nucleic Acid Hybridization with Reagentless Signal Amplification on a Paper-Based Platform Using Immobilized Quantum Dots as Donors", ANALYTICAL CHEMISTRY, vol. 86, no. 20, 16 September 2014 (2014-09-16), pages 10331-10339, XP055471006, ISSN: 0003-2700, DOI: 10.1021/ac502677n the whole document -/-	1-21



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

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Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2018/054583

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	RONGBIN XIE ET AL: "Graphene quantum dots as smart probes for biosensing", ANALYTICAL METHODS, vol. 8, no. 20, 21 April 2016 (2016-04-21), pages 4001-4016, XP055391754, GBR ISSN: 1759-9660, DOI: 10.1039/C6AY00289G cited in the application the whole document -----	1-21
A	US 2014/193839 A1 (CUNNINGHAM BRIAN T [US]) 10 July 2014 (2014-07-10) cited in the application figures 2A-3B, 5-6E, 12, 16 paragraph [0044] - paragraph [0057] paragraph [0072] paragraph [0090] - paragraph [0098] ----- -/--	10-21

INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	CARRILHO E ET AL: "Understanding Wax Printing, A simple Micropatterning Process for Paper-Based Microfluidics", ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 81, no. 16, 15 August 2009 (2009-08-15), pages 7091-7095, XP002585317, ISSN: 0003-2700, DOI: 10.1021/AC901071P [retrieved on 2009-07-15] the whole document -----	1-21

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International application No

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