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Revealing two chemical strategies to tune bright one- and two-photon excited fluorescence of carbon nanodots

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Carbon-based dots (CDs) are a novel class of luminescent carbon nanomaterials, attracting much interest as promising alternatives for cadmium-based quantum dots and fluorescent organic dyes. Although different strategies have been advanced to produce CDs with intense and tunable one-photon excited fluorescence (OPEF), the case of the analogous two-photon excited fluorescence (TPEF) has not been fully explored yet. By varying the synthesis conditions, we produced three types of phloroglucinol-based carbon nanodots (PG CNDs). Their remarkable OPEF and TPEF properties can be tuned by (la) the conjugated aromatic domains and (lb) the content of oxygenous moieties. In addition, the emission colour of the PG CNDs is strongly responsive to (ii) the hydrogen-bonding network, enabling colour-switching while maintaining excellent fluorescence yield (both OPEF and TPEF). Those three factors were evaluated for their suitability for the tuning of the emission colour. Our studies point out the advantages of the tuneable PG CNDs to use in optoelectronics and biological applications domains.

Introduction
Since their discovery in 2004,1 the carbon-based dots (CDs) have been extensively explored. A variety of CDs with various chemical composition and morphological forms have been prepared.2–9 They have been found to possess much potential for applications in a variety of fields, including biochemical and pharmaceutical assays,3, 6–10 photocatalysis,11–15 optoelectronics,6, 20–22 and photovoltaics.24–26

One of the most interesting properties of the CDs is their excellent fluorescence activity.4, 9, 22, 27 This makes the CDs interesting as replacement for inorganic, heavy metals based (e.g. Cd2+) nanoparticles and organic fluorescent dyes. A particular attention was paid to CDs’ one-photon excited fluorescence (OPEF). Two aspects were of special interest: (I) understanding of the fluorescence mechanism14, 22, 28, 29 and (II) rational CDs engineering leading to nanoparticles with bright and tunable emission properties.4, 9, 30

To provide high colour purity and tunability of the CDs fluorescence, two strategies have been mostly considered:

Variation of the design of CDs: the nanoparticles morphology and internal structure can be modified by changing the synthesis conditions. Yuan et al.4 reported the solvothermal way to produce highly-emitting CDs whose fluorescence properties depend on their size. Hola et al.7 demonstrated that the CDs fluorescence colour can be tuned by varying contribution of the graphitic nitrogen. Ding et al.9 prepared a set of carbon nanostructures whose emission colour could be tuned by adjusting both the graphitic nitrogen content and the size of a single nanoparticle.

Solvent effects: both the emission colour and performance of the CDs can be tuned by solvating media. The solvent-induced fluorescence of the CDs shows either the blue-green switching31–33 or broadband emission.33, 34 The most effective solvent-induced colour change was found in nitrogen-doped carbon nanodots (CNDs) by Sato et al.35

In both cases, the understanding of the structural origin of the emission mechanism is essential to adequately choose the best strategy to tune fluorescence colour.

Analogous extensive studies of tuning the multiphoton-excited emission of the CDs have not been reported yet. The multiphoton absorption and the corresponding multiphoton-excited fluorescence of CDs were reported first in 2007.10 To date, mainly two-photon excited fluorescence (TPEF) and two-photon absorption (TPA) of a few CNDs were briefly characterized.4, 36–45 Liu et al.46 prepared nitrogen-doped CDs which showed an intense emission related to two-, three-, and even four-photon excitation pathways.

Unfortunately, the existing publications have not provided extensive quantitative description of the relevant TPA parameters of the studied CDs.4, 36, 38, 40, 42, 45–47 The TPA performance has been usually quantified only at a single excitation wavelength37, 42, 44 (except in two papers).39, 41 In consequence, the factors influencing the TPA spectra of those CDs cannot be fully interpreted.
Additionally, while most of the papers have reported large TPA parameters, no detailed description of the applied methodology of their determination nor the indication of the molarity of CDs suspensions was provided.\textsuperscript{37, 41, 44} Finally, those studies have not fully described the actual TPEF performance of CDs which combines both the TPA cross-section and the emission quantum yield.

In our recent work we showed that acetone-derived polymeric dots (PDs) have remarkable TPA activity in the first biological window along with the pronounced TPEF properties.\textsuperscript{3} We have also reported on nitrogen-doped folic-acid CNDs (FA CNDs) exhibiting intense and nearly monochromatic TPEF.\textsuperscript{48}

Herein, we report on our study of three types of fluorescent phloroglucinol-derived CNDs (PG CNDs) suspended in colloidal solutions. First, we present two new, independent synthesis routines to produce PG CNDs, involving the novel thermal and modified solvothermal strategies.\textsuperscript{4} Both experimental strategies (varying synthesis conditions and the solvation) allowed us to tune the emission of the CNDs. Then we describe both absorption and fluorescence properties of the obtained PG CNDs, in one- and two-photon absorption (excitation) regimes, and show that PG CNDs demonstrate bright and narrowband emission. By combining theoretical (numerical) and experimental approaches we provide a deep insight into nanodots structure-property relationship. In consequence, we indicate three chemical strategies allowing the precise tuning of the colour of OPEF and TPEF of the carbon nanodots.

## Experimental Section

### Materials

Phloroglucinol (PG), boric acid (BA), potassium bromide (KBr), and silica gel (pore size 60 Å, 70-230 mesh, 63-200 μm) were purchased from Sigma Aldrich. High-grade purity dimethyl sulfoxide (DMSO), d₆-DMSO, methanol, d₆-methanol, n-butanol, n-pentanol, n-heptanol, isopropanol, ethylene glycol, dimethylformamide (DMF), acetone, and cyclohexanol were also provided by Sigma Aldrich. High-grade purity dichloromethane (DCM), methanol, ethanol, sulphuric acid, and sodium hydroxide were supplied from POCH S.A. The Milli-Q water was provided by an ultrapure water system. Prior to the structural characterization, the PG powder was dispersed in the DCM: methanol (v/v = 6/1) mixture and then dried. Other chemicals were used without any purification processes. All chemicals were stored in proper conditions.

### Solvothermal synthesis of CNDs

The PG CNDs have been synthesized here in a solvothermal way, according to a modified procedure elaborated by Yuan et al.\textsuperscript{4} This approach relies on the solvent-engineering method with the PG molecule as a carbon precursor. First, the PG powder (250 mg, 1.98 mmol) was dissolved in a proper polar medium (5.0 mL), followed by vigorous stirring (400 rpm) for 10 minutes in a round-bottom flask. Varying with reaction conditions, three fractions can be designed: cyan (\textit{CYAN}), green (\textit{GREEN}), and yellow-emitting (\textit{YELLOW}) CNDs. The precursor mixture of the \textit{CYAN} CNDs was prepared using Milli-Q water or ethanol. Meanwhile, the \textit{GREEN} CNDs were fabricated from the ethanol solution of PG. To produce the \textit{YELLOW} CNDs, the acid-mediated conditions were applied; briefly, the PG powder is dissolved in ethanol and then, a 125 μL of concentrated sulphuric acid was added dropwise, stirring constantly for 10 minutes. Subsequently, clear precursor solutions were transferred into a Teflon-lined autoclave reactor (12 mL) and heated at 200°C in a conventional oven. Solvothermal treatment was performed for 3 hours (\textit{CYAN} and \textit{YELLOW} CNDs) and 9 hours (\textit{GREEN} CNDs), respectively. After the reaction, the reactor was cooled down with air at room temperature and successively, with water.

In the case of \textit{YELLOW} CNDs, the excess sulphuric acid was neutralized by a fresh sodium hydroxide solution. As-obtained salt was separated from the reaction mixture with syringe filters (Nylon, pores size ~0.22 μm) and discarded. All crude products of CNDs were then dried using a rotary evaporator system, re-suspended in methanol, and subjected to further purification processes.

### Synthesis of CNDs: thermal decomposition

Alternatively, \textit{CYAN} and \textit{YELLOW} CNDs have been successfully produced through the one-step synthesis that involves the thermal carbonization of PG molecules mixed with proper media. Briefly, to fabricate \textit{CYAN} CNDs, the PG powder (250 mg, 1.98 mmol) was dissolved in ethanol (5.0 mL) under magnetic stirring for 10 minutes. In the case of \textit{YELLOW} CNDs, the BA granulate (98.1 mg, 1.59 mmol, 1 eq.) was ground and mixed with the PG precursor (200 mg, 1.59 mmol, 1 eq.) in the mortar. As-prepared precursor mixtures were then transferred into a ceramic crucible (15 mL) and heated up at 200°C for 30 minutes in the conventional oven. Finally, the reaction mixtures were quenched to room temperature with air. As-obtained solids were suspended in methanol and subjected to further purification.

### Purification of CNDs

Crude products were effectively purified, according to the following procedure. First, their methanol suspensions of PG CNDs were filtered with syringe membranes (polytetrafluoroethylene (PTFE), pore sizes ~0.22 μm). As-prepared samples were then purified with silica-column chromatography (SCC) using the mixture of DCM: methanol (v/v = 6/1) as an eluent. The separation process was monitored by the thin-layer chromatography (TLC) and the OPEF experiments (see Linear Optical Properties). To collect highly-pure fractions of PG CNDs, the SCC assays were repeated several times. Finally, the dispersions of PG CNDs were filtered again (PTFE, pore sizes ~0.22 μm).

Pure fractions of PG CNDs were dried with a rotary evaporator (40°C), followed by the methanol washing, resulting in dark yellow (\textit{CYAN}), bright brown (\textit{GREEN}), and red (\textit{YELLOW}) powders. All PG CNDs were stored in powder form for further investigation. In this work, the results of structural and optical characterizations were included for \textit{CYAN} (derived from the solvothermal treatment), \textit{GREEN} (the solvothermal treatment), and \textit{YELLOW} CNDs (derived from the thermal decomposition).

### Structural characterization

The structures of CNDs were studied by Fourier-Transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, X-ray photoelectron (XPS) spectroscopy, and Fourier-Transform Raman (FT-Raman) spectroscopy approaches. The chemical composition of CNDs was examined by energy-dispersive X-ray spectroscopy (EDX) in combination with scanning transmission...
electron microscopy (STEM). Their morphology was characterized by high-resolution transmission electron microscopy (HRTEM).

The proton (1H) NMR, carbon (13C) NMR, and heteronuclear single quantum coherence (HSQC) NMR spectra of CNDs and PG molecules were acquired on a Bruker Avance™ 600 MHz spectrometer. Prior to the NMR assays, analytes were dispersed in d₄-methanol to reach the concentration of 5 mg/mL. The NMR signals were then examined with the MestReNova software.

The measurements of the FTIR spectra were carried out in four ways. The spectral resolution values were set to 4 cm⁻¹ for all FTIR experiments. Each sample was measured over 64 accumulations. The solid-state FTIR spectra in attenuated-total reflectance mode (ATR-FTIR) were performed in the middle-infrared (MIR: 4000-400 cm⁻¹) and the far-infrared (FIR: 400-50 cm⁻¹) regions. The MIR ATR-FTIR signals were acquired on the IR TENSOR 27 Spectrometer (Bruker), consisting of a pyroelectric DTGS detector, a KBr beam splitter, and a black body source. The FIR ATR-FTIR spectra were recorded at vacuum conditions (2 mbar), using the IR IFS 66/s spectrometer (Bruker) with a Mylar beam splitter and silicon carbide (SiC) as an IR source. To measure the ATR-FTIR spectra, each powder was deposited on a diamond surface. The liquid-state transmission FTIR spectra in the MIR region (MIR TR-FTIR) were obtained on the IR TENSOR 27 Spectrometer (Bruker). The PG and PG CNDs were dispersed in d₄-DMso to reach the concentration of 0.2 mg/mL. As-prepared suspensions were placed into a sample holder, consisting of two CaF₂ plates and a 0.15 mm spacer.

The solid-state MIR TR-FTIR signals were collected on the IR IFS 66/s spectrometer (Bruker). Prior to this assay, each sample was mixed with KBr, and pellets were formed and dried.

The FT-Raman spectra of PG and PG CNDs were measured in the solid state in the range of 100-3500 cm⁻¹, using an RFS100 spectrometer (Bruker) in the macro configuration. The experimental setup was equipped with a continuous-wave Nd: YAG laser (λ laser = 1064 nm) as an excitation source and a nitrogen-cooled germanium detector. The Raman signals were collected over 300 scans. The EDX surveys of CNDs were recorded at different positions on the silicon drift EDX detector (SDD Oxford Instrument XMaxN 100 TL) connected with the STEM microscope setup (JEOL 2200FS).

The XPS spectra were measured on a PHI VersaProbell Scanning XPS system providing the monochromatic Al Kα X-ray beam (1486.6 eV). The photoelectron take-off angle was 45°. The X-ray beam was focused on a 100 μm spot. The pass energy in the analyser was 117.50 eV (survey scans) and 46.95 eV (the high energy resolution spectra). The 1 eV electrons and a dual beam charge compensation with 7 eV Ar⁺ ions were used to keep a constant sample surface potential. The XPS measurements were performed at vacuum conditions (with a pressure less than 3x10⁻⁹ mbar). The spectrum background was subtracted with the Shirley method. The XPS spectra were analysed with the Gaussian deconvolution method.

The HRTEM imaging of CNDs was carried out through the HRTEM microscope setup (JEOL 2200FS), consisting of the field emission gun working at the 200kV accelerating voltage. Prior to the HRTEM experiments, the fresh TEM samples were prepared by deposing a 10 µl drop of 5 µg/mL dispersion of CNDs on the copper grid (300 mesh) with the standard carbon cover and air-dried. To determine the size distribution of CNDs and their average diameters, tens of single nanoobjects were analysed with the ImageJ software.

**Computational details: DFT calculations**

The vibrational characteristics were calculated with the density functional theory (DFT), using Gaussian 16, Rev. C03.¹⁹ The PG molecules and the proposed molecular sub-units were optimized with the M06-2/L-def2-TZVP level of theory adopted for all atoms,²⁰,²¹ using the Mulliken population analysis. To take into account the long-range van der Waals forces, the Grimme-type (D3) empirical correction was used.²² The stationary points in the potential energy surface were evidenced not to have any imaginary frequencies. The studied molecular structures were modelled in the closed-shell singlet state. As-calculated vibration signals were fitted with the Lorentzian function; the width of the fitting curve was set to 4 cm⁻¹. The frequencies were scaled with an empirical scaling factor of 0.95187.

**Linear optical properties of CNDs**

Linear optical properties of PG molecules and CNDs were investigated by absorption spectroscopy, as well as steady-state and time-resolved fluorescence spectroscopy techniques. The ultraviolet-visible (UV-Vis) extinction spectra of all CNDs and the PG precursor were collected using a JASCO V-730 spectrophotometer (240-700 nm), equipped with a Peltier thermo cell holder to set and control the temperature (-10-80°C). To provide the relevant temperature, the sample was placed into the cell holder for 300 s before each scan. The one-photon excitation (OPE), OPEF, and one-photon excitation-emission maps were recorded on a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon) in wide wavelength ranges. The excitation/emission slits and spectral resolutions were set to 3.0 nm and 0.5 nm, respectively. The temperature-dependent OPEF spectra were measured on an FSS spectrofluorometer (Edinburgh Instruments). The spectrophotometric setup was equipped with a sample holder (SC-25) and a TC 1 temperature controller (Quantum Northwest) to tune the temperature from -5 to +60°C.

The absolute fluorescence quantum yields (FQYs) were estimated by measuring the excitation and emission signals of analytes and blank samples (i.e. pure solvents). Each spectrum was accumulated over 200 scans with an integration time of 100 ms. The home-built experimental setup consisted of an integrating sphere accessory, a high-sensitivity CCD array spectrometer (QE Pro, Ocean Optics), and a neutral density filter (Thorlabs).

The OPEF decays were measured following the time-correlated single-photon counting (TCSPC) technique. The TCSPC experimental setup was composed of an Acton SpectraPro SP-2300 monochromator (Princeton Instruments) and a high-speed hybrid detector HPM-100-50 (Becker&Hickl GmbH), controlled by a DCC-100 card. Each fluorescence signal was averaged over 6 accumulations with a collection time of 30 s.

To provide efficient excitation for the FQY and TCSPC assays, a BDL - 375 - SMN picosecond laser diode (377 nm) were used.

**Multi-photon fluorescence experiments**

The TPEF spectra were measured on a home-built experimental setup that combines a Shamrock 303i spectrometer (Andor) and a sensitive iDus camera (Andor). A femtosecond laser system (Ti: Sapphire Chameleon laser, Coherent Inc.) with a repetition rate of 80 MHz and a pulse duration =120 fs was used, operating from 690 nm to 1080 nm. The experimental setup contained also a Chameleon OPO optical parametric oscillator (Coherent Inc.) which provided an output laser signal tuneable from 1000 nm to 1600 nm.
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Figure 1. Synthesis of PG CNDs. Simplified scheme of two synthesis routes to produce PG CNDs: thermal treatment (left) and the solvothermal treatment (right). The differences in the internal structure are illustrated figuratively as nanoobjects with varying structural motifs: the size of the sp²-hybridized carbon domains, the content of the sp³-carbons, and oxygenous groups (red points).

The excitation laser beam was separated from its reflections with appropriate high-performance long-pass filters. Meanwhile, the emission signals were filtered by short-pass filters. The power of the laser beam was monitored by a PM100D Handheld Optical Power and Energy Meter (Thorlabs). To reduce undesirable events (i.e., inner filters and re-absorption effects), the TPEF signals were accumulated for diluted samples; their concentrations relate to absorbance values below 0.1 in the whole absorption and emission spectra ranges. All spectroscopic measurements were performed at room temperature, except for the temperature-controlled OPEF.

Results & Discussion

Synthesis of PG CNDs

The PG molecule (benzene-1,3,5-trioli, C₆H₃(OH)₃) exhibits three-fold symmetry: it possesses a phenyl ring with regularly distributed six substituents (three hydroxyls and three hydrogens, see Figure 1). PG can therefore act as precursor of highly graphitized CNDs, dispersible in polar solvents. For all these reasons, PG has been recently considered to be one of the most promising precursors of carbon nanostructures in the procedures involving solvothermal, thermal or microwave treatments. In the present study, we attempted to fabricate the PG CNDs following both solvothermal and thermal synthetic procedures, as illustrated in Figures 1 and S1-3.

First, the PG CNDs were synthesized according to the modified Yuan’s protocol, using ethanol as a solvating medium and the constant molarity of PG molecules (C₆H₃(OH)₃ = 0.397 M). To obtain CNDs showing either CYAN or GREEN emission, the solvothermal syntheses (at 200°C) were performed using varying reaction times. The fabrication of YELLOW CNDs required an addition of a catalyst (0.449 M sulphuric acid) to the reaction mixture.

In the second synthesis approach (thermal decomposition at 200°C), the reaction medium was changed from ethanol (for CYAN) to molten boric acid (BA; for YELLOW).

Both fabrication ways followed by the same purification procedure. The SCC with the TLC monitoring appeared to be the crucial step to purify the CNDs that efficiently replaced centrifugation or dialysis. The SCC allows the effective extraction of targeted CNDs from a mixture of molecular by-products and carbogenic contaminants, leading to ultrapure PG CNDs. The SCC assay of the mixture of CNDs’ fractions (characterized by different emission colours) also showed their different retention rates, suggesting that each PG CNDs’ fraction interacts with the same column system in a different way (Figure S3).

One-photon absorption and fluorescence properties

Linear optical properties (absorption and fluorescence) of the colloidal solutions of PG CNDs were investigated using methanol or d₂-DMSO as solvents. The UV-Vis extinction, OPE, and OPEF spectra are presented in Figures 2a (and S24-25) in Electronic Supporting Information (ESI). PG CNDs suspended in methanol show a complex, broad one-photon absorption (OPA) band with several maxima. The most prominent OPA peaks are located at 457 nm (CYAN), 497 nm (GREEN), and 468 nm (YELLOW); these locations are consistent with the corresponding OPE maxima (461 nm – CYAN, 500 nm – GREEN, and 478 nm – YELLOW). Although there is no systematic correlation between the OPA/OPE peak position of PG CNDs emitting at different wavelengths, there is a progressive redshift of the OPA tail. This reflects to the decreasing energy of the optical bandgap (2.39 eV, 2.30 eV, and 2.24 eV) (Figure S26). Moreover, a significant difference in their OPEF emission was also observed. The well-defined major OPEF components of the PG CNDs are centred at 478 nm, 518 nm, and 550 nm, respectively. They correspond to the Stokes’ shifts of 961 cm⁻¹ (CYAN), 816 cm⁻¹ (GREEN) and 3186 cm⁻¹ (YELLOW). All OPEF spectra contain also a weak emission shoulder at longer wavelengths. The emission spectrum of each emitter is relatively narrow at room temperature, with the full-width at half-maximum (FWHM) equal to 32 nm (CYAN), 33 nm (GREEN), and 72 nm (YELLOW). These relatively small values are comparable with the FWHM of other graphitic CDs, as well as the inorganic quantum dots. The narrow bandwidth spectra are responsible for almost monochromatic character of light emitted when PG CNDs are exposed to UV or blue light (Figure 2b).

To unravel the interplay between the OPE and OPEF wavelengths, the one-photon excitation-emission maps were recorded. As expected, each fraction of PG CNDs displays pronounced excitation-independent fluorescence when the excitation is varied in a wide wavelength range (Figures 2d and S27). Similar trends were reported previously for other CDs. The narrow bandwidth spectra are responsible for almost monochromatic character of light emitted when PG CNDs are exposed to UV or blue light (Figure 2b).

The fluorescence performance of PG CNDs was then analysed. The absolute FQYs were determined to be: 61.8% (CYAN), 29.7% (GREEN), and 25.2% (YELLOW), respectively (Figures 2f; Table S6). These values are of the same order of magnitude as the FQYs reported for PG-derived CDs (Yuan et al.), and for the green-emitting fraction of PDs (Moniruzzaman et al.).

The emission dynamics of PG CNDs was also analysed by measuring the fluorescence decays at the OPEF maxima, and at the corresponding emission shoulders (Figure S28). In each case, the fluorescence decay profile was fitted with a mono-exponential function, assuming a single lifetime component (Figures S29-32), i.e.
Figure 2. Linear and nonlinear optical properties of PG CNDs. a) The normalized UV-Vis extinction (black), OPE (violet), and OPEF spectra (blue, green, or yellow curves) of PG CNDs in methanol dispersion. The corresponding OPEF spectra of their d$_6$-DMSO suspensions are plotted for a comparison as bright blue (CYAN), bright green (GREEN), and red areas (YELLOW), respectively. b) The bright fluorescence of PG CNDs in methanol dispersion under UV and blue light. c) The log(I$_{\text{TPE FL}}$) vs. log(P) relations plotted for CYAN, GREEN, and YELLOW CNDs which were excited at 820 nm, 840 nm, and 980 nm, respectively. d-e) The one-photon and two-photon excitation-emission maps of CYAN CNDs as representative examples. The fluorescence intensity values are normalized with respect to the most intense peak. f) The fluorescence quantum yields of PG CNDs in methanol and d$_6$-DMSO suspensions.

only a single radiative pathway which accounts for PG CNDs fluorescence. The fluorescence lifetimes ($\tau$) were equal to 3.9 ns (CYAN), 3.2 ns (GREEN), and 2.8 ns (YELLOW), therefore decreasing with the longer emitted wavelength (Table S7).

The rate constants of the radiative ($k_r$) and non-radiative ($k_{nr}$) processes contributing to the relaxation after the excitation, were estimated as a combination of the FQY and $\tau$ parameters, and they indicate the significant role of the radiative relaxation pathways (Table S8).

The linear optical properties of the PG CNDs described above are independent of the synthesis routines of PG CNDs (but purified in the same way, see Section: Synthesis of PG CNDs). In addition, the fluorescence characteristics of the same type of PG CNDs are identical (see Figure S33). As-synthesized PG CNDs, coming from different preparation batches can be thus considered as identical. The OPEF spectra of PG CNDs suspended in d$_6$-DMSO remain very sharp (FWHM ~36 nm, 33 nm, and 70 nm, respectively). However, the positions of OPEF peaks are red-shifted (with respect to those observed in methanol suspensions) (Figure 2a). The OPEF maxima are peaked at 510 nm, 553 nm, and 571 nm, respectively. The peak position is not excitation-dependent (see e.g. the excitation-emission map for YELLOW dots in Figure S27). Such bathochromic shifts lead to the change of fluorescence colour of all dots: CYAN dots start to emit in green, (cyan → green), GREEN dots emit in yellow (green → yellow), and YELLOW dots emit in orange (yellow → orange). The use of d$_6$-DMSO enhances also the fluorescence performance of
PG CNDs as compared to the methanol samples; the FQYs of as-prepared dispersions were found to be 67.9%, 51.1%, and 44.9% (Figure 2f and Table S6). Similar solvent-induced variations in emission, i.e. colour change and high FQY values, between alcoholic and DMSO suspensions of PG-based CDs were described by Yoshinaga et al.; however, in the cited work the authors focused on the blue-emitting fraction of dots only. Therefore, our work extends the existing knowledge about the solvatochromic effect in heteroatom-free, ultrabright and monochromatic PG CNDs, and about tuneability of the emitted colour.

**Two-photon absorption and fluorescence properties**

Figures 2e and S37-38 show emission signals of methanol dispersions of PG CNDs upon femtosecond laser irradiation in the wide excitation wavelength range, consistent with the first biological window. The double logarithmic plots of the integrated intensity vs. the input laser power (log (I<sub>TPEF</sub>) vs. log (P)) are linear (Figures 2c and S34-36) with the slope of ca. 2 which evidences the two-photon nature of the laser-induced processes.

The TPEF spectra are slightly red-shifted (by 2-7 nm) with respect to their one-photon excited analogues. The spectral red-shifts were also observed for other CDs and fluorescent peptide assemblies, however, those bathochromic shifts were larger (~20-60 nm). In the case of PG CNDs, we can confirm the similar relaxation pathways in one- and two-photon regimes, with only minor modifications of the vibrational structure.

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The TPA activity of PG CNDs was quantified by a microscopic TPA cross-section normalized by the molar mass (σ<sub>TPA</sub>/M). The use of such structural merit factor was recently discussed to be crucial when the TPA properties are compared with the variety of disparate materials. The σ<sub>TPA</sub>/M parameters were estimated from the luminescence method, using relevant reference dyes (Table S9). More details are reported in the ESI. The multipeak and broad bandwidth TPA spectra of PG CNDs are shown in Figures 3a-c and S39. All spectra are blue-shifted with respect to the doubled-wavelength position of their OPA analogues.
Figure 4. Structure and morphology of PG CNDs. a-c) Size distribution of the PG CNDs. d-f) The zoomed HRTEM images of representative CYAN (d), GREEN (e), and YELLOW CNDs (f). The full-size pictures are included in ESI. g) The HRTEM projection of a single CYAN carbon nanodot with the approximated boundaries (dash lines) and the lattice fringes (0.23 nm; the 100 interplanar spacing). h) The $^1$H NMR spectra of PG and PG CNDs. i) The $^{13}$C NMR spectra of PG and PG CNDs. The peaks are grouped into the crucial kinds of sub-groups of PG and PG CNDs. The terms Ar-, Ar, and Ar+ correspond to the aromatic carbons with the oxygen-substitution (electron-rich), the conjugated aromatic domains, and the aromatic carbons near oxygen defects (electron-poor), respectively. The signals of the solvent (d$_4$-methanol) are marked with a red cross.

(Figure 3c), in contrast to nitrogen-doped CNDs.\textsuperscript{18} The profiles of TPA spectra of all three PG CNDs are similar (with, \textit{inter alia}, the peaks located at 860 nm and 885 nm). However, the TPA shoulder in the short-wavelengths (<800 nm), and the absorption peak at 820-830 nm progressively increase with the most prominent enhancement for the YELLOW CNDs. New TPA band components emerge in the long-wavelength region (>900 nm) for GREEN and YELLOW CNDs. Those bands tend to grow and red-shift considerably in the case of YELLOW CNDs (Figure 3a-b).

The TPA cross section of CYAN, GREEN, and YELLOW CNDs spectra are different. The $\sigma_{TPA}/M$ values reach $2.1\times10^{-3}$ (740 nm), $2.1\times10^{-3}$ (760 nm), and 0.31 GM-mol/g (740 nm) for the CYAN, GREEN, and YELLOW fractions, respectively. The TPA performances of CYAN and GREEN CNDs are similar to those found for acetone-derived PDs ($\sigma_{TPA}/M \sim 1.3\times10^{-3}$ GM-mol/g at 760 nm).\textsuperscript{3} The $\sigma_{TPA}/M$ parameters of
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**YELLOW** CNDs are of the same order of magnitude as those of nitrogen-doped CNDs and other most prominent two-photon absorbers in the near-infrared. To quantify the TPEF performance, we also estimated the normalized two-photon brightness which combines the $\Phi_{\text{TPA}}/M$ merit factor with the FQY value ($\Phi_{\text{TPA}}'$). As observed in the $\Phi_{\text{TPA}}/M$ merit factor, two-photon brightness values increase for **CYAN**, **GREEN**, and **YELLOW** respectively (Figure S40). This trend indicates **YELLOW** CNDs as the most efficient two-photon excited emitters.

**Morphology and structural properties**

Figures 4f-g and S4-6 display high-resolution transmission electron microscopy (HR-TEM) images of well-separated PG CNDs. In contrast to Yuan’s work, the PG CNDs have irregular shapes and they are nearly similar in size (Figure 4a-c). Their average diameters were estimated to be 3.9±0.9 nm (**CYAN** and **GREEN**) and 4.3±0.8 nm (**YELLOW**).

The HR-TEM imaging shows also the crystal fringes with a 0.23 nm lattice spacing (Figure S7), comparable with the inter-(100) planes distance in graphite (0.21 nm). This indicates the presence of the sp²-hybridized carbon domains in the internal structure of PG CNDs. The elemental composition of PG CNDs was examined with the **C** NMR; 0.81 ppm (13C NMR) with no hydrogen coupling (see Table S3). As depicted in Figure 5c, the peaks of the aromatic C=C stretching (the C=C stretching in PG CNDs can be found in the ATR-FTIR spectra (Figure Sb). The complex IR band in the range of 2300-3700 cm⁻¹ (i.e. the OH stretching) corresponds to stretching vibrations of hydroxyl groups. Its significant broadening indicates the formation of a new hydrogen-bonding network, not observed in the PG sample. The collective nature of this hydrogen-bonded assembly is evidenced by new complex and strong bands at ca. 165 cm⁻¹ (Figures S21).

Weak peaks at 1720-1760 cm⁻¹ (i.e. the C=O stretching) evidence the presence of carbonyl groups. The IR components at ca. 1020, 1080-1090, and 1260 cm⁻¹ (the C-O stretching which can be involved in the CO or COC stretching) suggest the formation of singly-oxygenated carbon groups (i.e. the hydroxyl and ether moieties). The particular change is observed for the aromatic sub-groups. Compared to narrow PG’s signals, the peaks of the C=C stretching of PG CNDs become broader: 1200-1500 cm⁻¹ (semicircle) and 1500-1680 cm⁻¹ (quadrant modes). The IR components at ca. 3070 cm⁻¹ (the C-H stretching, aromatic) are also broader for PG CNDs. New IR peaks at ca. 255 cm⁻¹ arise from the ring vibrations. Besides, the series of the characteristic vibration signals of a PG molecule (e.g. the C-O stretching at 520 cm⁻¹ and the ring stretching at 580 cm⁻¹) disappear. These findings reveal the conjugation of single phenyl rings of the PG into versatile polycyclic aromatic sub-units in PG CNDs. In contrast to the PG molecule, the PG CNDs’ structure possesses the significant amount of the saturated hydrocarbon chains. The representative IR signals can be found at 2840-2960 cm⁻¹ (the C-H stretching, aliphatic), 1440-1460 cm⁻¹ (the C-H bending, aliphatic), 1360-1380 cm⁻¹ (the C-H bending, aliphatic), and 1160-1190 cm⁻¹ (the C=C stretching).

As in the case of the PG molecules, some IR components of the C=C stretching are upshifted when CNDs are dispersed in d₈-DMSO (Figures 5a). For example, the peaks of **CYAN** CNDs shift from ca. 1665 cm⁻¹ (d₈-DMSO) to ca. 1645 cm⁻¹ (free CNDs and the KBr mixture). This unravels their sensitivity to the rearrangement of the hydrogen-bonding network (with the change in the average bond strength). Hence, those aromatic residues seem to be adjacent directly to polar groups (i.e. hydroxyls). This interpretation is consistent with the NMR spectra analysis.

The vibrational characteristics of PG CNDs were then investigated in the MIR using two experimental procedures: (i) the TR-FTIR mode in two dispersing media (d₈-DMSO or KBr) and (ii) the ATR-FTIR mode. The ATR-FTIR spectra were also recorded in the FIR. The ATR-FTIR is here an essential tool, since it allows to avoid the signals of absorbed water molecules. The experimentally observed signals were compared with the ones calculated using the DFT method.

We first analysed the precursor molecule, taking into account both enol and ketone tautomers (Figure S14). This analysis shows that the PG molecules appear mostly in the enol form, as depicted in Tables S1-2 and Figures S15, 19-20. The experimental spectra are dominated by peaks from the molecular vibrations of the phenyl ring and hydroxyl groups. Their peaks positions depend on the chemical environment; for example, the C=C stretching signal shifts from 1600 to 1630 cm⁻¹. Moreover, the peaks of the hydrogen-bonding vibration are confirmed at 60-155 cm⁻¹ (Figure S19). These results indicate the essential role of intra- and intermolecular hydrogen bonds.

The IR spectrum of the PG was then used as a reference to analyse IR spectra of PG CNDs (Figures 5a-b and S21). The PG CNDs are rich in polar moieties. A pure fingerprint of the CNDs of the OH stretching in PG CNDs can be found in the ATR-FTIR spectra (Figure Sb). The complex IR band in the range of 2300-3700 cm⁻¹ (i.e. the OH stretching) corresponds to stretching vibrations of hydroxyl groups. Its significant broadening indicates the formation of a new hydrogen-bonding network, not observed in the PG sample. The collective nature of this hydrogen-bonded assembly is evidenced by new complex and strong bands at ca. 165 cm⁻¹ (Figures S21).

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specific for CDs with the heterogenous architecture, as compared to graphene quantum dots (GQDs). Moreover, the characteristic signals of PG (i.e. the aromatic C-H vibrations at 1000, 1160, and 3070 cm⁻¹) decrease radically for PG CNDs (Figures 5c and S22). This confirms the complete consumption of the PG precursor during the synthesis of PG CNDs. Raman peaks at 1500-1540 cm⁻¹ may be assigned to the sp²-carbons, affected by structural distortions (e.g. termination with the oxygen groups or furan cyclization). These findings reveal the rearrangement of isolated phenyl rings of PG into more complex aromatic domains in PG CNDs; they appear in versatile structural forms, including the conjugated and the rich-in-oxygen aromatic motifs. The Raman spectra of PG CNDs unravel also the presence of the sp³-hybridized carbon groups in PG CNDs: the aliphatic CH vibrations are found at 1430-1460 cm⁻¹ and 2830-2950 cm⁻¹ while the peaks in the range of 1020-1120 cm⁻¹ can be assigned to the skeletal stretching. As a result, the diverse and heterogenous internal structure of PG CNDs can be evidenced; it is consistent with the FTIR and NMR studies.
With the Raman spectra, three crucial differences can be found between PG CNDs. The Raman peaks of aliphatic CH vibrations are strong and well-defined only for CYAN CNDs. The Raman bands of the C=C stretching of GREEN and YELLOW become broader as compared to narrow signals of CYAN. Moreover, the Raman signature of the structural distortions in the \( sp^2 \)-carbon domain is observed in YELLOW CNDs. These findings indicate that the character of the internal structure of CNDs evolves from the molecular (CYAN) to the assemblies with the conjugated aromatic domains (GREEN and YELLOW).

The nature of the chemical bonds of PG and PG CNDs was studied with the high-resolution C1s and O1s XPS peaks.\(^{19,103-106}\) The C1s peak of the PG precursor has only three components (i.e. the C=C, the C-O/C=O-C, and the \( sp^2 \) shake-up signals) and the O1s signal shows the predominant content of the C-O bonds. In contrast, as shown in Figures 5e-g, the C1s bands of PG CNDs are composed of six components. The most intense signals appear at 284.7 eV (C=C), 285.3 eV (C=C), 286.5 eV (C-O/C=O-C), and 287.4 eV (C=O). There are also weak C1s components at 289.5 eV (O=C=O) and 292.5 eV (\( sp^2 \) carbon shake-up). The formation of the different carbon-oxygen bonds in PG CNDs was also confirmed by the analysis of O1s bands (Figure S23). Therefore, the XPS results evidence that PG CNDs possess a complex internal structure with \( sp^2 \)/\( sp^3 \)-carbon domains and versatile oxygenous groups, as compared to the PG.

From the XPS data we found that the amount of the aliphatic carbon (the C-C band) decreases with the red-shifting emission spectrum (from blue to yellow region). The content of the C=C bonds remains nearly constant while the contribution of the C-O/C=O-C peaks grows (Tables S4). The gradual increase in the C=C:C:C and the C-O/C=O-C:C ratios were then observed for CYAN, GREEN, and YELLOW CNDs, respectively (Table S5). As a result, the overall ratios between the carbon-oxygen and carbon-carbon bonds also rise. The above results underline the increasing role of the oxygenous groups and the significant rearrangement of the aromatic domains and in the redshift of the emission spectra of PG CNDs.

**Solvatochromic effect on OPA and OPEF properties**

As shown above, the OPA and OPEF properties of PG CNDs can be strongly affected by their solvating media (i.e. methanol and d\(_6\)-DMSO). This correlation is further explored, using YELLOW CNDs as a case study. Their OPA and OPEF spectra were recorded for a set of solvents (11 conventional and 2 deuterated ones); these solvents provide remarkable dispersibility of the CNDs. The UV-Vis extinction spectra of those suspensions are different, especially in the blue-green region (Figure S44). In ethylene glycol and methanol, the YELLOW CNDs show narrow major OPA peaks at 462 nm and 468 nm. The OPA spectrum tends to be slightly broadened (from blue to yellow region). The content of the C=C bonds remains nearly constant while the contribution of the C-O/C=O-C peaks grows (Tables S4). The gradual increase in the C=C:C:C and the C-O/C=O-C:C ratios were then observed for CYAN, GREEN, and YELLOW CNDs, respectively (Table S5). As a result, the overall ratios between the carbon-oxygen and carbon-carbon bonds also rise. The above results underline the increasing role of the oxygenous groups and the significant rearrangement of the aromatic domains and in the redshift of the emission spectra of PG CNDs.

The OPEF maxima evolve gradually when the dispersing media are changed from simple alcohols (ca. 550 nm) to their longer-chain analogues (i.e. n-heptanol, ca. 559 nm). The last group of organic solvents (DMF, DMSO, and acetone) leads to the most significant redshift of the OPEF peaks to 572 nm. It is worth noting that for deuterated solvent analogues only minor variations in the position of the OPEF maxima can be identified: d\(_6\)-methanol (\( \Delta \lambda = 2 \) nm) and d\(_6\)-DMSO (\( \Delta \lambda = 1 \) nm).

A significant solvent-induced change was also observed in the fluorescence dynamics. In contrast to a single fluorescence lifetime observed in the alcohol dispersion (Table S7 and Figure S31), the fitting of the fluorescence decays of a d\(_6\)-DMSO dispersion leads to two lifetime components (Table S7 and Figure S32). The average fluorescence lifetime (\( \tau \)) of 4.4 ns is around 1.5 ns longer than the lifetime value of a methanol suspension. Long \( \tau \) and high FQY values confirm also an increasing role of the k\(_{tr}\) parameter of a d\(_6\)-DMSO dispersion of YELLOW CNDs; the k\(_{tr}\) is comparable with its k\(_{tr}\) analogue (Table S8). Note that YELLOW CNDs in methanol possess k\(_{tr}\) which is nearly 3-fold larger than k\(_{tr}\). More complex and long-living fluorescence indicates that various new solvation interactions appear when a protic solvent is replaced by an aprotic one.

To better understand the chemical factors governing the solvatochromic effect, major parameters were chosen to be examined. First, the influence of the solvating media can be analysed in terms of their relative polarity/non-polarity. Hence, we considered two parameters used in previous studies on solvatochromic effects of CDs: \(^{34}\) the dielectric constant (\( \varepsilon \)) and the Reichardt’s scale parameter (E\(_z\); see ESI and Table S10).\(^{107}\) No clear correlation between the OPEF maxima and dielectric constant was observed (Figure S45). On the other hand, the OPEF peaks plotted as a function of the E\(_z\) trend to decrease (Figure 6d). A similar relationship was reported by Yoshinaga et al.\(^{35}\) In contrast, the reverse trends were found so far for nitrogen-doped CDs,\(^{35,108}\) as well as other heteroatom-free PDs.\(^{31}\)

Our results indicate also that the fluorescence colour of PG CNDs is related to the overall solvation capacities of solvating media; however, it cannot explain exhaustively the interactions occurring between them and the CNDs.

Therefore, we further analysed the OPEF maximum positions in a function of the Hansen Solubility Parameters (\( \delta \), see ESI and Table S10).\(^{109,110}\) A distinct correlation was found in the case of the hydrogen-bonding component (\( \delta_{h,b} \)); the OPEF maxima reach a plateau in the aprotic solvents’ region, corresponding to the hydrogen-bond acceptors (Figure 6c); then, the emission maxima decrease nearly linearly (\( R^2 = 0.959 \)) when the \( \delta_{h,b} \) parameters of protic solvents (i.e. the hydrogen-bond donors) increase. In contrast, the OPEF peak positions are not correlated with the other two parameters (i.e. \( \delta_{polar} \) and \( \delta_{polar} \), Figure S46). The hydrogen-bonding is therefore a dominating factor of the solvatochromatic response of PG CNDs, and may be used for the precise tuning of the emission colour. This statement is confirmed by the comparison of fluorescence of PG CNDs suspended in the standard protic solvent and in its deuterium-substituted analogue; for example, the use of d\(_6\)-methanol red-shifts slightly the fluorescence peak (\( \Delta \lambda = 2 \) nm).

The influence of the hydrogen bonding can be quantified with the parameters of the solvents’ basicity (acceptor; \( \alpha \)) and acidity (donor; \( \beta \)) from the Kamlet-Taft equation (see ESI and Table S10).\(^{111}\) OPEF maxima plotted as a function of the \( \alpha \) parameter keep the linear tendency for donors (Figure S47), which is consistent with the \( \delta_{h,b} \). On the contrary, no trend was observed for the acceptors. This evidences that the varying donor abilities of solvents play here a


Figure 6. The solvatochromic effect vs. linear optical properties. a) The evolution of the temperature-dependent integrated fluorescence intensity of YELLOW CNDs’ dispersions after normalization with the respect to the intensity value obtained for dispersions at room temperature. Note that the temperature ranges had to be different because the solvents have various melting and boiling points. b) The OPEF spectra of YELLOW CNDs in different solvents. c) The OPEF maxima of YELLOW CNDs in the organic media plotted vs. the Hansen Solubility Parameters. This relation was fitted with a linear function in the case of the hydrogen-bond donors. d) The alternative correlation between the OPEF maxima and Reichardt’s Scale of the polarity. The solvents are numbered as follows: 1 - ethylene glycol, 2 – methanol, 3 – d$_6$-methanol, 4 - ethanol, 5 – isopropanol, 6 – n-butanol, 7 – n-pentanol, 8 – cyclohexanol, 9 – n-heptanol, 10 – dimethylformamide (DMF), 11 – d$_6$-DMSO, 12 – DMSO, and 13 – acetone. The hydrogen-bonding acceptors and donors are grouped with red and blue colours, respectively. The blue arrow indicates the increasing donor ability of the solvents.

crucial role in the gradual tuning of the emission colour. Meanwhile, each aprotic medium causes colour-switching in the same manner. The essential role of hydrogen bonding in the solvent-induced one-photon fluorescence response of CDs has already been discussed in the literature. The most attention was initially paid to nitrogen- and sulphur-doped nanostructures. For example, Reckemeier et al. reported that the correlation between excitation and emission wavelengths of CDs can depend on the hydrogen-bond nature of the solvent (acceptor: DMSO and donor: water).$^{3}$ Mukherjee et al. identified the significant shift of the OPEF maxima, indicating their relocation from blue (donor, in water) to green (acceptor, in ethyl acetate) regions.$^{112}$

In the case of heteroatom-free CDs, Basu et al. indicated that the donor and acceptor capacities of solvating media modulate the position of the edge-state emission. However, those CDs provide broadband and excitation-dependent OPEF spectra which precludes high purity of emitted colour.$^{31}$ The prominent solvent effect on the emission of PG CNDs reflects their structural features. As described above, PG CNDs are decorated
with the protic (hydroxyl and carbonyl) and aprotic (ether and carbonyl) groups. The high amount of these polar sub-units leads to the rearrangement of the hydrogen bonding network in various media. Therefore, PG CNDs can interact with solvent molecules via hydrogen bonds in both ways: as acceptors (e.g. with methanol) and donors (e.g. with DMSO).

**Temperature effects on one-photon absorption and fluorescence activities**

The OPEF of **YELLOW** CNDs was also studied in a function of temperature (Figures 6b and S48-49). It is worth noting that OPEF responses of **YELLOW** CNDs suspended in methanol and in d<sub>6</sub>-DMSO are different. The integrated fluorescence intensity of methanol-dispersed sample gradually decreases as temperature grows, which is consistent with previous observations. A d<sub>6</sub>-DMSO-dispersed sample shows a decreasing trend only in the range of 20-40°C and then, a sharp increase, reaching the strongest emission enhancement at 70°C. Nonetheless, both systems maintain their excellent OPEF properties in the wide range of temperature (methanol: -5°C - 45°C, and d<sub>6</sub>-DMSO: 20°C - 75°C) – at least 90% of the room temperature values.

The temperature effect on OPEF intensity can be also discussed in terms of the influence of the solvating agents, and the stabilizing role of the hydrogen bonding. When the temperature rises, the relaxation rate of solvent molecules increases. This causes the gradual weakening and breaking of the hydrogen-bond network (the δ<sub>HB</sub> is known to decrease at elevated temperatures). The CNDs tend then to relocate faster (intramolecular motions are enhanced), interact with each other, and aggregate. As a result, the OPEF signal is slightly quenched. On the other hand, the rising of the OPEF signal for the d<sub>6</sub>-DMSO sample (at 40-70°C) suggests that weak hydrogen bonds can also account for specific aggregation forms in the aprotic environment, leading to a minor fluorescence enhancement. The formation of new agglomerates is confirmed when monitoring the evolution of the extinction spectra; the main absorption peaks appear to be red-shifted at high temperatures (in methanol: Δλ = 5 nm; in d<sub>6</sub>-DMSO: Δλ = 2 nm, see Figure S50).

Moreover, in both solvents we observed the changes in the width of OPEF signals. Their progressive broadening at the increasing temperature was found for the methanol sample (ΔFWHM: +5 nm); the d<sub>6</sub>-DMSO suspension reveals the opposite trend (ΔFWHM: -3 nm) (Figure S49).

All the results described above indicate the formation of the different hydrogen-bonding networks between CNDs and solvent molecules, depending on the donor/acceptor nature of the solvent.

**Solvent-triggered colour switching in TPA and TPEF activities of **YELLOW** CNDs**

The solvent-induced colour-switching in the OPEF of PG CNDs is also reflected in their TPEF properties. To give an insight into the underlying colour tuning in the non-linear regime, the TPA and TPEF were studied for representative system of **YELLOW** CNDs.

The TPE band of **YELLOW** CNDs in d<sub>6</sub>-DMSO red-shifts with respect to the methanol suspended CNDs to reach its maximum at 576 nm (λ<sub>TPE, methanol</sub> = 552 nm). This result is consistent with the one observed for the OPEF (λ<sub>OPEF, d6-DMSO</sub> = 572 nm and λ<sub>OPEF, methanol</sub> = 550 nm). Moreover, the two-photon excitation-emission map of the d<sub>6</sub>-DMSO dispersion reveals a pronounced extension of the two-photon excitation region up to 1100 nm, as compared to the methanol sample (Figures 7a and S38).

To quantify the TPA activity, the σ<sub>TPA</sub>/M and Φ<sub>TPA</sub>/M parameters...
were estimated and plotted in Figures 7b and S41-42. In the range of 800-1000 nm, the \( \sigma_{\text{TPA}} \)-DMSO-dispersed sample shows a narrow and strong TPA peak at 820 nm instead of the broad TPA band observed in the methanol suspension (Figures 7b). Two new TPA peaks emerge at ca. 1040 nm and 1150 nm. Nevertheless, the \( \sigma_{\text{TPA}} \)-DMSO suspension retains the strong TPA shoulder in the low-wavelength window (<800 nm).

The TPA performance of both dispersions were then compared. The \( \sigma_{\text{TPA}} \)-DMSO suspension possesses the lower values of the TPA parameters than its methanol-dispersed analogue (Figure S43): the \( \sigma_{\text{TPA}} \)/M and \( \phi \sigma_{\text{TPA}} \)/M reach 1.7·10\(^{-2}\) GM·mol/g and 7.8·10\(^{-3}\) GM·mol/g at 760 nm, respectively. The minor enhancement of both TPA parameters of the \( \sigma_{\text{TPA}} \)-DMSO sample is observed in the high-wavelength range (1040-1080 nm). These results are still comparable with other efficient two-photon absorbers. Moreover, the \( \sigma_{\text{TPA}} \)-DMSO dispersion maintains the \( \sigma_{\text{TPA}} \)/M and \( \phi \sigma_{\text{TPA}} \)/M values above 1080 nm where the TPA response of the methanol suspension cannot be detected.

Such a large variation of the TPA activity can be again attributed to the hydrogen bonding within the solvent. As discussed above, changing the solvating medium from a donor to acceptor-like accounts for the charge modification in polar moieties and then, the overall charge distribution within a single nanodot.

To the best of our knowledge, it is the first report on the TPEF colour tuning which is induced by solvent, keeping remarkable TPA and TPEF performances.

**Fluorescence mechanism**

The fluorescence of PG CNDs is expected to originate from the radiative recombination of the exciton (the electron-hole pair; e-h).\(^4\),\(^114\) The energy of the e-h recombination is related to the bandgap energy. This process is localized within the finite-sized sp\(^3\)-carbon domains - surrounded by defects (e.g. sp\(^3\)-carbon matrix).\(^114\) Hence, it is typical for CDs with aromatic sites.\(^4\),\(^115\) In principle, it involves mainly the \( \pi-\pi^* \) electronic transitions;\(^114\) however, it may also be modified by the \( n-\pi^* \) electronic transitions by incorporating electron-rich moieties (e.g. polar groups).\(^115\)

Therefore, it is crucial to understand better the tuning strategies for the OPEF and TPEF colours from the chemical point view. We intend to find the chemical factors which could affect the distribution of electronic orbitals and account for manipulating the energy of the e-h recombination. In contrast to previous studies,\(^116\)-\(^118\)
the fluorescence properties of PG CNDs do not depend on their size; hence, the quantum size effect cannot be considered here. Nevertheless, we identified two other significant chemical factors. The strategy I relates to the structure of CNDs itself. We found that the conjugation of the aromatic domains (with the decreasing content of the sp³-carbon) and the increasing contribution of the oxygenous groups resulted jointly in the redshift of the fluorescence. These findings may be confirmed by correlating the fluorescence parameters (OPEF and TPEF energies, and the OPEF lifetime) and the signal ratios from the XPS assays; they exhibit the linear trends with the ratio of the C=C:C=O (Figure 8a) and the ratio of the C-O:C=C bonds (Figure 8a).

The combination of small molecular sp²-carbon units into the large aromatic domains changes the displacement of π-electrons within the CNDs. As a result, the separation energy between the π and π* orbitals may decrease, leading then to the narrowing of the bandgap.114, 115, 119-122 Meanwhile, the charge distribution in the n-electron system of the CNDs can be also modified by decorating the heterogenous structure of CNDs with the external and internal oxygenous groups. Among them, hydroxyls and ether bridges dominate within CNDs, acting as donors of non-bonded (n) electrons. As a consequence, the intermediate n-orbitals are formed between the π and π* orbitals, causing the formation of midgap states.115, 123 Both the narrowing of the bandgap and the intraband states result in the decrease of the energy from the exciton recombination. Therefore, the OPEF and TPEF spectra are red-shifted. We should note that the stability of the electron and hole tends to be weaker when the electron is trapped in the low-energy states. Therefore, they recombine faster as confirmed by decreasing fluorescence lifetime (3.9 ns → 2.8 ns) and ks values (0.16 ns⁻¹ → 0.090 ns⁻¹) (Tables S7-8).

The strategy II involves the interplay between the oxygenous groups of the CNDs and the surrounding media. Their chemical nature may modify the electron distribution within the CNDs. We expect PG CNDs to exchange electrons with solvent molecules via hydrogen bonds.124 If the polar moieties (e.g. hydroxyls) receive electrons by interacting with the hydrogen-bond acceptors (i.e. electron-donating groups), the content of n-electrons in CNDs structure will increase. As a result, new n-orbitals can be formed, as compared to the case with the hydrogen-bond donors as a solvent. This accounts for the low-energy intraband states for the e-h recombination and the redshift of OPEF and TPEF spectra. A new long-lifetime component (~9 ns, Table S7) signifies complex interactions in this case. The simplified fluorescence mechanism is represented in Figure 8c. It is worth noting that the strategies described above can be used to tune both the OPEF and TPEF of PG CNDs; therefore, they extend the application potential of multicolour PG CNDs.

Conclusions

Using the PG as the precursor molecule and following two synthesis methods: solvothermal treatment and thermal decomposition, we synthesised three types of CNDs. All PG CNDs have bright and narrow bandwidth OPEF and TPEF spectra. However, they differ in the emission colour – it ranges from blue to orange. The excellent TPEF performance is due to broad and strong TPA in the red and NIR regions (including the first biological window). The combination of theoretical and experimental studies allowed the detailed description of heterogenous internal design of PG CNDs. PG CNDs are found to be composed of a variety of aromatic/aliphatic groups, and enriched with oxygen-containing polar units (e.g. hydroxyl and ether groups). The ratios of the sp²- and sp³-carbons can be used to identify the particular kind of PG CNDs. The emission colours of CNDs were tuned by changing donating/accepting character of the dispersing media; it indicates the crucial role of hydrogen bonds in the process. Finally, a deep insight into the fluorescence mechanism of PG CNDs was gained. We recognized two chemical strategies: the content of the oxygenous groups with the conjugation of aromatic sites (strategy I) and the donor/acceptor character of the solvents (strategy II) in the formation of the hydrogen bonds. They were found to regulate the content of the π- and n-electrons within CNDs' structure and in consequence, govern the emission colour of the CNDs (both OPEF and TPEF).

The above interpretation is essential for the potential application of PG CNDs in various fields, including bioimaging (the conventional and multiphoton) and optoelectronics. In the first case, a thorough understanding of the structure-property relations can help in the analysis of interactions between PG CNDs and the biological species (with a complex composition). Second, it can facilitate the informed selection of the synthesis protocol and the chemical environment for the synthesized CNDs (e.g. the polymeric matrix) to produce the light-emitting layers with a particular emission colour (e.g. for the light-emitting-diodes, LEDs). The relevant conditions enable especially the fabrication of the LEDS with a pure blue signal.

Author Contributions

Sebastian G. Mucha – conceptualization, methodology, investigation, formal analysis, writing – original draft, writing – review & editing, visualization, validation; Lucyna Firlej – conceptualization, validation, writing – review & editing, funding acquisition, and supervision; Filip Formalik – software and formal analysis; Jean-Louis Bantignies – formal analysis, resources, and writing – review & editing; Eric Anglaret – formal analysis, resources, and writing – review & editing; Marek Samoć – formal analysis, and writing – review & editing; Katarzyna Matczyszyn – conceptualization, validation, resources, writing – review & editing, supervision, and funding acquisition;

Conflicts of interest

There are no conflicts to declare.

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