Towards red emissive systems based on carbon dots

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Abstract: Carbon dots (C-dots) represent an emerging class of non-toxic nano-emitters that show excitation wavelength dependent photoluminescence (PL) with high quantum yield (QY) and minimal photobleaching. The vast majority of studies focus on C-dots that exhibit the strongest PL emission in the blue/green region of the spectrum, while longer wavelength emissions are ideal for applications such as bioimaging, photothermal and photodynamic therapy and light emitting diodes. Effective strategies to modulate the PL emission of C-dot based systems towards the red end of the spectrum rely on extensive conjugation of sp² domains, heteroatom doping, solvatochromism, surface functionalization and passivation. Those approaches are systematically presented in this review, while emphasis is given on important applications of red-emissive suspensions, nanopowders and polymer nanocomposites.

Keywords: fluorescence; carbon-dots; red-emitting; bioimaging; light emitting diodes.

1. Introduction

The dynamic presence of carbon dots (C-dots) in the field of nanoemitters over the last 15 years is directly related to their unique combination of three desired characteristics: inexpensive preparation, non-toxic nature and superior photophysical properties in terms of light absorption, chemiluminescence, electroluminescence, phosphorescence and up-conversion [1]–[5]. Typically, C-dots are spherical nanoparticles with size 2-20 nm that are composed of C, O, H, along with heteroatoms such as N, P, S. Their carbogenic core can be predominantly amorphous or highly graphitic in nature [6], while the term graphene quantum dots (GQDs) is typically reserved for nanodisks of single or few-layered graphene sheets [7]. In that sense, GQDs are considered as a distinct subgroup of C-dots and are synthesised by various strategies including chemical vapor deposition on hexagonal boron nitride substrates [8], all-organic synthesis starting from polyphenylene dendrimers [9] and ring opening of fullerenes adsorbed to Ru metal teraces [10]. Alternatively, GQDs are prepared by oxidation or electrooxidation of carbon-rich sources such as coal [11], carbon black [12], graphite [13], carbon fibres [14] and carbon nanotubes (CNT) [15].

Well-defined C-dots are prepared at a large scale following hydrothermal, microwave and calcination protocols of a wide range of feedstock materials, including renewable and abundant resources such as crude biomass [16], coffee beans [17], fruit juice [18], etc. Pyrolytic decomposition of suitable precursors gives rise to molecular fluorophores that are embedded within the carbogenic cores or are adsorbed on their surface [19], [20]. For example, thermal treatment of urea and citric acid (CA) generates blue-emissive citra-zinic acid and green emissive 4-hydroxy-1H-pyrrolo(3,4-c) pyridine-1,3,6(2H,5H)-trione [21].

The PL properties of aqueous solutions of C-dots have been systematically investigated with emphasis on theragnostics [22], gene delivery [22], drug delivery [23], antimicrobial applications [24], bioimaging [25] and biosensing [26]. At the same time the PL properties of polymer-based nanocomposites [27], [28] and powder formulations based
on C-dots [29], [30] are directly relevant to nanoforensics [29], [30], light emitting diodes [31] and catalytic platforms [32].

In contrast to conventional semiconducting quantum dots that are based on heavy metals, C-dots are considered as non-toxic, biocompatible and environmentally benign nanomaterials. Nevertheless, it has been demonstrated that the surface functionalization can dramatically modify their cytotoxicity, the cell uptake and the intercellular trafficking [33], [34].

Although a large variety of blue/green emissive C-dots with advanced PL properties have been developed, it has been demonstrated that extensive π-conjugated domains, high levels of surface oxidation, incorporation of heteroatoms such as N, S, P and solvatochromism can give rise to red-shifted emissions [35], [36]. It has been recently suggested that the red/NIR emissive signals recorded on C-dot based systems are stemming from organic-like dye molecules encapsulated within carbonaceous nanostructures and might not be related to the intrinsic properties of nanoparticles [37]. This approach however might not explain the behaviour observed in GQDS and in related systems not prepared via pyrolysis. There is general consensus that pyrolytic treatments of precursor molecules give rise to complex mixtures of carbonaceous nanoparticles, crosslinked systems and organic molecules, necessitating further systematic studies in order to accurate deconvolute and identify the emissive contributions of each one of those components.

In this review we present promising strategies for the synthesis of C-dots based systems with predominantly red-shifted optical signals. Particular attention is given to the preparation of red-emissive liquid suspensions, polymer nanocomposites and nano-powder formulations. The distinct advantages of those nanomaterials in emerging applications such as bioimaging, biosensing, photothermal and photodynamic therapy, light emitting diodes, pollutant detection and nanoforensics are highlighted.

2. Discussion

2.1. Strategies to generate red-shifted C-dots.

The main strategies that give rise to red-shifted C-dots rely on: i) Heteroatom doping, ii) Extensive conjugation length, iii) Surface functionalization and passivation and iv) Solvatochromism.

**Heteroatom Doping**

Heteroatom doping is a well-explored strategy to modify the electronic and optical properties of C-dots, ultimately leading to red-shifted C-dots [38]. To that end, Hola et al. [39] synthesized N-doped graphitic C-dots following the solvothermal treatment of urea and CA in formamide (Figure 1a) that were subsequently subjected to column chromatography yielding blue (b-C-dots), green (g-C-dots), yellow (y-C-dots) and red (r-C-dots) emissive fractions as shown in Figure 1b, c, d, e, respectively. In water, at $\lambda_{\text{ex}}=440$ nm the QY of b-C-dots, g-C-dots, y-C-dots and r-C-dots were 13.3%, 10.0%, 11.6%, and 4.0%, respectively. Transmission electron microscopy (TEM) imaging suggested that their average diameter ($d_{\text{av}}$) was 2-3 nm (Figure 2a-d). X-ray photoelectron spectroscopy (XPS) indicated a lower concentration of oxygen, hydroxyl and carboxyl groups and an increased content of graphitic nitrogen for y-C-dots and r-C-dots (Figure 2e-l), while nitrogen stemming from surface amide groups was dominant in b-C-dots and g-C-dots. Fourier transform infrared spectroscopy (FT-IR) confirmed the presence of carboxylic C=O bonds and surface C–N bonds for b-C-dots and g-C-dots, whereas the most pronounced FT-IR peaks were ascribed to C–N= and C=N bonds for y-C-dots and r-C-dots, verifying the dominant role of graphitic nitrogen for the red-shifted fractions.
Moreover, Ding et al. [40] fabricated red graphitic C-dots (QY=28% at λ_{ex}=533 nm) with d_{av}=4.6 nm by means of solvothermal treatment of lemon juice in ethanol (λ_{ex} stands for excitation wavelength). Their PL spectra suggests λ_{ex}-dependent behaviour, with the maximum of their emission peak at λ_{max}=615 nm. FT-IR suggests the presence of O–H, N–H, –COOH and C–O on their surface, while C=C/C≡N and C–N vibrations were attributed to the polyaromatic structures of the carbogenic cores. XPS confirmed the presence of C–C/C=C, C–N, C–O, C=O/C≡N, and COOH groups along with the presence of pyridinic C=N–C, pyrrolic C=C–N–C, and graphitic N–C3 groups.

Capitalizing on similar concepts, Guo et al. [41] used various ratios of two types of functionalized polythiophene derivatives to prepare a series of graphitic C-dots with tunable PL emissions ranging from blue to near infra-red (NIR). All C-dots consisted of C, O, S, while the N content was higher for the red-shifted fractions. Pan et al. [42] synthesized graphitic C-dots with d_{av}=6.8 nm, through microwave heating of CA and formamide solution in an autoclave with QY close to 11.9%, 16.7%, and 26.2% at λ_{ex}=360, 450 and 540 nm, respectively. Likewise, Pan et al. [43] followed microwave-assisted heating of
glutathione formamide to synthesize graphitic NIR-emitting C-dots with $d_{av} = 2.9$ nm and QY = 16.8% at $\lambda_{ex} = 420$ nm.

In terms of multi-heteroatom doping, Huang et al. [44] followed a hydrothermal method using 3-aminobenzeneboronic acid and 2,5-diaminobenzenesulfonic acid as precursors to synthesize N, B, S co-doped C-dots (NBS-C-dots) with $d_{av} = 2.8$ nm and QY = 11.6% at $\lambda_{ex} = 520$ nm. NBS-C-dots show $\lambda_{ex}$-independent behavior with $\lambda_{max} = 605$ nm for $410 \text{nm} \leq \lambda_{ex} \leq 530$ nm (Figure 3a). The high-resolution C 1s XPS spectrum indicates the presence of C-B (283.89 eV), C-C/C=C (284.78 eV), C-O/C-S (285.55 eV), and C-N/C-O (286.00 eV), respectively (Figure 3b). The N 1s XPS spectrum can be deconvoluted into three main peaks at 401.95 eV, 401.20 eV, and 399.48 eV which are related to N-H, C-N, and C-N-C, respectively (Figure 3c). The B 1s XPS spectrum reveals three peaks at 192.23 eV, 191.10 eV, and 190.43 eV, corresponding to B-S, B-O, and B-C, respectively (Figure 3d). The S 2p XPS spectrum indicates two peaks at 167.90 eV and 168.45 eV which relate to S 2p1/2 and S 2p3/2 spectra of the C-S-C bond and one peak at 167.9 eV that is attributed to -C-SOx (x=2, 3, and 4) (Figure 3e). The O 1s XPS spectrum shows three peaks at 532.93 eV, 532.08 eV and 531.15 eV, ascribed to O=C-O, C-O and C=O, respectively (Figure 3f). FT-IR spectroscopy suggests the presence of O-H, N-H bonds, -SCN- groups, C-O, C=C, C-N, C=C, C-S, C-O-C bonds, along with B-O, B-O-H, C-B and B-O-H bonds.

Xu et al. [45] followed the hydrothermal treatment of L-methionine and urea using water and dimethylformamide (DMF) as the solvent to produce red (N,S-dopped) and blue emitting (N-dopped) graphitic C-dots, respectively. Miao et al. [46] followed hydrothermal treatment of CA and thiourea in acetone to synthesize graphitic red-emitting N,S-doped C-dots with QY = 22% in water. Ge et al. [47] followed an hydrothermal treatment of polythiophene phenylpropionic acid (PPA) to synthesize graphitic, red-emissive S-doped C-dots with $d_{av} = 10$ nm exhibiting $\lambda_{ex}$-independent properties with QY = 2.3%. Yang et al. [48] followed hydrothermal treatment of 2,5-diaminobenzenesulfonic acid to synthesize graphitic, red-emissive N,S-doped C-dots with $d_{av} = 4.9$ nm, having QY = 2.7% at $\lambda_{ex} = 500$ nm.

In situ chromophore doping can lead to red shifted C-dot, by introducing new electron transition pathways that alter their highest occupied molecular orbital and the lowest unoccupied molecular orbital (HOMO–LUMO) levels. Karami et al. [49] developed a synthetic procedure to synthesise chromophore-doped graphitic C-dots with $d_{av} = 5$ nm via a hydrothermal treatment of glucose and 3-nitroaniline dissolved in sulfuric acid. The C-dots exhibited three $\lambda_{ex}$-independent emissions (blue, red, and yellow) under acidic,
neutral and alkaline environment, respectively. At pH=2 the C-dots showed $\lambda_{\text{max}}$=385 nm (QY =43.0%), but at pH=3 a weak red peak appeared along with the dominant blue peak. Upon increasing the pH, the intensity of the blue emission peak declined, whereas the red emission peak was enhanced and at pH= 7 there was a single emission peak at $\lambda_{\text{max}}$=610 nm (QY =14.4%), which at pH =12 shifted to $\lambda_{\text{max}}$=565 nm (QY =24.3%). FT-IR spectra showed peaks related to O−H and N−H groups, C−H stretching vibrations, C=O band of amide and carbonyl groups, C=C stretching/N−H bending bands and C−S/C−N/C−O stretching vibration bands. At the same time, a pH-dependent FT-IR peak centred at 1545 cm$^{-1}$ was attributed to the presence of azo groups (-N=N), showing maximum intensity at neutral pH and the lowest intensity at acidic pH.

**Extensive Conjugation length**

Early studies indicated the crucial role of quantum confinement in π-bonded hexagonal carbon clusters on the broadband emission of carbon-based films [50]. More recently, it has been shown that the HOMO–LUMO gap energy decreases as the size of GQDs increases [51]. Moreover, it has been demonstrated that the emission wavelength shows a linear size dependence in defect free, functionality free, zig-zag edged GQDs and the fluorescence emission covers a broad spectrum from deep UV ($\lambda_{\text{em}}$=235.2 nm) to NIR ($\lambda_{\text{em}}$=999.5 nm) as the size varies from 0.46 nm to 2.31 nm [52]. At the same time, the presence of armchair edge widens the band gap in GQDs and blue shifts their emissive signal [53].

Yeh et al. [54] relied on the oxidation of GO sheets to generate GQDs that were separated (via polyethersulfone membranes with different cut-off molecular weights) to fractions with $d_{\text{av}}$=1.0 nm (QD10), 1.6 nm (QD16), 2.6 nm (QD26), 5.4 nm (QD54), 6.1 nm (QD61), 7.9 nm (QD 79); all of which exhibited excitation-wavelength independent PL with $\lambda_{\text{max}}$ at 460 nm, 490 nm, 520 nm, 540 nm, 560 nm and 580 nm, respectively (Figure 4).

![Figure 4](image-url). Normalized PL spectra ($\lambda_{\text{ex}}$=350 nm) of QD10, QD16, QD26, QD54, QD61, QD79. Reprinted (adapted) with permission from [54]. Copyright 2021 American Chemical Society.

Li et al. [51] explored an alkali-assisted electrooxidation of graphite rod applying different current densities to fabricate a series of C-dots and demonstrated that small sized-C-dots (1.2 nm), medium-sized C-dots (1.5–3 nm) and large-sized C-dots (3.8 nm) emit within the UV (350 nm), visible (400-700 nm) and NIR (800 nm) region, respectively (Figure 5a,b). FT-IR spectroscopy indicated the presence of carbonyl (C=O) groups on their surface that can be eliminated via hydrogen plasma treatment, but without altering the PL properties of C-dots, an effect that points to the dominant role of the quantum confinement for this class of materials. Figure 5c suggests that as the size of the C-dots increases, the HOMO-LUMO energy gap decreases and therefore, the PL emission is shifted towards the NIR region of the spectra.
Yuan et al. [55] followed solvothermal treatment of CA and diaminonaphthalene in ethanol and allowed different reaction times in the presence of concentrated sulfuric acid, yielding GQDs with d_{av}= 1.9 nm, 2.4 nm, 3.8 nm, 4.9 nm and 6.7 nm emitting respectively within the blue, green, yellow, orange and red region, in agreement with the expected behaviour due to quantum confinement effects. Similar trends were confirmed by Tian et al. [56] who explored the solvothermal treatment of CA and urea in water, glycerol, DMF and their mixtures to generate C-dots with tuneable size and PL emissions.

**Surface functionalization and passivation**

Surface functionalization strategies have been shown to dramatically modify the PL properties of C-dots via defect and intrinsic state emission mechanisms [57] and charge transfer effects [58]. In principle, the presence of functional groups on C-dots generates structural defects and mid-gap states facilitating π→mid-gap states→π transitions, thus enhancing the red components on their PL spectra [59]. An interesting study showed that within a series of C-dots with similar size distribution and graphitization degree, the bandgap was gradually reduced upon increasing the degree of surface oxidation, leading to a pronounced red shift from 440 to 625 nm [60]. In electrochemically oxidized C-dots it was shown that the PL mode stemming from the organic fluorophores was blue-shifted, while the PL component originated by the carbogenic cores was red-shifted [61].

Xiong's group [60] synthesized a series of graphitic C-dots via hydrothermal treatment of urea and p-phenylenediamine (p-PD) yielding a mixture of four fractions (A, B, C, D in Figure 6a) that could be separated via silica column chromatography due to their different polarities. TEM imaging indicated that all fractions consisted of C-dots with d_{av}=2.6 nm, while FT-IR spectra revealed the presence of O−H, N−H, C−O and -COOH groups on their surfaces. XPS indicated a systematic enhancement of the oxygen content when moving from fraction A to D, a trend that is accompanied by a systematic shift from blue to red PL emission at λ_{ex}=365 nm. It has been suggested that as the population of surface oxygen atoms increases, it results in a gradual narrowing of the HOMO-LUMO bandgap, ultimately facilitating red PL emission (Figure 6b).
Liu et al. [62] followed a microwave-assisted pyrolysis of m-PD, o-PD and p-PD in formamide to generate three types of C-dots that exhibit λ\textsubscript{ex}-independent emission with λ\textsubscript{max} at 360 nm, 450 nm and 530 nm, respectively. On the basis of FT-IR and XPS data, the authors demonstrated that the red-shifted C-dots exhibited a higher level of surface oxidation and possessed a larger population of amino groups on their surface. Zhang et al. [63] reported that blue emitting C-dots (derived via pyrolysis of polyethylene glycol (PEG)) can be turned into green and orange emissive systems when treated thermally with PEG and 2,20-(ethylenedioxy)-bis(ethylamine), respectively.

Pang et al. [64] suggested that the PL of C-dots is directly related to surface-state emission, but the energy gap is controlled not only by the nature of the surface, but also by the size of the carbogenic core. In particular, they reported the synthesis of C-dots via HNO\textsubscript{3} assisted oxidation of carbon fibres (CFs). By means of ultrafiltration and using membranes with molecular cut-offs <3 kDa, 3–10 kDa and 10–30 kDa, three different fractions were isolated with d\textsubscript{av} = 2.7, 3.3 and 4.1 nm, respectively. Evidently, low concentrations of HNO\textsubscript{3} facilitate the commencement of exfoliation from the defect sites of CFs, leading to small and blue-shifted C-dots. In contrast, higher concentrations of HNO\textsubscript{3} and larger oxidation times give rise to larger red-shifted C-dots with a higher degree of surface oxidation. By accurately controlling the oxidation conditions (reaction time, size of cut-off membranes, concentration of nitric acid) a series of well-defined C-dots were prepared that exhibit λ\textsubscript{ex}-independent emission but their λ\textsubscript{max} vary from 430 nm to 610 nm (λ\textsubscript{ex}=360 nm).

Solvatochromism

Solvatochromism effects are common in C-dots dispersions and have been attributed to extensive nanoparticle-solvent dipole-dipole interactions and H-bonding [65]. By virtue of their highly responsive nature, solvatochromic C-dots have been explored as sensitive optical sensors to detect volatile organic compounds [66]. Interestingly, emissive contributions arising from edge and surface bands might induce solvatochromic shifts in the opposite directions [67]. At the same time, pyrrolic nitrogen and amino nitrogen enriched C-dots show wider solvatochromic shifts and impart higher QY [68].

Wang et al. [69] used p-PD and diphenyl ether as precursors to synthesise spherical C-dots with d\textsubscript{av}=2.6 nm. FT-IR revealed the presence of –OH, N–H, C–H, C=N, C=C, C–N and C–O groups. C 1s XPS indicated the presence of C–C/C=, C–N, C–O, and C=N, N 1s XPS indicated the presence of pyridinic, amino and pyrrolic N, while O 1s XPS suggest the presence of C–O bonds. When the C-dots were dispersed in CCl\textsubscript{4}, toluene, CHCl\textsubscript{3}, acetone, DMF, CH\textsubscript{3}OH and H\textsubscript{2}O their λ\textsubscript{max} was at 511, 525, 545, 554, 568, 602 and 615 nm, respectively. In other words, the PL of C-dots systematically red-shifts, as the polarity of the aprotic solvents increases. In protic solvents (such as methanol, ethanol, benzyl alcohol, 1-hexanol, and 1-octanol), the C-dots emitted identical red PL, regardless of the nature of the solvent, an effect that has been attributed to the formation of hydrogen bonds between the N and O atoms on the surface of C-dots and the -OH groups of the solvent molecules, that stabilise the excited states.

In addition, Li et al. [70] followed thermal treatment of CA, DMF and urea to synthesise C-dots that showed enhanced red (λ\textsubscript{max} =640 nm) and NIR (λ\textsubscript{max} =760 nm) emission in aprotic solvents such as DMSO, DMF and N-methyl-2-pyrrolidone, but in water they exhibited weaker signal in the red region and show no NIR emission. This solvatochromic effect was ascribed to electron-acceptor groups (S=O/C=O) of the aprotic solvents attached to the outer layers and the edges of the C-dots. Yang et al. [71] synthesised triphenylphosphine (P(Ph)\textsubscript{3}) functionalised C-dots (p-C-dots) with d\textsubscript{av}=4 nm and QY up to 58%. It was observed that λ\textsubscript{ex} of p-C-dots systematically red shifts from 431 to 641 nm.
upon increasing the dielectric constant (ε) of the dispersion medium (Figure 7), an effect that has been attributed to the reduced electron-donating capacity of the P(Ph)3 groups in environments with increased polarity.

2.2. Applications of aqueous dispersions of red C-dots

Bioimaging

Red/NIR emissive C-dots are considered to be ideal for bioimaging applications because they allow larger penetration depth without damaging the surrounding tissue. To that end, Liu et al. [72] suggested a rapid synthesis of red emitting C-dots (λ<sub>max</sub>= 630 nm) with QY= 10.8% in water and 31.5% in ethanol (λ<sub>ex</sub>=540 nm), using o-PD as a precursor in diluted HNO₃ via a one-step hydrothermal procedure. The C-dots showed low level of toxicity against mice osteoblasts MC3T3-E1 and bone marrow stromal cells (BMSCs) (Figure 7 a, d). The bright field and FL images (λ<sub>ex</sub>=530 nm) of MC3T3-E1 and BMSCs incubated with C-dots at a concentration of 200 μg/ml for 24 h at 37 °C are displayed in Figure 7 b, c, e, f, suggesting that C-dots are predominantly distributed into the cytoplasm and not the nucleus, thus facilitating high resolution cell imaging without affecting the replication or the transcription of DNA.

Figure 7. Viability of MC3T3-E1 (a) and BMSCs cells (d) after incubation with C-dots for the 24 h uptake process. FL images of (b, e) MC3T3-E1 and (c, f) BMSCs, respectively; (b, e) bright field and (c, f) under λ<sub>ex</sub>=530 nm. Reprinted (adapted) with permission from [72]. Copyright 2021 John Wiley and Sons.

Tan et al. [73] followed electrochemical exfoliation of graphite in 0.01 M K₂S₂O₈ solution to synthesize red C-dots with d<sub>av</sub>= 3 nm, λ<sub>max</sub>=610 nm at λ<sub>ex</sub>=500 nm. Those C-dots were used without any further surface treatment and showed minimal toxicity against Hela cells and were able to stain both their cell membrane and their cytoplasm offering clear images under the FL microscope, while exhibiting excellent photostability over a prolonged period.

Sensing/Biosensing

Oftentimes, heteroatom doping is coupled with surface patterning strategies and sp<sup>2</sup> domain engineering in order to optimise red-shifted emissions. Along those lines, Li et al. [74] followed hydrothermal treatment of CA and thiourea to synthesize red-emitting S, N co-doped C-dots that were functionalised with phenylboronic acid tags yielding S,N-C-dots-PBA with QY=23% and λ<sub>max</sub>=593 nm under λ<sub>ex</sub>=550 nm. The S, N-C-dots-PBA showed excellent biocompatibility and were able to stain both their cell membrane and their cytoplasm offering clear images under the FL microscope, while exhibiting excellent photostability over a prolonged period.
was 73% and 96% for 1.0 μM and 5.0 μM Fe$^{3+}$ ions, respectively. This behaviour indicates that S, N-C-dots-PBA can be explored for the highly sensitive intracellular detection of Fe$^{3+}$.

Figure 8. FL images of living PC12 cells stained with 5.0 μM S, N-C-dots: (a) in the absence of Fe$^{3+}$ (control sample), (b) in the presence of 1.0 μM Fe$^{3+}$, (c) in the presence of 5.0 μM Fe$^{3+}$. (d) The PL intensity of samples described in a, b, c. Reprinted (adapted) with permission from [74]. Copyright 2021 Elsevier.

In addition, Gao et al. [75] followed hydrothermal treatment of CA and neutral red dye (NR) (ratio of 1000:1) to prepare red-emissive C-dots whose PL quenches in the presence of Pt$^{2+}$ (ethanol as solvent) due to a rapid electron-transfer process between the metal ions and the C-dots’ surface. Moreover, the zebrafish (ZF), that were loaded with Pt$^{2+}$ and C-dots, exhibited PL properties, in a manner that critically depends upon the concentration of the internalised Pt$^{2+}$ (Figure 9), indicating that C-dots can be used as sensors to quantify the Pt$^{2+}$ levels present in living organisms.
Photothermal therapy (PTT)

Photothermal therapy (PTT) relies on radiation-absorbing particles to cause thermal ablation of tumor cells and subsequent cell death. Red-shifted C-dots are ideal candidates as PTT agents due to their low phototoxicity, great photothermal conversion effectiveness and deep tissue penetration.

As mentioned in the Heteroatom doping section, Ge et al. [47] developed an hydrothermal approach of PPA to fabricate red-emissive C-dots (u-C-dots) that showed a wide absorption range from 400 to 750 nm and 38.5 % photothermal conversion. Those properties make u-C-dots suitable for PL and photoacoustic (PA) imaging as well as PPT. In Figure 10a the IR images of a mouse that has been subjected to intertumoral injection of u-C-dots indicate that the temperature of the tumor cells raises up to 58.4 °C within 10 min of laser radiation, thus causing permanent damage to cancer cells. Following a 16 days course, the tumor disappeared and the mouse had fully recovered, while the mouse receiving saline treatment failed to do so (Figure 10b, c). At the same time, no distinct inflammation, cell necrosis, or apoptosis in the heart, liver, spleen, lung, and kidney were observed, confirming the absence of undesired side effects induced by u-C-dots (Figure 10d).
Photodynamic therapy (PDT)

Photodynamic therapy (PDT) has distinct advantages over traditional cancer treatments (surgery, chemotherapy and radiotherapy) such as low toxicity, minimal damage to healthy tissue and mild side effects. In PDT, a laser beam excites the photosensitizer to generate reactive oxygen that eventually destroys the tumour cells.

Jia’s group [76] followed solvothermal treatment of *hypocrella bambusae* to synthesize red-emissive HBC-dots with $d_{av}$=4.8 nm and $\lambda_{max}$=610 nm at $\lambda_{exc}$=540 nm. After 10 minutes of laser irradiation (635 nm at 0.8 W/cm$^2$), the HBC-dots solution (200 μg/ml) temperature rose by 26.9 °C, while it only increased by 3.7 °C upon irradiation at 0.1 W/cm$^2$. The results confirmed that the HBC-dots at 0.1 W/cm$^2$ could produce $^{1}$O$_{2}$ facilitating PDT, but at 8 W/cm$^2$ they could generate both $^{1}$O$_{2}$ and thermal energy facilitating both PDT and PTT treatments. Upon a 6-hour incubation of HeLa cells with HBC-dots, it was observed that the cytoplasm emitted red PL (Figure 11a). It can be seen that the co-incubation of HeLa cells with HBC-dots and 2',7'-Dichlorofluorescin diacetate (DCF-DA), a green dye that is commonly used to probe intracellular $^{1}$O$_{2}$ formation, followed by 635 nm irradiation gives rise to strong green PL emission (Figure 11b), thus confirming the presence of $^{1}$O$_{2}$. Moreover, standard MTT assay showed that HBC-dots exhibit low toxicity against HeLa cell under dark conditions (black bars in Figure 11c). The cell viability is compromised at power density at 0.1 W/cm$^2$ (PDT system, red bars in Figure 11c) and the effect is more pronounced at power density at 0.8 W/cm$^2$ (PDT+PTT system, blue bars in Figure 11c), given that 99% of HeLa cells incubated with 200 mg/mL HBC-dots do not survive. After intravenous injection of HBC-dots in mice, the PL intensity in cancer sites rose with time, reaching its maximum value after 8 h (Figure 11d).
Ge et al. [77] synthesized C-dots with excellent water solubility using polythiophene derivatives as the precursor via a hydrothermal method. The C-dots showed interesting properties such as broad absorption region from the visible to the NIR, superior biocompatibility and photostability, high $^1$O$_2$ yield and strong emission with $\lambda_{\text{max}} = 680$ nm at $\lambda_{\text{ex}} = 488$ nm.

2.3. Solid-state red C-dots and their applications

Significant progress has been made in solid-state red C-dots based systems such as hybrid powders and polymer nanocomposites typically derived via in situ polymerisation, mechanical and melt mixing and solution blending.

**WLEDs (white light-emitting diodes)**

Yuan et al. [78] fabricated graphitic, excitation-independent, green-emitting GnC-dots ($d_{\text{av}}=6.5$ nm, $\lambda_{\text{max}}=515$ nm, $\lambda_{\text{ex}}=460$ nm, QY=81%) following solvothermal treatment of perylene that were further treated with NaOH to produce red-emitting RdC-dots ($d_{\text{av}}=6.45$ nm, $\lambda_{\text{max}}=610$ nm, at $\lambda_{\text{ex}}=560$ nm, QY=80%), as shown in Figure 12a, b. FT-IR proved the presence of $-\text{NO}_2$ and $-\text{NH}_2$ for the GnC-dots and the C=O in the quinone structure for the RdC-dots. The dispersion of GnC-dots into methyltriethoxysilane (MTES) and RdC-dots into 3- triethoxysilylpropylamine (APTES) gives rise to transparent nanocomposite gel glasses that under UV radiation appear green and red, respectively (Figure 12c). To
form WLEDs, the GnC-dots/MTES gel was deposited (and allowed to dry) on a blue LED chip (27 lm/W at 20 mA, $\lambda_{ex}=460$ nm), followed by the deposition of RdC-dots/APTES gel (Figure 12d). The electroluminescence (EL) spectra of both cold and warm WLED lamps revealed three emission peaks located at 460, 508, and 615 nm, corresponding to the emission of the LED chip, GnC-dots and RdC-dots (Figure 12e), respectively. Moreover, the warm WLED lamp showed a high colour rendering index (CRI) of 92.9, compared to CRI=81.1 for the cold one.

**Figure 12.** PL spectra of (a) GnC-dots and (b) RdC-dots under different $\lambda_{ex}$ [inset: images under daylight (left) and 365 nm light (right)]. (c) Photographs of GnC-dots/MTES and RdC-dots/APTES gels bearing various C-dot loadings under daylight (up) and 365 nm excitation (down). (d) Fabrication of WLEDs from GnC-dots/MTES and RdC-dots/APTES gel glasses. (e) EL spectra (up: cold WLED, down: warm LED, and insets: the picture below WLED). Reprinted (adapted) with permission from [78]. Copyright 2021 American Chemical Society.

Jin et. al [79] followed hydrothermal treatment of l-tyrosine, o-PD, l-tyrosine/o-PD mixture to generate blue ($\text{d}_{av}=5.1$ nm, QY=8.6% at $\lambda_{ex}=365$ nm) green ($\text{d}_{av}=5.7$ nm, QY=12.6% at $\lambda_{ex}=430$ nm) and orange/red C-dots ($\text{d}_{av}=4.4$ nm, 20.9% at $\lambda_{ex}=405$ nm). Subsequently, the C-dots were dispersed in polyvinyl alcohol (PVA) to generate blue, green and orange/red emissive films. The films were deposited on a UV-chip to generate WLED with **Commission Internationale de L’Eclairage** (CIE) chromaticity coordinates (0.30, 0.33), compared to pure white light (0.33, 0.33).

As mentioned above Wang et al. [69] followed a solvothermal treatment of p-PD into diphenyl ether to synthesise graphitic C-dots that exhibit $\lambda_{max}$ at 511, 525, 545, 554, 568, 602 and 615 nm in CCl$_4$, toluene, CHCl$_3$, acetone, DMF, CH$_3$OH and H$_2$O, respectively. To prepare liquid LEDs, C-dot dispersions in toluene, DMF and methanol were encapsulated in silica glass boxes, packed above a UV-LED chip ($\lambda_{em}=370$ nm) in order to generate green, yellow and red PL contributions respectively. Moreover, the green emissive C-dots in methyl methacrylate nanocomposite and the red emissive C-dots in PVA nanocomposite were deposited on a UV-LED chip to assemble solid LEDs.
Zhang et al. [80] synthesized a colour-tuneable solid-state luminescent material via a hydrothermal procedure using CA, urea and Eu (DPA); and a 2D layered structure-nanoclay. The hybrid material obtained showed tuneable emission colours from red to blue under different λ_{ex} where the Eu^{3+} and C-dots mainly contribute to the red and blue emission, respectively, making them potential candidates for WLED applications. Yuan et al. [81] synthesized amorphous, red emissive C-dots using 1,2,4-triaminobenzen as carbon source and PEG200 as passivation agent via a solvothermal method. The C-dots showed λ_{ex}-independent behaviour, with a QY up to 25% in ethyl acetate (λ_{ex}=460 nm) and were combined with silica to fabricate red PL powder in order to construct warm WLEDs.

**Pollutant sensing**

Liang’s group [82] followed the carbonization of sugarcane bagasse in a mixture of concentrated sulfuric and phosphoric acid to synthesise red-emitting C-dots that were subsequently coated on polyvinylidene fluoride (PVDF) membranes. The PVDF/C-dots membranes exhibited red PL (λ_{ex}=365 nm) that is quenched in the presence of ammonia, but not in the presence of acetonitrile, o-nitrotoluene, toluene, cyclohexylamine, hydrazine, ethanediamine, dimethyl sulfoxide, acetic acid, acetone, chloroform, hydrochloric acid (Figure 13 a, b). The PVDF/C-dots system was shown to be a highly selective and highly sensitive sensor for ammonia with detection limit 1.7 ppm and a response time of 30 s (Figure 13c).

**Nanoforensics**

Li et al. [83] synthesized red emissive, λ_{ex}-independent, graphitic C-dots (pC-dots) with d_{av}=12.5 nm following solvothermal treatment of p-PD. FT-IR of pC-dots showed the stretching vibrations of N-H, C=N, C=C and C=C and XPS spectra revealed they consist of C (79.56%), N (15.61%), and O (4.83%). As shown in Figure 14a incorporation of pC-dots to starch gives rise to highly PL powders (the PL spectra of pC-dots in ethanol is also displayed for comparison). The pC-dot/starch powder was used to develop latent fingerprints deposited on a glass substrate and was able to reveal well defined ridges with minimal background interference (Figure 14b).
Figure 14. (a) PL emission spectra of pC-dots in ethanol and pC-dots-starch powder. Inset: pC-dots in ethanol solution (right) and pC-dots-starch powder (left) ($\lambda_{ex}$=365 nm). (b) Image of fingerprints developed with pC-dots-starch on glass substrate ($\lambda_{ex}$=365 nm). Reprinted (adapted) with permission from [83]. Copyright 2021 Elsevier.

3. Conclusions

Although the large-scale production of well-defined, red-emissive C-dots remains an open challenge, a number of promising approaches towards this direction have been demonstrated. Those synthetic strategies rely on the heteroatom doping, surface functionalization, the formation of extensively conjugated domains, while a combination of two or more of those engineering principles is usually more effective in terms of PL performance. In contrast to their blue and green counterparts, the synthesis of red C-dots based systems is rather tedious, energy intensive and largely depends on the use of aromatic precursors, organic solvents and toxic compounds. Overcoming those barriers will allow the development of a new generation of photoactive materials that can potentially advance crucial biomedical treatments, sensing applications and lighting technologies.

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References


