Crimson Publishers
Wings to the Research

Perspective

Perspective On Nanoherbals + Carbon Dots for Theranostic Applications

Ahmad F and Ahmad S*

Eros Garden, Charmwood Village, India

ISSN: 2770-6613

***Corresponding author:** Shamim Ahmad, 508, BWT, Eros Garden, Charmwood Village, Faridabad, Haryana, India

Submission: April 08, 2022

Published: May 02, 2022

Volume 3 - Issue 4

How to cite this article: Ahmad F, Ahmad S. Perspective On Nanoherbals + Carbon Dots for Theranostic Applications. Polymer Sci peer Rev J. 3(4). PSPRJ. 000567. 2022. DOI: 10.31031/PSPRJ.2022.03.000567

Abstract

A novel possibility of conjugating nano herbal formulations and carbon dots is proposed in this note for their theranostic applications. This protocol, once developed, will certainly offer the possibility of including bioimaging during animal trials of the nano herbal biomedical formulations with better compatibility and minimum toxicity. Our earlier development of nano herbal formulations involving about a dozen herbs of common use will be used in carrying out *in vitro* and *in vivo* as the second phase of developing nontoxic biomedical formulations.

Keywords: Carbon dots; Nanoformulations; Theranostic; Synthesizing; Materials; Polymer

Abbreviations: CDs: Carbon Dots; QY: Quantum Yield; TADF: Thermally Activated Delayed Fluorescence; GQDs: Graphene Quantum Dots; CQDs: Carbon Quantum Dots; CPDs: Carbonized Polymer Dots; MR: Magnetic Resonance; PET: Photo-Induced Electron Transfer; EDTA: Ethylenediaminetetraacetic Acid

Introduction

There are numerous local herbs possessing medicinal properties which have been used in traditional Indian medicines in Unani and Ayurveda system of treating different ailments. The main drawback has mostly been the lack of data regarding animal trials to assess their efficacies and fix the dosages. For taking care of these generic problems, nanoformulations of the extracts from a chosen set of herbs were studied way back in 2006 at Hamdard University, N Delhi, India. These studies were confined to preparing nano colloidal form of the herbal extracts that were tested again different disease cell lines to identify the specific ailment by screening in which they could be used. Thereafter, standard animal disease models were taken as per approved procedures available in the university for deciding their effective dosages in terms of per Kg body weight of the animal. These experimental assays conducted at that time did confirm the enhancements in their efficacies with much lower dosages in case of about a dozed herbs commonly used in Indian households. Encouraged by these observations, the research-team continued studying further resulting into few publications and patents. It was further envisaged to transform these nanoherbal formulations into the form of targeted delivery of nano herbal medicines by combing appropriate herbal extracts suitably. Accordingly, a tentative roadmap was prepared and reported for possible follow up. Out of a number of herbs, especially, curcumin was found very versatile in taking care of a number of ailments in human beings [1-3].

In the context of attempting the targeted drug and gene deliveries, based on nanoherbal formulations was not much focused at that time due to lack of some neutral carrier. However, the current reports on a new family of Carbon Dots (CDs) could possibly be explored for developing theranostic applications of the combined form of nano herbal species and CDs with the specific advantages of green processes and materials. Herbal species could very well be tried as precursors for synthesising different forms of CDs as reported in several cases.

In this brief note regarding the emerging perspective, the subject of converting nanoherbal formulations has been re-examined afresh to highlight the importance of developing hybrid forms of nanoherbals loaded onto CDs for their theranostic applications in affordable form with minimum toxicity [2,3]. For confirming the suitability of different forms of CDs, their salient features are briefly summarised here. It may be noted that there is a recent report of confirming the advantages of using nanocurcumin as antiviral combine with CDs [4].

Development of Carbon Dots - Overview

Carbon Dots (CDs) are being extensively studied for their useful adjustable Photoluminescence (PL) properties with high Quantum Yield (QY), besides their low toxicity, small size, better biocompatibility, and low-cost precursor requirements. These characteristic features of CDs are finding applications in fields like biomedicine, catalysis, optoelectronic devices, and anticounterfeiting. CDs are sub-20nm diameter species with fluorescence as their intrinsic property. In 2004, carbon NPs with fluorescence were noted during purification of SWCNTs. In 2006, nanoscale carbon particles were synthesized by laser ablation of carbon target and named CDs, but the QY of these surface passivated CDs was only ~10%. The associated low QY and complex syntheses, impeded the further development of CDs until 2013 when using alternate precursors like Citric Acid (CA) and ethylenediamine, CDs were synthesized with QY up to 80% (hydrothermal). These CDs have been used as printing inks and functional nanocomposites. Thereafter, different strategies were proposed for synthesizing CDs resulting in breakthroughs including multicolor/deep red/Near-Infrared (NIR) emission of narrow full width at half maximum (FWHM), two-/multiphoton PL, Room Temperature Phosphorescence (RTP), and Thermally Activated Delayed Fluorescence (TADF) features finding several more useful applications [5]. Currently available CDs contain Graphene Quantum Dots (GQDs), Carbon Quantum Dots (CQDs), and Carbonized Polymer Dots (CPDs) according to their formation mechanisms, micro/nanostructures, and their associated characteristic properties.

GQDs, for instance, possess single/multiple-layer graphite structures with connected chemical groups on the surface/edge or within the interlayer defects. The presence of graphene lattices come as a result of oxide cutting of larger graphitized carbon materials such as graphite powder, carbon rods, carbon fibers, carbon nanotubes, carbon black, or GO into smaller pieces. Their optical properties are dominated by the size of pi-conjugated domains and the surface/edge structures. GQDs are anisotropic, but CQDs and CPDs are typically spherical, and comprise of small molecules, polymers, or biomass by assembling, polymerization, crosslinking, and carbonization via combustion, and thermal treatment. CQDs exhibit multiple-layer graphite structures connected via surface groups. Intrinsic state luminescence and the quantum confinement due to size causes PL [5]. Aggregated/crosslinked and carbonized polymer hybrid nanostructures based CPDs were first introduced in 2018 based on their synthesis route, structures, and PL mechanism. They possess special "core-shell" nanostructures, consisting of carbon cores less than 20nm with highly dehydrated crosslinking polymer frames or slight graphitization and shells of abundant functional groups/polymer chains, which endow higher stability, better compatibility, easier modification, and functionalization. The optical properties of CPDs mainly originate from the molecular state and crosslinking enhanced emission effect, which make the relationship between structure and performance of CPDs more controllable [6,7]. CDs prepared using different carbon sources and synthetic routes, invariably present different absorption characteristics. However, they typically exhibit strong absorption in UV-region (200-400nm), with a tail extending into the visible range. Some CDs with red or NIR emission usually possess pi-conjugated electrons in the sp^2 domains and/or the connected surface groups/polymer chains resulting in their long-wavelength absorption in 500-800nm range. The absorption features of CDs are mainly affected by types and content of surface groups, size of pi-conjugated domains, and variation of the oxygen/nitrogen content in C-cores [5,8].

Photoluminescence (PL) in CDs is of importance from both fundamental and applied research point of view. Compared with other fluorescent materials such as semiconductor Quantum Dots (QDs) containing cadmium/lead, rare-earth nanomaterials, and organic dyes, CDs show better stability, higher QY, lower toxicity, low-cost precursor requirement, and excellent biocompatibility. PL brightness being directly related to QY-value, is greatly influenced by carbon sources, synthesis protocols, and post-passivation. Broadly, CDs prepared by top-down routes exhibit relatively lower QY compared with bottom-up ones. Thus, the QY of GQDs is always lower than those of CQDs and CPDs. From the perspective of PL mechanism, more defects are generated during the processing of oxide cutting carbon resources resulting in lower QY. However, surface modifications for reducing the nonradiative recombination and enhancing the integrity of the pi-conjugated system of GQDs has been used to improve the PL intensity. For CPDs, the CEE effect of crosslinked sub-fluorophores, supramolecular interactions, as well as molecular state emissions contribute to their higher QY as compared to completely carbonized CDs (GQDs and CQDs). Now, the 99% (QY) CPDs have already been reported in solution [9]. In most cases, CDs producing blue or green fluorescence restrict their further applications in biomedicine, while recent studies have successfully demonstrated red and NIR emissive CDs via adjusting reaction conditions or carbon sources. For example, red emissive CPDs with a QY of 31% were reported by modulating the dosage of HNO_3 before the reaction and this resulted in synthesizing full-color light-emitting CPDs from p-phenylenediamine and urea. By regulating the reaction temperature and CA/urea ratio, CPDs were produced with multicolor emissions. Cyanine dye and poly (ethylene glycol) were transformed into NIR CPDs with a PL peak at 820nm using solvothermal route. There are some differences among the morphology, surface groups, or nanostructures of CPDs, CQDs, or GQDs, while most of them show similar excitation-dependent emission; that is, the PL emission commonly red-shifts with the increase of excitation wavelength. Thus, the PL of CDs

can be easily regulated by controlling the excitation wavelength, without changing their chemical structure or size, which is helpful in multicolor bioimaging. The excitation-specific PL behavior and broad PL profile of CDs may come from the multiple PL centers and wide distribution of different energy levels. For preparing CDs for bioimaging applications, efforts have been made to narrow bandwidth emission. Recently, CDs with FWHM of 20-40nm were prepared showing excitation independent emissive CQDs with FWHM of 30nm. The unique rigid triangular structure, molecular purity, crystalline perfection, and weak electron-phonon interaction of the CQDs surrounded by hydroxy groups resulted in the high color-purity. Alternately, uniform size distribution of CDs gave narrow bandwidth emission. Very recently, the deep red emissive CPDs with unprecedented FWHM of 20nm and QY up to 59% were prepared from dry taxus leaves, and then purified via silica gel column chromatography. The purity, uniform size distribution, single PL center, and simple energy levels play significantly roles in preparing the narrow emissive CDs [5].

CDs have been used as fluorescent probes for detecting analytes in the environment or biosystems due to their intrinsic properties, high sensitivity, quick response, low-cost, and simple synthesis. The small size, large specific surface area, and abundant surface functional groups enable CDs to be sensitive to the surrounding environment such as temperature, ionic strength, and solvent, resulting in changes to their optical properties. For instance, the enhancement/activation (turn-on) and quenching (turn-off) of fluorescence may employ Photo-Induced Electron Transfer (PET), Fluorescence Resonance Energy Transfer (FRET), and the Inner Filter Effect (IFE) for detection purposes [5]. CDs are used in detecting cations and anions like Pt^{2+}, Hg^{2+}, Zn^{2+}, Fe^{3+}, $ONOO^{-}$, and ClO^{-}, which can bind to the surface groups of CDs including carboxylate and amino groups, through coordination/electrostatic interaction or free radical reaction. A reversible "off-on" fluorescent nanosensor was reported for selectively detecting Ethylenediaminetetraacetic Acid (EDTA) and Zn^{2+} through depassivation and repassivation of Zn^{2+}-passivated CPDs, which were prepared from zinc gluconate. Orange-emissive CPDs with pH-sensitive fluorescence immobilized in medical cotton cloth was used for wound pH monitoring via both fluorescence and visible colorimetric changes. Benefiting from the dual-model response to pH changing as well as the established analytical method, wound pH could be both predicted theoretically and estimated visually in the case of blood contamination and long-term observations. CDs show selectivity toward a wide range of biomolecules including amino acids (cysteine), glutathione, vitamins (such as vitamin B_{12}, vitamin B_7, ascorbic acid), formaldehyde, glucose, DNA, and proteins, which are associated with diseases. Therefore, CDs can provide valuable insights into the diagnostics and early precaution of diseases [5].

CDs with low toxicity, biocompatibility, and photostability are used as probes for targeting and imaging cancer cells, identifying and detecting bacteria. For example, CPDs with green emission were reported to distinguish the cancer cells from normal cells because of differences in their mitochondrial membrane potentials and substance uptake capabilities. CPDs-aptamer-conjugates have been used for selective detection of living cancer cells. Acidophilic CPDs showed blue and red emission in case of four bacterial strains namely: *Porphyromonas gingivalis*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli* with different environments and cell walls. After labeling with CPDs, compared with *Porphyromonas gingivalis*, *Streptococcus mutans* showed stronger green and red fluorescence and clearer contour, while *Staphylococcus aureus* and *Escherichia coli* only presented one color bright red and green fluorescence, respectively [5]. Most promising application of CDs is in biomedicine for *in vitro* cytotoxicity assays showing low toxicity or no toxicity and excellent biocompatibility even at a high concentration. *In vivo* experiments indicated that CDs are rapidly excreted via the kidney and/or hepatobiliary system and no symptoms of inflammation were noted in the brain, heart, lung, liver, spleen, kidney, testicle, and bladder in rats based on blood biochemistry and hematological analysis [5]. CDs are safe in biomedical applications at affordable costs. Factors like small size, controlled surface functions, high photostability, unique down-conversion PL, multiphoton PL, and high brightness make CDs behave as photo-/nanomaterials to traditional fluorescent materials in disease diagnosis, therapy, and healthcare. Fluorescence imaging has become a powerful tool in clinical diagnosis due to its characteristic features including low cost, high sensitivity, noninvasiveness, simple to use and long-term observation. However, conventional fluorophores like QDs and organic dyes suffer from toxicity and poor performance. In this context, higher photostability, excellent biocompatibility, simple synthetic routes, flexibility, multicolor emissions, deep red/NIR emission, and two-/multiphoton PL make CDs the next-generation fluorescent probes for *in vitro* and *in vivo* bioimaging.

CDs have been used for imaging cells, microorganisms, and plant tissue as they enter the cells through energy/temperature-dependent macropinocytosis-, clathrin-, caveolae-, and/or lipid raft-mediated endocytosis and are distributed into mitochondria, lysosomes, endoplasmic reticulum, Golgi apparatus, and/or nucleolus based on the different nanostructures of CDs and types of cells. Imaging organelles like mitochondria and/or nucleolus is profitable to understand and study organelle-related diseases such as cancer, Alzheimer's disease, Parkinson's disease, diabetes, and cardiac dysfunction. CPDs prepared from mPD and L-cysteine could realize nucleus-targeted imaging in both fixed and living cells. Conjugated with protoporphyrin IX, CPDs obtained a nucleus-targeted PDT ability causing effective tumor ablation without toxicity effects after laser irradiation. Lactobacillus plantarum derived CPDs served as staining agents for imaging biofilm-encased microorganisms, which could give information about the morphology and physiological state of bacteria in a biofilm. L. plantarum derived CPDs displayed better photostability than commercial dye SYTO 9, and this imaging method was much easier and universal as compared to fluorescent proteins as probes [5]. Taking advantages of minimum autofluorescence and light scattering by tissues, great imaging contrast and spatial resolution are offered by CDs with red/NIR emission or two/multiphoton

PL for *in vivo* fluorescence tracking. The first *in vivo* imaging was reported by adopting three routes in mice. The clearance rate of CDs was ranked as intravenous (tail vein), intramuscular (muscle of left leg), and subcutaneous (under the skin of left leg. A two and three-photon-induced deep red emissive CPDs were reported using surface engineering for *in vivo* deep red fluorescence imaging of the stomach of a living mouse. Recently, deep red emissive CPDs were reported with 59% QY as an efficient probe for both one-photon and two-photon bioimaging [5].

Experimental results on CPDs exhibited rapid entry in the whole body of mice in a few minutes accumulating in the liver, lung, and kidney and then were gradually cleared via both the kidney and hepatobiliary system within 24h. The red emission from CPDs prepared from oPD could easily cross the blood brain barrier of healthy mice without targeting agents, which provided a new material for prevention and theranostics of brain diseases via real-time tracking. The fluorescence imaging of CDs shows a high contrast ratio and high sensitivity, but the spatial resolution is still not satisfactory for clinic applications because of the limited penetration depth. Thus, there is a need to develop multimodal imaging probes through the combination of other imaging modalities including Photoacoustic (PA), Magnetic Resonance (MR), and Computed Tomography (CT) imaging's. Other multimodal CDs can be obtained by doping MRI/CT probes with Gd, Mn, and Yb doping into CDs [5]. Red/NIR emitting CPDs prepared from CA and urea, polythiophene, and diphenyl diselenide were recognized as effective theranostic agents for PTT with high conversion efficiency >50% due to their unique red/NIR absorption, which were superior to that of conventional photothermal agents. PTT usually requiring high-power and long-time laser irradiation to produce adequate heat to kill cells, and the up-regulated expression of heat shock proteins greatly decreases the treatment effect. To avoid drawbacks of the mono-mode therapy, synergistic PDT and PTT were adopted to cancer therapy.

Theranostic Formulations – Newer Opportunities

Targeted drug delivery carrying the medicine to a specific location in the body and releasing it in a sustained manner requires provision for controlled drug release and robust selectivity for enhancing local therapeutic effects and minimizing side effects of non-infectious and/or non-cancerous tissue. CDs have advantages in visualizing drug accumulation at the pathological sites via their fluorescent properties. Through tracking the green emission from folic acid-modified CPDs (FA-CPDs) combined with chloroquine was reported in this context. The encapsulation of GQDs/anticancer drug docetaxel in a nanosponge exhibited effective drug delivery, imaging, and photolytic abilities against deep tumors. To improve tumor-specific imaging and drug delivery performance, deep red emissive CQDs were prepared with multiple paired α-carboxyl and amino groups, which could target tumors including glioma due to their multivalent interactions with large neutral amino acid transporter. Loaded with topotecan hydrochloride, the CQDs were used for fluorescence/PA imaging and the treatment of brain cancer, showing clinical applications in imaging and drug delivery of the CNS [5]. The vectors employed in gene therapy to deliver genetic materials into cells with high gene transfection efficiency generally deploy viral vectors to invade and deliver their genetic material as effective gene carriers. However, severe safety risks due to their immunogenicity and their oncogenic potentials have not yet allowed them for clinical uses. The small size CDs, on the other hand, contribute to adequate cellular uptake of vectors, enhancing gene transfection efficiency. In addition, their unique fluorescence can be used for the tracking the gene internalization. CDs as such possess antibacterial, anticancer, antiviral, and antioxidant activities. Using drug molecules like metronidazole, gentamicin sulphate, and glycyrrhizic acid as precursors, the as-prepared CPDs possess similar or superior therapeutic performances as compared to pristine drugs due to the retention of pharmacophores in their structures or the formation of new active structures. Compared to the drug molecules, these drug-CPDs complexes show better biocompatibility and water solubility besides stronger fluorescence for bioimaging in theranostics. For instance, Met-CPDs prepared by hydrothermal treatment of metronidazole, a wide-spectrum antibiotic against anaerobes showed better aqueous solubility and excellent biocompatibility because of the formation of new functional groups like carboxyl, hydroxyl, and amino groups. The experimental data demonstrated that the Met-CPDs showed excellent selective antibacterial activity against obligate anaerobes due to nitro group, a pharmacophore, which was in accordance with the main mode of action of metronidazole [5]. A combined curcumin-CD-NP behaved as antiviral agent against enterovirus 71, while curcumin as such had no activity against EV71 in RD cells. However, further studies are required to identify the exact molecular mechanism of these drug-CPDs in the antibacterial, anticancer, antiviral activities [2-4].

Facile, green, and simpler synthesis of producing excellent optical and electrical properties, low cost, as well as good biocompatibility make CDs popular in optical, and biomedical fields besides others. CDs are still at nascent stage facing the fundamental obstacle due to lack of scalable synthesis to produce CDs with desirable structures. Their exact reaction, nucleation, and formation processes are also unclear due to non-standard synthetic pathways and impurities. Therefore, for large-scale production of CDs with high performance through an efficient route, effects of precursors and reaction conditions including temperature, time, and pH on the performance of CDs should be explored, and a purification scheme based on size or polarity also needs to be developed. Notably, developing in situ techniques is necessary for characterizing the formation mechanism of CDs, which contributes to controllable syntheses. CD-sensors for quantitative estimation of metal ions, organic molecules, or biomolecules, exhibit poor sensitivity/selectivity as compared to the other probes. CD-nanocomposites, exhibit improved selectivity in sensing and imaging. CDs emitting deep red to NIR (650–1700nm) excited by deep red to NIR light are highly desirable due to less light scattering, less damage, and deeper light penetration into the tissues in clinical applications

involving phototheranostics and smart healthcare devices like wearable brain imaging system, and skin temperature monitoring setup.

Conclusion

In the context of developing hybrid nanoformulations of involving nanostructured herbal species and CDs, systematic research on toxicity and metabolic pathways of CDs in animal models are necessary to undertake for their future clinical applications based on exact molecular mechanism of drug-CPDs interactions. In the context of CDs-based materials' real applications, a lot of work is yet to be done regarding preparation, mechanism, structures, properties, and their applications. Further developments in technology and characterizations, controlled syntheses, large-scale production, and improved understanding of the structure-performance relationship once arrived at, will extend the applications CD-based materials in conjunction with nanoherbal medicines for better health care [5].

References

1. Ahmad S, Hashim U (2012) Nanoherbals in human healthcare: A proposed research and development - roadmap I, and II. ASEAN J Sc Technol Dev 29(1): 55-75.
2. Ahmad S (2016) Engineered nanomaterials for drug and gene deliveries - A review. J Nanopharmaceutics and Drug Delivery 3(1): 1-50.
3. Ahmad S (2016) Current status and future prospects of application specific engineered nanocurcumin compounds. Int J Pharmaceutical Sciences and Nanotechnology 9(5).
4. Lin C, Chang L, Chu H, Lin H, Chang P, et al. (2019) High amplification of the antiviral activity of curcumin through transformation into carbon quantum dots. Small 15(41): 1902641.
5. Liu J, Li R, Yang B (2020) Carbon dots: A new type of carbon-based nanomaterial with wide applications. ACS Cent Sci 6(12): 2179-2195.
6. Liu J, Li D, Zhang K, Yang M, Sun H, et al. (2018) One-step hydrothermal synthesis of nitrogen-doped conjugated carbonized polymer dots with 31% efficient red emission for *in vivo* imaging. Small 14(15): 1703919.
7. Xia C, Tao S, Zhu S, Song Y, Feng T, et al. (2018) Hydrothermal addition polymerization for ultrahigh-yield carbonized polymer dots with room temperature phosphorescence via nanocomposite. Chem Eur J 24(44): 11303-11308.
8. Lou Y, Hao X, Liao L, Zhang K, Chen S, et al. (2021) Recent advances of biomass carbon dots on syntheses, characterization, luminescence mechanism, and sensing applications. Nano Select 2(6): 1117-1145.
9. Zhang Y, Liu X, Fan Y, Guo X, Zhou L, et al. (2016) One-step microwave synthesis of n-doped hydroxyl-functionalized carbon dots with ultra-high fluorescence quantum yields. Nanoscale 8(33): 15281-15287.