

The Importance of the Purification Step and the Characterization of the Products in the Synthesis of Carbon Nanodots

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In the synthesis of carbon nanodots (CNDs), the critical step of the purification from the starting materials and unwanted side products is faced. In the exciting race toward new and interesting CNDs, this problem is often underestimated, leading to false properties and erroneous reports. In fact, on many occasions, the properties described for novel CNDs derive from impurities not completely eliminated during the purification process. Dialysis, for instance, is not always helpful, especially if the side products are not soluble in water. In this *Perspective*, the importance of the purification and characterization steps, in order to obtain solid reports and reliable procedures, is emphasized.

is that (at least) part of the properties of the starting materials may be retained/transferred to the nanoparticle structure. For instance, it is possible to prepare chiral CNDs starting from enantiopure chiral organic compounds,^[4,5] or heteroatom doping can be realized by adding specific passivating agents.^[6] On the other hand, the use of high temperature/pressure conditions may trigger many simultaneous reactions with no selectivity, often resulting in the formation, along with CNDs, of many possible side products,

1. Introduction


Carbon nanodots (CNDs) are photoluminescent nanoparticles, with a characteristic size below 10 nm and a quasi-spherical morphology.^[1,2] They have attracted a lot of attention in the materials science field, mainly owing to their easy preparation and exciting properties. However, these properties are not always correctly attributed to the right components, mostly due to incomplete purification. In the preparation of CNDs, solvothermal methods have become the procedure of choice, relying on the high-temperature treatment of small organic molecules, such as amino acids, carboxylic acids, and aromatic derivatives, able to undergo condensation/decarboxylation/carbonization reactions.^[3] An appealing advantage of this synthetic approach

whose structure has nothing to do with CNDs. When these undesired compounds are not correctly and completely eliminated from CNDs, they can be responsible for properties erroneously attributed to nanoparticles.^[7] For example, molecular fluorophores formed during the solvothermal synthesis, that is, following condensation reactions, were identified as strongly photoluminescent, interfering with the properties of CNDs.^[8] Alternatively, chiral starting materials can lead to chiral side products, exhibiting chiroptical properties not deriving from CNDs.^[9] Furthermore, the commonly employed characterization techniques such as transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), and optical spectroscopies cannot confidently claim that CNDs are the only or the primary product of the synthesis nor that the observed properties derive exclusively from CNDs. Due to these critical issues, the purification and characterization processes represent fundamental steps before studying the properties (**Figure 1**). *The publication and diffusion of erroneous results inhibit the proper development and slow down the maturity of the field.* This *Perspective* aims at raising awareness on the issue of purification and correct characterization of CNDs and stresses the urgency to draw out general, reliable, and reproducible protocols for the materials preparation.^[10] In this context, the available purification techniques and novel characterization tools are presented to standardize nanoparticle production. Eventually, this work wishes to stimulate a critical re-evaluation of the state of the art for CNDs: in fact, in many instances, due to purification issues, some valuable properties have been incorrectly attributed to CNDs.

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2. The Origin of the Purification Problem

The high temperature treatment involved in the solvothermal synthesis of CNDs triggers multiple reaction pathways, which

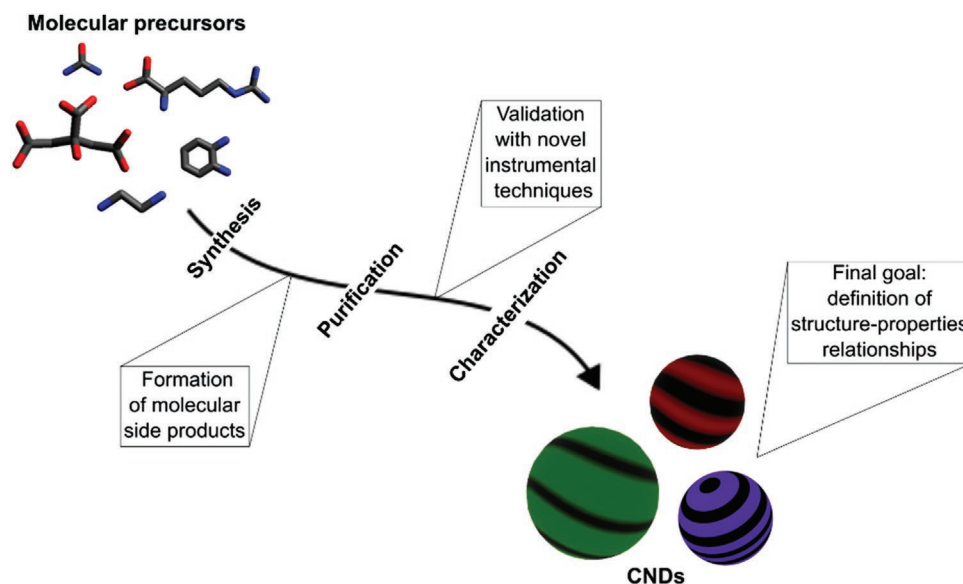


Figure 1. From molecular precursors to CNDs: crucial steps for future development.

can not only promote both the carbonization of the precursors but also simple chemical transformations. Indeed, it is known that many organic reactions are performed at high temperature, especially those including transformation of stable aromatic scaffolds, without involving the carbonization of the substrates (for an example, see ref. [11]).^[11]

Most of the time, a crude reaction mixture may contain both CNDs and molecular side products which can strongly affect the properties of the sample in terms of photoluminescence, chirality, catalytic activity, etc. Let us consider, for example, the following situation: during the synthesis, both the formation of a highly emissive species and non-fluorescent CNDs occurs. The sample, consisting of CNDs and the molecular side products, will present the emission of a solution of the fluorescent dye. Meanwhile, TEM/AFM images will reveal the presence of nanoparticles, without suggesting the presence of the dye. At that point, it is easy to believe that the synthesis of fluorescent CNDs was successful, but in fact the insufficient purification has led to erroneous conclusions. This apparently simple concept has actually been the source of many flaws present in the literature of carbon nanoparticles, where molecular features were incorrectly attributed to CNDs due to an inadequate purification.^[7,9] One of the reasons why this purification problem is so widespread is that the commonly employed characterization techniques are not able to reveal and discriminate the presence of residual molecular entities.

To further complicate the picture, we have to consider that both TEM and AFM are not always able to differentiate between aggregates of organic molecules and carbon nanoparticles.^[12] For example, Nandi et al. have demonstrated that the hydrothermal treatment of citric acid produces methylene succinic acid, which gives rise to hydrogen-bonded nano-assemblies, which, under TEM inspection appear as spherically shaped nanoparticles with an average diameter of 3.6 nm and a lattice spacing of 0.21 nm, which are common features reported for CNDs.^[13] In another example, reported by our group, we have demonstrated that a pure solution of *l*-tryptophan (*l*-Trp)

deposited on a mica substrate, in AFM appears as spherically shaped nanoobjects with a mean diameter around 2 nm.^[9] These two examples reveal that microscopic investigations cannot provide direct evidence of nanoparticle formation nor they can confirm the purity of the sample. Building on this, the general feeling is that the efficient purification of the reaction crude is mandatory, along with the introduction of novel characterization techniques for unquestionably verifying the purity of the CND samples.

3. Purification Strategies

Baker et al. investigated more than 550 literature examples for the preparation of CNDs, and found out that half of them employed insufficient purification procedures.^[7] A universal recipe for the CND purification does not exist, but some protocols can be developed in accordance with the product solubility (Figure 2). Indeed, water soluble samples can be easily filtered and dialyzed. However, the molecular weight cut-off (MWCO) of the employed dialysis membrane and the step-by-step procedure—in terms of time and water replacement—requires a proper optimization for the best outcome. A detailed investigation about these aspects was reported by Chang et al., who synthesized CNDs starting from citric acid and employed dialysis for their purification.^[14] Following the dialysis process over time, it resulted that the photoluminescence quantum yield of the retained species inside the dialysis bag decreased from 2.2% to 0.6% while, in the meantime, the sample concentration decreased from about 580 to <1 mg mL⁻¹. This result suggests that, in this case, the CND solution was mainly composed of small molecular side products and the production yield of actual CND materials was very low. Moreover, via high performance liquid chromatography (HPLC), the authors monitored the composition of both the retentates and dialysates following their UV-vis absorption and fluorescence signals, demonstrating that many side products remain in the retentate after

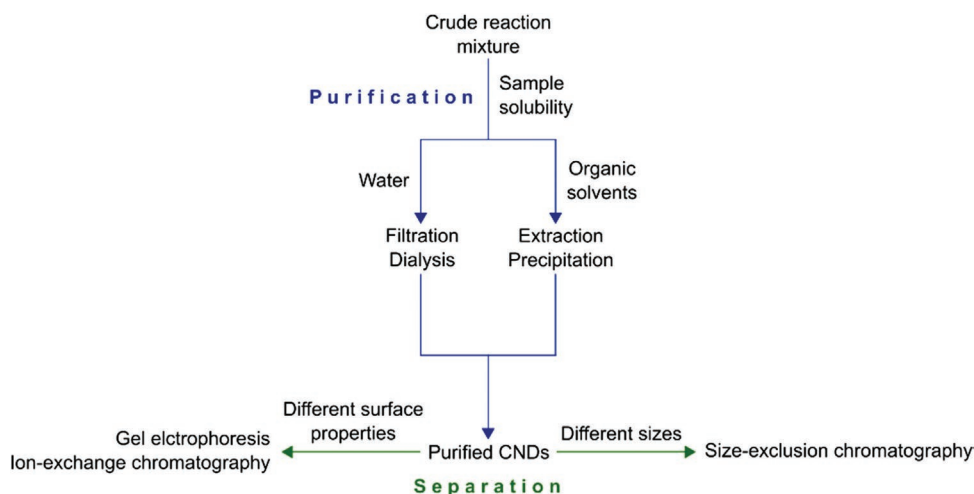


Figure 2. Summary of the main procedures available for CND purification and separation.

24 h, and about 72–120 h of dialysis are required to completely remove the molecular impurities. This example clearly highlights the importance of purification but also stresses the necessity to customize the dialysis parameters in accordance with the investigated sample. On the other hand, if CNDs are soluble in organic solvents, extraction and/or precipitation with *ad hoc* solvents can be used to get rid of residual molecules. Importantly, the abovementioned techniques can be used to remove molecular-like side products, but they are incapable of separating CNDs with different sizes or surface functional groups. For these specific points, gel electrophoresis, ion-exchange chromatography, size-exclusion chromatography, and HPLC are suggested.^[10]

4. Novel Characterization Techniques

The characterization techniques recalled in the introduction (TEM, AFM, XPS, etc.) present severe limitations in the assessment of the CND purity. Starting from that, we urgently need to broaden the spectrum of techniques used for the characterization of these materials. In this context, a valuable role can be played by nuclear magnetic resonance (NMR). Indeed, the

expected NMR signals for CNDs are very different from those of simple organic molecules. CNDs present a complex and heterogeneous structure and so they should not present sharp and resolved signals. Protons experience different environments resulting in broad and non-resolved peaks, similar to those usually reported for polydispersed polymers.^[15] Moreover, NMR spectra of macromolecules/nanoparticles present an increase in the linewidths associated with slower tumbling.^[16] On the contrary, small molecules typically display sharp signals, which should make it possible to identify their residual presence in CND samples. Basically, the development of CNDs should follow the outline sketched in the **Figure 3**.

To demonstrate the important role that NMR can play in this research field, our group has recently investigated two case studies.^[9] The first one deals with the preparation of chiral CNDs starting from *l*-arginine (*l*-Arg) and (*R,R*)-1,2-diphenylethylenediamine (*RR*-Dphen). After the hydrothermal reaction and dialysis, the final solution presents a blue emission and a circular dichroism (ECD) band. The ¹H-NMR spectrum shows broad aliphatic signals, which are compatible with the formation of CNDs, but in the aromatic region the clear presence of the starting *RR*-Dphen can be observed. The sample was further purified performing an extraction

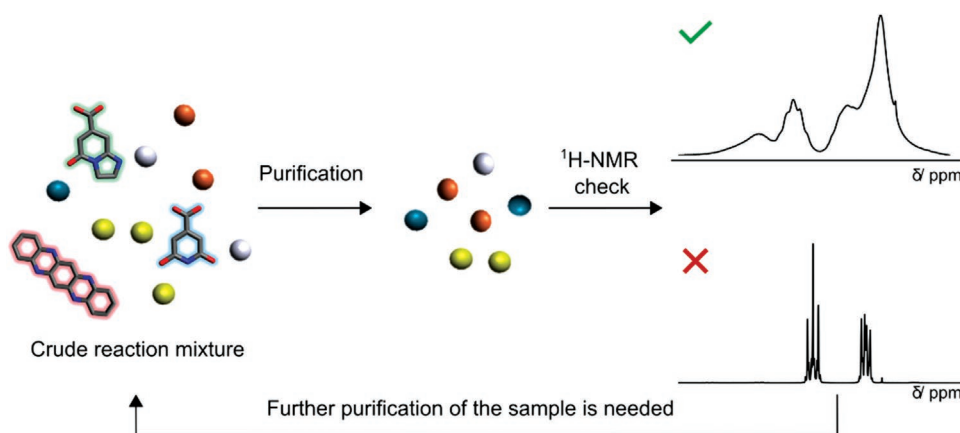


Figure 3. Flow chart associated to purification and validation of a CND sample.

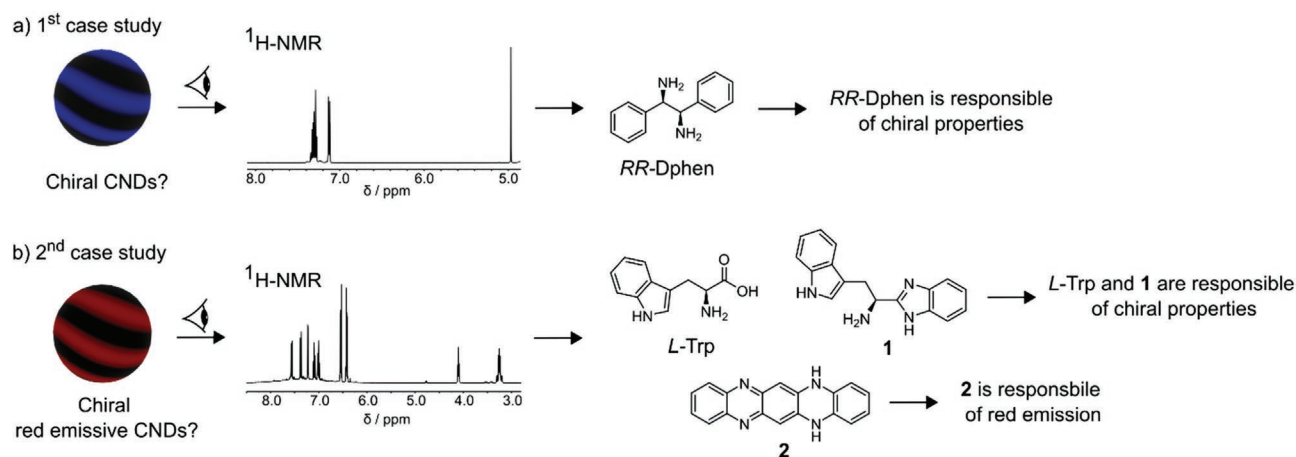


Figure 4. Structure and properties of species identified after purification of CNDs. a) CNDs synthesized from *l*-Arg and *RR*-Dphen. b) CNDs synthesized from *o*-PDA and *l*-Trp.

that allowed the removal of *RR*-Dphen, but contemporarily this caused the disappearance of the ECD band. This means that the chiral properties associated to CNDs at first glance were in fact due to the presence of residual starting material (Figure 4a).

The second case study deals with chiral red emissive CNDs.^[9] The synthesis of the material was performed starting from *o*-phenyldiamine (*o*-PDA) and *l*-Trp. After dialysis, the ¹H-NMR spectrum revealed the presence of molecular species, so that an additional purification step was performed using HPLC, which showed that the chiral properties and the red emission come from molecular species present in the sample. In particular, the chiral properties are related to the unreacted *l*-Trp and its derivative α -(2-benzimidazolyl)- β -(3-indolyl)-ethylamine (**1**), while the red emission is attributed to 5,14-dihydro-5,7,12,14-tetraazapentacene (**2**) (Figure 4b). This latter result is important, because *o*-PDA is a commonly used starting material for the synthesis of red emissive CNDs. Often, the red emission attributed to CNDs is in reality due to the presence of this stable aromatic molecule. Moreover, as **2** was separated by the sample via HPLC, there is no experimental evidence that this species is in some way embedded or attached to the carbon nanoparticles.

NMR can be further used to obtain information on the structure and surface composition of CNDs. ¹³C-NMR was used to get insight into the structure of CNDs synthesized from *l*-Arg and ethylenediamine (EDA) by using ¹³C enriched starting materials.^[17] Through a detailed mono- and bidimensional ¹³C-NMR analysis, we observed a core/shell type of structure where the aromatic core mainly originates from *l*-Arg while EDA mostly contributes to the formation of the shell, confirming its surface doping and passivation role in the synthesis. For the same type of material, NMR was subsequently used to differentiate the types of amines present on the surface of the nanoparticles.^[18] In doing that, we exploited the condensation of amino groups with *p*-fluorocinnamaldehyde to generate the corresponding imine and iminium ion species, which could be detected through ¹⁹F-NMR. Comparing the chemical shift of the ¹⁹F-NMR signals of CNDs with that of model amines it was possible to map the presence of both primary and secondary

amines on CNDs, unlocking the use of these materials as nano-aminocatalysts.^[19]

Additionally, NMR can be employed to get information on the size homogeneity of the CND samples. Diffusion-ordered spectroscopy (DOSY) reveals the diffusion coefficient of the signals present in the NMR spectrum. The DOSY technique is commonly used in the field of polymers and gives fundamental information on the macromolecular nature of the species involved, based on their diffusion rate. The higher is the molecular weight, the less the species will diffuse, with a strong difference between polymeric species and monomers.^[20] Figure 5 reports a typical example of a DOSY spectrum of three compounds: the top compound is a low weight molecular species, which has a typical high diffusion coefficient. The middle compound is a medium molecular weight polymer (or a carbon dot), which diffuses in a slower way with respect to the small molecule. Finally, the bottom compound is higher molecular weight polymer (of a bigger nanoparticle), with still lower diffusion coefficient. Building on this, DOSY could be employed to investigate CND samples both to check the presence of residual

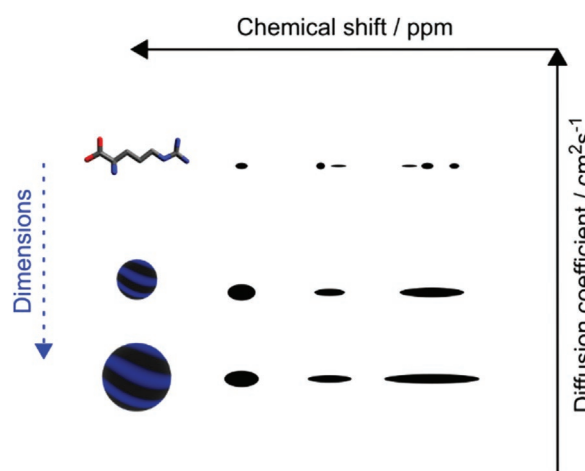


Figure 5. Typical DOSY spectrum with three components, with low, medium, and high molecular weight. Their diffusion coefficients are inversely proportional to their molecular weight.

molecules, to estimate the CND molecular weight and also to validate the post-functionalization of CNDs.^[21–23]

In addition to NMR, other techniques can be introduced in the characterization toolbox of CNDs. For example, thermogravimetric analysis (TGA) can be employed to determine the weight loss of the precursors and therefore guide the temperature choice for the solvothermal synthesis. Another example is fluorescence correlation spectroscopy (FCS) which can be utilized to estimate the translational diffusion coefficient of the emitting species, thereby providing direct access to their average hydrodynamic size via the Stokes–Einstein relation.^[24] As an example, Ferrante et al. have used FCS to investigate CNDs synthesized from *o*-, *m*-, and *p*-PDA demonstrating that the fluorescence of these samples is driven by small species, down to one order of magnitude smaller than the compounds observed under TEM.^[25]

5. Conclusions and Future Perspectives

The simple bottom-up preparation of CNDs through a multitude of precursors and methodologies has given rise to an exponential growth of this research field but, at the same time, purification and characterization issues have created a lot of concerns. In particular, the field suffers the lack of general purification protocols and specific characterization techniques that can confirm the purity of the new materials. These two issues prevent the maturation of the field, making it difficult to compare literature data along with the impossibility to understand the fundamental features of these new materials. On the other hand, this *Perspective* provides simple and general guidelines to design novel CNDs with the idea to create a common ground for the development of these nanoparticles. In particular, the present article summarizes the fundamental purification strategies which can be used to separate CNDs from molecular side-products and proposes additional characterization techniques, which can be used to deeply investigate the nature of the samples and to check their purity. A particular emphasis is given here to NMR, which stands as a novel and promising technique in the CND field, due to its ability to reveal the presence of residual side products and unreacted starting materials in a fast and easy operative way. The examples shown in this *Perspective* indicate that inconsistencies and errors in published works may be widespread and a re-evaluation of the state of the art for these materials is necessary. Often, the properties associated to CNDs are not supported by adequate experimental data.

Eventually, only with a collective commitment and a rigorous methodology it will be possible to understand how the features of the starting materials can be translated into CNDs with tailored properties, unlocking the full potential of these nanoparticles in materials science.

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Conflict of Interest

The authors declare no conflict of interest.

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