LVEM for Carbon Dot Research

Background

Carbon dots (CDs) are of emerging interest in areas including medicine and pharmacy applications. CDs are spherical carbonaceous nanomaterials typically with small diameters. CDs are chemically stable, water dispersible, photostable, and intrinsically photoluminescent. Their fluorescent and stability properties make exciting potential applications for optical imaging alternates to quantum dots. CDs are also being explored for gene delivery systems, and theranostics (materials that offer diagnostics, drug delivery, and imaging in one platform).

CDs can be made by both bottom-up synthesis and top-down approaches. Bottom-up methods include pyrolysis of organic matter such as fruit juice, glucose or citric acid in the presence of catalysts and passivating agents, with reaction activation energy coming from hydrothermal or microwave sources. Top down approaches take carbon powders, carbon nanotubes or graphene and break down these structures either electrochemically, by chemical oxidation or solvothermal synthesis.

CDs, as nanoparticles, are intrinsically defined by their measured size. Thus, the ability to quickly, easily and accurately measure their size is of critical importance to the research community and manufacturers making or utilizing nanomaterials.

This report will examine how low voltage electron microscopy and in particular how the LVEM 5 have been utilized in the field of carbon dot research. Highlights of the recent research findings, the strengths of the LVEM instrumentation technique, and approaches for sample preparation methods will be presented.

LVEM Characterization of Nanoparticles

LVEM was recently demonstrated to be incredibly accurate and consistent with traditional TEM for nanoparticle sizing applications (Dazon, 2019). The lower accelerating voltage provides a darker contrast with lower atomic number (Z) elements. This is a strong advantage for carbon-based materials, including carbon dots (Figure 1).

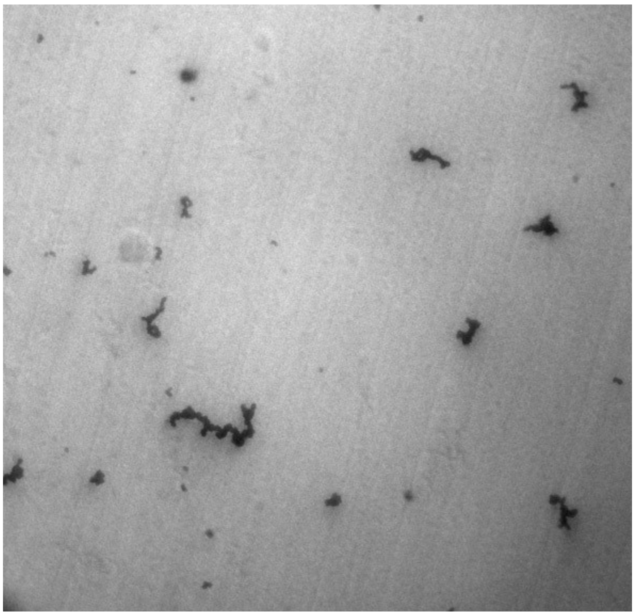

Figure 1. Dendrimer polymer nanoparticles imaged on an LVEM 5.

Libraries of Carbon Dots

One impressive application of LVEM is high-throughput size analysis, recently demonstrated on a library of 35 CD materials (Fan, 2019). This work characterizing a library of 35 different carbon dots defines the “size” of the CDs by the TEM size collected by LVEM, and refers to the hydrodynamic diameter of the CDs when reporting the measurements by dynamic light scattering.

Fan and colleagues are able to achieve significant insights about CDs based on this synthesized library of materials. Structure-toxicity relationships are thus able to be determined from data on well-characterized materials. This enables much stronger conclusions and insights to be drawn. For example, consistent with other nanomaterials, the size, surface charge and aggregation state in culture medium influence the observed toxicity results. This is consistent with previous literature highlighting the importance of the nanoparticle surface (Walczyk, 2010) and aggregation state of nanomaterials (Zook, 2011) on the observed toxicological results. Additionally, other parameters such as the nitrogen content, carbonization procedure, and passivation agent all interplay to determine the toxicity, with no single factor being the most important. The conclusions highlight the complexity of predicting the safety of CDs, and reinforces the need to determine the size of materials using LVEM.

Methods

Sample preparation methods reported in the literature vary based on unique sample properties and the preferences of the researchers.

Carbon dots were deposited onto Cu-300HD grids using 5 × 2 µL drops of a 1 mg/mL dispersion in 50% by volume 18MΩ water and 95% ethanol. (Yarur, et al., 2019) In the same study for other materials, these authors used three 2 µL aliquots of 500 µg/mL ZnO NP dispersions in 95% ethanol. No less than 200 nanoparticles were examined to determine the size distribution.

Other authors performed glow discharge at 90 V and 2 mA for 15 s on the Cu-300HD carbon-coated TEM grids, before deposition of the sample. (Fan, et al., 2019; Ronzani, 2019) Samples were deposited in the amount of 0.5 µL from a 1mg/mL dispersion in 1.5 mM NaCl at a pH of 7.4. The grids dried at room temperature for at least 2 h before imaging. These authors analyzed sets of 300–1000 particles to determine the size distribution.

CDs have been used developing engaging educational laboratory exercises based on the formation of carbon nanoparticles from lactose and baking soda. (Klobes and Koch, 2020) These safe materials provide a captivating color-change reaction that draws students in, and allows introduction of additional concepts ranging from fluorescence to measuring the size of nanoparticles using a Delong Instruments LVEM 5 transmission electron microscope (TEM).

Glycine-citric acid CDs were prepared for LVEM analysis by depositing a 10 µL drop of a 10 mg/mL concentration CD solution onto a grid and allowing the droplets to evaporate. (Macina, 2019) Samples were imaged at 5 kV.

Comparison of results

Table 1. Comparison of sample prep methods for recent LVEM carbon dot studies:

STUDY	GRIDS	DROPS	CD conc.	SOLVENT	# NPs sized
Yarur 2019, & Ronzani 2019	Cu-300HD	5 × 2 µL	1 mg/mL	1:1 18MΩ H_2O + 95% EtOH	> 200
Fan 2019	Cu-300HD	1 × 0.5 µL	1 mg/mL	1.5 mM NaCl, pH 7.4	300–1000
Macina 2019	Cu-300HD	1 × 10 µL	10 mg/mL	MeOH	Not listed

LVEM Instruments

There are two models of LVEM offered by Delong Instruments, offering a much smaller footprint than traditional TEM (Figure 2). The original model, the LVEM 5, offers a small benchtop footprint and easy operation. The LVEM 25 provides a compact footprint and imaging of thicker samples.

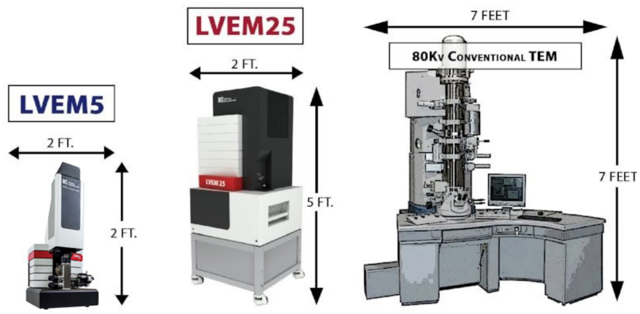

Figure 2. The LVEM 5 fits on a 2 ft wide benchtop, and the LVEM 25 footprint is about 2 ft by 3 ft, compared to 7 ft by 8 ft for conventional TEM.

The LVEM 5 is a versatile tool for characterizing nanomaterials. By being able to operate in either SEM, TEM or STEM modes, and with the smallest footprint available for this suite of capabilities, the LVEM 5 is powerfully positioned to improve research efficiency. The LVEM is primarily operated at an accelerating voltage of 5 kV from a field emission gun (FEG). Typical sample thickness is 20–50 nm, when nanoparticles are embedded in other material matrices. Feature resolutions of 2 nm and down to 1.2 nm in boost mode are possible.

LVEM Advantages

There are several well-established advantages to LVEM compared to traditional TEM instruments.

- Lower initial cost
- Lower operating cost
- Easier operation
- Easier maintenance
- Smaller laboratory footprint
- No specialized site prep required

The significantly lower initial cost of a new LVEM instrument compared to even a used TEM is a tremendous advantage, allowing routine access to electron microscopy images when otherwise unobtainable and freeing up larger budgets for other critical tasks.

Additionally, placement of an LVEM is possible in many laboratories, making for much more efficient collection of routine characterization data, as was demonstrated in the CD nanoparticle library work (Fan, 2019). Much as low-cost instruments are ubiquitous in synthesis labs for initial screening characterization, LVEM enables electron microscopy to now become a rapid, affordable and easy screening tool for nanoparticle size characterization, reducing the burden on or entirely eliminating the need for costly core user facilities.

Conclusion

Low voltage transmission electron microscopy is a powerful tool for characterizing the size of carbon dots with great accuracy and fidelity compared to traditional high voltage TEM. Added benefits of LVEM include lower costs, easier operation, and rapid results. The world's best benchtop electron microscope, the Delong LVEM 5, continues to contribute to important areas of nanotechnology, biology, and materials science research.

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About the author:

Robert I. MacCuspie, Ph.D., has over twenty years of experience in nanotechnology and materials characterization. Career highlights include leading the team that developed the silver nanoparticle reference materials at the National Institute of Standards and Technology, the first faculty and Director of Nanotechnology and Multifunctional Materials Program at Florida Polytechnic University, and over five years of consulting at the business-science interface from MacCuspie Innovations, helping companies commercialize and educate on technologies to improve human health.