Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Fabrication, Characterization, and Spectral Properties of Indigo Blue Nanocrystals

Alex Johnson-Buck [a], Gwangseong Kim [a], Shouyan Wang [a], Hoe Jin Hah [a] & Raoul Kopelman [a]
[a] Department of Chemistry, University of Michigan, Ann Arbor, Michigan, USA

To cite this article: Alex Johnson-Buck, Gwangseong Kim, Shouyan Wang, Hoe Jin Hah & Raoul Kopelman (2009): Fabrication, Characterization, and Spectral Properties of Indigo Blue Nanocrystals, Molecular Crystals and Liquid Crystals, 501:1, 138-144

To link to this article: http://dx.doi.org/10.1080/15421400802697756

Mol. Cryst. Liq. Cryst., Vol. 501, pp. 138–144, 2009
Copyright © Taylor & Francis Group, LLC
ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400802697756

Taylor & Francis
Taylor & Francis Group

Fabrication, Characterization, and Spectral Properties of Indigo Blue Nanocrystals

Alex Johnson-Buck, Gwangseong Kim, Shouyan Wang, Hoe Jin Hah, and Raoul Kopelman
Department of Chemistry, University of Michigan, Ann Arbor, Michigan, USA

Organic nanocrystals have been extensively studied in recent years for their unique optoelectronic properties and for applications in medicine. We report the first synthesis of nanocrystals from the dye compound indigo by reprecipitation. Scanning electron microscopy and dynamic light scattering reveal the particles to be approximately 10–20 nm in size, among the smallest organic nanoparticles reported, while powder X-ray diffraction shows similarities to previously reported bulk crystals of indigo. The intense visual absorption peak of the nanoparticles is blue-shifted relative to bulk crystals, consistent with previous observations for nanoscale materials. Potential medical applications are discussed.

Keywords: indigo blue; nanocrystals; organic nanoparticles; reprecipitation

INTRODUCTION

Recently, there has been much interest in the fabrication of nanostructured materials for applications in biology and medicine [1] as well as for structural [2], nanoelectronic [3], and analytical [4] purposes. In addition to the inorganic and polymer-based materials traditionally used in this field [1–6], organic materials have also been studied extensively for their unique electronic and optical properties [7]. More recently, organic nanocrystals have found application in medicine as a means to deliver pure hydrophobic drugs in concentrated form to cancer cells [8].

Hydrophobic organic nanocrystals are usually prepared by the reprecipitation method [9] in which a rich solution of the compound

Address correspondence to Raoul Kopelman, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, USA. E-mail: kopelman@umich.edu

of interest is diluted into a large volume of poor solvent, such as water. The "infinite dilution" results in the formation of crystals ranging from tens to thousands of nanometers in size. Organic nanocrystals have also been prepared using emulsion techniques [10] and, in the case of hydrophilic compounds, reprecipitation from a saturated salt solution or within a polymer matrix [11]. Using these methods, organic crystals from ca. 15 nm to 1 μm have been prepared from compounds ranging from polycyclic aromatic hydrocarbons to hydrophilic chromophores [8–14].

Here, we report the first synthesis of nanoparticles smaller than 20 nanometers from the commercially available hydrophobic food and textile dye indigo, prepared by reprecipitation from a DMSO-water system. The properties and a potential biomedical application of these particles as a contrast agent are also discussed.

MATERIALS AND METHODS

Indigo (synthetic, 95%), dimethyl sulfoxide (DMSO, 99.9+%, anhydrous), and Tween 80 were purchased from Aldrich and used as-is. Indigo is quite insoluble in most solvents, including water, and after investigating several solvents with varying polarity and functionality, we found that the most concentrated indigo solution (ca. 0.1 mg/mL) could be achieved in DMSO.

To prepare the nanoparticles, a saturated solution of indigo in DMSO was filtered through a Whatman Anodisc membrane (silica, pore size 20 nm) to remove any remaining large particulates, then 400 μL of this solution were injected through a 22-gauge needle into 10 mL of deionized water while stirring at 800 rpm. The resulting mixture was a light blue, transparent dispersion with the appearance of a solution. After stirring the dispersion for 5 minutes at room temperature, DMSO was removed by centrifugation at 2000 × g in an Amicon filter tube with 30 kDa MWCO and dilution in deionized water; this resulted in some product loss due to aggregation, but most could be re-dispersed by sonication for 60 minutes. Alternatively, the nanoparticles were filtered from the dispersion through a Whatman Anodisc membrane (20 nm pore size) under suction, then re-dispersed in deionized water by sonication in a Fisher Scientific FS30 Ultrasonic Cleaner. No significant differences were noted between the outcomes of these two methods, but the latter is better-suited to large-scale fabrication. The nanoparticle dispersion was stable over several weeks.

UV-Vis absorption spectra were obtained using a Shimadzu UV-1601 spectrometer. The particles were sized using dynamic light scattering (DLS, Particle Sizing Systems Nicomp 380) and scanning electron microscopy (SEM, Philips XL30 FEG). Powder X-ray diffraction measurements were carried out on a freeze-dried sample of indigo nanoparticles at room temperature using a Rigaku R-AXIS SPIDER diffractometer with an imaging plate detector using graphite monochromated Cu-Kα radiation (1.5406 Å).

RESULTS AND DISCUSSION

Particle sizing by DLS shows the indigo nanoparticles to be about 11 ± 3 nm in diameter and nearly monodisperse (97.6% of particles fall within this range by volume-weighted distribution using Nicomp software; data not shown). By SEM, the particles appear approximately spherical (see Fig. 1) with an average diameter of about 17 ± 8 nm after accounting for uncertainties in topological contrast caused by sputter-coating with gold. Powder X-ray measurements yielded a diffraction pattern consistent with previously determined structures of indigo crystals [15,16], though a significant portion of the sample appears to be in amorphous phase (see Fig. 2). This suggests that either the rapidity of precipitation or the small particle dimensions hampers the formation of well-ordered crystallites.

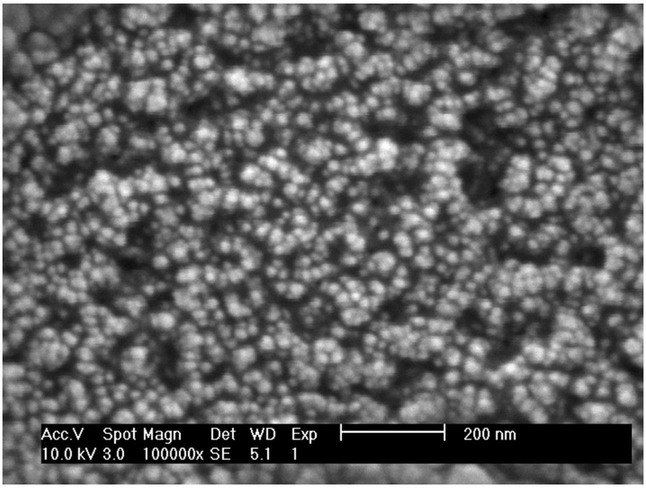

FIGURE 1 SEM micrograph of indigo nanoparticles prepared from deionized water dispersion. The 10–20 nm particles appear to form aggregates with features on the order of hundreds of nanometers.

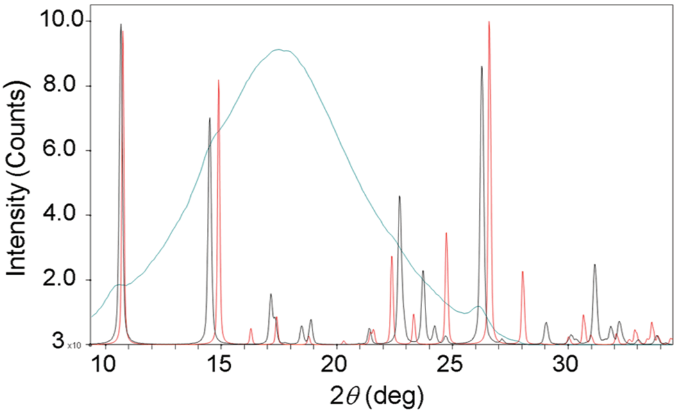

FIGURE 2 X-ray diffraction pattern of indigo nanoparticles (green) is consistent with the principal peaks of simulated diffraction patterns from indigo Type A (red) and Type B (black), the two known polymorphs of indigo [20,21]. The substantial peak broadening and weak signal in the measured pattern are likely the result of small dimensions and/or infrequency of true crystallites in the sample.

The absorption maximum (λ_{max}) of indigo in the range of 600–700 nm varies quite dramatically, depending on the chemical environment of the dye (see Fig. 3). For aqueous nanoparticles, a typical λ_{max} is 660 nm, in agreement with the value reported previously for indigo in KBr pellet form [17] which likely contained some of the smallest previously characterized crystallites. In contrast, the suspension of bulk indigo crystals in Tween 80 has a λ_{max} of 678 nm, in precise agreement with the value for solid indigo reported by Sadler [18]. Finally, a DMSO solution of indigo exhibits a λ_{max} of 620 nm. Thus, the absorption maximum of the indigo nanoparticles is between that of bulk crystals and that of free dye in DMSO solution, which is consistent with the intermediate, mesoscopic size of the nanoparticles. The blue-shift of the nanoparticles relative to bulk crystals is consistent with trends reported previously for organic nanocrystals [12].

More concentrated (ca. 1 mg/mL) indigo nanoparticle dispersions were prepared by scaling up the same method, isolating the nanoparticles by suction filtration on a silica membrane, and redispersing them in a smaller volume of water; this produced a dark navy-blue liquid with an inky appearance. Although this procedure produced aggregates of the particles larger than 1 µm in diameter, after sonicating the sample for one hour and letting it sit for two days, most of these aggregates had redispersed into particles smaller than 200 nm.

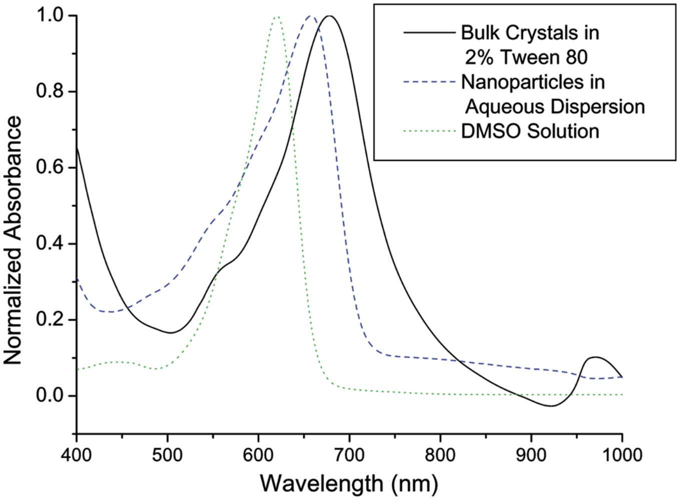

FIGURE 3 Absorption spectra of indigo as aqueous nanoparticles, bulk crystals suspended in 2% Tween 80, and dissolved in DMSO. The absorption maxima occur at 658 nm for nanocrystals, 678 nm for bulk crystals in Tween 80, and 620 nm for the DMSO solution. Absorbance is normalized to a maximum value of 1.

The hydrophobic nanoparticles are more prone to forming aggregates of size >1 µm in a dilute salt solution (5 g/L NaCl, Sigma-Aldrich) or RPMI cell culture medium (RPMI 1640, + L-Alanine, – Phenol Red, Invitrogen) than in pure water, suggesting that ionic strength modulates the equilibrium in favor of microscale aggregation. These aggregates do not disperse with sonication alone; however, they do, to some extent, upon suspension in pure water or with the addition of Bovine Serum Albumin (0.05 wt% final concentration) followed by sonication. Microscopic aggregation is also observed when the indigo nanoparticle concentration is increased above 0.1 mg/mL in pure water, and is evident in SEM micrographs (see Fig. 1). It may be possible to mitigate this aggregation by coating the particles with an inert material such as functionalized polyacrylamide, as has been done with inorganic crystals of similar dimensions [19].

Given the intense color of the concentrated dispersions, we hope to implement these nanoparticles as visual biomedical contrast agents. For example, the nanoparticles might be used as a targeted stain, providing the surgeon with a visual guide for the excision of malignant tumors. Although we have reported some preliminary work towards such staining applications using methylene blue embedded in silica nanoparticles [20], it has been challenging to load a sufficient concentration of dye into such nanoparticles for practical in vivo use. On the other hand, indigo nanoparticles may provide sufficient contrast to bypass this hurdle. The particles may localize to tumors via the enhanced permeability and retention effect, as reported for other organic nanocrystals [8], possibly with the aid of an albumin coating, as in the recently reported surfactant-free hydrophobic drug formulation Abraxane [21], or by use of another functionalized surface coating. Investigations in these directions are currently underway.

ACKNOWLEDGMENT

This work was supported by NIH/NCI Grant R21CA125297 (RK) and by NSF/DMR 0455330 (RK).

REFERENCES

[1] Salata, O. V. (2004). *J. Nanobiotechnology*, *2*, 3.

[2] Hannink, R. H., & Hill, A. J. Eds. (2006). *Nanostructure Control of Materials*, Woodhead Publishing Limited: Cambridge, MA.

[3] Wang, B. L., Yin, S. Y., & Wang, G. H. (2001). *Phys. Rev. Lett.*, *86*, 2046–2049.

[4] Clark, H. A., Hoyer, M., Philbert, M. A., & Kopelman, R. (1999). *Anal. Chem.*, *71*(21), 4831–4836.

[5] Clark, H. A., Kopelman, R., Tjalkens, R., & Philbert, M. A. (1999). *Anal. Chem.*, *71*(21), 4837–4843.

[6] Koo, Y. E., Cao, Y., Kopelman, R., Koo, S. M., Brasuel, M., & Philbert, M. (2004). *Anal. Chem.*, *76*, 2498–2505.

[7] Bosshard, Ch., Sutter, K., Prêtre, Ph., Hulliger, J., Flörsheimer, M., Kaatz, P., & Günter, P. (1995). *Organic Nonlinear Optical Materials, Advances in Nonlinear Optics*, Gordon and Breach: Basel, Switzerland, Vol. 1, p. 139–179.

[8] Baba, K., Pudavar, H. E., Roy, I., Ohulchanskyy, T. Y., Chen, Y., Pandey, R. K., & Prasad, P. N. (2007). *Molecular Pharmaceutics*, *4*(2), 289–297.

[9] Kasai, H., Nalwa, H. S., Oikawa, H., Okada, S., Matsuda, H., Minami, N., Kakuta, A., Ono, K., Mukoh, A., & Nakanishi, H. (1992). *Jpn. J. Appl. Phys.*, *31*, 1132–1134.

[10] Kwon, E., Oikawa, H., Kasai, H., & Nakanishi, H. (2007). *Crystal Growth and Design*, *7*(4), 600–602.

[11] Kamatani, H., Kasai, H., Okada, S., Matsuda, H., Oikawa, H., Minami, N., Kakuta, A., Ono, K., Mukoh, A., & Nakanishi, H. (1994). *Mol. Cryst., Liq. Cryst.*, *252*, 233.

[12] Katagi, H., Oikawa, H., Okada, S., Kasai, H., Watanabe, A., Ito, O., Nozue, Y., & Nakanishi, H. (1998). *Mol. Cryst., Liq. Cryst.*, *314*, 285–290.

[13] Katagi, H., Kasai, H., Okada, S., Oikawa, H., Matsuda, H., & Nakanishi, H. (1997). *J. Macromol. Sci. A*, *34*, 2013.

[14] Nalwa, H. S. (2000). *Handbook of Nanostructured Materials and Nanotechnology*, Academic Press: San Diego, CA, Vol. V, 433–470.

[15] Süsse, P., Manfred, S., & Vladimir, K. (1988). *Z. Kristallogr.*, *54*, 269–273.

[16] Süsse, P., & Wolf, A. (1980). *Naturwissenschaften*, *67*, 453.

[17] Weinstein, J., & Wyman, G. M. (1956). *J. Am. Chem. Soc.*, *78*, 2387.

[18] Sadler, P. W. (1956). *J. Org. Chem.*, *21*, 316.

[19] Moffat, B. A., Reddy, G. R., McConville, P., Hall, D. E., Chenevert, T. L., Kopelman, R. R., Philbert, M., Weissleder, R., & Rehemtulla, A. (2003). *Molecular Imaging*, *2*(4), 324–332.

[20] Koo, Y.-E. L., Fan, W., Hah, H. J., Kopelman, R., Xu, H., Philbert, M. A., Orringer, D., Ross, B., & Rehemtulla, A. (2007). *Applied Optics*, *46*(10), 1924–1930.

[21] Green, M. R., Manikhas, G. M., Orlov, S. et al. (2006). *Ann. Oncol.*, *17*, 1263–1268.