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ORIGINAL ARTICLE

Fluorescent carbon dot embedded polystyrene particle: an alternative to fluorescently tagged polystyrene for fate of microplastic studies: a preliminary investigation

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Abstract
Laboratory-based experiments using fluorescently labeled microplastic particles are common techniques for studying the fate of microplastic in the environment. However, the stability of fluorescent dyes used to label microplastic particles becomes an issue and can create artifacts due to the leaching of dyes. Here, we synthesize fluorescent polystyrene (PS) particles by embedding carbon dots (CDs), thus eliminating problems associated with the stability of dyes. Polystyrene particles were synthesized by mini-emulsion polymerization. Hydrophobic CDs were obtained from candle soot and were dispersed in the monomer before the mini-emulsion polymerization. The carbon dot embedded polymer particles (CDPS) show blue fluorescence on UV 370 nm excitation. This material could be a viable and more reliable alternative to fluorescently labeled PS particles in microplastic studies.

Keywords Microplastics · Carbon dot embedded polystyrene · Mini-emulsion polymerization · Hydrophobic carbon dots

Introduction

Plastic is a versatile material having a wide range of applications such as packaging, cosmetics, electrical insulations, medical tools, etc. However, the mismanagement in the production of plastic and its improper disposal has created substantial environmental pollution. It has been estimated that around 80% of plastics end up in landfills and the natural environment (Geyer et al. 2017; Hernandez et al. 2017). The weathering and degradation of these discarded plastics lead to the formation of micro/nano plastics, which possess severe concerns due to their long presence in the environment and lack of knowledge about their adverse effects on the environment (Campanale et al. 2020; Corsolini et al. 2021; Ragusa et al. 2021; Holzinger 2022; Padha et al. 2022; Yao et al. 2022).

MPs are known to have an impact on marine organisms as well as soil biota. The ingestion and associated effects on various flora and fauna are being studied. The possibility and extent of MPs' cytotoxicity on cells in living organisms due to the hydrophobic nature and smaller size are yet to be studied in depth. Thus, studying MPs in a simulated environment using standard materials is of great significance in understanding the fate of microplastic in the environment. Tracing the MPs in laboratory-based studies is limited due to the lack of proper identification techniques (Schirinzi et al. 2017; Eitzen et al. 2019; Pikuda et al. 2019; Pinto da Costa et al. 2019; Natarajan et al. 2020; Oliveri Conti et al. 2020; Schins et al. 2021; Zhang et al. 2021).

Fluorescence microscopy, due to its easier identification and quantification capabilities, is the most preferred choice among researchers for the studies of MPs. The use of fluorescently labeled MPs aids in easily identifying MPs in various systems, and lipophilic dyes such as Nile red are commonly used for fluorescent tagging of MPs (Jiang et al. 2019; Li et al. 2020; Marco et al. 2022). Polystyrene (PS) attached with different dyes is frequently used in laboratory-based microplastic studies as it is one of the freshwater standard test species suggested in several international test guidelines and due to commercial availability (Cui et al. 2017; Liu et al. 2020; Xu et al. 2020). However, the leaching of dyes in the

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altering media (due to salts, pH, or biological affinity) can create artifacts by fluorescence of the detached fluorophores (Catarino et al. 2019; Schür et al. 2019). Even after dialysis of fluorescently labeled polystyrene for removing the excess loosely bound fluorophores, we cannot ensure that the dyes will not elute from nanoparticles upon contact with cells and tissues in altering media (Catarino et al. 2019).

To address the limitation of dyes in fluorescence microscopy, quantum dot embedded polystyrene particles could be a reliable alternative. Carbon dots, fluorescent material with simple synthesis techniques and easy functionalization, is a sought-after material for fluorescence applications (Ramanan et al. 2018; Li et al. 2018; Das et al. 2020). Even though carbon dots assembled on polystyrene surfaces were used for white light-emitting devices, CDs or other types of fluorescent quantum dots embedded within polystyrene were never explored for use in microplastic studies (Wang et al. 2019). A novel technique for synthesizing fluorescent PS nanoparticles for usage in the fate of MPs remediation studies by embedding carbon dots (CDs) in PS is presented in this work. Mini-emulsion polymerization is a simple and efficient way to embed hydrophobic carbon dots within a uniform polymer nanoparticle with low polydispersity and good size distribution. The hydrophobicity of CDs ensures the good dispersibility of carbon dots in the organic phase. The CDs are sterically trapped within polystyrene upon polymerization. The current method, where fluorescent dyes are attached by lipophilic interaction between dye and polymer, is prone to leach, leading to artifacts. Carbon dots embedded in polystyrene (CDPS) gives off fluorescent emission with less chance of leaching fluorophores into the altering media, eliminating possible artifacts in the experiment. As fluorescently labeled microplastics are sought after in laboratory-based fate of microplastic studies, further investigation using the CDPS materials in laboratory-based microplastic studies could eliminate the current ambiguity in microplastic studies due to dye leaching.

Experimental

Materials

Styrene and sodium dodecyl sulfate (SDS) were purchased from Sigma Aldrich. L-ascorbic acid and hydrogen peroxide were procured from Merck. Styrene was washed with 2% w/v aqueous sodium hydroxide and passed through an alumina column to remove the inhibitor. All other chemicals were used as received without further processing.

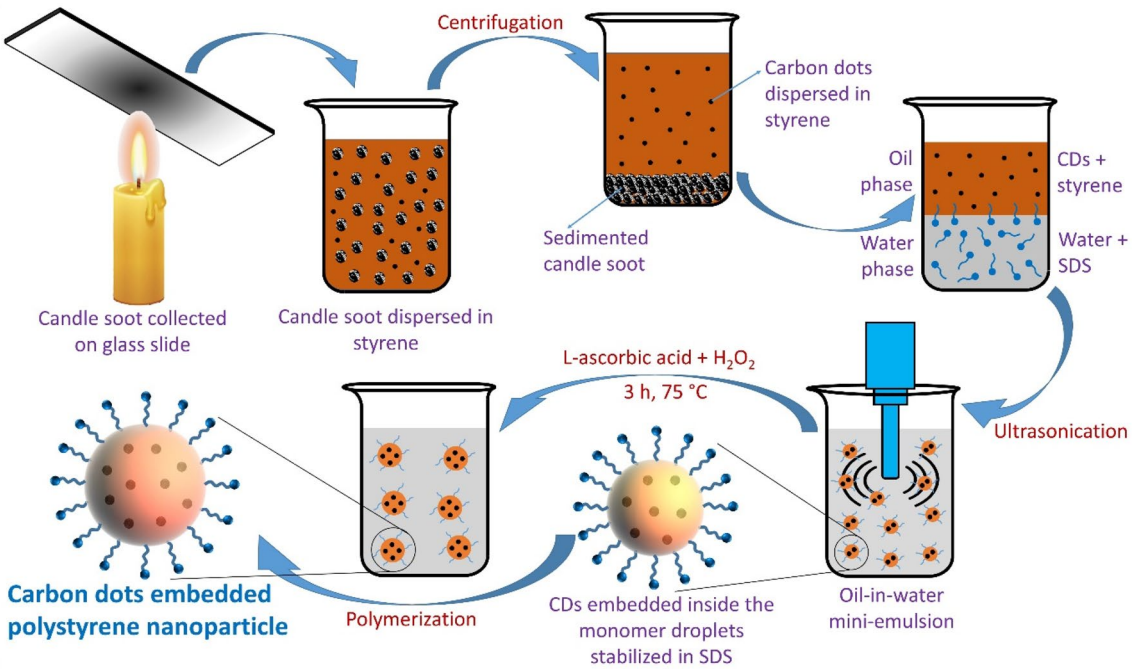
Synthesis of carbon dots and carbon dots embedded in polystyrene nanoparticles

Hydrophobic carbon dots were obtained from candle soot. The candle soot was collected on a glass slide kept above the tip of the flame (Liu et al. 2007; Russo et al. 2019). To separate CDs from candle soot, soot was dispersed in styrene and sonicated for 30 min without allowing the solution to heat up using an ice bath to eliminate premature initiation of polymerization. Upon centrifugation of this solution (7000 rpm, 15 min), the soot is settled, leaving a light brown supernatant solution. The supernatant was taken as the stock solution, which was then diluted and used as the oil phase in oil-in-water mini-emulsion polymerization.

In the mini-emulsion polymerization process, initially, the CDs dispersed styrene (4 wt.%) was introduced to an aqueous phase containing deionized water and SDS (20 mM concentration) (Anderson et al. 2002). The mini-emulsion was prepared by ultrasonication of the mixture using a probe sonicator for 30 min, and the emulsion was then transferred to a round bottom flask. 20 mg of L-ascorbic acid and 0.1 mL hydrogen peroxide were added to the emulsion as free radical initiators and kept for stirring for 3 h at 75 ℃. A slow initiation rate could lead to droplet degradation (Oswald ripening) after the formation of mini-emulsion. Hence, to attain a faster initiation rate, polymerization was initiated using a combination of L-ascorbic acid and hydrogen peroxide. The emulsion was cooled down to room temperature to complete the polymerization. The CDPS dispersion was taken for microscopic, particle size distribution, UV–vis spectroscopy, and photoluminescence (PL) spectroscopy characterization. The dispersion was freeze-dried using a lyophilizer, and the obtained CDPS powder was subjected to Raman spectroscopy, FTIR spectroscopy, and fluorescence microscopy analysis. Styrene without CDs was polymerized to obtain pure PS using the same procedure for comparison purposes.

Characterization of CDPS nanoparticles

High-resolution transmission electron microscope (HR-TEM, Jeol/JEM 2100) was used to obtain the structural features and morphology of CDs and CDPS nanoparticles and to check whether the CDs are visible over the edges in TEM images. The particle size distribution and the zeta potential of nanofluid were estimated using Dynamic light scattering (Malvern Zetasizer Nano ZS). Raman spectra (Horiba Labram HR Evolution CCD Model 1024X256-OE) were obtained using a 532 nm unpolarized light source to confirm the polymerization of PS and the



Scheme 1 Schematic diagram showing the mechanism of mini-emulsion polymerization synthesis of carbon dot embedded polystyrene

presence of CDs in the CDPS nanoparticles. FTIR Spectroscopy was done for PS and CDPS nanoparticles using an FTIR spectrophotometer (Perkin Elmer Frontier MIR) to identify various functional groups present in the polymers and confirm the completion of the polymerization reaction. The optical properties of the nanoparticles were characterized using UV–Vis spectroscopy (Peak Instruments C-7200). Photoluminescence spectroscopy analysis (Agilent Cary Eclipse) was done to detect the fluorescence property of CDPS nanoparticles at different excitation wavelengths. Fluorescence imaging was done using an inverted fluorescence microscope (Olympus IX73) to understand the proposed material's fluorescence property. Carbon dot labeled MPs were observed under fluorescence microscopy with UV (U-FUW), blue (U-FBW), and green (U-FGW) filter sets, using a 40 × objective.

Results and discussion

Mechanism of mini-emulsion polymerization

Mini-emulsion polymerization process was successfully employed to prepare carbon dots embedded in polystyrene nanoparticles. Mini-emulsion polymerization is an excellent technique for synthesizing composite nano-polymers, hybrid polymer nanomaterials, encapsulation of inorganic solids as protection in the dispersion media, etc. A schematic diagram depicting the mini-emulsion polymerization mechanism of CDPS nanoparticles is shown in Scheme 1. The emulsion is prepared by providing high shear force to the two-phase mixture, which is then stabilized using surfactants. Ultrasonic energy is supplied to the two-phase mixture, and the shearing leads to the formation of smaller droplets of minor phase from bigger ones. The interfacial tension drives the droplets to form spherical droplets. But the increased interfacial energy of the mini-emulsion can drive the system towards Oswald ripening to decrease the interfacial energy. The surfactants help reduce the interfacial tension and prevent the aggregation of droplets through steric and/or electrostatic stabilization. SDS is an excellent anionic surfactant giving good stability to the two-phase emulsion of styrene and water. While using CDs dispersed styrene as an oil phase, the hydrophobic nature of CDs ensures that the particles are embedded within the monomer droplet, and the spherical morphology of CDPS particles confirms this.

The possibility of droplet degradation (Oswald ripening) is avoided using fast-reacting free-radical initiators. L- ascorbic acid and H_2O_2 have a fast OH- free-radical formation tendency, which monomer captures. The polymerization of monomer droplets occurs through the formation of secondary free radicals. Nitrogen purging of deionized water removes the dissolved oxygen to increase free radical formation efficiency. The polymerization of surfactant stabilized spherical monomer leads to the formation of polymer nanoparticles with spherical morphology and excellent size distribution.

Characterization of CDPS nanoparticles

The structural morphology and size distribution of the synthesized carbon dots and CDPS nanoparticles were analyzed using HR-TEM, and the results are provided in Fig. 1. The formation of carbon dots within the 2–5 nm size range is visible from the TEM images (Fig. 1a–d). The CDPS nanoparticles (Fig. 1e–g) show a spherical morphology with high monodispersity in the 40–60 nm size range. It can be inferred that carbon dots are embedded within the polystyrene spheres since the surface of PS is smooth, and no CDs are visible near the periphery of PS, ensuring the absence of interaction of CDs with altering media. The TEM image is insufficient to provide a good contrast difference between CDs and polymer since both CDs and PS contain carbon as the primary component. Hence, CDs are not distinguishable from polymer.

The particle size distribution of CDPS was obtained using the dynamic light scattering (DLS) technique, and the size distribution graph is provided in Figure S1 (see supporting information). An average hydrodynamic diameter of 57 nm is observed for the CDPS nanoparticles, which agrees with the HR-TEM results. The Zeta potential is found to be -79.3 ± 2.27 mV which shows the excellent suspension stability of CDPS nanoparticles.

Raman spectroscopy was used to confirm the formation of CDs, PS, and CDPS nanoparticles, and the results are provided in Fig. 2. The peaks corresponding to polystyrene are present in Raman spectra of PS, confirming the completion of polymerization reactions. The characteristic aromatic ring chain vibrations of polystyrene at 1000 cm^{-1} and 1600 cm^{-1} are present in PS (Käppler et al. 2016). The completion of polymerization of styrene is assessed by the reduction in the intensity of the 1630 cm^{-1} peak corresponding to C = C stretching. As the 1630 cm^{-1} peak is absent in the Raman spectra of PS, the completion of polymerization can be ensured (Brun et al. 2013). The peaks corresponding to the D band (1348 cm^{-1}) and G band (1581 cm^{-1}) of carbon dots are present in the synthesized hydrophobic CDs. The peaks corresponding to both PS and CS are present in CDPS, confirming the formation of carbon dot embedded polystyrene.

FTIR spectra confirmed the completion of polymerization of styrene in both PS and CDPS samples (See supporting information Figure S2). The characteristic peaks at 3059 cm^{-1} and 3030 cm^{-1} due to the aromatic C-H stretching are present in both CDPS and PS samples. The peaks at 2920 cm^{-1} and 2849 cm^{-1} due to the existence of methylene and aromatic C = C stretching vibration at 1445 cm^{-1}, 1497 cm^{-1}, and 1595 cm^{-1} are all present in the spectra of both PS and CDPS samples (Fang et al. 2010; Zolotarev 2017). As the carbon dots within the polymer are of very

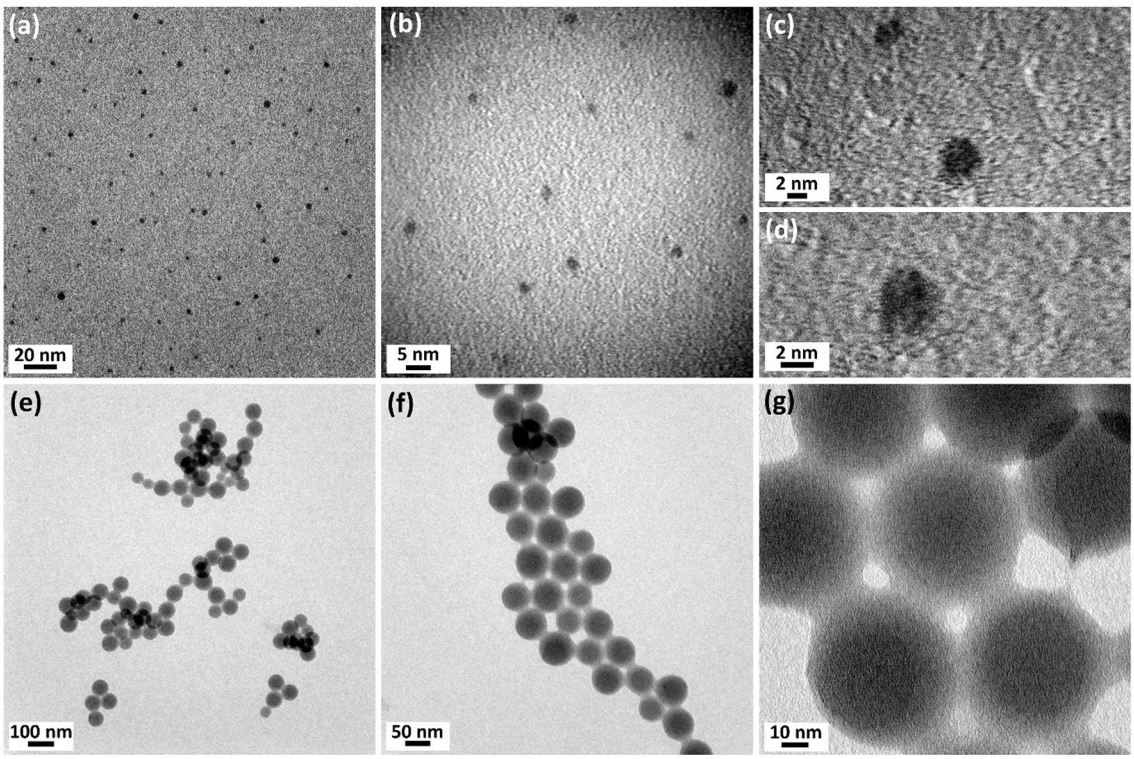


Fig. 1 HR-TEM images of **a**–**d** Carbon dots. **e**–**g** Carbon dots embedded in polystyrene

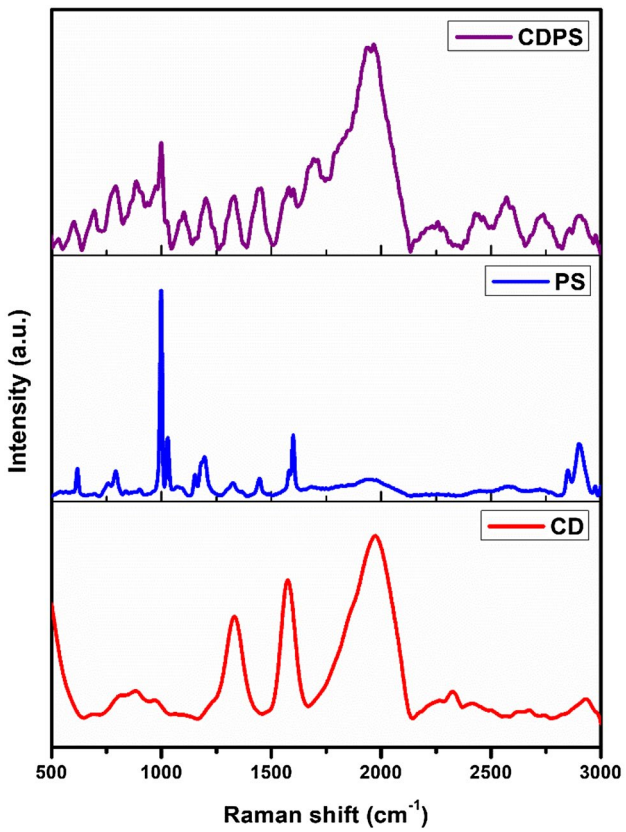


Fig. 2 Raman spectra of CD, PS, and CDPS

low concentration, the presence of any distinct peaks or enhancement of peaks is absent. Hence, there are no characteristic changes in the spectra of CDPS and PS.

Optical properties of CDPS nanoparticles

Figure S3 (See supporting information) shows the UV–Vis spectra of PS and CDPS dispersion. The UV–Vis spectroscopy of both PS and CDPS indicate absorption in the 240–250 nm range. However, the absorption peak corresponding to the fluorescence of CDs is not visible in the spectra (Wang et al. 2014). Photoluminescence spectroscopy was used to confirm the fluorescence property of the CDPS nanoparticles, and the PL spectra for PS and CDPS nanoparticles at different excitation wavelengths are depicted in Fig. 3a. The CDPS nanoparticles show a clear emission peak at 422 nm corresponding to 370 nm excitation, confirming the fluorescence of CDPS in the blue region upon excitation with a UV source. No emission is detected for PS nanoparticles in this region which leads to the inference that the fluorescence observed in CDPS is due to the presence of fluorescent CDs. Lower intensity emissions are present for other excitation wavelengths closer to the 370 nm excitation, with a slight red shift in emission wavelength, a characteristic property of carbon dots (Li et al. 2018; Ramanan et al. 2018; He et al. 2019; Das et al. 2020). Attempts were not made for the purification of synthesized CDs; hence, the presence of particles showing different fluorescence emissions based on particle size is not considered. The fluorescence of CDPS particles of lower intensities is present in the range of blue excitation (See supporting information Figure S4), which is due to such carbon dots of varied sizes. The photoluminescence spectra of CDs dispersed in styrene show a shift in fluorescence of CDs (See supporting information Figure S5). This is due to the solvent-dependent fluorescence property of carbon dots. A fluorescence peak at 381 nm is observed for 325 nm excitation. Similar fluorescence is detected for excitation wavelengths closer to 325 nm. This indicates the

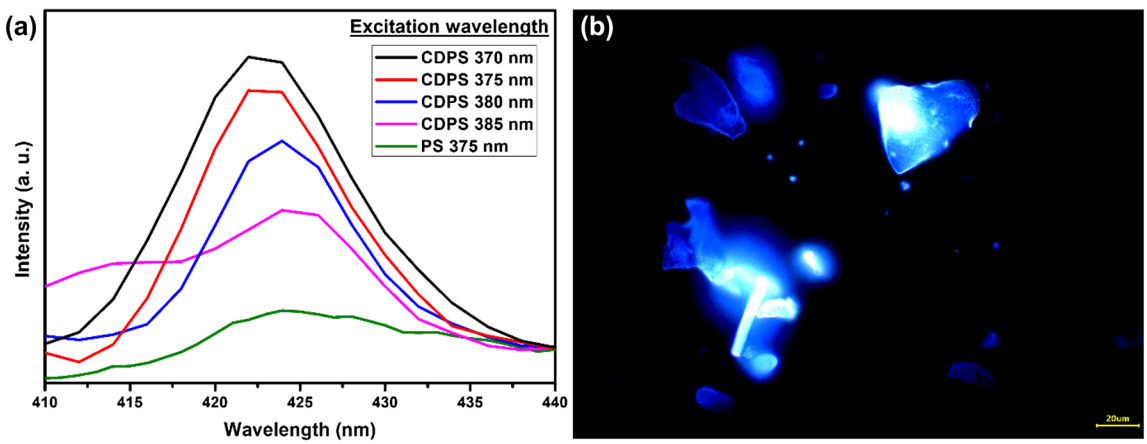


Fig. 3 **a** Photoluminescence spectra of PS and CDPS nanoparticles. **b** Fluorescence microscopy image of freeze-dried CDPS nanoparticles using UV excitation (U-FUW filter; excitation: 340–390 nm; exposure 24 ms).

changes in fluorescence of CDs depending on the dispersed media.

Figure 3b shows the fluorescence microscopy image of CDPS nanoparticles. The fluorescence image taken using an inverted fluorescence microscope shows blue fluorescence upon UV excitation using a UV filter set (U-FUW; excitation: 340–390 nm; exposure 24 ms). The particles were agglomerated upon freeze-drying; hence, microscopic images displayed particles of different sizes and shapes. Green fluorescence of lesser intensity is observed upon changing to a blue filter set (U-FBW; excitation: 460–495 nm). The exposure of the camera was increased from 24 to 240 ms to capture the fluorescence image. The lower intensity fluorescence from the blue filter set was confirmed using PL spectroscopy. The results indicate the fluorescence in the blue filter excitation range (see supporting information S5). Similarly, changing to a green filter set (U-FGW; excitation: 530–550 nm) results in red fluorescence with intensity similar to green fluorescence (exposure 240 ms). These fluorescence microscopy images are shown in Figure S7 (see supporting information). The blue fluorescence of CDPS is of higher intensity than green and red fluorescence since the un-purified CDs contain more blue fluorescent CDs than green and red fluorescent CDs.

Fluorescent labeled polystyrene particles are sought after by researchers in laboratory-based microplastic studies due to their easier visual identification using fluorescence microscopy. The CDPS nanoparticles have excellent fluorescence due to the presence of carbon dots; hence, they could replace the labeled polymer particles and eliminate artifacts. The spherical morphology with the smooth surface of CDPS confirmed that CDs are embedded within the polymer, ensuring the absence of any interaction with altering media. Consequently, the leaching of fluorophore, a major source of artifacts in fluorescently labeled polystyrene, is absent in CDPS. Moreover, our cheap and facile synthesis technique for CDPS brings a viable alternative for fluorescently tagged microplastics.

Conclusions

We synthesized carbon dots embedded in polystyrene particles, exhibiting blue fluorescence upon excitation with a UV source. The CDs were synthesized from candle soot which was dispersed in styrene. CDPS was prepared using a mini-emulsion polymerization technique that employed oil-in-water emulsion. The CDPS nanoparticles were characterized using HR-TEM and DLS, which showed a spherical morphology with a mean diameter of 57 nm. The presence of CDs in CDPS was confirmed using Raman spectroscopy. The material shows green and red fluorescence corresponding to blue and green excitation, respectively. The fluorescence microscopy was done on CDPS for visualization and confirmed using photoluminescence spectroscopy. CDPS is a reliable and cheaper alternative to fluorescently labeled polystyrene particles having inherent problems like leaching, leading to false information regarding the uptake of microplastic particles. As the CDs in CDPS are embedded within the polymer, the possibility of leaching of fluorophores is absent; hence uptake of MPs could be easily identified without the possibility of artifacts. Further studies are required to confirm the efficacy of the suggested material for different applications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13204-022-02566-8.

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