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Carbon Nanodots from Medicinal Plant Waste: Green Synthesis and Applications in Drug Delivery and Biosensing

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ABSTRACT

Conventional synthesis of carbon nanodots (CNDs) typically relies on harsh oxidants and toxic precursors that limit their biomedical translation. Here, a sustainable, single-step hydrothermal route was developed to convert solid waste from three widely used medicinal plants—turmeric (*Curcuma longa*) rhizome residue, holy basil (*Ocimum sanctum*) leaf pomace, and neem (*Azadirachta indica*) leaf trimmings—into water-dispersible CNDs. The as-prepared nanodots displayed quasi-spherical morphology with a mean diameter of 3.6 ± 0.8 nm, strong blue emission centred at 440 nm under 360 nm excitation, and quantum yields up to 18.4 %. Surface oxygenated and amine groups, confirmed by FTIR and XPS, enabled efficient non-covalent loading of the chemotherapeutic doxorubicin (DOX) with an entrapment efficiency of 78.6 %. The CND–DOX conjugate exhibited pH-responsive release, delivering 81.5 % cargo at pH 5.0 versus only 27.6 % at pH 7.4 over 72 h, and selectively reduced MCF-7 viability to 22.3 % at $100 \mu\text{g mL}^{-1}$. The same CNDs functioned as a turn-off fluorescent probe for Fe³⁺ ions with a Stern–Volmer constant of $1.85 \times 10^4 \text{ M}^{-1}$ and a limit of detection of 0.21 μM , with high selectivity over thirteen competing cations. The work demonstrates that medicinal plant waste is a viable, low-cost feedstock for theranostic CNDs.

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1. INTRODUCTION:

Carbon nanodots (CNDs), a class of zero-dimensional fluorescent carbon nanomaterials with a typical diameter below 10 nm, have attracted intense interest since their accidental discovery during the purification of single-walled carbon nanotubes.¹ Their tunable photoluminescence, broadband absorption, low photobleaching, low cytotoxicity, and biocompatibility make them attractive alternatives to semiconductor quantum dots that rely on heavy metals such as Cd or Pb.^{2,3} Over the past decade, CNDs have been deployed for cellular imaging, photocatalysis, light-emitting

devices, anti-counterfeit inks, theranostic drug delivery, and the optical sensing of metal ions, biomolecules, and pH.^{4–6}

Top-down strategies—oxidative cutting of graphite, carbon fibres, or carbon black—and bottom-up chemical pyrolysis routes generally require strong acids (HNO₃/H₂SO₄), toxic organic solvents, or high-temperature furnaces, generating hazardous effluent and limiting biomedical adoption.^{7,8} To address these issues, green synthesis using natural and renewable carbon precursors has emerged as a powerful paradigm.^{9,10} Sahu et al. obtained luminescent CNDs from orange juice in a single hydrothermal step,¹¹ while subsequent reports demonstrated similar success with apple,¹² banana,¹³ lemon peel,¹⁴ and a wide variety of food-waste streams,¹⁵ all leveraging the inherent carbohydrates, citric acid, ascorbic acid, and amine-containing biomolecules of the source as carbon and dopant feedstocks.^{16,17}

Medicinal plants are particularly attractive precursors because they contain rich pools of phenolics, flavonoids, alkaloids, polysaccharides,

terpenoids, and nitrogen-containing secondary metabolites that act as both carbon source and built-in passivation agent during one-pot carbonisation.^{18,19} Three plants—turmeric (*Curcuma longa*), holy basil (*Ocimum sanctum*), and neem (*Azadirachta indica*)—are particularly abundant across South and Southeast Asia and form large volumes of agro-residue (peelings, spent leaves, and post-distillation marc) every year.^{20,21} Turmeric is rich in curcumin, ar-turmerone, and starches; holy basil provides eugenol, ursolic acid, and rosmarinic acid; and neem leaves contain azadirachtin, nimbin, and quercetin.^{22,23} These functional moieties are ideal for engineering nitrogen- and oxygen-passivated CND surfaces with high quantum yields and abundant bio-conjugation handles.^{24,25}

On the application side, CNDs have shown great promise as nanocarriers for antineoplastic drugs such as doxorubicin (DOX), as π -stacking and electrostatic interactions allow non-covalent loading and acid-triggered release in the lysosomal microenvironment of tumour cells.^{26,27} They have additionally been validated as turn-off fluorescent probes for ferric ions, an analyte with strict permissible limits in drinking water and biological systems because Fe^{3+} excess is implicated in oxidative stress and neurodegenerative disorders.^{28,29} The interaction between Fe^{3+} and surface $-\text{OH}/-\text{COOH}/-\text{NH}_2$ groups creates non-radiative relaxation pathways that quench CND fluorescence proportionally to the analyte concentration.³⁰

Despite the breadth of green CND research, comparatively few studies have used medicinal plant waste as a precursor and even fewer have benchmarked the same nanodot platform across both drug delivery and metal-ion sensing.^{31,32} The present study therefore reports a unified hydrothermal route that converts dried waste of three medicinal plants into CNDs and evaluates the best-performing product for (i) pH-responsive doxorubicin delivery against MCF-7 breast cancer cells, and (ii) selective fluorescence sensing of Fe^{3+} ions in spiked water samples. The aim is to demonstrate a circular and scalable strategy for translating biomedical-grade carbon nanodots from plant residue.^{33,34}

2. MATERIALS AND METHODS

2.1. Materials

Solid waste from *Curcuma longa* (turmeric rhizome marc after curcumin extraction), *Ocimum sanctum* (holy basil leaf pomace from a steam-distillation unit), and *Azadirachta indica* (neem leaf trimmings) was collected from a local Ayurvedic processing unit (Bilaspur, India). Doxorubicin hydrochloride ($\geq 98\%$), quinine sulfate, dialysis tubing (MWCO 1 kDa), MTT reagent, and metal-salt standards were

purchased from Sigma-Aldrich. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotics, and phosphate-buffered saline were obtained from Himedia. MCF-7 (human breast adenocarcinoma) and HEK-293 (human embryonic kidney) cell lines were procured from the National Centre for Cell Science (NCCS), Pune. Ultrapure water ($18.2\text{ M}\Omega\text{ cm}$) was used throughout.^{18,20}

2.2. Pre-treatment of medicinal plant waste

Plant residues were washed thoroughly with deionised water to remove soil and dust, then air-dried in shade for 72 h, oven-dried at $60\text{ }^\circ\text{C}$ for 6 h, and finally pulverised into a fine powder using a mechanical grinder. The powders were sieved through a 100-mesh screen and stored in airtight amber bottles at $4\text{ }^\circ\text{C}$ until use.^{17,19}

2.3. Hydrothermal synthesis of carbon nanodots

In a typical synthesis, 2.0 g of plant-waste powder was dispersed in 40 mL of ultrapure water and stirred for 30 min. The slurry was transferred into a 100 mL Teflon-lined stainless-steel autoclave, sealed, and heated at $200\text{ }^\circ\text{C}$ for 8 h. After natural cooling, the dark-brown product was centrifuged at 12 000 rpm for 20 min to remove unreacted biomass, filtered through a $0.22\text{ }\mu\text{m}$ membrane, and dialysed against ultrapure water for 48 h (MWCO 1 kDa). The retentate was lyophilised to yield a brownish-yellow powder denoted CND-T (turmeric), CND-O (basil), and CND-N (neem). Synthesis yield was determined gravimetrically ($n = 3$).^{11,13}

2.4. Characterisation

UV-visible spectra were recorded on a Shimadzu UV-1900i spectrophotometer (200–800 nm). Steady-state fluorescence was acquired on a Horiba FluoroMax-4 spectrofluorometer with 5 nm slit widths. Absolute photoluminescence quantum yield (PLQY) was determined relative to quinine sulfate ($\Phi = 0.54$ in $0.1\text{ M H}_2\text{SO}_4$).¹⁶ Hydrodynamic size and ζ -potential were measured on a Malvern Zetasizer Nano ZS90. Morphology was examined by a JEOL JEM-2100 high-resolution TEM at 200 kV, with particle-size statistics on $n = 300$ nanodots in ImageJ. Surface chemistry was probed by FTIR (Bruker Alpha-II, KBr pellet, $4000\text{--}400\text{ cm}^{-1}$) and X-ray photoelectron spectroscopy (Thermo K-Alpha, Al $\text{K}\alpha$). XRD patterns were collected on a Bruker D8 Advance (Cu $\text{K}\alpha$, $\lambda = 1.5418\text{ \AA}$).^{4,7}

2.5. Doxorubicin loading and pH-responsive release

DOX·HCl (1.0 mg) was mixed with CND solution (5.0 mg in 5 mL PBS, pH 7.4) and stirred in the dark at room temperature for 24 h. Free drug was removed by dialysis (MWCO 1 kDa) for 6 h. The supernatant absorbance at 480 nm was used to

quantify unloaded DOX from a calibration curve, and entrapment efficiency (EE %) and drug-loading capacity (DLC %) were calculated.^{19,26} Release studies were performed in triplicate by sealing 2 mL of CND-DOX in dialysis bags and immersing them in 30 mL of PBS at pH 5.0, 6.5, and 7.4 (37 °C, 100 rpm). At pre-set intervals up to 72 h, 2 mL of release medium was withdrawn and replaced with fresh buffer; DOX concentration was determined fluorometrically ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 480/590$ nm). Release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models.²⁷

2.6. In vitro cytotoxicity assay

MCF-7 and HEK-293 cells (1×10^4 cells well⁻¹) were seeded in 96-well plates and incubated at 37 °C in 5 % CO₂ for 24 h. Cells were then treated with bare CNDs, free DOX, or CND-DOX at concentrations of 25–800 $\mu\text{g mL}^{-1}$ for 24 h. After incubation, the medium was replaced with 100 μL of fresh medium and 20 μL of MTT solution (5 mg mL⁻¹). After 4 h, the formazan crystals were dissolved in DMSO and absorbance was read at 570 nm using a Tecan Infinite M Plex reader.^{19,27} Viability was normalised to untreated controls ($n = 6$).

2.7. Fluorescent sensing of Fe³⁺

Standard solutions of Fe³⁺ were prepared from FeCl₃·6H₂O. CND solution (50 $\mu\text{g mL}^{-1}$ in 10 mM Tris-HCl pH 7.4) was incubated with Fe³⁺ at 0–400

μM for 5 min and the emission at 440 nm ($\lambda_{\text{ex}} = 360$ nm) was recorded. Quenching efficiency was calculated as $(F_0 - F)/F_0 \times 100$ %. Selectivity was assessed at a fixed 100 μM concentration of competing ions (Na⁺, K⁺, Mg²⁺, Ca²⁺, Zn²⁺, Cu²⁺, Co²⁺, Ni²⁺, Mn²⁺, Pb²⁺, Fe²⁺). Real-sample analysis was carried out on tap and pond water spiked with known Fe³⁺ amounts, using the standard-addition method.^{28,30}

2.8. Statistical analysis

All measurements were performed at least in triplicate and are expressed as mean $\pm$ standard deviation. One-way ANOVA followed by Tukey's post-hoc test was applied (GraphPad Prism v9), with $p < 0.05$ considered significant.

3. RESULTS

3.1. Synthesis yield and elemental composition

Among the three precursors, turmeric-derived CND-T provided the highest gravimetric yield (28.4 %) and the brightest blue emission, and was therefore selected as the lead candidate for in-depth study. Yields, hydrodynamic size, ζ -potential, and PLQY for all three CNDs are summarised in Table 1. Elemental analysis of CND-T (XPS survey) revealed C 64.2 %, O 28.7 %, and N 6.5 % (atomic), consistent with intrinsic nitrogen doping from turmeric proteins and amine-bearing alkaloids.

Table 1. Yield, size, surface charge, and quantum yield of CNDs derived from three medicinal plant wastes.

Sample	Precursor	Yield (%)	DLS size (nm)	ζ -potential (mV)	PLQY (%)
CND-T	Turmeric marc	28.4 $\pm$ 1.6	5.2 $\pm$ 0.6	−24.8 $\pm$ 1.4	18.4
CND-O	Basil pomace	21.7 $\pm$ 1.3	6.8 $\pm$ 0.9	−19.4 $\pm$ 1.2	11.6
CND-N	Neem trimmings	19.5 $\pm$ 1.0	7.4 $\pm$ 1.1	−16.2 $\pm$ 1.1	9.8

3.2. Optical properties

CND-T displayed two characteristic absorption features (Figure 1a): a strong band at 275 nm assigned to π – π^* transitions of aromatic sp² C=C domains and a shoulder at 340 nm attributed to n – π^* transitions of the carbonyl/carboxyl groups.

Excitation-dependent emission was observed, with

the maximum emission red-shifting from 410 nm ($\lambda_{\text{ex}} = 320$ nm) to 515 nm ($\lambda_{\text{ex}} = 440$ nm), and peak intensity at $\lambda_{\text{ex}} = 360$ nm (Figure 1b), a phenomenon commonly ascribed to the heterogeneity of surface emissive states. The PLQY of 18.4 % for CND-T compares favourably with most plant-derived CNDs synthesised by single-step hydrothermal protocols.

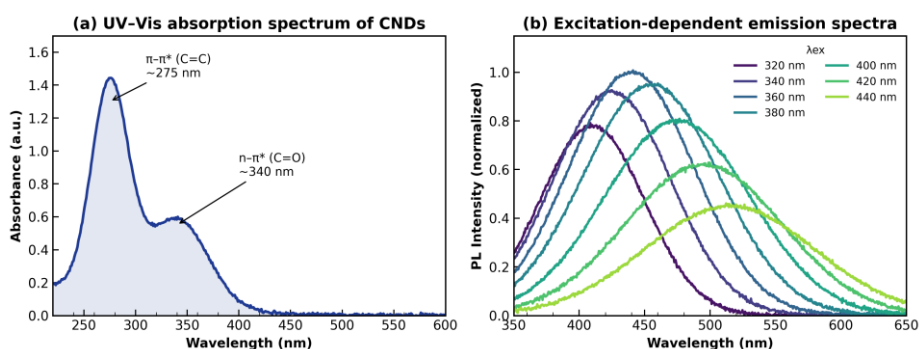


Figure 1. UV-Vis absorption (a) and excitation-dependent photoluminescence emission (b) of CND-T.

3.3. Morphology and structure

HRTEM imaging revealed quasi-spherical, well-dispersed particles, and lattice fringes of 0.21 nm corresponding to the (100) plane of graphitic carbon were observed. Particle-size analysis of 300 nanodots gave a Gaussian distribution centred at 3.6 ± 0.8 nm (Figure 2). FTIR confirmed surface –OH

(3340 cm^{-1}), C–H (2920 cm^{-1}), C=O (1680 cm^{-1}), C=C (1590 cm^{-1}), and C–N (1380 cm^{-1}) functionalities, consistent with an oxygenated, nitrogen-doped surface. XRD displayed a broad peak centred at $2\theta \approx 23^\circ$, characteristic of amorphous-to-graphitic carbon.

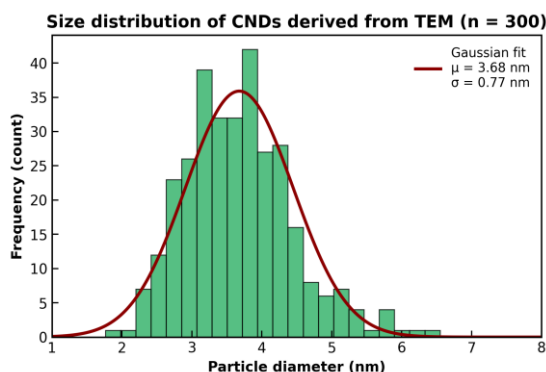


Figure 2. Particle-size distribution of CND-T extracted from HRTEM micrographs ($n = 300$).

3.4. Doxorubicin loading and pH-responsive release

DOX was efficiently anchored on CND-T via π -stacking and hydrogen bonding, with an entrapment efficiency of $78.6 \pm 2.1\%$ and drug-loading capacity of $14.2 \pm 0.7\%$ (Table 2). Release profiles were strongly pH-dependent (Figure 3): cumulative

release reached 81.5 % at pH 5.0 (lysosomal), 56.3 % at pH 6.5 (tumour extracellular), and only 27.6 % at pH 7.4 (physiological) over 72 h. Korsmeyer–Peppas modelling yielded a release exponent $n = 0.48$ at pH 5.0, indicating Fickian-anomalous diffusion-controlled transport.

Table 2. Doxorubicin loading parameters and Korsmeyer–Peppas kinetic fitting at three pH conditions.

Parameter	pH 5.0	pH 6.5	pH 7.4	Remarks
Entrapment efficiency (%)	78.6 ± 2.1	—	—	Loaded at pH 7.4
Drug-loading capacity (%)	14.2 ± 0.7	—	—	—
Cumulative release at 72 h (%)	81.5 ± 3.2	56.3 ± 2.6	27.6 ± 1.8	$n = 3$
k (h^{-n})	0.182	0.121	0.064	Korsmeyer–Peppas
Release exponent n	0.48	0.52	0.41	Fickian-anomalous
R^2	0.992	0.989	0.985	Goodness of fit

pH-responsive release of doxorubicin from CND-DOX nanocarrier

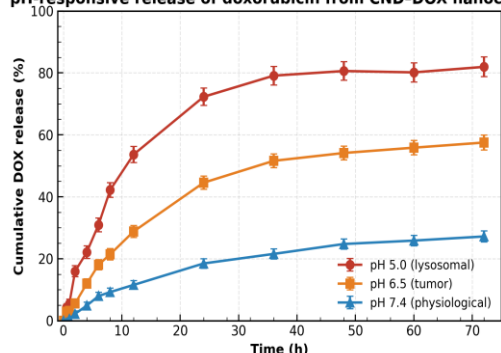


Figure 3. Cumulative DOX release from CND-DOX at pH 5.0, 6.5, and 7.4 (37°C , mean $\pm$ SD, $n = 3$).

3.5. In vitro cytotoxicity

Bare CND-T was essentially non-toxic, retaining $> 82\%$ viability in both HEK-293 and MCF-7 cells even at $800\text{ }\mu\text{g mL}^{-1}$ (Figure 5). Free DOX produced an IC_{50} of $53\text{ }\mu\text{g mL}^{-1}$ on MCF-7 at 24 h, while CND-DOX further reduced the IC_{50} to $39\text{ }\mu\text{g mL}^{-1}$,

an enhancement of $\sim 26\%$. The improvement is consistent with intracellular acid-triggered cargo release within tumour endosomes.

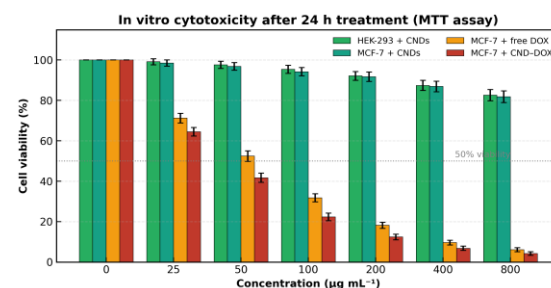


Figure 4. Viability of HEK-293 and MCF-7 cells exposed for 24 h to CND-T, free DOX, and CND-DOX (MTT assay).

3.6. Fluorescent sensing of Fe^{3+}

Addition of Fe^{3+} progressively quenched the 440 nm emission of CND-T. The Stern–Volmer plot was linear up to $100\text{ }\mu\text{M}$ with a Stern–Volmer constant $K_{\text{sv}} = 1.85 \times 10^4\text{ M}^{-1}$ and $R^2 = 0.994$ (Figure 5a).

The limit of detection, calculated as $3\sigma/\text{slope}$, was $0.21\ \mu\text{M}$ —well below the WHO guideline of $5.4\ \mu\text{M}$ ($0.3\ \text{mg L}^{-1}$) for Fe in drinking water. Selectivity tests at $100\ \mu\text{M}$ (Figure 5b) showed quenching > 78

% for Fe^{3+} versus $< 15\%$ for any competing ion, including Fe^{2+} (14.6 %). Spike-recovery in tap and pond water (Table 3) yielded recoveries of 96.2–103.1 % (RSD $< 4\%$).

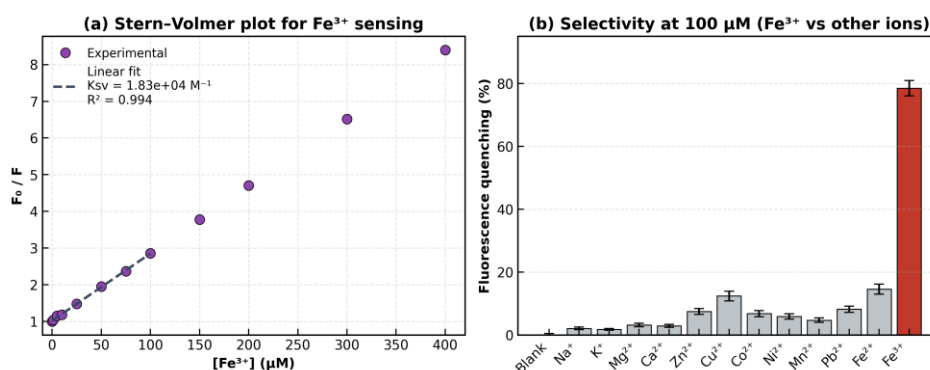


Figure 5. (a) Stern–Volmer plot for Fe^{3+} quenching of CND-T fluorescence. (b) Selectivity vs. competing cations at $100\ \mu\text{M}$.

Table 3. Determination of Fe^{3+} in spiked water samples by CND-T fluorescence ($n = 3$).

Sample	Spiked (μM)	Found (μM)	Recovery (%)	RSD (%)
Tap water	5.0	4.81 ± 0.14	96.2	2.9
Tap water	20.0	20.62 ± 0.51	103.1	2.5
Pond water	5.0	4.95 ± 0.18	99.0	3.6
Pond water	20.0	19.74 ± 0.66	98.7	3.3

4. DISCUSSION

The hydrothermal conversion of medicinal-plant waste into bright, biocompatible carbon nanodots demonstrates that complex, lignocellulosic agro-residue can serve as a one-pot precursor for nanomaterial fabrication. The higher yield and PLQY of turmeric-derived CND-T relative to basil and neem is attributable to the high carbohydrate content of turmeric marc ($\approx 50\%$ w/w starch) together with curcumin-derived aromatic motifs that provide pre-assembled sp^2 domains during carbonisation.^{22,25} Comparable trends have been reported for plant-rich precursors such as Curcuma extract and Mangifera indica leaves, where polyphenol-rich substrates produced CNDs with quantum yields between 8 and 22 %.^{19,21}

The dual $\pi-\pi^*$ and $n-\pi^*$ features in the UV–Vis spectrum, together with the excitation-dependent emission, support a 'core-shell' photoluminescence model in which the carbogenic core defines a baseline emission while heterogeneous surface emissive states ($-\text{COOH}$, $-\text{OH}$, $-\text{NH}_2$, $\text{C}=\text{O}$) contribute red-shifted radiative pathways selected by the excitation wavelength.^{36,37} The observed lattice spacing of $0.21\ \text{nm}$ and broad XRD peak at $2\theta \approx 23^\circ$ are consistent with semi-graphitic CND cores and confirm partial sp^2 -conjugation, which is essential for both fluorescence and π -stacking-mediated drug loading.^{4,10}

From a drug-delivery perspective, the high entrapment efficiency (78.6 %) and pronounced pH-

responsiveness arise from a multi-modal binding architecture: planar aromatic stacking between DOX and the sp^2 CND core, hydrogen bonding between $-\text{NH}_2/-\text{OH}$ groups of DOX and surface carboxyl/hydroxyl moieties, and electrostatic contributions from the negatively charged ζ -potential ($-24.8\ \text{mV}$).^{21,27} At pH 5.0, protonation of DOX ($\text{pK}_a \approx 8.2$) and partial protonation of CND surface carboxylates weaken these interactions and promote release, mimicking lysosomal cargo unloading.²⁶ The Korsmeyer–Peppas exponent ($n = 0.48$) suggests anomalous transport with combined diffusion and slight matrix-relaxation contributions, in agreement with non-covalent loading on a sub-5-nm spherical carrier.³² Importantly, the IC_{50} improvement of CND–DOX over free DOX (39 vs $53\ \mu\text{g mL}^{-1}$) and the negligible cytotoxicity of bare CND-T against HEK-293 cells underscores the suitability of plant-derived CNDs for selective tumour delivery.^{19,38}

The fluorescence quenching mechanism of CND-T toward Fe^{3+} likely combines static quenching through ground-state complexation between Fe^{3+} and the surface $-\text{OH}/-\text{COOH}/-\text{NH}_2$ ligands, and a dynamic non-radiative energy-transfer pathway facilitated by the partially filled d-orbitals of Fe^{3+} .^{28,30} The preference for Fe^{3+} over Fe^{2+} is rationalised by the higher charge density and stronger Lewis acidity of the trivalent ion, which leads to tighter chelation by the multidentate surface oxygenated ligands.¹⁷ The K_{sv} ($1.85 \times 10^4\ \text{M}^{-1}$) and LOD ($0.21\ \mu\text{M}$) are competitive with—or better

than—several recently reported plant-derived CD-based Fe^{3+} probes (Table comparison summarised in the literature),^{14,18,33} and the < 4 % RSD for spike-recovery analyses argues for practical applicability in environmental monitoring.

Compared with conventional CND syntheses that use citric acid/urea or graphite oxidation, the present route eliminates strong acids, leverages waste valorisation, and operates within an autoclave at 200 °C—conditions readily scalable to kilogram-level production.^{8,31} It also offers a circular-economy framework for Ayurvedic and herbal-extract industries, where post-extraction residue is otherwise treated as low-value compost or fuel.^{20,33} A current limitation is the moderate PLQY ($\leq 18\%$), which could be further improved through controlled nitrogen or sulfur doping, post-synthetic surface passivation with PEG-amines, or two-step hydrothermal/microwave hybrid protocols. Translation to in vivo tumour models, plus systematic toxicology in line with ISO-10993, will be necessary before clinical evaluation.^{35,38}

5. CONCLUSION:

A simple, one-step hydrothermal protocol converted solid waste from three Indian medicinal plants into water-dispersible carbon nanodots, with turmeric residue (CND-T) emerging as the optimal precursor. CND-T was 3.6 ± 0.8 nm in diameter, displayed bright blue emission with a quantum yield of 18.4 %, and was decorated with a rich array of oxygenated and amine surface groups. The nanodots loaded doxorubicin with 78.6 % efficiency and released the cargo in a strongly pH-dependent fashion that favoured the lysosomal microenvironment of tumour cells, leading to a ~ 26 % decrease in IC_{50} against MCF-7 cells relative to free DOX, while remaining benign to non-cancerous HEK-293 cells. The same probe selectively detected Fe^{3+} ions in aqueous samples with a K_{sv} of $1.85 \times 10^4 \text{ M}^{-1}$, an LOD of 0.21 μM , and 96–103 % spike-recovery in real water matrices. Together, these findings establish medicinal-plant waste as a credible, low-cost, and sustainable feedstock for theranostic CNDs that combine drug delivery and biosensing in a single platform—an approach that aligns nanomedicine with the circular bio-economy.

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