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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(10): 33-43

www.thepharmajournal.com
Received: 22-07-2022
Accepted: 25-09-2022

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Mangifera indica (cv. Alphonso) leaf extract mediated bluish fluorescent carbon dots: An approach for green nanotechnology

Samarth R Patel and Rameshchandra M Patel

Abstract
In green nanotechnology, carbonaceous nanomaterials have been widely used due to their potential for non-toxic, biocompatible, varieties of physico- chemical attributes and ease of functionalization. The varieties of carbon and graphane-based nanodots, nanoparticles, nanofibers and nanotubes have been widely used. The chemical and physical methods of carbon dots synthesis have limitations like toxicity, high cost and complexity to obtain pure CDs. Therefore, in the present study, the carbon dots were synthesized from mango leaf extracts of local cv. Alphonso via Hydrothermal method as a green approach. Because mango leaf is rich in phytochemicals like phenolics and flavonoids it can be used to functionalize the fluorescent carbon dots (CDs) via the hydrothermal method. Thus, two different temperatures 150 °C and 180 °C were used for synthesising the CDs. The UV-Visible spectra revealed the different optical reactivity of CDs in an aqueous solution. The fluorescence spectrum showed the excitation-dependent emission at different excitation levels of CDs. The highest emission of CD 180 after 24 hr dialysis was used to measure quantum yield, which was 5.94%. DLS analysis revealed the average diameter was 1.32 nm along with a zeta potential of -18.3 mV. The outcome indicated that the highly fluorescent and blue colour carbon dots were successfully synthesized from mango leaf extracts. Furthermore, the highly functionalized CDs can be used in the further application of nanoscience and in many other fields because of safe, non- toxic and cost-effective.

Keywords: Green synthesis, mango leaf, extracts, carbon dots, characterizations

Introduction
Recently, carbon-based nanomaterials have been recognized as a potential candidate due to their biocompatible, non-toxicity, excellent physic- chemical and optical properties and biocompatibility ease of functionalization. Among them, carbon nanomaterials such as carbon nanotubes, carbon dots, graphene sheets, graphene dots, fullerene and carbon nanofibers have been widely used in different applications. Additionally, carbon dots have promising characteristics, including water solubility, low toxicity, high chemical stability and easy derivatization (Iravani, 2011; Mohammadinejad *et al.,* 2019) [9, 20]. Carbon dots are usually defined as a class of core-shell composites with an average size of 1-10 nm which comprise a carbon core and surface passivation with various functional groups such as hydroxyl, carboxyl, amine, etc. These functional groups provide functionalization and passivation resulting in hydrophilic. Surface passivation is usually attained by the production of a thin insulating layer of passivated materials on a carbon dot surface which yields high fluorescence intensity and quantum yield (Campuzano *et al.,* 2019) [4].
Moreover, CDs are called as fluorescent carbon because of their excellent fluorescence including photostability, resistance to photobleaching and non-blinking (Xu *et al.,* 2014; Wang *et al.,* 2019) [28, 26]. Furthermore, as compared to post-synthetic alterations of traditional fluorescent dyes, CDs with functional groups such as amines and carboxyl's can impose various defects on the CDs surface which operate as excitation energy traps and lead to an extensive deviation in fluorescence emissions (Liu *et al.,* 2016; He *et al.,* 2019) [14, 8].
Till today, many diverse green carbon precursors have been used for the synthesis of CDs. For instance, Apple juice (Mehta *et al.,* 2015) [18], pasteurized milk (Mehta *et al.,* 2017) [16], Saccharum officinarum Juice (Mehta *et al.,* 2014a) [17], Tuber of Solanum tuberosum (Mehta *et al.,* 2014b) [19], Leaf extracts of plants (Kumawat *et al.,* 2017; Zare *et al.,* 2020; Omran *et al.,* 2021) [11, 29], Pseudo-stem of banana plant (Vandarkuzhali *et al.,* 2017) [24], Sucrose in from of Table sugar (Ansi *et al.,* 2021) [2]. CDs are considered to have crystal lattices with sp^2/sp^3 carbons (Nie *et al.,* 2014; Lin and Zhang, 2012) [32, 12].

Therefore in the present study, the CDs were synthesized from mango leaf extracts to check their fluorescence and optical properties at different temperatures.

Materials and Methods

Mango (2n = 40, Anacardiaceae family, order Sapindales) fully matured green leaf samples of local cv. Alphonso (Alternate Bearing) was collected from the orchard of Athwa Farm, Navsari Agricultural University, Ghod Dod Road, Surat, West province of India.

All glassware was cleaned with acetone (Samir Tech-Chem Pvt. Ltd.) and rinsed several times with distilled water followed by deionized (DDW-Extrapure, ACS Chemicals, conductivity <0.5, neutral pH) water. pH adjustments of solutions were performed using 0.1 M HCL and 0.2 M NaOH. All solutions were prepared in deionized water.

For proper washing out of metal ions from used glassware, the aqua regia solution (corrosive acid) mixture is made by combining Nitric acid (HNO_3-65%, MW-63.01 g/mol, Merk) and Hydrochloric acid (HCl-37%, MW-36.46 g/mol, Applichem) at ratio 2:6 in a fume hood with most of the precautions. It was also used for the removal of organic salt substances from glass wares followed by rinsing with DI water several times. In the end, all the glassware were dried in a hot air oven before being used in the biogenic synthesis of Carbon dots.

To remove dried black slag from the wall of the Teflon container in a highly pressurized autoclave system, a Di-acid solution was used. It was a mixture of perchloric acid and nitric acid (10:4). This mixture was completely filled up in the container and was left overnight in well-ventilated areas. The next day it was drained away carefully followed by several times washing with DI water.

Extraction of pool of phytochemicals from mango leaves

The collected healthy dark green leaves of mango was thoroughly washed three times using normal tap water followed by deionized water to remove impurities. Further, the leaf extract was prepared by suggested method of Anoop *et al.,* (2018) [1] with slight modification. After cooling, at room temperature the as-prepared solution was filtered through a Whatman filter paper. Furthermore, the extract was stored at chilling temperature in an amber colour glass bottle. This extracted solution of the leaf was used as the stabilizing and functionalizing agent in the synthesis of carbon dots.

Green Synthesis of Carbon Dots

It was synthesized by hydrothermal methods with mango Leaf extract as a carbon source. Teflon-lined autoclave vessel was cleaned with acetone and rinsed with DI water several times before being used to remove earlier reaction's slag. The 30 ml leaf extract was transferred into a clean Teflon-lined autoclave vessel and heated at two different temperatures. i.e.150 °C and 180 °C for a time period of 6 hr in the highly pressurized reactor (PSI ~ 4.0, Bar ~ 60) with a rotor speed of 700 rpm. After completion of the reaction, the heater was switched off followed by a switch to manually cooling and the temperature was set to 28 °C for cooling down the reactor. Then after reactor setup allowed it to stable for 2 hr followed by releasing of pressure by outlet pressure valve. The reactor assembly was opened by unscrewing and detaching different parts to obtain the mixture in the vessel. Further obtained mixture of the CDs solution was cleaned by removing carbide slag via centrifugation at 10,000 rpm for 10 min at room temperature.

As prepared dark brown supernatant was further purified through a dialysis membrane (Himedia, capacity- 2.41 ml/cm, MW 12-14 kDa) for 24 hr. The 10 ml supernatant was taken into a 15 cm long dialysis bag for membrane filter (wrapped by rubber band of both sides ends) submerged in deionized water in another clean beaker. The CDs were collected after 6 hr, 12 hr, and 24 hr presented in deionized water and stored at chilling temperature for further use. For analysis UV-vis. spectra, Fluorescence under UV light and Excitation-Emission activity, it was diluted five times with DI water. FL spectra file was saved in asc. Format for further analysis in software.

Hydrothermal carbonization is a single-step greener approach with the use of renewable biomass as a carbon source and at the low reaction, temperatures to attain functionalized CDs (Wang *et al*., 2013) [25]. Furthermore, the produced CDs required complex separation techniques to get mono-dispersed CDs from mixed sizes (Liu *et al.*, 2007) [13]. In the present study, we used dialysis followed by centrifugation as post- synthesis separation techniques (Mehta *et al.*, 2015; Zheng *et al*., 2015) [18, 30].

Furthermore, fluorescence quantum yield (ΦF) is a characteristic property of CDs and is denoted as the ratio of the number of photons emitted through fluorescence, to the quantity of photons absorbed by the fluorophore (Atkins *et al.*, 2018) [3]. The direct method is often applied to liquid samples because they are likely to exhibit isotropy in their fluorescence emission, resulting in identical quantum yields when measured using direct and indirect excitation (Faulkner *et al*., 2012) [7]. According to Xu *et al.* (2015) [27], the quantum yield of the CDs was calculated by using five times dilution of 200 ppm quinine sulphate in 0.1N H_2SO_4 (Refractive index = 1.346, QY=0.54) solution as a reference and based on Faulkner *et al.* (2012) [7]. The fluorescent quantum yield of the most effective CD based on characterization synthesized with temperatures 180 °C was measured.

Instrumentation and techniques

Samples were weighed on an Ohaus E- 225D weighing balance machine. All the UV-Vis absorbance spectra were recorded on a UV-Vis spectrophotometer (UV-3000+ LABINDIA® Analytical). In the scan spectrum curve menu, the baseline was adjusted with a reference sample (DI water) prior to sample analysis.

Particle size distribution curves were obtained using the dynamic light scattering technique (DLS) and recorded using a Malvern Zetasizer Nano series followed by Zeta potential (mV). All measurements were carried out with a temperature equilibration time of 1 min. The fluorescent behaviour of CDs and quantum yield (%) was recorded using a Perkin Elmer FL 8500 Fluorescence Spectrophotometer (FL85K8112001).

Result and Discussion

The UV-Visible analysis of fresh leaf extract cv. Alphonso exhibited slight peak at 360 and intense peak at 370 nm (Fig. 1). This finding is supported by Costa *et al.* (2015) [6] that plant extracts usually are prominant in flavonoids efficient in absorbing ultraviolet rays, typically indicated by two peaks of ultraviolet absorption at ranges of 300-550 nm. Mango leaf consists of a variety of phenolic compounds such as phenolic acids, phenolic esters, flavanols and mangiferin ($C^{19}H^{18}O^{11}$)

were reported predominant (Masibo and He 2008; Jhaumeer Laulloo *et al.*, 2018) [15, 10]. Those phytochemcials can reduce cations and stabilize the as-formed NPs to prevent agglomeration (Anoop *et al.*, 2018) [1]. The charactrization techniques utilized for present study of the synthezsized CDs is given in table 1.

Table 1: Characterizations techniques used for synthesized CDs

Sr. No./ Techniques		CDs	Leaf extract
1	UV-Visible Spectroscopy	✓	✓
2	FL-Spectroscopy	✓	-
3	DLS and Zeta Potential	✓	-

Green Synthesis of Fluorescent CDs and Its Characterization

The CDs were successfully synthesized by the hydrothermal route using leaf extract of Mango *cv.* Alphonso as a carbon precursor since it contains a wide variety of organic molecules (carbohydrates, polyphenols and volatile organic compounds).

In the present study, CDs were preared with one step hydorothermal method at two distinct temperatures i.e. 150 °C and 180 °C and coded as 150 CD and 180 CD. The different three time intervals (6 hr, 12 hr and 24 hr) were used for dialysis to obtain CDs in ultrapure water.

UV-Visible spectroscopy analysis and FL-emission properties of synthesized CDs

Table 2 represents the UV-Visible spectra synthesized from CDs at different temperatures with different dialysis times. Furthermore, the peaks at 275 nm and 268 nm, corresponding to the 150 °C and 180 °C temperature, respectively after 6 hr of dialysis (Fig. 2 and 4). After 12 hr dialysis, the UV-Visible peaks were observed at 270, peak at 210 nm followed by 265 nm for 150 °C and 180 °C temperature, respectively (Fig. 6 and 8). The UV-Visible peaks were observed at 235 nm, di peak at 212 nm followed by 260 nm for 150 °C and 180 °C temperature, respectively with the dialysis time of 24 hr (Fig. 10 and 12).

The peaks at 200 to 300 nm to π-π* interaction of C=C and C–C bonds. The red-shift of the peaks confirms the formation of carbon nanostructures with larger conjugation, which indicates that the as-prepared CDs have quite extraordinary stability (Mehta *et al.*, 2017; Mehta *et al.*, 2015) [16, 18].

Table 2: UV-Visible spectra of green synthesized CDs at two distinct temperatures after dialysis for 6 hr, 12 hr and 24 hr time period, respectively

Two distinct temperature for green synthesized CDs via Hydrothermal reaction		
Dialysis ^time (h)	**150 °C (CD150)**	**180 °C (CD180)**
6 hr	275 nm	268 nm
12 hr	270 nm	210 nm and 265 nm
24 hr	235 nm	212 nm and 260 nm
^Dialiysis period time after obtained crude of CDs		

The fluorescence intensity of various CDs i.e. 150CD and 180 CD exhibited different maximum emission intensities as described in Table 3.

In order to know the excitation-dependent fluorescence of the CDs, the wavelength of the CDs was excited in the range of 300 to 550 nm. The different emission spectra of CDs were obtained by increasing the excitation value (Table 3) from 300 to 550 nm with a 10 nm interval (Fig. 3, 5, 7, 9, 11, 13). It was observed that the emission peak of CDs was red-shifted with decreasing emission peak intensity at excitation wavelength from 300 to 550 nm.

The scan analysis of FL spectra of CDs was carried out in the 300–700 nm wavelength range. From the FL spectra, the synthesized CDs (CD150, 6 hr dialysis) showed the highest emission peak at 445 nm when excited at 370 nm (Fig. 3, Table 3). The synthesized CDs (CD180, 6 hr dialysis) exhibited a strong emission peak at 395 nm when excited at 310 nm (Fig.5, Table 3).

Furthermore, the synthesized CDs (CD150, 12 hr dialysis) exhibited a strong emission peak at 441 nm when excited at 360 nm (Fig. 7, Table 3). The synthesized CDs (CD180, 12 hr dialysis) showed the maximum emission peak at 398 nm when excited at 320 nm (Fig. 9, Table 3).

Table 3: Fluroscence data of Mango leaf extract derived CDs at different temperature 150 ºC and 180 ºC after dialysis for certain time period (6 hr, 12 hr and 24 hr)

Ex. (nm)	After 6 hr Dialysis				After 12 hr Dialysis				After 24 hr Dialysis			
	150CD		180CD		150CD		180CD		150CD		180CD	
	Em.(nm)	Int.	Em.(nm)	Int.	Em.(nm)	Int.	Em.(nm)	Int.	Em.(nm)	Int.	Em.(nm)	Int.
300	436	8944	392	39319	435	10197	393	38027	427	15823	394	38196
310	437	11782	**395**	**41130**	437	13692	396	40962	**429**	**16951**	**397**	**41879**
320	437	15566	398	40982	437	17478	**398**	**41290**	430	16595	399	39558
330	438	19152	403	39828	436	20688	403	38015	432	15420	403	37086
340	438	22727	415	34136	437	23714	416	33521	433	14029	420	33067
350	440	27815	428	30462	438	27879	429	21050	437	13385	430	30470
360	441	31734	435	29506	**441**	**30530**	435	30379	438	13036	437	30047
370	**445**	**32616**	439	27144	445	30462	439	28506	443	12239	439	28575
380	453	29872	442	24252	451	27476	442	25715	449	10943	440	25823
390	484	28985	447	20086	485	25655	447	21727	464	9689	447	21689
400	494	28768	450	17408	493	25052	450	19235	484	9295	449	18788
410	500	30315	455	13692	499	26275	457	15930	495	8937	452	15599

420	504	27950	487	10997	506	24088	485	12812	500	8787	490	13453
430	514	24646	502	10491	514	21149	498	11831	513	9651	505	12511
440	524	23893	507	9060	525	19808	507	11092	520	9420	518	12012
450	534	23544	507	9031	535	19430	504	10447	532	8377	509	10496
460	540	25078	511	8077	541	21234	509	9373	542	7790	502	9360
470	543	23993	521	7587	543	20284	516	8473	547	7477	515	8789
480	545	24676	527	8166	545	21427	524	9162	547	8528	517	9564
490	547	25880	527	8500	547	22569	526	9548	548	9086	520	9971
500	547	25321	539	9688	547	25411	536	10652	548	10308	534	11203
510	546	28843	-	-	547	28915	-	-	548	10862	531	11545
520	548	30479	-	-	548	30680	-	-	549	11160	585	11658
530	551	26639	-	-	551	27314	-	-	540	11125	588	12163
540	550	22244	-	-	550	20003	-	-	549	10775	578	12416
550	560	14796	-	-	-	-	-	-	-	-	-	-

Where Int: Intensity, Ex.- Excitation wavelength, (Bold digits and values indicated highest emission wavelength with intensity of respective CD

Moreover, the synthesized CDs (CD150, 24 hr dialysis) showed a strong emission peak at 429 nm when excited at 310 nm (Fig. 11, Table 3). The synthesized CDs (CD180, 24 hr dialysis) exhibited the maximum emission peak at 397 nm when excited at 310 nm (Fig. 13, Table 3).

Zhu *et al*. (2015) [31] observed that all CDs have chemical groups on their surface, such as oxygen- or amino-based groups, and polymer chains and which play a crucial role in the photoluminescence behaviour. Further, CDs are effective in photon- harvesting in the short-wavelength region because of the π–π* transition of C = C bonds. It typically expresses potent optical absorption in the ultraviolet region (~260– 320 nm), with a tail extending into the visible range. The absorption band located in 230–270 nm is typically ascribed to π–π* transition of C = C bonds and the peak/shoulder located around 300–390 nm is attributed to n–π* transition of C = O bonds (Zheng *et al*., 2015) [30]. The obtained sharp peak indicates the monodispersity of the system. The formation of CDs was further confirmed by the presence of intensive peaks obtained at different excitations level due to the π-π* transition of the graphitic sheets and the emission peak was attributed due to the presence of conjugated π domains. CDs were also characterised to identify excitation-dependent emission properties. The developed CDs were effective in showing unique bluish fluorescence in all the excitation range (Fig. 14 and 15). The QY of synthesized CDs were calculated considering the QY of quinine sulphate as a standard reference. The QY was found around 5.94% of CD180 (24h dialysis) (Mehta *et al*., 2017, Mehta *et al*., 2014a) [16, 17].

The aqueous solution of CDs appeared as a light yellow colour under daylight (Inset Fig. 2, 4, 6, 8, 10, 12, 14A and 15A), but exhibited bright blue fluorescence under UV light at 365 nm (Fig. 14D and 15D). The UV-illumination at 302 and 365 nm produced a bright blue fluorescence with different emission spectra of respective CDs and in accordance with dialysis time (Fig. 14C, 15C, 14D and 15D, respectively), confirming that the formation of fluorescent CDs (Mehta *et al.*, 2015). The CDs exhibited excitation-dependent FL behaviour (Fig. 3, 5, 7, 9, 11 and 13) due to different sized nanoparticles and functional groups on the surface of the CDs as well as defects of CDs (Chen *et al.*, 2014) [5].

DLS and Zeta Potential analysis of synthesized CDs

DLS and zeta potential characterization of the CDs represent the colloidal morphologies of nanodots in ultra-pure water. The hydrodynamic diameters of CD180 were determined after 24hr dialysis. Because it was found as exhibited the highest intensities of selected excitation levels and considered as best among other CD samples. The average diameter was 1.32 nm (Fig. 16A) along with a zeta potential of -18.3 mV, respectively (Fig. 16B). The negative zeta potential may be because of hydroxyl and carbonyl functional groups on the surface of CDs (He *et al*., 2019) [8].

Conclusion

As presented here, CDs from mango cv. Alphonso leaf extract were synthesized via the hydrothermal method. The two different temperatures were used to synthesise two different CDs. The pure form of carbon dots was obtained from the dialysis of as-synthesized crude CDs. The different three times of dialysis to obtain pure CDs were selected to check the different optical and fluorescence activity. The highest fluorescence activity was obtained in CD180 after 24hr dialysis of obtained crude in de-ionised water. The obtained CDs exhibited 5.94% QY and 1.32 nm average diameter with -18.3 mV zeta potential. Thus, the cost-effective, green, non-toxic and efficient bluish fluorescence CDs were prepared from mango *cv*. Alphonso leaf extracts. Furthermore, these synthesized CDs will be used in different applications of nanoscience and related fields.

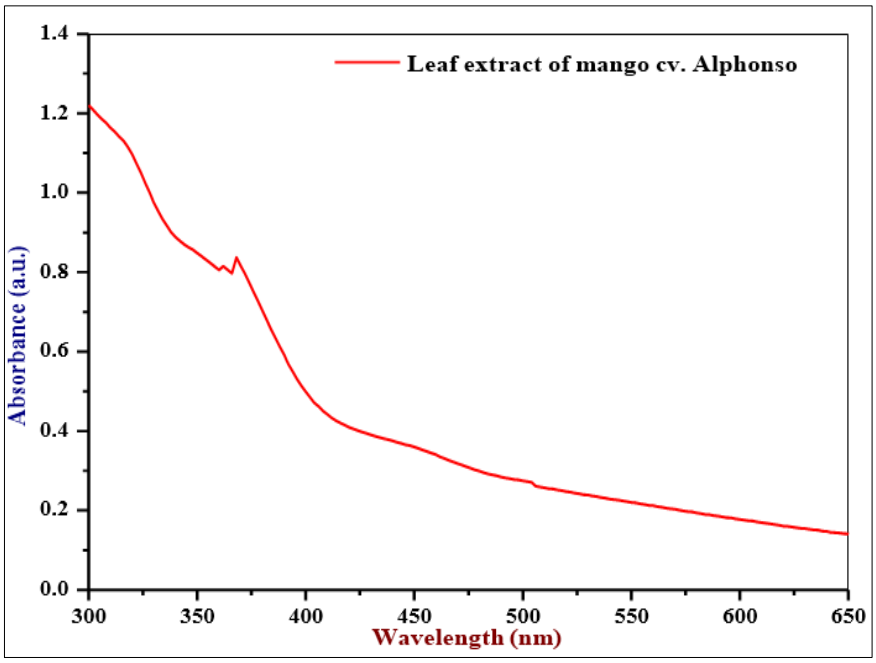

Fig 1: UV-Visible spectroscopy of fresh leaf extract of *M. indica* cv. Alphonso

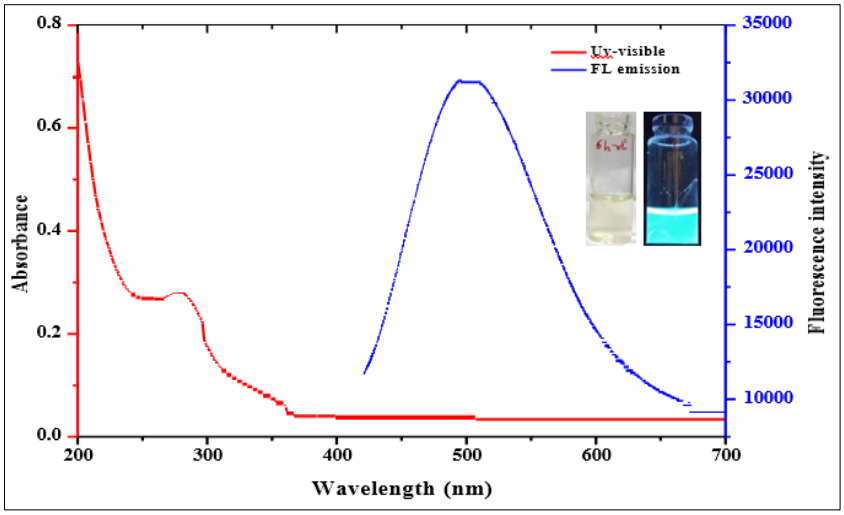

Fig 2: UV-visible absorption and fluorescence emission spectra of CDs (1.0 mg/ml) synthesized at 150 °C. Inset picture shows the carbon dots under daylight (left) and UV light at 365 nm wavelength (right) dialysis for 6 hr

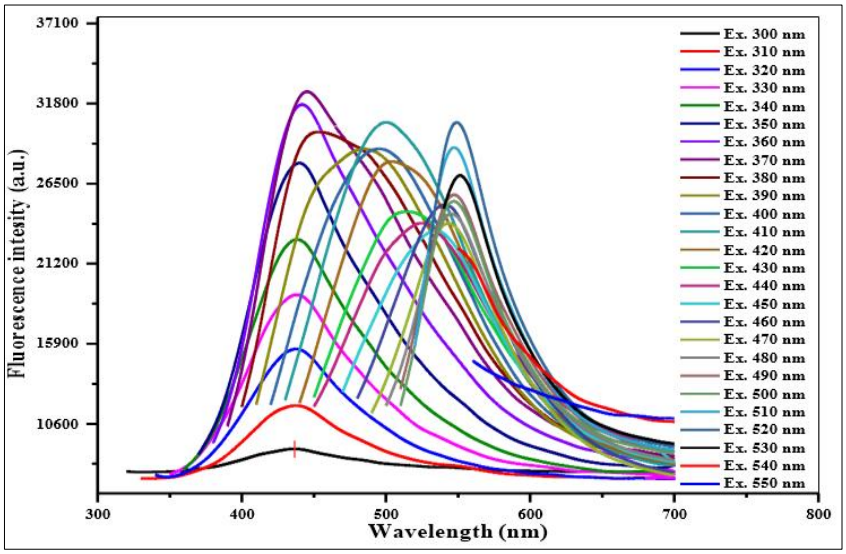

Fig 3: Fluorescence emission spectra of the CDs synthesized at 150 °C followed by 6 hr dialysis

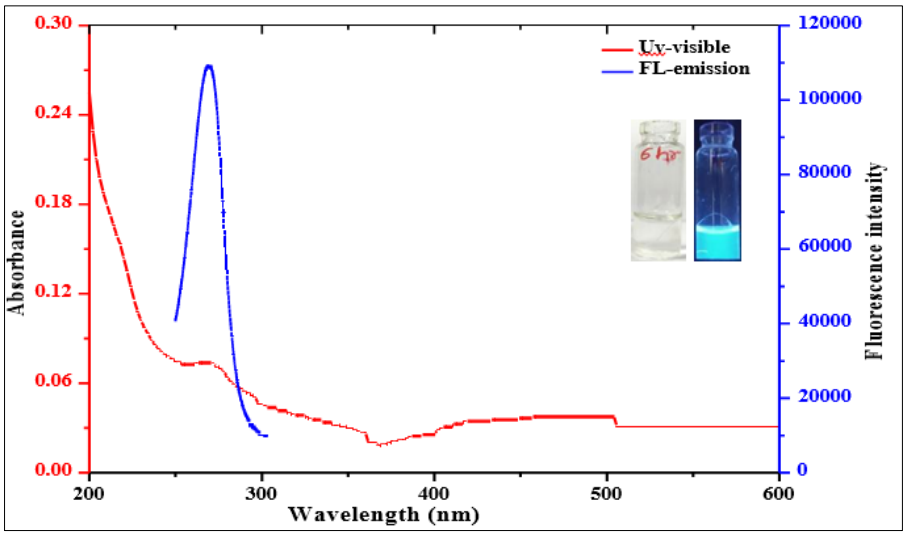

Fig 4: UV-visible absorption and fluorescence emission spectra of CDs (1.0 mg/ml) synthesized at 180 °C. Inset picture shows the carbon dots under daylight (left) and UV light at 365 nm wavelength (right) dialysis for 6 hr

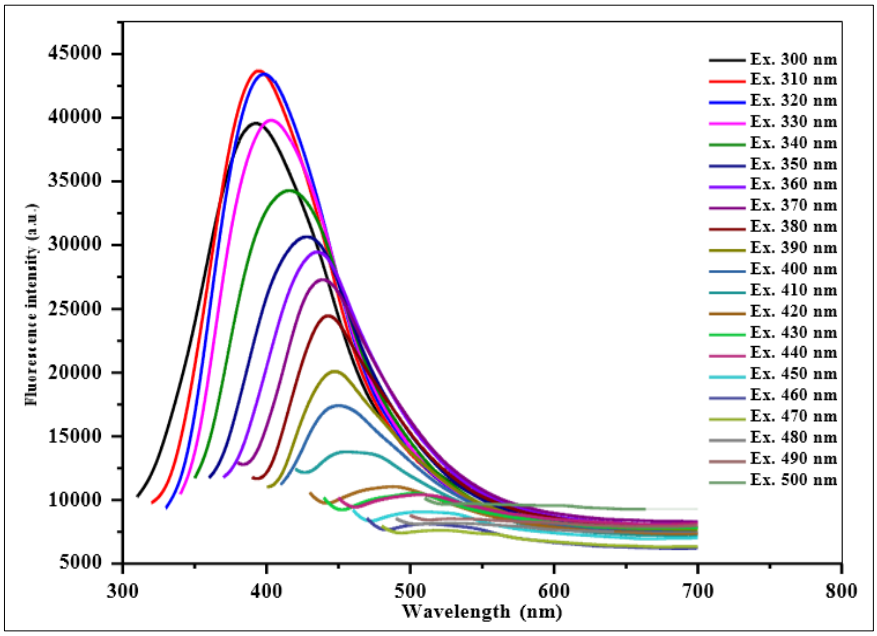

Fig 5: Fluroscence emission spectra of the CDs synthesized at 180 °C followed by 6 hr dialysis

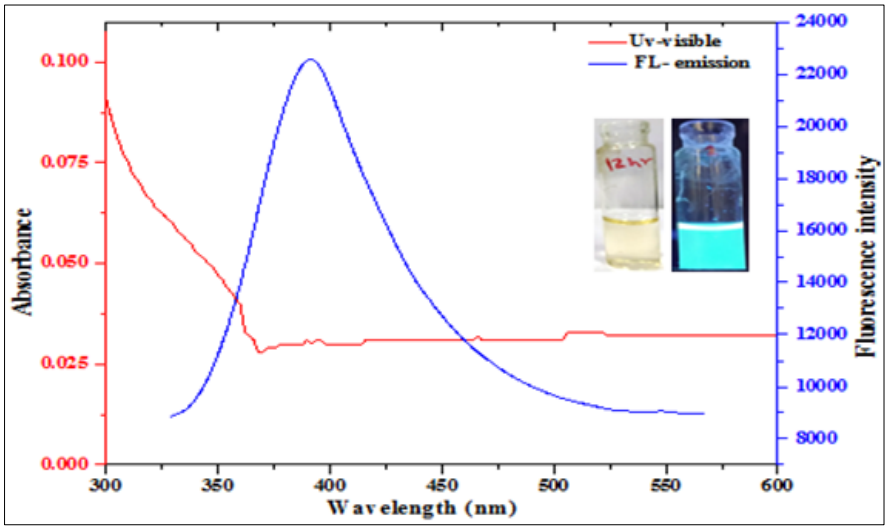

Fig 6: UV-visible absorption and fluorescence emission spectra of CDs (1.0 mg/ml) synthesized at 150 °C. Inset picture shows the carbon dots under daylight (left) and UV light at 365 nm wavelength (right) dialysis for 12 hr

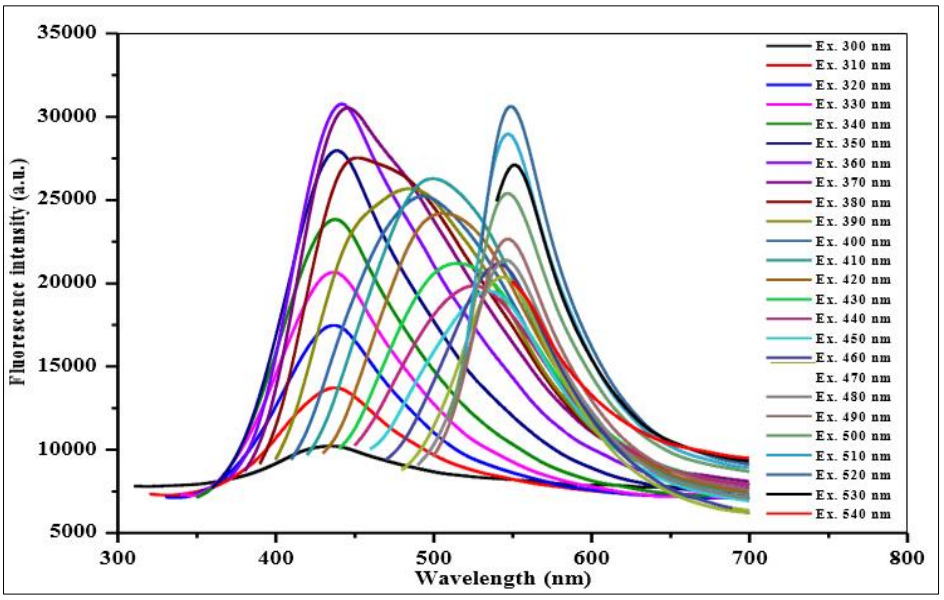

Fig 7: Fluorescence emission spectra of the CDs synthesized at 150 °C followed by 12 hr dialysis

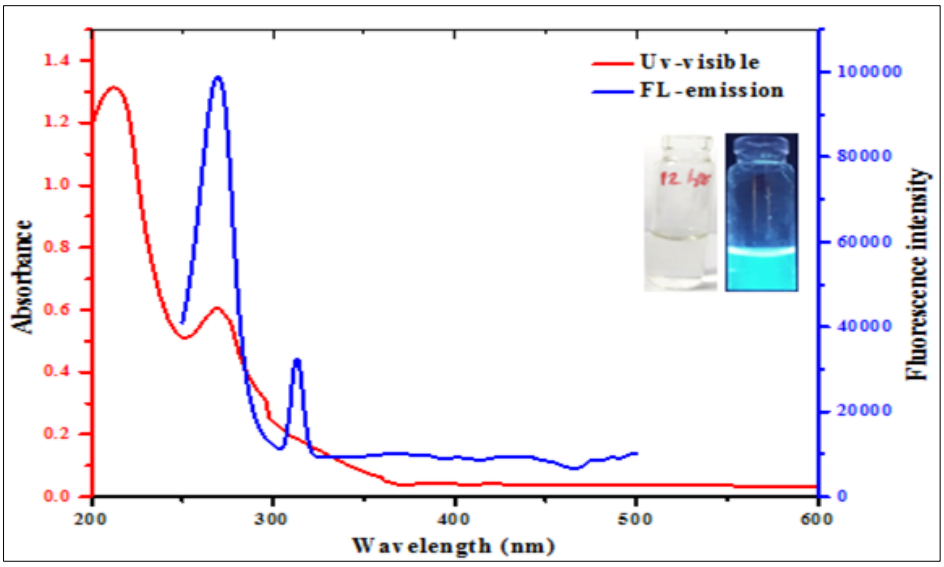

Fig 8: UV-visible absorption and fluorescence emission spectra of CDs (1.0 mg/ml) synthesized at 180 °C. Inset picture shows the carbon dots under daylight (left) and UV light at 365 nm wavelength (right) dialysis for 12 hr

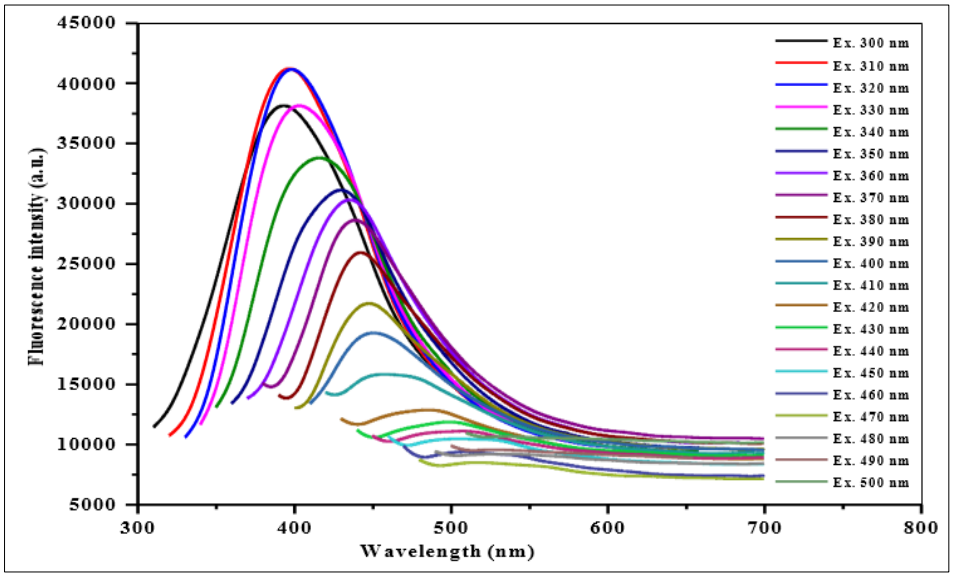

Fig 9: Fluorescence emission spectra of the CDs synthesized at 180 °C followed by 12 hr dialysis

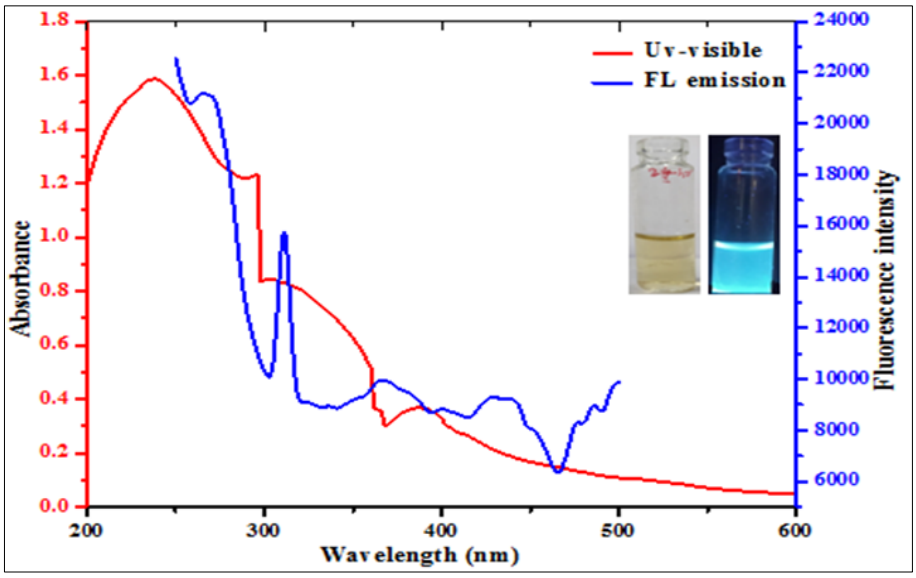

Fig 10: UV-visible absorption and fluorescence emission spectra of CDs (1.0 mg/ml) synthesized at 150 °C. Inset picture shows the carbon dots under daylight (left) and UV light at 365 nm wavelength (right) dialysis for 24 hr

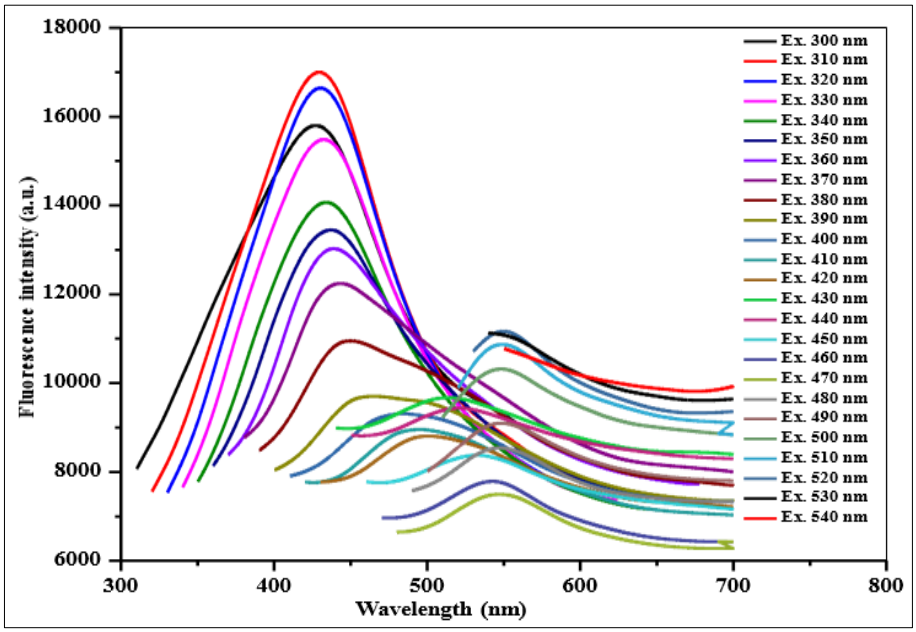

Fig 11: Fluorescence emission spectra of the CDs synthesized at 150 °C followed by 24 hr dialysis

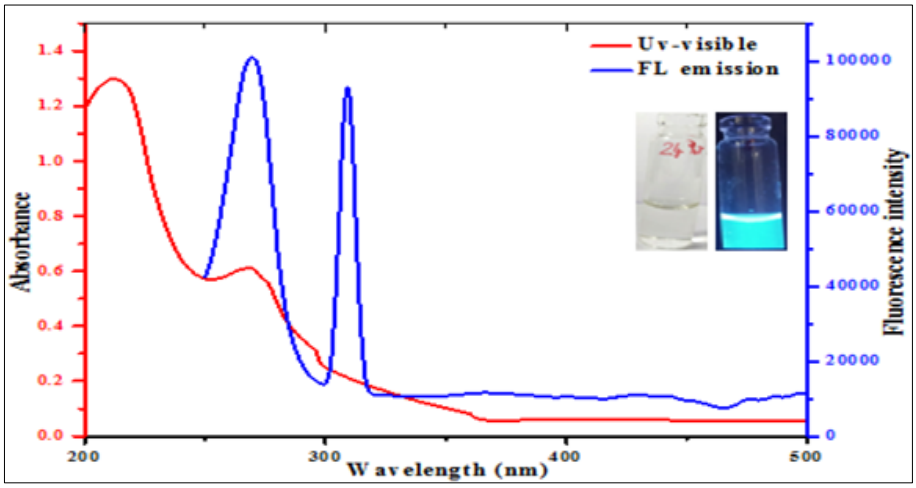

Fig 12: UV-visible absorption and fluorescence emission spectra of CDs (1.0 mg/ml) synthesized at 180 °C. Inset picture shows the carbon dots under daylight (left) and UV light at 365 nm wavelength (right) dialysis for 24 hr

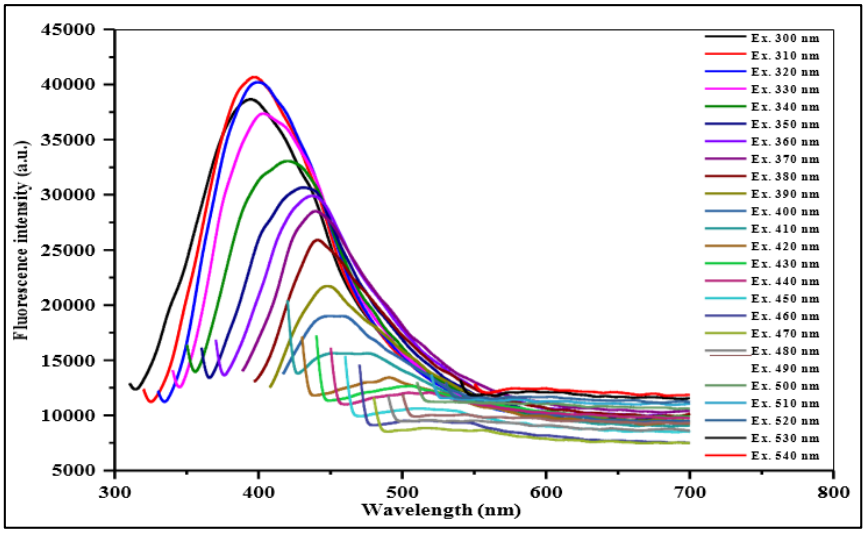

Fig 13: Fluorescence emission spectra of the CDs synthesized at 180 °C followed by 24 hr dialysis

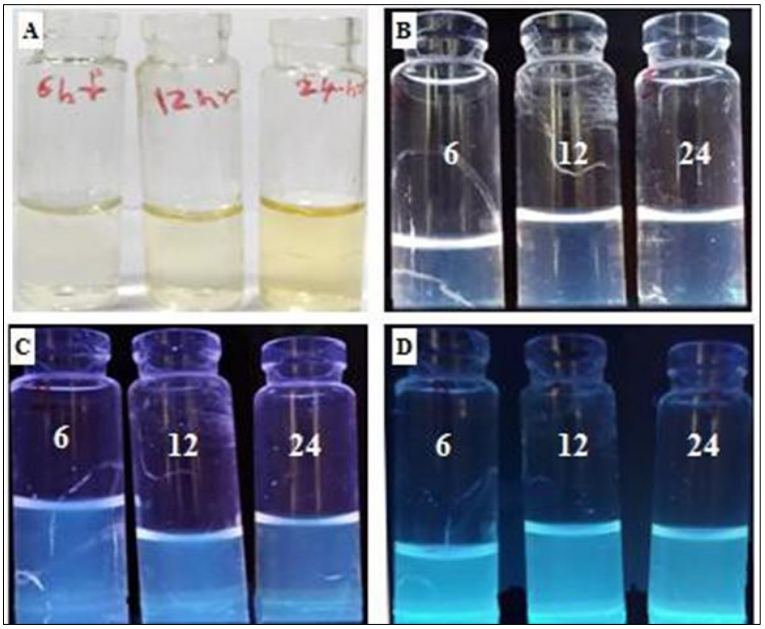

Fig 14: Fluorescence behavior of CDs (A) Day light, (B) 254 nm, (C) 302 nm and (D) 365 nm synthesized at 150 ºC (6 hr, 12 hr and 24 hr of dialysis, respectively)

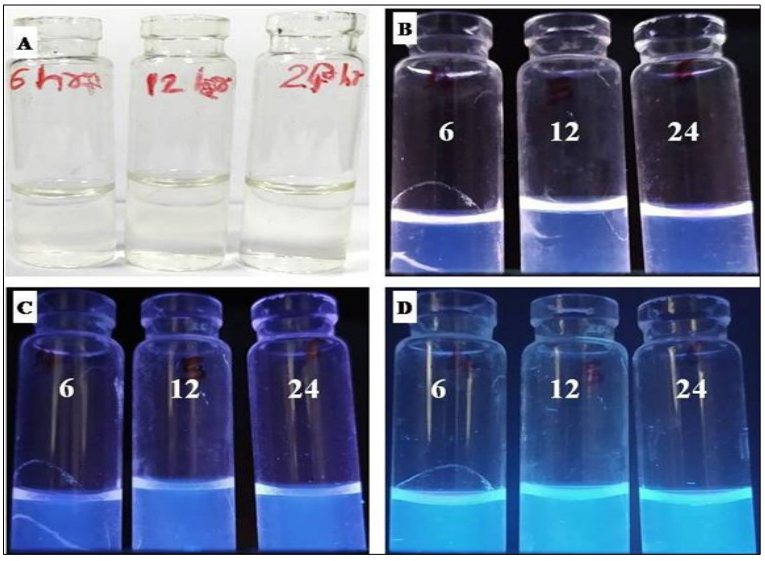

Fig 15: Fluorescence behavior of CDs (A) Day light, (B) 254 nm, (C) 302 nm and (D) 365 nm synthesized at 180 ºC (6 hr, 12 hr and 24 hr of dialysis, respectively)

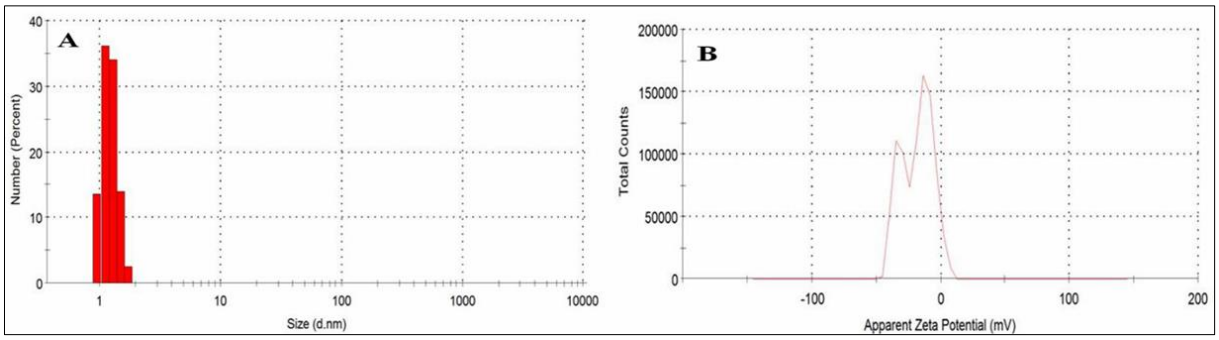

Fig 16: (A) DLS analysis (B) zeta potential of CDs synthesized at 180 ^{o}C followed by 24 hr dialysis

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