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Spectrophotofluorometric Study of Amaranth with Fluorescent Carbon Dots and its Analytical Application

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Hydrothermal carbonization of bottle gourd as the carbon source has been performed to synthesize fluorescent carbon dots . The carbon dots were formed in the form of yellow brown aqueous solution, soluble in water and stable towards light. Characterization of fluorescent carbon dots was done using TEM, UV-vis and fluorescence spectroscopy. Spectrophotofluorometric method has been followed for the study of common food colorant Amaranth present in foodstuffs using fluorescent carbon dots. Conditions optimized during analysis were reaction pH, concentration of Amaranth, concentration of C-dots, thermal effect, time period, *etc*. Results showed that Amaranth could result in fluorescence quenching of carbon dots due to interaction of surface –COOH, $–SO_3H$ group with Amaranth dye. The present method offers good linear range of 0.20-25.0 µM with LOD 0.019 µM and highly selective and therefore, method have been applied successfully for the detection of Amaranth in foodstuffs.

Keywords: Carbon dots, Hydrothermal process, Amaranth dye, Fluorescence quenching, Food samples.

INTRODUCTION

Food industries use colorants to colour their food products to make them look attractive and more tasty. Synthetic colours are added to beverages, chocolates, cake mixes, jams, jellies, *etc*. [1-4]. Amaranth is reddish brown synthetic azo dye used to colour food in large excess [5-7]. The daily acceptable intake of the dye is 0-1.5 mg/kg as percibed by Joint Food and Agriculture Organization (FAQ) and World Health Organization (WHO) [8]. However, beyond this limit, Amaranth is linked to hyperactivity in children, genotoxicity, cytotoxicity and seriously affect individuals allergic to aspirin [9]. Therefore, food safety concerns have raised serious issues towards determination of contents of the Amaranth dye in various foodstuffs.

Various analytical procedures have been followed to analyze the contents of food dyes in different food samples such as UV-visible spectroscopy [10], electrochemical method [11], diffuse reflectance FTIR spectroscopy [12], solid phase extraction [13] and high-performance liquid chromatography [14]. But all these reported techniques are not efficient due to requirement of tedious sample pretreatment and delicate instrumentation. Therefore, there is urgent need of development of method, which requires easy sample preparation, simple and reliable instrumentation.

Currently, carbon dots (C-dots), a new kind of fluorescent carboneous nanomaterial are emerging in wider research areas to limit the increasing environmental pollution due to their unique characteristics. These nanosized C-dots consist of sp^2 hybridized carbon core and surface hydroxyl and carboxyl group [15], which owes to their vast applications in research fields such as small size, quantum effects and high surface area to volume ratio. Wider applications are found in the field of food packaging and processing, biosensors, *etc*. [16-18]. Other applications of C-dots includes nanodevices, photocatalysts, tumor cell detection and in biomedical applications [19-21]. Abundant carbon sources provide excellent raw materials for synthesis of C-dots such as small organic molecules like caesin [22], cabbage [23], lemon and onion [24], biomass [25], pork [26], cysteine [27], vegetables [28] have been reported for the C-dots production.

Single step hydrothermal treatment was proven as an effective tool to produce C-dots, from complex food mixture under thermal conditions have been highlighted in different reviews [29]. Hydrothermal carbonization attributed a facile route towards

production of fluorescent C-dots over other reported methods in terms of synthesis strategies, renewable carbon sources and no specific equipment [30,31].

In current work, fluorescent C-dots were applied for the analysis of Amaranth. The C-dots were synthesized using bottle gourd as raw material which has excellent water solubility and exhibit strong fluorescence. Based on these unique characteristics of C-dots, an effective nanosensor was applied successfully for Amaranth detection in the beverages. The proposed method based on fluorescence quenching is highly sensitive and selective towards practical applications.

EXPERIMENTAL

A Shimadzu UV-vis 2500 absorption spectrophotometer with 10 mm quartz cell for spectrophotometric measurements. High resolution transmission electron microscopy (HRTEM) image of the synthesized carbon dots were recorded on Hitachi H-8100 transmission electron microscope (Tokyo, Japan). Shimadzu RF-5301 PC spectrofluorophotometer (Tokyo, Japan) was used to carry out fluorescence analysis. All the pH levels were measured using Digital century pH-meter (Cp-901).

Amaranth dye (95%), urea, quinine sulfate, dibasic sodium phosphates, sodium hydroxide, sodium dihydrogen phosphate, potassium bromide, starch and all other basic chemicals were purchased from Sigma-Aldrich Chemicals Pvt. Ltd. (India). Fructoses, sucrose, glucose, alanine, glycine, lysine, vitamin C, vitamin B were purchased from Puja Science House (Patiala, Punjab). All reagents were of analytical grade and used without any further purification. Millipore water was used all over the experiments. Buffer solutions of different pH 2-12 were obtained by adding different amounts of NaOH or HCl (1 M) to 0.01 M mixture solution of Na_2HPO_4 and NaH_2PO_4. Amaranth standard solution (2 mM) was prepared by using millipore water as solvent and stored at 4 ºC in refrigerator for experimental use.

Bottle gourd was obtained from local fields and washed thoroughly with distilled water before use. Confectionery samples like gems and biscuit were collected from the local grocery store of Punjabi University, Patiala and pretreated before use. These samples were crushed to fine powder followed by dissolution in hot water and filtered through 0.45 µm membrane to remove impurities. Further the resulting filtered solution was stored for experimental use.

Synthesis of fluorescent carbon dots (C-dots): Fresh bottle gourd was thoroughly washed with water to remove any dust particles. After washing, bottle gourd was peeled and the peelings were heated in microwave for 3 h at 200 ºC to obtain dried residue. Finely dried residue (6 g) was mixed with 30 mL of millipore water and then the contents were passed into a 250 mL titration flask, kept in Teflon-lined autoclave heated for 4 h at 120 ºC. Afterwards, the autoclave was cooled at room temperature and the resulting light brown coloured solution was filtered through 0.22 µm membrane. Finally, the supernatant containing C-dots was kept at 5 ºC for experimental use.

Fluorescence studies: An aliquot of 400 µL C-dots was added to the buffer solution of pH 4.0 and made up the volume to 5 mL with Millipore water. And the spectrum was recorded after reaction time of 10 min with excitation wavelength of 461 nm and slit width of 10/10 nm. % Recovery values were calculated by the following equation as:

$$\text{Recovery (\%)} = \frac{C_{measured} - C_{initial}}{C_{added}}$$

RESULTS AND DISCUSSION

Characterization of C-dots was done using absorption and emission study. Maximum absorption peak at 298 nm, which is due to $n \rightarrow \pi^*$ transition of C=O group and $\pi \rightarrow \pi^*$ transition of C=C group [14]. Fluorescence emission peak was observed at 505 nm, when excited to 461 nm which shows that C-dots are highly fluorescent and therefore, excitation wavelength depends upon emission wavelength and intensity (Fig. 1).

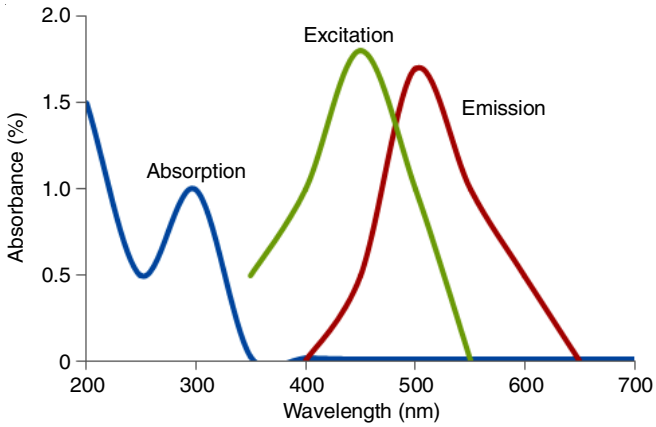

Fig. 1. UV-vis absorption and fluorescence emission spectra of C-dots

Transmission electron microscopy (TEM) results revealed the morphological studies of particle size distribution of the C-dots (Figs. 2 and 3). TEM image results showed that C-dots with size 200 nm are spherical and uniform in shape, size. Further FT-IR of synthesized C-dots was also performed (Fig. 4). The spectrum revealed the sharp peaks at 3294 cm^{-1} and 1077 cm^{-1} due to –OH stretching, -C-H stretching occurs at 2924 cm^{-1} and asymmetric and symmetric vibration modes of COO^- occurs at 1420 cm^{-1}.

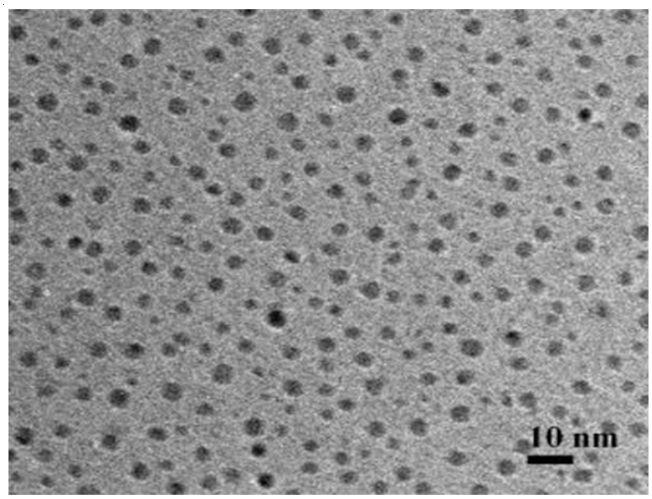

Fig. 2. TEM of C-dots

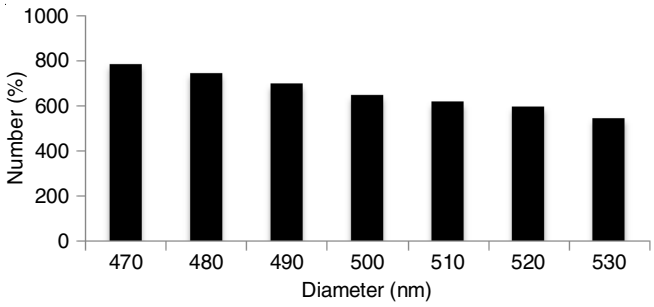

Fig. 3. Particle size distribution of C-dots

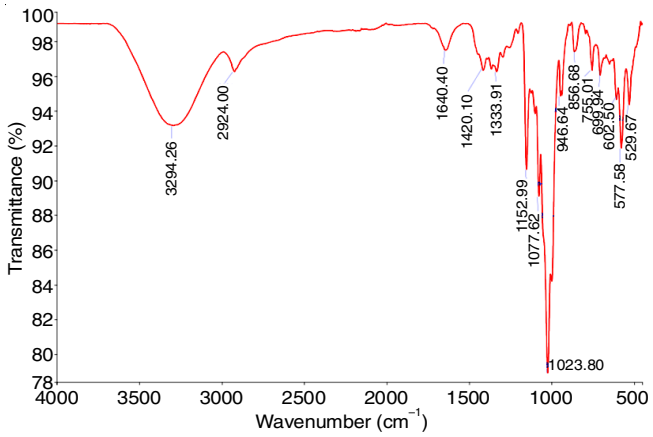

Fig. 4. FT-IR of C-dots

Fluorescence studies: Fluorescence emission was performed under different excitation wavelengths from 440 to 520 nm. The results showed that with increase in the excitation wavelength, fluorescence intensity decreases. Therefore, it is clear from Fig. 5 that absorption occurs at higher intensity as the concentration of Amaranth increases, which indicates formation of Amaranth-C-dots complex. Thus, C-dots fluorescence intensity depends on excitation wavelength. These results are in accordance with the previous reports [32,33].

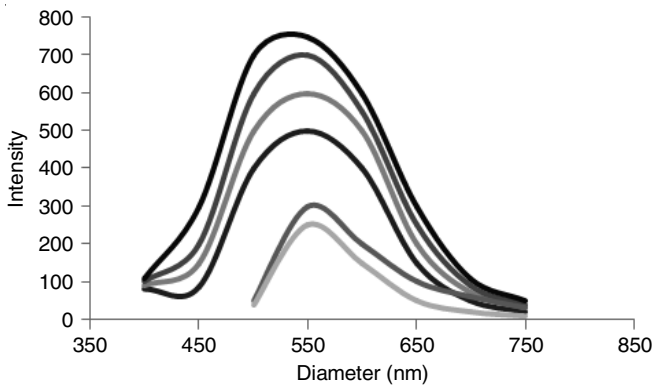

Fig. 5. Emission spectra of C-dots under different excitation wavelengths

Fluorescence studies were applied for amaranth detection in foodstuffs. The results showed that C-dots solution (F) exhibits strong peak at 440 nm and addition of Amaranth results in decrease in fluorescence intensity (F_0) due to quenching of C-dots fluorescence intensity given by Stern-Volmer equation [34]:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q]$$

where F_0 is the fluorescence intensity of C-dots and F is the fluorescence intensity of C-dots-Amaranth complex. K_{SV} is Stern-Volmer quenching constant can be calculated from the plot of F_0/F *vs*. Q at four different temperatures (Fig. 6). K_q is the quencher coefficient and Q is amaranth concentration and τ_0 is the average lifetime of C-dots in the absence of amaranth and has general value 10^{-8} s. Therefore, it is clear from Table-1 that K_{SV} decreases as temperature increasesand K_q is larger than maximum collision quenching constant (2.0×10^{10} L mol^{-1} s^{-1}).

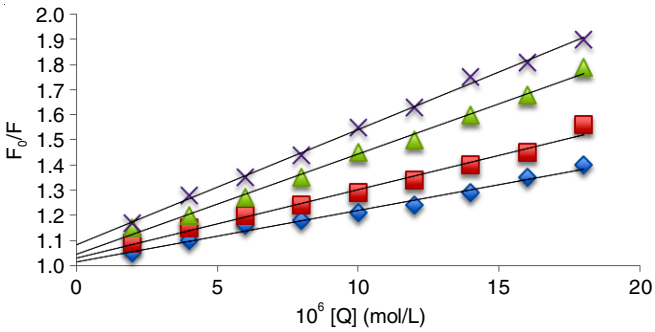

Fig. 6. Stern-Volmer plots at 278, 288, 298, 308 K for C-dots and amaranth at pH 4.0, conc. of C-dots 400 µL

TABLE-1
CALCULATION OF K_{SV} AND K_q AT DIFFERENT TEMPERATURES FOR C-DOTS-AMARANTH

Buffer	T (K)	K_{SV} (L mol^{-1})	K_q (L mol^{-1} s^{-1})	R
4.0	278	4.663×10^4	4.663×10^{12}	0.9929
4.0	288	4.193×10^4	4.193×10^{12}	0.9944
4.0	298	4.165×10^4	4.165×10^{12}	0.9954
4.0	308	3.576×10^4	3.570×10^{12}	0.9934

Optimization of various parameters

Effect of pH: Fluorescence quenching (F_0/F) increases with increase in pH upto 4.0 and thereafter decreases with further increase in pH (Fig. 7). Thus, pH 4.0 was selected as optimum pH for reaction medium.

Effect of C-dots concentration: The effect of C-dots concentration on the fluorescence intensity is shown in Fig. 8.

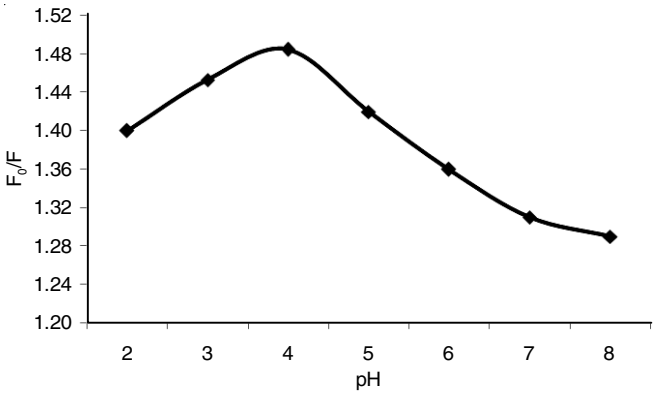

Fig. 7. Effect of pH on interaction of C-dots and amaranth

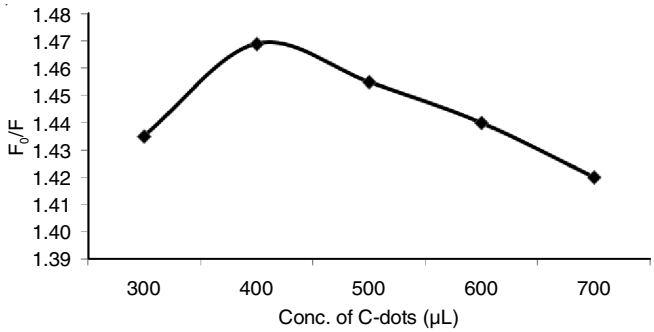

Fig. 8. Effect of C-dots conc. on quenching intensity

Fluorescence quenching efficiency increases upto 400 µL and then decreases with when the concentration of C-dots beyond 700 µL. Thus, 400 µL was selected as optimal concentration of C-dots for the experimental work.

Effect of temperature: To evaluate the effect of heat on F_0/F of C-dots, temperature was raised from 0-40 ºC. Maximum fluorescence occured at 10 ºC and thereafter decreases with increase in the temperature (Fig. 9). Thus, 10 ºC was selected as reaction temperature for experimental work.

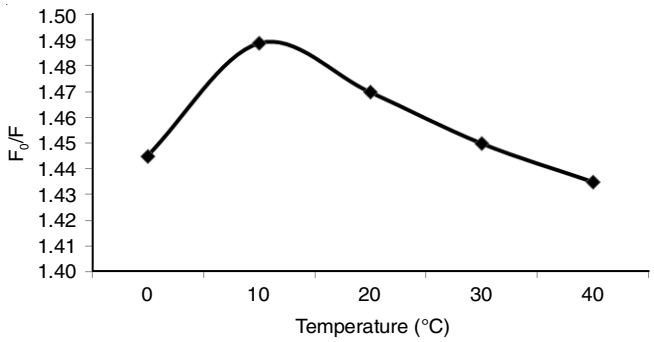

Fig. 9. Effect of temperature on F_0/F of C-dots

Effect of time: The F_0/F of C-dots also depends upon the duration of reaction. Therefore, reaction time was varied from 1-12 min. Fluorescence intensity was maximum at 1 min and further becomes constant with increase in reaction time (Fig. 10). Therefore, 5 min is selected as accurate reaction response.

With increase in concentration of amaranth, F_0/F of C-dots deceases (Fig. 11). As clear, fluorescence intensity varies linearly with concentration of amaranth dye.

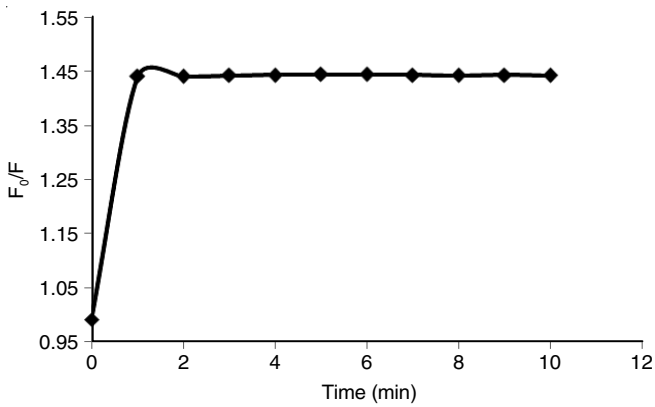

Fig. 10. Effect of time on interaction between C-dots and amaranth

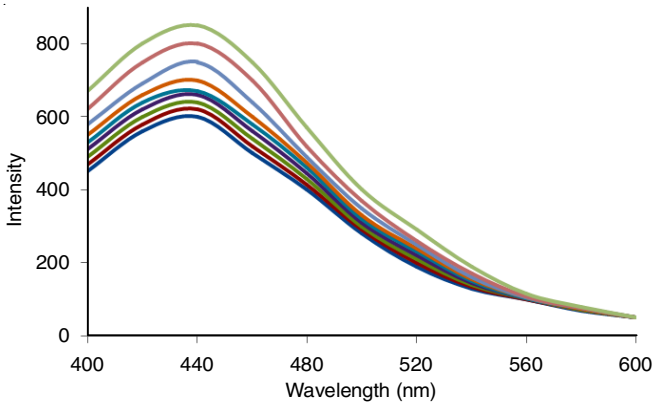

Fig. 11. Fluorescence emission spectra of C-dots with amaranth with C-dot concentration varying from 0.00 to 25.5 µM

Interference studies: The fluorescence intensity of Amaranth in the concentration of 4.0 µM was studied in the presence of different interferences like fructose, sucrose, glucose, alanine, glycine, lysine, vitamin C and vitamin B with concentration higher than that of Amaranth as shown in Fig. 12. And the concentrations of these interferences were lower than the required permissible limit. Therefore, present method offers high potential towards analysis of Amaranth.

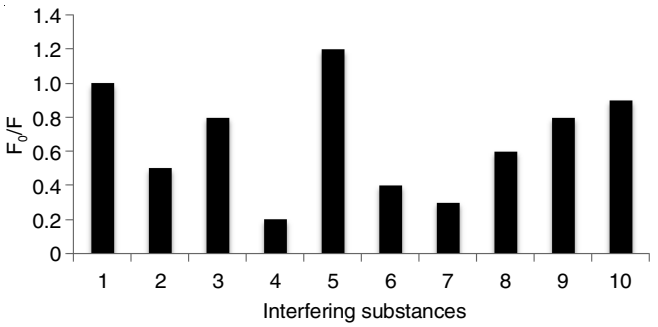

Fig. 12. Effect of interfering substances (1) blank (2) fructose (3) sucrose (4) Amaranth (5) glucose (6) alanine (7) glycine (8) lysine (9) vitamin C (10) vitamin B

Applications: The above method was applied under optimized parameters in food products available locally for trace analysis of Amaranth. For this, samples were pretreated followed by addition of known amount of Amaranth. Results (Table-2) show recovery values of 99.2% and 100% and the RSD values ranges between 0.1% to 2.2%, which further shows the accuracy of developed method.

Comparison with reported methods: Table-3 shows comparison of the experimental results to that of literature for Amaranth analysis. As clear that present method of Amaranth detection shows good linear range and RSD values to those reported in literature. Therefore, fluorescence method offers alternate to other methods of amaranth analysis in food samples. Moreover, this method is simple and economical.

Conclusion

Hydrothermal preparation of carbon dots using bottle gourd is simple, green and economical for the trace analysis of Amaranth food dye in commercial food products with good

TABLE-2
RESULTS OF AMARANTH ANALYSIS WITH METHODS REPORTED IN LITERATURE

Method of analysis	Linear range (μM)	R^2	LOD (μM)	RSD (%)	Ref.
Spectrophotometry method	0.05-4.8	0.999	0.01	–	[35]
Diffuse reflectance spectroscopy	0.1-5.0	0.999	1.13	–	[36]
Fabricated electrochemical sensor	5.0-4.0	0.999	0.1	< 3.1	[37]
Graphene nanomeshes	0.005-1.0	0.995	7.0	3.1	[38]
Voltametric method	1.0-1.1	0.999	1.7	0.23	[37]
HPLC		0.997	0.017	0.08	[39]
Ion-pair HPLC	0.3-3.6	0.993	0.01	0.04	[40]
CZE	3.0-6.0	0.995	0.61	1.1	[41]
Indirect ELISA method	3.0-243.0	0.999	3.35	–	[42]
SPE	0.5-1.5	0.997	0.15	10	[43]
HPLC-UV diode array detector	1.0-20	0.999	5.9	–	[44]
Carbon paste electrode	0.004	0.998	0.0001	4.2	[45]
Electrochemical sensor	0.1-1.0	0.999	6.8	–	[7]
Electrochemical method	8.0-4.0	0.999	36	2.70	[46]
HPLC coupled with DAD and MS/MS	1.0-10	0.999	0.008	< 15	[47]
Spectrofluorimetry	0.20-25.0	0.998	0.019	0.1	Present work

TABLE-3
APPLICATIONS OF AMARANTH IN BAKERY PRODUCTS

Food sample	Detected (μM)	Spiked (μM)	Found (μM)	Recovery (%)	RSD (%)
Gems*	ND	4.00	3.96 ± 0.07	99.0	0.1
		6.00	5.96 ± 0.06	99.3	0.3
		8.00	8.00 ± 0.05	100.0	0.5
Biscuit**	ND	3.00	3.01 ± 0.04	100.0	0.4
		5.00	4.48 ± 0.15	89.6	1.6
		7.00	6.97 ± 0.02	89.9	2.2

*Cadbury gems; **Parle G biscuit (Both purchased from local grocery store, Punjabi University campus, Patiala).

linear range of 0.20-25.0 μM with LOD 0.019 μM and high selectivity even in the presence of different interferences. The method is based upon interaction of C-dots–COOH and $–SO_3H$ group of Amaranth dye, which is responsible for fluorescence quenching. The Stern-Volmer equation was used to calculate the analytical data and the results revealed the occurrence of the static quenching mechanism.The calculated RSD value of 0.35% shows that the present method is easy and reliable for Amaranth detection in food samples. Moreover, this method is also easy, rapid, cost effective and therefore advantageous over most other reported methods for the Amaranth detection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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