6.1 Introduction

Fish is an important part of our diet which serve as an important source of protein (supplies 16 % of animal protein throughout the world) and other nutrients (fats (omega-3 and 6), vitamins (B, D), minerals (Ca, K, Mg, P, I, Zn, etc.), etc.) (Kaur et al., 2024; Chutia et al., 2024; Jinadasa, Elliott & Jayasinghe, 2022). Nevertheless, fish is an abundant source of omega-3 polyunsaturated fatty acids which plays important role in regulating heart rhythm alongside working as structural component of cell and precursor of eicosanoids (Mai et al., 2022; Willett et al., 2019). The intake of fish can reduce the chance of heart attack by lowering the blood pressure (Jinadasa, Elliott & Jayasinghe, 2022).The nutritional benefit of fish increases their value and demand as well throughout the world which can perpetuate food and nutrition security globally (Body, McNevin & Davis, 2022). However, the perishable nature of fish restricts the preservation of its overall quality as exhibited after harvesting. Moreover, it is very challenging to maintain freshness, texture and overall quality of fish with time. This may be due to high susceptibility of fish towards postmortem change which closely depends on various parameters like, source, environmental condition, harvesting technique, storage methods, type, composition, etc. (Yumnam et al., 2024). Therefore, it became an open challenge to all including, local sell person, vendors, fish merchants, researchers, scientists, etc. to overcome this serious issue.

Freezing, ice cooling, chilling, etc. are some of the conventional techniques usually adopted to preserve fish with time. However, with the aforesaid techniques quality of fish is compromised by default which reflects loss of money to the fish merchants. To get rid of this loss and maximizing profit fish merchants thrives for a technique through which they can escalate shelf life of fish. They started applying harmful chemicals like sodium benzoate and formalin (FA) in fish illegally (Mehta et al. 2023). Formalin reduces the bacterial load along with enhancing the muscle flexibility which results in fresh appearance and delayed spoilage, though it decreases essential and non-essential amino acids in fish (Wongniramaikul et al., 2018; Kaur et al., 2024).

FA is basically a colourless, transparent solution of formaldehyde (37 – 40 % w/w) which is a highly flammable, extremely sensitive, colorless gas that readily polymerizes in normal temperature and pressure. The consumption of FA causes serious health issues like damage in

liver, kidney, lung, neurological systems etc. International Agency for Research in Cancer (IARC) has listed formaldehyde as Group I carcinogen. According to WHO (World Health Organization), the estimated daily intake of FA is in the range of 1.5 to 14 mg (Thepchuay et al., 2022) however, EFSA (European Food Safety Authority) limits the level of FA up to 100 mg/day (European Food Safety Authority, 2014). FSSAI (Food Safety and Standard Authority of India) has set a maximum permissible limit (MPL) of FA in fresh water fish and sea fish as 4 mg/kg and 100 mg/kg respectively (FSSAI, 2019). Chinese Ministry of Agriculture has set the MPL of FA in aquatic products to 10 mg/kg (Zhang et al., 2015). Moreover, according to Malaysian Food Regulation the MPL of FA in fish is 5 mg/kg (Malaysian Food Regulation, 1985). Generally, FA is widely used for biological preservation purpose (Thepchuay et al., 2022). Moreover, FA is used as preservative and disinfectant, bacteria inhibitor in agricultural sector and sugar industry respectively. It is noteworthy to mention that, the illegal addition of FA is not only limited to fish (Mohanty et al., 2018) but also in milk (Veríssimo et al., 2020), mushroom (Mason et al., 2004), fruit juice (Kundu et al., 2019), fruit and vegetables (Wahed et al., 2016), etc. However, the enzymatic breakdown of TMAO (trimethylamine oxide) endogenously produce FA in the fish muscle. The level of TMAO and its breakdown depends upon several factors like feeding quality, preservation techniques, harvesting methods, handling methods, storage practices, etc. (Bhowmik et al., 2020; Mohanty et al., 2018).

Boiling fish for several minutes and rinsing fish in tap water are some effective methods of minimizing FA proposed by several group of researchers (Kundu et al., 2020; Bhowmik et al., 2020) however, the complete removal of artificially added FA is very challenging as it is adsorbed in the fish muscles and it causes serious health issues. Therefore, detection of formalin in fish become crucial to extenuate the risk. The conventional analytic detection methods [spectrophotometric (Das et al., 2018; Naksen et al., 2022), gas chromatography-mass spectrometer (GC-MS) (Bianchi et al., 2007), high performance liquid chromatography (HPLC) (storey et al., 2015; Kundu et al., 2020), etc.] of FA are complex, vexatious and costly (Fappiano et al., 2022). Therefore, simple, advance and economic techniques of FA is highly needed to cope with the rising concern of illegal addition of FA in food (fish, meat, vegetables, etc.).

It is noteworthy mentioning, that the colorimetric and fluorescence based methods are emerging in this field (Seebunrueng et al., 2024; Chutia et al., 2024; Singseeta et al., 2023; Zhang et al., 2023; Wongsing et al., 2023).Therefore, in this study, a CDs based aerogel was developed considering the concept of fluorescence and colorimetry to detect FA in fish easily.

6.2 Materials and methods

6.2.1 *Materials*

Native corn starch (amylose content: 13.32%) was purchased from LOBA CHEMIE PVT. LTD. Ethanol (C_2H_5OH: ≥ 99.5% purity) was purchased from Changshu Hongsheng Fine Chemical Co. Ltd. Calcium chloride ($CaCl_2$), citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$), ammonium hydroxide (NH_4OH; 25 %), sodium hydroxide (NaOH), silver nitrate ($AgNO_3$), ammonium acetate ($NH_4CH_3CO_2$), acetylacetone ($C_5H_8O_2$), acetic acid (CH_3COOH), silica gel, and glycerol were procured from Merck Life Science Private Limited (Mumbai, India). Fresh fish samples were purchased from local market of Tezpur, Assam, India.

6.2.2 *Development of CDs based functional aerogel (CDFA)*

The development process of CDFA (**Fig. 6.1**) consists of four crucial steps (i) synthesis of CDs, (ii) preparation and selection of optimum concentration of Tollens reagent (TR), (iii) development of aerogel, and (iv) loading of CDs with TR and development of CDFA. All these four steps involved were explained briefly in the following sections.

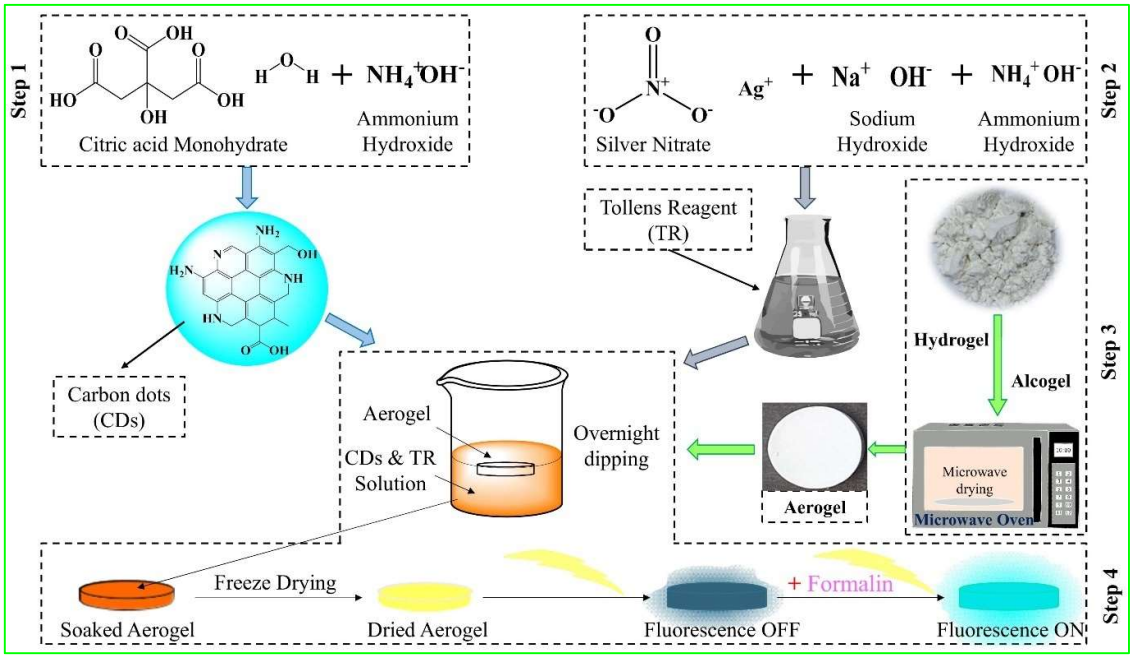

Fig. 6.1 Development process of carbon dots based functional aerogel (CDFA)

6.2.2.1 *Synthesis of CDs*

Hydrothermal method of synthesis as reported by Zhou et al. (2015) and Naksen et al. (2022) was followed with some modifications to synthesize nitrogen doped CDs. Citric acid monohydrate and ammonium hydroxide was used as precursor material of CDs and nitrogen

respectively. In brief, 1.05 g of citric acid monohydrate was mixed with deionized (DI) water to dissolve it properly, then 2.68 mL ammonia solution was added to the solution and the volume was maintained to 50 mL by adding DI water. The mixture was kept in a magnetic stirrer at 450 rpm to stir for 30 min. The mixture was then transferred to a Teflon lined autoclave and kept in a muffle furnace at 200 ℃ for 5 h. The autoclave was cooled in room temperature and the solution was centrifuged (10000 rpm for 15 min) and filtered through membrane filter to remove large impurities. The CDs were obtained through freeze drying from the yellow solution containing CDs. Then, CDs solution was prepared using powdered CDs and DI water for further analysis.

6.2.2.2 *Preparation and selection of optimum concentration of TR*

The TR was prepared by following the method given by Naksen et al. (2022) with some modifications. First, 0.5 M $AgNO_3$ and 0.1 M NaOH solution was prepared. The 0.1 M NaOH solution was mixed with 0.5 M $AgNO_3$ solution which turned the color of the solution from transparent to hazy white. Then, NH_4OH solution was added to the mixture which formed a brown precipitate, after excess addition of NH_4OH the solution became transparent again. Finally, the TR was prepared. To get the optimum concentration of TR to be added in CDs solution, different volume (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL) of TR was added with CDs solution and the solution was mixed properly. The fluorescence intensity of each solution was measured in a fluorescence spectrophotometer (Cary Eclipse, Agilent, USA). Based on the saturation in fluorescence intensity, the concentration of TR was fixed for further use.

6.2.2.3 *Development of aerogel*

The aerogel was developed by following the method described in Section **4.2.2**. Corn starch (7.5 % w/v) was mixed with distilled water to prepare a solution. The solution was gelatinized at 93 ℃ using a laboratory stirrer at 450 rpm for 20 min. Then, 6 mL of 0.01 % $CaCl_2$ and glycerol (5 %) was added to the gelatinized solution and stirred at 10000 rpm for 10 min. The solution was poured into cylindrical polypropylene mold and incubated at 4 °C for 2 days. The cylindrical shaped hydrogel was cut into small pieces (5 mm) and submerged into 30 to 100 % ethanol solution for ethanol substitution and to form alcogel. The alcogel were dried in a microwave oven (240 W for 280 min) to develop aerogel. The developed aerogel were stored in a desiccator (desiccant: silica gel) for further use.

6.2.2.4 *Loading of CDs with TR and development of CDFA*

The TR with optimum concentration was added in CDs solution and the concentration of CDs was maintained to 1.0 wt %. The CDs and TR was loaded into the aerogel matrix by dipping the aerogel into the mixture for overnight. The wet aerogel obtained after soaking was kept in a freezer at -18 °C for 16 h. Then the frozen samples were dried through sublimation using a freeze drier (-50 °C for 3 h). Finally, the CDFA were developed. The developed CDFA were stored properly in an amber color desiccator (desiccant: silica gel) for further experiments and characterization.

6.2.3 *Calibration of the CDFA using colorimetric method*

The developed CDFA was calibrated by adding standard FA solutions ranging from 0 to 150 mg/L for 30 min on aerogel. The aerogel was exposed under UV light (wavelength: 365 nm) in a close chamber to check the fluorescence color developed and the images were taken. The images were processed through Image J software to obtain green-blue (G-B) values and then the G/B values with respect to each standard FA concentration were calculated. A calibration curve of G/B values against standard formalin concentrations was made and the linear equation acquired from the calibration curve was used to detect FA in fish (**Eq. 6.1**). The performance of the functional aerogel as formalin sensor was evaluated.

$$\text{Formalin concentration} = \frac{\left({}^{\text{G}}/_{\text{B}}\,\text{value} - \text{b}\right)}{\text{S}} \tag{6.1}$$

Where, G/B value, b, and S represents the green divided by blue value, intercept and slope of the calibration curve respectively.

The uncertainty of measurement was also calculated in terms of each parameter (slope (S), intercept (b), and coefficient of determination (R^2)) related to concentration measurement available in the linear curve. The average value of each parameter and their respective standard deviation was calculated (Irnawati et al., 2021).

6.2.4 *Performance evaluation of CDFA*

Limit of detection (LOD), limit of quantification (LOQ), accuracy and precision, and % recovery was measured to evaluate the performance of the CDFA (Yumnam et al., 2023). Standard FA solutions of 0, 50, 70, and 90 mg/L was added to CDFA and the images were captured after 30 min under UV-light. The G/B value and concentration was calculated with respect to each standard FA concentrations.

The LOD and LOQ was measured using the following equations (**Eq. 6.2** and **Eq. 6.3**):

$$\mathrm{LOD} = \frac{3\sigma}{\mathrm{S}} \tag{6.2}$$

$$\mathrm{LOQ} = \frac{10\sigma}{\mathrm{S}} \tag{6.3}$$

Where, σ represents the standard deviation of the response of the blank sample and S represents the slope of the calibration curve

Generally, accuracy and precision of sensor is expressed by % Bias (mean of individual difference) and % RSD (relative standard deviation). The % Bias and % RSD was measured by following equations (**Eq. 6.4** and **Eq. 6.5**):

$$\%\ \mathrm{Bias} = \frac{(\mathrm{K_c} - \mathrm{M_c})}{\mathrm{K_c}} \times 100 \tag{6.4}$$

$$\%\ \mathrm{RSD} = \frac{\mathrm{SD}}{\mathrm{M_c}} \times 100 \tag{6.5}$$

Where, K_c, M_c, and SD represents the known concentration, mean concentration, and standard deviation respectively.

The % recovery was calculated by using following equation (**Eq. 6.6**):

$$\%\ \mathrm{Recovery} = \frac{\mathrm{Estimated\ Concentration}}{\mathrm{Actual\ Concentration}} \times 100 \tag{6.6}$$

6.2.5 *Sample preparation*

Fresh live Common Carp (*Cyprinus carpio*) fish (**Fig. 6.2**) were procured from the local market of Tezpur, Assam, India. On the same day of fish harvesting, the experiment was conducted. The samples were prepared in two different ways. To prepare the first type of sample, the head, gut, scale, and middle bones were removed and washed under tap water, and fish fillets of uniform size were prepared in a sterile environment. The fresh fillet (5 g) was kept with 50 mL of de-ionized (DI) water for 30 min and the extract was filtered twice through a Whatman No. 1 filter paper (Chaiendoo et al., 2018). The fish extract was made in contact with the aerogel. To prepare the second type of sample, whole fish was kept in contact with 50 mL DI water for 30 min. The surface fluid mixed with DI water was filtered through filter paper. The samples (fish extract) were prepared an interval of 0, 3, and 6 h for the fish fillet

and whole fish stored at ambient temperature. The pH of the extract was measured using pH electrode (Yumnam et al., 2023). The fish extract was made in contact with the aerogel.

Fig. 6.2 Common Carp (Cyprinus carpio) fish

6.2.6 *Experimental analysis of fish samples using CDFA*

The fish fillet extract and whole fish extract was brought in contact with the CDFA for 30 min at ambient temperature (28 ± 1 °C). The images of CDFA were taken under UV-light (wavelength: 365 nm). The images of CDFA was captured at an interval of 0, 3, and 6 h for the samples stored at ambient temperature. The images were processed through Image J software to obtain green-blue (G-B) values and then, the G/B values with respect to each sample were calculated. The concentration of FA was calculated using the linear equation obtained from calibration curve of G/B values against standard formalin concentrations. The linear equation obtained from the curve was also used to calculate the unknown concentration and to compare between the calculated and spiked concentration (50 and 150 mg/L) of fish extract samples. The experimental method was validated with acetylacetone method.

6.2.7 *Validation method*

The experimental method was validated by acetylacetone method (Karasz et al., 1974). To perform the validation experiment, Nash's reagent was used. First, 3.0 g ammonium acetate was dissolved in 20 mL DI water, then 60 µL of acetylacetone and 40 µL acetic acid was added into the solution to prepare Nash's reagent. Then the pH of the solution was maintained between 6.0 and 6.5 by adding NH_4OH. The freshly prepared Nash's reagent was kept in dark for future use. For detection, 3 mL Nash's reagent and 0.3 mL FA standards (0, 20, 40, 60, 80, and 100 mg/L) was added in test tubes and the total volume was made to 5 mL by adding DI water. The test tubes were placed in a hot water bath for heating at 60 °C for 15 min. The absorbance of the samples were measured immediately after cooling at 412 nm using a spectrophotometer. A standard curve was prepared by plotting absorbance values against

standard FA concentrations. The linear equation obtained from the curve was used to calculate the unknown concentration and to compare between the calculated and spiked concentration (50 and 150 mg/L) of fish extract samples. The percentage deviation of CDFA response from the spectrophotometric response of acetylacetone method was calculated by using following equation (**Eq. 6.7**) to check the overall acceptability of the sensor.

$$\% \text{ Deviation} = \frac{(V_c - A_c)}{V_c} \times 100 \quad (6.7)$$

Where, V_c and A_c represents the concentration obtained from validation method and CDFA respectively.

6.2.8 *Statistical analysis*

All the analyses were performed in triplicates and the results data were represented as mean ± standard deviation. Statistical analysis was performed using SPSS software (SPSS 17.0, SPSS Inc., Chicago, IL). The data were analyzed by one-way analysis of variance test and to determine the significant differences among individual results DUNCAN's multiple range test (DMRT) ($p < 0.05$) was performed. The level of significance was set to $p < 0.05$ to determine the statistical significance. Microsoft Excel 2017 and Origin Pro 2018 (OriginLab, USA) were used for graphing and principal component analysis.

6.3 Results and discussion

6.3.1 *Development of CDFA*

The presence of TR in CDs solution used to quench the fluorescence of CDs (Naksen et al., 2022). Therefore, the quantity of the TR to be added in CDs solution was optimized on the basis of saturation on quenching. It was observed that the fluorescence intensity decreased with the increase in TR volume as a consequence of increased concentration of Ag^+. This may be due to transfer of electrons from CDs to Ag^+ based on photoinduced electron transfer (PET) process (Zu et al., 2017). However, after addition of 1.5 to 3.0 mL TR, the fluorescence intensity did not change significantly (**Fig. 6.3a** and **Fig. 6.3b**) therefore, 1.0 mL TR was selected as the optimum volume of TR. The concentration of Ag^+ corresponds to 1.0 mL TR was used for further analysis. The significant reduction in fluorescence intensity may be due to binding of Ag^+ on the surface of CDs. The flexible coordination sphere of Ag^+ acted as the driving force towards its selection by the CDs among other metal ions (Cayuela et al., 2016). Moreover, the existence of functional groups (like –COOH, –OH, and $–NH_2$) on the surface of CDs escalate the interaction between Ag^+ and CDs which may be attributed to formation of

energy gap between CDs and Ag^+ that drives the PET process (Algarra et al., 2014; Zu et al., 2017; Naksen et al., 2022). Moreover, the presence of excess ammonia in TR formed a complexation ($[Ag(NH_3)_2]^+$) with Ag^+ on the surface of CDs (Naksen et al., 2022).

To observe the fluorescence regain, varying concentration of FA (10 to 100 mg/L) was added with the mixture of optimized TR and CDs solution. It was found that the fluorescence intensity increased with the increase in FA concentration. The re-enhancement in fluorescence intensity may be attributed to the reduction of Ag^+ to Ag^0 and release from the surface of CDs as a consequence of FA addition due to its very high oxidation capability (Adkins et al., 1949; Wongsing et al., 2023). The selectivity of FA over other aldehyde compounds and other functional group containing compounds (benzaldehyde, acetaldehyde, acetone, acetylacetone, n-butanol, formic acid, and glucose) may be attributed to its highest oxidation capability which can reduce best through reaction with TR as an oxidizing agent (Adkins et al., 1949; Naksen et al., 2022). Naksen et al. (2022) reported the interference of afore mentioned compounds in terms of tolerance concentration. The adequate tolerance for other aldehydes can be attributed to the selectivity of the silver mirror reaction towards FA. Specifically, the selectivity towards FA over other compounds depends on the reducing ability of the interference compounds. The increase in fluorescence intensity with respect to control sample (sample without FA) was plotted against different FA concentrations (**Fig. 6.4a**).

The selected concentration of TR was mixed with CDs solution and the concentration of CDs was maintained to 1 wt % in the mixture. The mixture of CDs and TR was loaded into aerogel through overnight dipping. The CDFA was obtained through freeze drying of wet aerogel. The fluorescence behaviour of the developed CDFA was observed under UV-light (wavelength: 365 nm). The apparent blue fluorescence was not observed in CDFA as observed in the case of CDs loaded aerogel (**Fig. 6.4b**). The loading of TR with CDs quenched the fluorescence in CDFA. However, the addition of FA in CDFA re-enhanced its apparent blue fluorescence under UV-light (**Fig. 6.4b**).

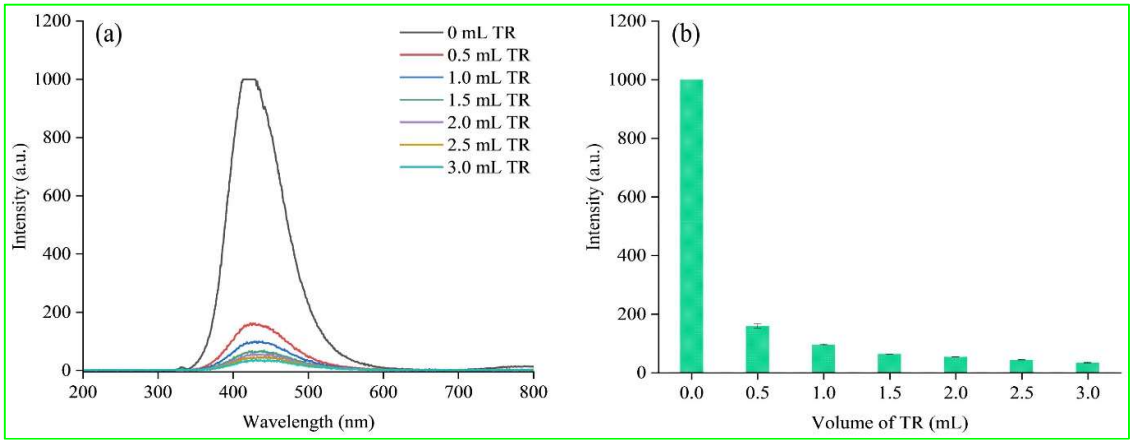

Fig. 6.3 Fluorescence spectra (a) and fluorescence intensity (b) of CDs in the presence of Tollens reagent (TR)

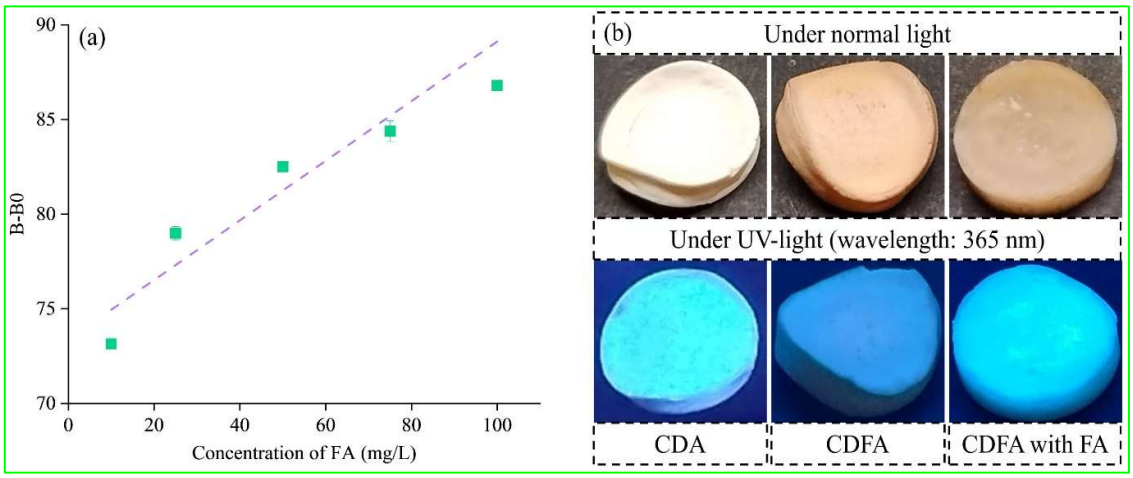

Fig. 6.4 Plot between intensity difference (B–B0) and concentration of FA (a) and images of CDs based aerogel (CDA) (left) and CDs based functional aerogel (CDFA) without (middle) and with FA (right) (b)

Note: B and B0 is the fluorescence intensity with FA and without FA respectively

6.3.2 *Calibration and performance evaluation of CDFA based on G/B*

Standard FA solutions having concentration of 0, 20, 40, 60, 80, 100, and 150 mg/L was added to CDFA. The G/B value obtained from the images of CDFA under UV-light was plotted against respective FA concentration (**Fig. 6.5**). The plot between G/B value and FA concentration exhibited a linear relationship with a coefficient of determination (R^2) of 0.96. The value of each parameter related to uncertainty of measurement of the calibration curve were calculated and tabulated in **Table 6.1**.

Table 6.1 The linearity and the uncertainty of measurement of the calibration curve

Parameters	CDs based functional aerogel
Coefficient of Determination (R^2)	0.9521
Slope (S)	0.00157
Intercept (b)	0.6022
Standard deviation of Slope (S_S)	0.00006
Standard deviation of Intercept (S_b)	0.0023
Standard deviation of Regression (S_r)	0.0104

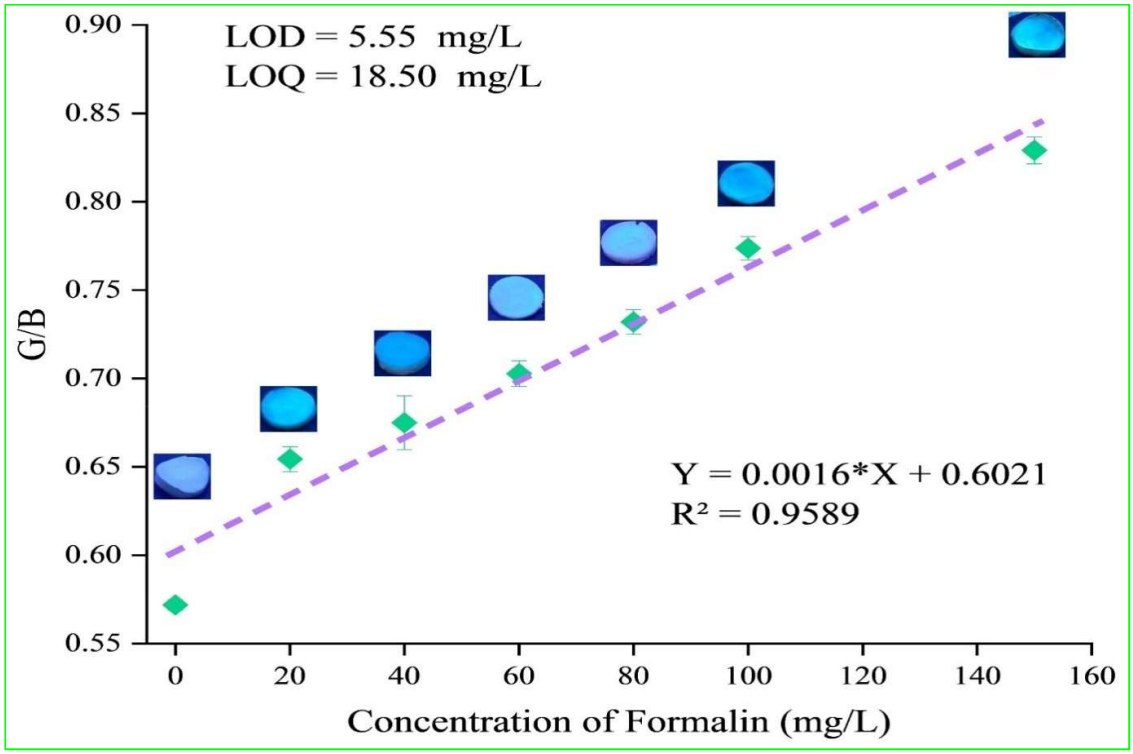

Fig. 6.5 Calibration curve of CDFA response to different formalin concentration

The developed CDFA was exhibited LOD and LOQ of 5.55 mg/L and 18.50 mg/L respectively. The accuracy and precision of the developed CDFA in terms of % bias and % RSD was found in the range of – 0.39 to – 2.02 % and 4.71 to 6.43 % respectively (**Table 6.2**). The low % bias and low % RSD of the developed CDFA confirmed that it is reliable and it generates nearly accurate results when it is subjected to spiked concentrations of FA. The % recovery was calculated to measure the accuracy of CDFA in the detection of FA. In brief, %

recovery suggests the closeness of estimated value in comparison to added value. The CDFA exhibited % recovery (**Table 6.2**) of spiked FA solutions in the range of 100.39 to 102.02 % which lies in the common range of % recovery i.e. 80 to 120 % (Irnawati et al., 2021). The findings justified that the CDFA is effective in the detection of FA in fish.

Table 6.2 Accuracy, precision, and % recovery of CDFA

Formalin solution (mg/L)	% Recovery	% Bias	RSD (%)
50	100.39	-0.39	4.71
70	101.80	-1.80	5.07
90	102.02	-2.02	6.43

6.3.3 *Performance evaluation of CDFA with respect to fish sample*

The presence of any trace of FA in the stored common carp was tested using the developed CDFA. The test was conducted in the form of fish fillets and whole fish. The extract prepared from both fish fillets and whole fish was made in contact with CDFA for 30 min. The CDFA containing fish extract was exposed under UV-light (wavelength: 365nm) to capture fluorescence images of CDFA. The images were further process through Image J software to obtain green (G) and blue (B) value. The obtained values were used to calculate the concentration of FA using the calibration curve. It was observed that the unspiked fish fillet extract showed an increase in concentration of FA at 3 h however, a decrease was also observed at 6h. The extract spiked with 50 mg/L FA was also showed an increase in FA concentration till 3 h however, it showed no change in FA concentration at 6 h. The 150 mg/L FA spiked extract exhibited increase in FA concentration over time. The extract prepared from whole fish showed an increase in FA concentration value over time. The increase in FA concentration over time may be due to enzymatic breakdown of TMAO which produces FA indigenously (Mohanty et al., 2018). The obtained results were further compared with the existing conventional FA detection techniques to justify the applicability of CDFA for FA detection.

6.3.4 *Method validation of CDFA*

The calibration curve plotted between the absorbance and standard FA concentrations was obtained from the acetylacetone method. The calibration curve was used to calculate the unknown concentrations of FA by putting absorbance value on the curve. It was observed that the unspiked fish fillet extract showed similar trend of changing in FA concentration with the advancement of time from 0 to 6h. Moreover, for the spiked (50 mg/L and 150 mg/L) fish fillet extracts, the results showed an increase in FA concentration till 3 h however, after 6 h of storage

it exhibited almost constant value. Moreover, the extract prepared from whole fish exhibited increase in FA concentration over time. Therefore, the performance of the CDFA was validated with the % deviation values with reference to acetyl acetone method. It was observed that % deviation ranges from – 6.24 to 10.28 %, when the experiment conducted with the extract obtained from fish fillets (**Table 6.3**). The % deviation was observed to be varied from – 11.72 to 7.98 % for the extract obtained from whole fish (**Table 6.4**). These findings supported the compatibility of CDFA in real sample analysis.

Table 6.3 Comparison of formalin determination in fish fillets through CDFA and Acetylacetone method

Storage time (h)	Added FA (mg/L)	CDFA	Acetylacetone method	% Deviation
		Found (mg/kg)	Found (mg/kg)	
0	0	360.68 ± 14.00	388.70 ± 0.35	7.21
3		671.13 ± 36.30	631.70 ± 1.35	-6.24
6		499.77 ± 37.97	530.68 ± 4.63	5.83
0	50	747.73 ± 16.58	763.12 ± 1.09	2.02
3		983.37 ± 35.47	1039.60 ± 0.13	5.41
6		986.52 ± 4.16	983.33 ± 4.28	-0.32
0	150	1498.50 ± 59.56	1506.70 ± 0.31	0.54
3		1626.73 ± 32.20	1813.18 ± 1.79	10.28
6		1855.40 ± 93.17	1836.82 ± 2.75	-1.01

Table 6.4: Comparison of formalin determination in raw fish through CDFA and Acetylacetone method

Storage time (h)	CDFA	Acetylacetone method	% Deviation
	Found (mg/kg)	Found (mg/kg)	
0	3.56 ± 0.42	3.19 ± 0.05	-11.72
3	4.90 ± 0.29	5.32 ± 0.03	7.98
6	10.85 ± 0.23	10.05 ± 0.01	-8.03

6.4 Conclusion

The developed CDFA have successfully applied for formalin detection. The detection was based on the silver mirror method. More specifically, the detection method works on the principle of fluorescence quenching (OFF) and re-enhancing (ON). TR in combination with CDs was loaded into the aerogel matrix which results in decreased fluorescence behaviour of aerogel under UV-light (wavelength: 365 nm). Standard FA solutions were added to CDFA to observe the fluorescence re-generation behaviour. The calibration curve plotted between G/B value and standard FA concentration exhibited a broad linear range with a coefficient of determination (R^2) of 0.96. The developed CDFA was exhibited LOD and LOQ of 5.55 mg/L and 18.50 mg/L respectively. The low % bias and low % RSD of the developed CDFA have confirmed its reliability and accuracy towards the detection of FA. The CDFA exhibited % recovery of spiked FA solutions lies in the common range of % recovery i.e. 80 to 120 %. The presence of any trace of FA in fish fillets and whole fish of stored common carp was tested using the developed CDFA. The observations made through CDFA have been compared with the existing acetyleacetone (Nash's Reagent) method. The developed CDFA have showed comparable and reliable results to existing method. The performance of the CDFA was validated with the % deviation values with reference to acetyl acetone method. It was observed that % deviation ranges from – 6.24 to + 10.28 %, when the experiment conducted with the extract obtained from fish fillets. The % deviation was observed to be varied from – 11.72 to + 7.98 % for the extract obtained from whole fish. These findings supported the compatibility of CDFA in real sample analysis. However, the developed CDFA possesses limitation in multiple time use, as it is very difficult to brought CDFA to its original stage after use. Therefore, to enhance CDFA's reproducibility and multiple use, future study on CDFA is needed. Future research can be done on CDFA so that it can detect very low level of FA.

6.5 References

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