Development of multiresponsive carbon dots based aerogel and its application in food analysis

ABSTRACT

As an emerging material bio-polymer based aerogel are being used to develop functional materials (delivery vehicle for bioactive compounds, nutraceuticals, etc. and sensing material for the detection of adulterants, pesticides, spoilage, etc.). Bio-polymer based aerogel exhibit tremendous potential in food packaging based applications as a moisture absorber, bioactive compound releaser, carrying material, preserver etc. It also have the potential to serve as target based delivery vehicle, improves bioavailability of loaded materials, protects them from adverse environment and essential oil incorporated oleogel etc. Nevertheless, bio-polymer based aerogel are also emerging in the field of sensing pollutants, adulterants, pesticides, spoilage, etc. Now a days nanomaterials have gained interest from the researchers due to their exciting properties like high bioavailability, surface reactivity and specific surface area. Among all, carbon dots (CDs) are very unique nanomaterials due to their unique characteristics like nano size range (<10 nm), surface activity, photo luminescent behaviour, non-toxicity, etc. Since, last decades CDs grab attention in the field of functional aerogel preparation. Functional CDs based aerogel is an emerging material which combines the characteristics (high porosity, high specific surface area, and very low density) of bio-polymer based aerogel (polysaccharide, protein and mucilage based). The CDs based aerogel's luminescent behaviour at different wavelength, fluorescence quantum yield, and fluorescent quenching and re-enhancing capability have found huge application in the field of sensing. Moreover, the application of CDs based aerogel in the area food contaminants detection and sensing is limited. Currently, the high rise of formalin (FA) application in fish preservation become a fright to the consumers as it causes serious health issues like, cardiac arrest, damage of kidney, liver, etc. Therefore, detection of formalin in fish become crucial to extenuate the risk. The conventional techniques are very complex, time consuming, and costly. To cope with these challenges and find an alternative solution, this research was formulated with four objectives.

Firstly, corn starch-based aerogel was successfully developed through supercritical CO_2 ($SCCO_2$) drying method. The corn starch and water was mixed thoroughly to produce a homogeneous solution. The solution was gelatinized by heating (93 °C) to obtain a gel. The gel was kept for incubation at 4 °C for 2 days to produce hydrogel. Then, the hydrogel was converted to alcogel through ethanol substitution. The alcogel was dried through $SCCO_2$ drying

(pressure: 150 bar, temperature: 40 °C and time: 2.5 h) to develop aerogel. The impact of glycerol on the physico-functional, morphological, mechanical, and rehydration properties of corn starch-based aerogel has been investigated. The Glycerol-infused aerogel had a more connected, denser structure (381.08 – 451.34 kg/m^3), enhanced hygroscopic behavior, and was reusable up to eight times in terms of its capacity to absorb water after being drawn from the soaked sample. However, the inclusion of glycerol reduced the aerogel's porosity (75.89 - 69.91 %) and water absorption percentage for 30 min ($WAC_{30\ min}$; 118.53 - 84.64 %) but enhanced its percentage shrinkage (75.03-77.99 %) and compressive strength (26.01 - 295.06 N). The glycerol added aerogel were recompressible up to 10 times. Glycerol addition improved the internal strength of the aerogel so could be recycled without significant change in the physical characteristics of the aerogel. The glycerol-based aerogel has the potential to be employed as a carrier matrix for various chemicals and a moisture scavenger. The development of aerogel was further investigated using other drying techniques (freeze drying and microwave drying).

Secondly, corn starch-based aerogel was successfully developed using freeze drying and microwave drying method. The hydrogel was prepared from the homogenous mixture of corn starch and water through gelatinization (temperature: 93 °C) followed by an incubation at 4 °C for 2 days. The freeze dried aerogel was developed directly from hydrogel through sublimation in a freeze drier (condenser temperature: - 80 °C and time: 18 h). The microwave dried aerogel was developed through microwave drying (microwave power: 240 W and time: 28 min) of alcogel prepared through ethanol substitution in hydrogel. The effect of glycerol on both microwave and freeze-dried aerogel has also been investigated. Microwave dried aerogel possesses higher total shrinkage (76.09 - 87.00 %), density (330.70 – 764.74 kg/m^3), and lesser water absorption capacity (144.03 – 213.75 %), porosity (49.02 – 77.95 %) as compared to freeze dried aerogel. However, microwave dried aerogel showed higher stability to thermal degradation and mechanical compression than freeze dried aerogel. The microwave dried aerogel showed good recompressibility as well as good reusability as compared to freeze dried aerogel. Less time taking process (28 min), ease of availability and operation, and less power consumption, etc. are the most important advantages of microwave dried aerogel development over freeze dried aerogel. Microwave dried aerogel smaller pores (nano range) than the freeze dried aerogel. The 5 % glycerol added microwave dried aerogel exhibited more uniform pores than the control aerogel. The 5 % glycerol added aerogel was then further investigated for the loading of CDs to develop a functional CDs based aerogel.

Thirdly, CDs were synthesised through hydrothermal method using citric acid monohydrate and ammonium hydroxide as precursor solution. The solution was put in a Teflon lined autoclave and was kept at 200 °C for 5 h for CDs synthesis. Characterization of synthesised CDs was done in terms of yield (0.60 %), quantum yield (≈ 46 %), particle size (2.21 nm), FTIR, NMR, UV-absorbance, fluorescence intensity, etc. The 5 % glycerol added aerogel were developed through microwave drying approach. The aerogel was dipped in the CDs solution (0.5 – 4.0 wt %) for overnight to get loaded inside the aerogel matrix. Then the wet aerogel was dried through freeze drying (-50 °C, 3 h) to get CDs loaded aerogel. On the basis of fluorescence intensity and green/blue (G/B) value obtained through processing the images in Image J software, 1.0 wt % CDs loaded aerogel was selected for further use. The CDs based aerogel was then characterized for the presence of functional groups, thermal properties and crystalline behaviour. The developed CDs based aerogel have retained stable fluorescence characteristics as exhibited by CDs. The CDs based aerogel showed uniformity in CDs dispersion inside the aerogel matrix. The FTIR spectra confirmed that the CDs based aerogel have retained both the native qualities of corn starch and CDs. The aerogel was used as pH sensor. The aerogel was calibrated with different pH (3, 6, 9, and 12) tuned CDs solutions, then the G/B value was obtained. A calibration curve between G/B values and different pH was plotted and the linear equation obtained from the plot was used to find an unknown pH from its G/B value. The CDs based aerogel exhibited good results with a deviation of only 9% while determining pH through spiking approach (spiked pH: 3). The CDs based aerogel was further examined for its competency to detect FA in fish.

Finally, the CDs based functional aerogel (CDFA) was developed to detect formalin in fish. 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). Tollens reagent (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). The standard FA solutions having concentration of 0 - 150 mg/L was added to CDFA. A calibration curve was plotted between G/B value and standard FA concentration which exhibited a linear relationship 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 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. The CDFA exhibited % recovery of spiked FA solutions in the range of 100.39 to 102.02 %. The presence of any trace of FA in fish fillets

and whole fish of stored common carp was tested using the developed CDFA. The extract prepared from both fish fillets and whole fish was made in contact with CDFA for 30 min. 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 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.