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Synthesis of fluorescent carbon dots via simple acid hydrolysis of bovine serum albumin and its potential as sensitive sensing probe for lead (II) ions

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ABSTRACT

Carbon dots have great potential to be utilised as an optical sensing probe due to its unique photoluminescence and less toxic properties. This work reports a simple and novel synthesis method of carbon dots via direct acid hydrolysis of bovine serum albumin protein in a one-pot approach. Optimisation of the important synthetic parameters has been performed which consists of temperature effect, acid to protein ratio and kinetics of reaction. Higher temperature has promoted better yield with shorter reaction time. The carbon dots obtained shows a strong emission at the wavelength of 400 nm with an optimum excitation of 305 nm. The potential of the carbon dots as optical sensing probe has been investigated on with different cations that are of environmental and health concern. The fluorescence of the carbon dots was significantly quenched particularly by lead (II) ions in a selective manner. Further analytical study has been performed to leverage the performance of the carbon dots for lead (II) ions sensing using the standard Stern–Volmer relationship. The sensing probe has a dynamic linear range up to 6.0 mM with a Stern–Volmer constant of 605.99 M^{-1} and a limit of detection (LOD) of 5.05 μM. The probe performance was highly repeatable with a standard deviation below 3.0%. The probe suggested in this study demonstrates the potential of a more economical and greener approach that uses protein based carbon dots for sensing of heavy metal ions.

1. Introduction

The finding of alternative optical receptors has been an on-going process in the area of optical sensors development. The progress has started with the utilisation of naturally abundance dyes isolated from the environment, followed by synthesis of organic fluorophores in labs, and to the most recent one that uses of nano-sized particles known as the quantum dots (Q-Dots). Q-Dots portray some remarkable properties including high emission quantum yields, size-tunable emission, chemically and physically more stable, narrow spectral bands, and can be surface modified for a specific sensing application [1,2]. However, majority of the Q-Dots suffer from toxicity concern since these Q-Dots are colloidal semiconductors comprising elements from the periodic groups II–VI, III–V or IV–VI, which are harmful to health [3–5]. This has limited the scope of usage especially for those related to biomedical and health diagnostic applications. Leaching of the heavy metal ions such as the cadmium ions will be catastrophic towards the biological system and the environment. In the mist of overcoming this limitation, it has accidentally found that nanoparticles comprise of majority carbon element can also portray similar property analogue to the Q-Dots. This finding is definitely of advantage since the carbon nanoparticles is far less toxic and cheaper, which can be a good candidate to replace the use of Q-Dots. Carbon based starting precursors used for the synthesis as compared to the heavy metal ions is definitely a better option since it is greener to the environment and having lower health risk to living organisms.

Carbon nanoparticles were first discovered accidently by Xu and co-workers [6] during the electrophoretic purification of carbon nanotubes and since then, the research activity in this area has been increasingly active. It is not only less toxic, but also reported to have less fluorescent blinking effect, an easier method for synthesis compared to Q-Dots, and remarkably cheaper in handling and production costs [7–9]. Till date, these nanoparticles are more well-known as the carbon dots (C-Dots). The current research focuses of the C-Dots are on exploring of novel synthetic routes, more economical synthesis approaches, utilising greener chemistry, and diversifying possible starting precursors [7]. Basically, the synthesis of C-Dots falls into three main stages; carbonisation, passivation, and surface functionalisation [8]. Carbonisation refers to the process of converting starting organic precursors into its

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basic carbon element or carbon containing residues via pyrolysis, strong dehydration, or destructive distillation. For the synthesis of C-Dots, the starting precursors are often in the nano-size range. However for those in bulk, physical grinding is required after carbonisation to obtain the carbon in the nanometre size range. In terms of optical property, pure carbon nanoparticles are usually non-fluorescent and the chemical treatment of the surface also known as passivation operation is usually required. This step introduces an insulating shell on the surface that minimises the impact of surface-defect, trap sites, and direct quenching from the surrounding, which all will enhance the fluorescence emission. Finally, the functionalisation step is performed to confer a specific chemical or biological reactivity on the surface of the C-Dots. This is important to introduce specific sites that can be used for bio-imaging or sensing purposes. Although major synthesis steps are similar, C-Dots obtained often show variation in terms of optical and physical properties when compared among batches produced via different starting precursors, carbonisation conversion methods, and/or the pre-treatments performed. For instance, Chin and co-workers [10] have synthesised C-Dots via sago starch that emitted fluorescence at 435 nm when excited at 360 nm, while the C-Dots prepared by Qu and co-workers [11] using urea show shifting emissions peaking from 440–570 nm when excited at 340–500 nm. This demonstrates that each batch of the C-Dots has specific and unique identity, which can be potentially leveraged into useful applications.

This study reported hereby will demonstrate a novel approach in converting bovine serum albumin (BSA) protein, a potential biocompatible starting precursor into C-Dots. Simple and economical wet chemistry with the one-pot approach has been adopted. BSA was selected due to its biocompatibility nature, which later will stand a better chance in applications that require low toxicity tolerance. This avoids the possibility of having contaminants that can bring harmful effect to the biological system. The carbonisation process has been accomplished using strong acid, acted as dehydrating and burning agents in converting BSA into the carbon residue. For this instance, no further passivation step was required since the strong acid has sufficient capability to oxidise the carbon surface, which is evident from the observed strong fluorescence emission. Various parameters related to the synthesis have been investigated and optimised to obtain the most economical system with the best optical property of the C-Dots. C-Dots obtained were then tested with a series of different metal cations. This was with intention to discover a potential application for the C-Dots synthesis from this study in sensing field. Till date, there are very few reports on metal ions sensing utilising C-Dots that appear in the literature compared to Q-Dots. The potential in this area is still huge to be explored.

2. Experimental

2.1. Reagents

All chemicals used in this study were of analytical grade unless otherwise stated. Double-distilled deionised water (prepared from Elga MC:DS Micromeg ioniser) was used as solvents throughout the study. Bovine serum albumin of biotechnology grade (Amresco) in powder form was dissolved into stock solution and freezed during storage. Concentrated sulphuric acid (H_2SO_4), sodium hydroxide (NaOH), silver nitrate ($AgNO_3$), copper nitrate ($Cu(NO_3)_2$), cobalt nitrate ($Co(NO_3)_2$), mercury chloride ($HgCl_2$), nickel nitrate ($Ni(NO_3)_2$), calcium nitrate ($Ca(NO_3)_2$), magnesium nitrate ($Mg(NO_3)_2$), tin chloride ($SnCl_2$), and lead nitrate ($Pb(NO_3)_2$), were purchased from R&M marketing, Malaysia and used as received without further purification.

2.2. Instrumentation

All fluorescence measurements were carried out using a fluorescence spectrophotometer (CARY Eclipse, Varian) with automatic emission intensity normalisation mode. Diluted solution of C-Dots in aqueous medium was placed in a quartz cuvette with four polished windows and a path length of 1.0 cm. Emission spectrum of the C-Dots was recorded in the range of 300–600 nm with fixed emission energy at 305 nm. Both the emission and excitation slits were set at 10 nm. The physical morphology of CDs was observed by using a transmission electron microscope (TEM) operated at 80 kV (JEOL-2000). A pH metre (Mettler Toledo SevenEasy) was used for accurate adjustment of pH and water bath (Memmert WNB 14) was used to control the temperature during the study.

2.3. Synthesis of C-Dots

The C-Dots were prepared using the direct one-pot carbonisation approach via concentrated strong acid in aqueous medium. The approach adopted in this study was modified from the previous method by Chin et al. (2012) [10] that produced the C-Dots using starch nanoparticles. In general, accurately 1.0 ml of BSA in stock solution of 105 g l^{-1} was pipette into a Schott bottle followed with the addition of 3.0 ml of concentrated sulphuric acid drop wise into the same bottle under constant stirring condition. The homogenous mixture was then transferred into water bath at 50 °C for at least 2 h. A yellowish-brown solution was obtained at the end of 2 h. Sodium hydroxide solution (2 M) was used to neutralise the solution and dialysis was performed for the removal of salts and other possible low molecular weight contaminants. The sample was kept in opaque bottle and stored in dark cool place for further study.

2.4. Optimisation of synthetic and analytical variables

Important parameters during the synthesis affecting the final photo-luminescence of the C-Dots were investigated. The optimum concentration for the starting precursor (BSA) was determined by adding a fixed amount of the concentrated acid into different concentrations of BSA and the fluorescence of the products was monitored respectively at 400 nm. The concentration of acid used to carbonise the BSA was varied from 3.0 to 18.0 M in this study. The kinetic of the conversion of the BSA into C-Dots was also monitored at two different temperatures in water bath (at 25 and 50 °C). This was performed by recording the emissions of the mixture at predetermined intervals over a period of time after the BSA was mixed with the acid.

The C-Dots were suspended in aqueous solution for all subsequent analytical studies. The dilution factor for the C-Dots into aqueous solution (vol/vol%) was determined to give the highest emission intensity. This ensures a wider analytical window for the quenching of fluorescence to occur during the detection of heavy metal cations. Besides, the fluorescence emission of the C-Dots in different pHs was also studied by adding the same amount of C-Dots into different pH solutions. To investigate its sensing potential for some predetermined metal cations, the standard aqueous stock solutions of cations were prepared by dissolving the respective metal salts in deionised water to obtain the concentration of 0.1 M. To a prefixed volume of C-Dots from stock solution, an appropriate volume of the metal ions from the stock was added together. The mixture was diluted to a final volume of 3.0 ml with deionised water and mixed thoroughly. The fluorescence spectrum of the mixture was recorded with the excitation wavelength of 305 nm.

3. Results and discussion

3.1. Morphological characteristics

A drop of the C-Dots sample was dried on a sample holder that is to be viewed under the TEM and the image taken is as shown in Fig. 1. The image clearly indicates that the C-Dots aggregated into small carbon grains sized of hundreds of nanometres. This suggested the presence of surface double layers on the C-Dots that could have been introduced due to surface oxidation during the hydrolysis of BSA by strong acid into carbon based materials. Often, it is reported that oxygen rich functional groups such as the carboxylic acid are the predominant groups grafted on the surface [12]. Thus, this surface functional layer can induce intermolecular forces that cause the C-Dot to aggregate together especially during the drying process where solvent was evaporated eventually to total dry state at the sample holder of the TEM. Aggregation often causes the loss of fluorescence and thus proper dispersion should be made with fresh solvent to minimise the aggregation problem in later intended application. The size of the C-Dots was homogenous throughout with an average estimated size of 1–2 nm, which is very consistent with the C-Dots reported by other groups [13].

3.2. Fluorescence property

In this study, it was aware that the starting precursor of BSA by itself is a fluorescent compound due to the two tryptophan (Trp) residues located in the protein surface; in domain 1 (Trp-134) and in the hydrophobic pocket in domain 2 (Trp-213) [14]. The emission peak of the BSA in this study was recorded to be around 345 nm under the excitation of 305 nm as shown in Fig. 2(a). As the concentrated sulphuric acid was slowly added into the BSA, the intensity of the initial peak at 345 nm has been observed to be weakened while a new and far stronger emission peak compared to the initial one was recorded at around 400 nm (Fig. 2(b)). At the same excitation energy and experimental condition, pure sulphuric acid used for this study did not show any significant fluorescence within the same emission range (Fig. 2(c)). Thus, the strong peak at 400 nm was associated to be originated from the C-Dots. This is matching with the literature that C-Dots are well-known of fluorescing in this emission range [13,15]. This fluorescence can be observed clearly via naked eye as bright emission under the shortwave ultraviolet light (inset of Fig. 2). There have been several mechanisms proposed on the origin of the emission for carbon nanoparticles such as surface defects [16] and band gap-like transition [17]. In this study, the excitation maximum at 305 nm was very close to the shoulder absorption peak of the C-Dots, which reasonably suggested that the fluorescence emission recorded should be ascribed due to the band gap transition. The full width at half maximum (FWHM) of the fluorescence band that has been evaluated to be around 70 nm, which was far narrower than those reported elsewhere (>90 nm) [12,15]. This implies the method proposed in this study has produced C-Dots of narrow distribution in size, which also matches with the observation obtained from the previous TEM image (Fig. 1). Smaller distribution of size for sure will be of greater advantage in later applications such as for sensing to obtain consistent results.

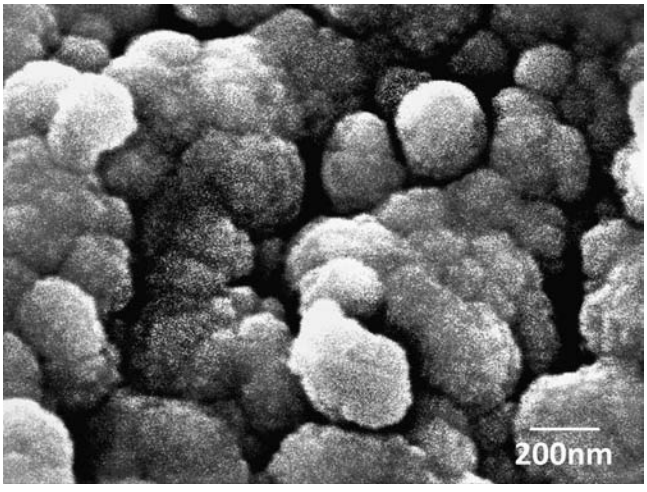

Fig. 1. TEM image of the C-Dots produced by the carbonisation of BSA using strong sulphuric acid at 50 °C for 2 h.

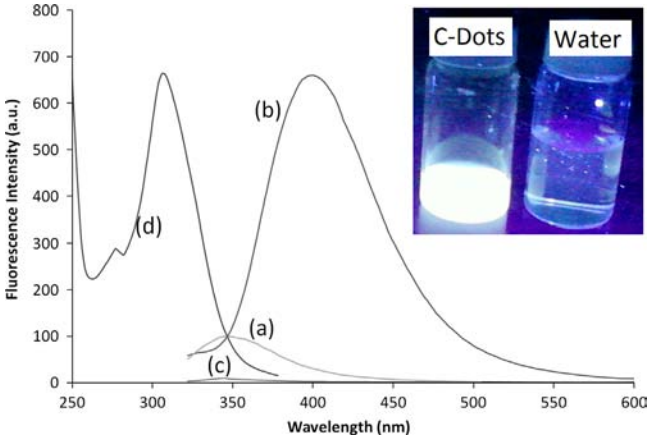

Fig. 2. Fluorescence spectra recorded where (a) is the emission of BSA, (b) is the emission of the C-dots, (c) is the emission of sulphuric acid, and (d) is the emission intensity at 400 nm correspond to different excitation wavelengths. Inset is the photograph of the C-Dots in aqueous solution and water (as comparison) taken under the irradiation of UV lamp.

3.3. Effects of starting precursors

Generally the C-Dots were produced from the BSA and the optimum amount required has to be investigated to minimise the wastage and to ensure complete carbonisation. This was performed by varying the weight of BSA used for the carbonisation process with a fixed amount of concentrated acid. The emission was recorded at 400 nm for all final isolated C-Dots. This study has assumed that the amount of C-Dots present in the sample was directly proportional to the intensity recorded, with higher intensity representing more concentrated C-Dots. The result obtained is shown in Fig. 3(a) and it was observed that the production of C-Dots was proportional with BSA added at the lower concentration range, while started to get saturated at around 105 mg/ml of BSA. The saturation maybe due to the limiting agent of sulphuric acid, where further carbonisation of BSA was not possible. Thus, the amount of BSA was fixed at 105 mg/ml for all further studies.

The effect on concentration of the sulphuric acid used was also studied with the motivation to look into the possibility of using less concentrated concentration during the synthesis. This will be good in reducing the cost of the synthesis besides modifying the chemistry involved to be greener. However, the result showed that the effectiveness of the carbonisation has decreased exponentially once the concentration of the acid was reduced (Fig. 3(b)). This can be rationalised by looking at the dehydration power of sulphuric acid on the BSA that get reduced due to the increasing amount of water added for the dilution of acid. However, it is positive that there will be still possibilities of using less concentrated acid such as adopting non-aqueous solvents of high polarity to dilute the

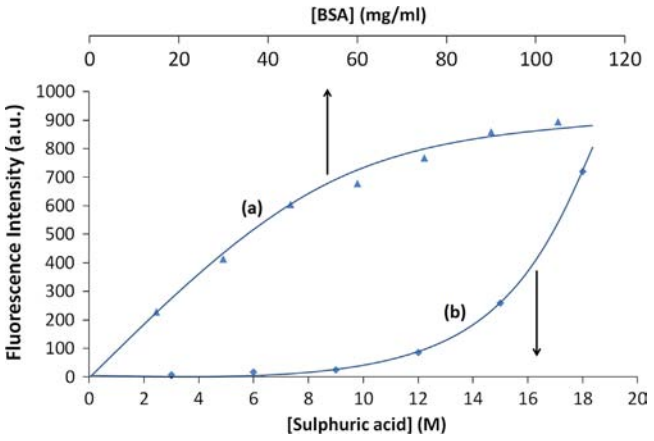

Fig. 3. Effect of the starting precursors on the emission of C-Dots synthesised with the variation in (a) concentration of BSA and (b) concentration of sulphuric acid during the carbonisation process in producing C-Dots.

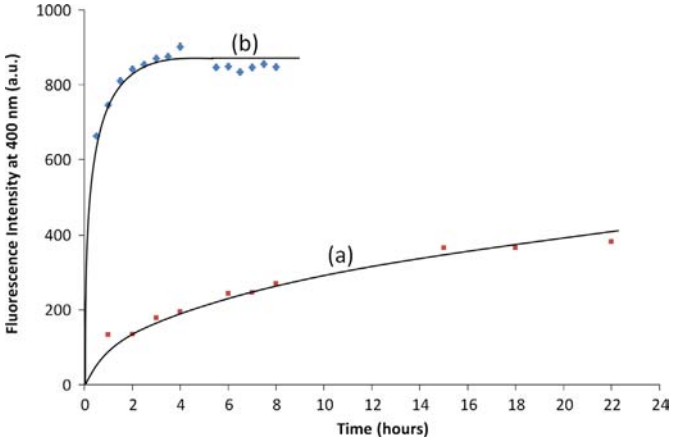

Fig. 4. Formation of C-Dots over time monitored at 400 nm during the synthesis stage at (a) room temperature and (b) 50 °C in water bath.

acid. This requires further investigation that is out of this study scope.

3.4. Effect of reaction kinetics and temperature

During the preparation of the C-Dots, it is important to ensure that conversion process of BSA into C-Dots has reached its completion under the experimental condition before isolating the final C-Dots product. This is to avoid inconsistency in the physiochemical and analytical properties of the C-Dots samples prepared with similar condition but of different batches. To explore further into this aspect, the conversion process has been monitored over a period of time at prefixed intervals at room temperature. Assumption was made that the emission recorded at 400 nm after the mixture of BSA and acid has to be due to the formation of C-Dots and its intensity will be correlating to the concentration of the C-Dots formed. This means that more of the C-Dots produced will be projected on with higher emission intensity. The result shows that initial conversion rate at the first hour was quite high, but then gradually proceeded at a steady slower rate continuously within the time frame of the study (Fig. 4(a)). It was observed that the intensity has continuously risen even after 30 h at a similar rate (result not shown). This concludes that the average time for the complete formation of C-Dots using BSA was in the manner of days. Thus to reduce the synthesis time, the factor of temperature has been investigated. An experiment has been performed at an elevated temperature of 50 °C and the kinetic trend obtained is as shown in Fig. 4(b). Rising in temperature has definitely improved significantly the initial carbonisation conversion rate compared to the one at room temperature. No significant raise in the intensity was observed after 2 h of reaction, which can be deduced that all convertibles BSA used have been converted into C-Dots. Higher temperature can promote greater motion of the precursors that directly can improve the efficiency of the carbonisation process. Since the ingredients and amount of the starting precursors used for both temperatures were the same, theoretically the amount of C-Dots produced for both conditions after the end of the reaction should be similar. So by extrapolating the trend of graph obtained at room temperature, the carbonisation was expected to reach completion after approximately 63 h (2.6 days), with the assumption of a consistent trend of the kinetic throughout. As a result, all the C-Dots produced for this study was performed in water bath at 50 °C for at least 2 h before being isolated for other studies. Although the temperature was set higher, it was still comparatively far greener and more energy efficient compared to those approaches that utilise thermal carbonisation that require the temperature to be raised up to 200–300 °C. These high temperatures usually will decompose any organic functional groups on the surface layer of C-Dots that causes the decrease in the fluorescence quantum yield. Thus further passivation process is required to reactivate back the surface in order to obtain a good fluorescence property for those C-Dots.

3.5. Sensing potential

Once the C-Dots have been isolated and characterised, its sensing potential for metal ions detection for those commonly encountered in the environment was investigated. The evaluation was made by monitoring on the initial fluorescence intensity of the C-Dots for possible significant changes once added with foreign species, which in this case metal ions. In this study, a series of 8 different metal ions of the same concentration has been selected for the test. Of the 8 tested, only lead (II) ions caused a significant drop ($>50\%$) in the intensity of C-Dots, while the others maintain up to more than 90% of the initial intensity (Fig. 5). This reflects that the C-Dots have high potential to be used for further development of lead (II) ion sensing receptor. The specificity of this sensing probe towards lead (II) ions was evaluated to be high since obviously the interference from other metal ions was low. This includes no significant effect caused by calcium (II) ions, where it is often found to be problematic due to its high abundance in the nature and biological systems. Since this was the initial finding, a detailed study has been performed to

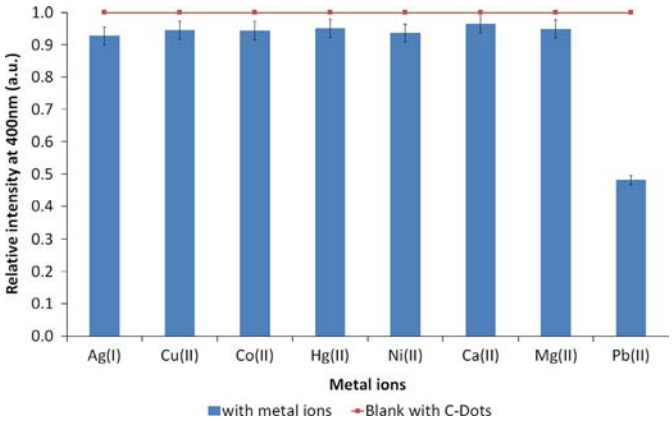

Fig. 5. The effect of different metal ions at the concentration of 3.5 mM respectively on the emission intensity of the C-Dots at 400 nm.

investigate further on the potential of the C-Dots for the specific detection of lead (II) ions in aqueous solution.

3.5.1. Effect of C-Dots concentration

Fluorescence of the C-Dots was found to be dependent with its concentration. Thus to adopt the C-Dots for sensing, the baseline fluorescence intensity needs to be set to ensure that maximum analytical window is available for the later analyte detection. In general, the fluorescence intensity recorded from this study showed an increment with increasing amount of the C-Dots at low concentration range, but gradually showed a decrease once exceeded the volume ratio limit of 1:14 vol/vol (C-Dots stock solution:deionised water). The loss of fluorescence intensity at high concentration is quite common as self-quenching can occur due to higher frequency of molecular collision. In this study, the C-Dot was intended for lead (II) ions detection based on the quenching effect. Therefore, the initial intensity of the system was optimised to the highest instrumentation scale in order to achieve a wider detection dynamic for lead (II) ions. Since the highest intensity before self-quenching occurs was determined to be 1:14 vol/vol (C-Dots stock solution:deionised water), this dilution factor was employed for the rest of the studies in the analytical potential characterisations.

3.5.2. Effect of pH

Since the C-Dots were dispersed in aqueous solution, the effect of pH towards the fluorescence intensity was investigated. In general, pH is one of the major variables reported to be altering the intensity of fluorescent species such as those non-organic nanoparticles or organic dyes. In this study, the intensity of the C-Dots showed a decrease after neutralised with sodium hydroxide solution. The decrease of intensity can be caused by the presence of the ionic species within the solution, especially due the formation of salt during neutralisation process. This was evidence with the observation of partial recovery of the emission from sample after dialysis being performed, although this study failed to recover back the intensity to its initial level. Using the neutralised sample, further alteration of pH ranging from 2 to 12 using acid and base showed no significant huge change in the intensity, rather a slight increment with the raise of the pH value (Fig. 6(a)). This indicates that the baseline intensity of the C-Dots prepared by this method was stable towards the pH factor. However, it is still of concern whether the pH will alter the detection performance of the C-Dots towards the lead (II) ions. Thus, an additional test has been performed by carrying out quenching study of the C-Dots with lead (II) ions of the same concentration under different pH conditions. The result shows that the degree of quenching dropped slowly as the pH increases from 2 to 10 and recorded a high degree of quenching in the extreme basic region of pH > 12 (Fig. 6(b)). This trend correlates with the solubility of lead (II) ions under hydroxide rich solution. As the pH is recorded to be higher, more hydroxide ions will be available to form complex with the lead (II) ions causing less interaction with the C-Dots. Thus it reduces the degree of quenching accordingly that can be observed by the intensity drop. However at higher pH, the solubility of the lead hydroxide increases due to the formation of charged complex and can promote quenching of C-Dots. Although the sensitivity can be improved at the high pH region, it is not practical to adopt such basic condition in real application. Thus, the pH has been controlled at the neutral range in between 6 and 7 for the subsequent studies.

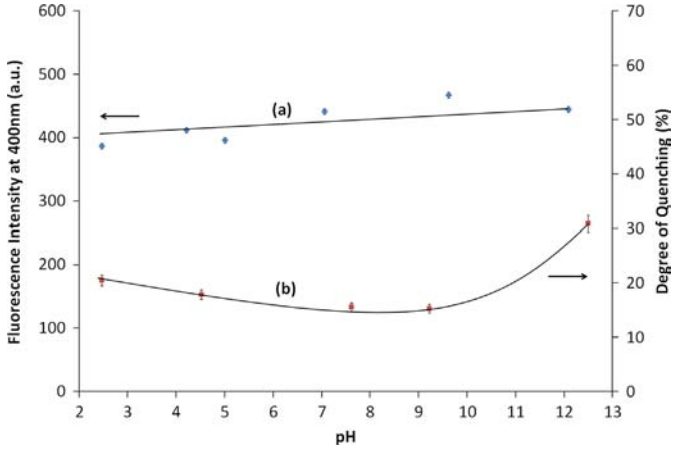

Fig.6. (a) The effect of pH on the fluorescence intensity of C-Dots in aqueous and (b) the degree of quenching of C-Dots in the presence of lead (II) ions at different pH values.

3.5.3. Sensing characteristic

In this study, it was found that the intensity of the C-Dots was quenched by the lead (II) ions in a concentration-dependent manner (Fig. 7). Considering this significant and consistent data quenching pattern, the possibility of developing these C-Dots into a sensing probe for lead (II) ions was evaluated. In this case, the standard Stern–Volmer relationship (Eq. (1)) that best describes the quenching profile of a fluorescence species was adopted.

$$F_o/F = Ksv[C] + 1 \quad (1)$$

where F_o and F are the fluorescence intensities recorded in absence and presence of Pb (II) ions, respectively, [C] is the concentration of Pb (II) ions and Ksv is the Stern-Volmer quenching constant. The raw data (Fig. 7) from the calibration study particularly at 400 nm was fed into Eq. (1) and it generated a linear relationship within the analytical window up to 6.0 mM of Pb (II) ions. The linear relationship obtained was $y = 606x + 1.0145$ with a R^2 value of 0.9976. Under this optimum condition, the limit of detection (LOD) for Pb (II) ion was evaluated to be 5.05 μM using Eq. (2).

$$LOD = 3\sigma/s \quad (2)$$

where σ is the standard deviation of the blank C-Dots sample recorded ($n = 6$) and s is the slope of the calibration plot. The probe performance was highly repeatable with a standard deviation below 3.0% under the controlled study condition. Besides the photostability of the C-Dots tested under continuous exposure of UV for 30 min showed no significant drop in the intensity, and it was observed that the C-Dots maintained its performance after storage in dark condition for at least 7 days. This is of advantage

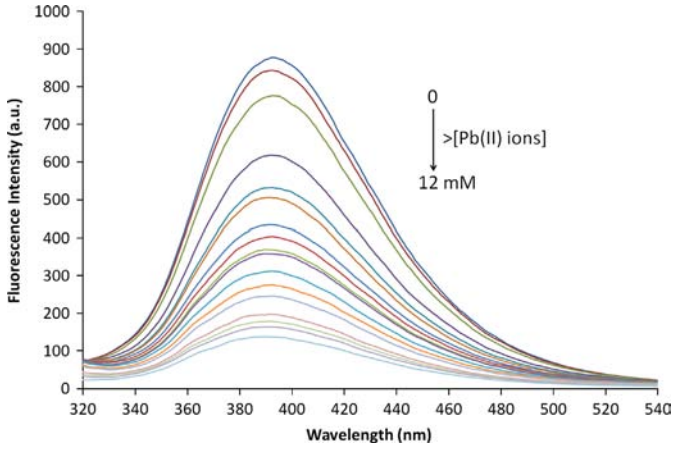

Fig. 7. The quenching effect on the C-Dots in the presence of different concentrations of Pb (II) ions.

when the C-Dots is utilised as commercial sensing probe as it has long shelf-life.

The origin and quenching mechanism for inorganic fluorescent nanoparticles is yet to be fully understood, but there have been several suggestions from the literature such as inner filter effect, electron transfer process, Forster energy transfer, hole-trap, etc. [1,18–20]. The information of such mechanisms is more for those established quantum dots, while not very detailed yet for C-Dots. However, both are assumed to be of high similarity since the physiochemical properties are derived from the size effect that is due to the energy confinement below the size of the Bohr radius. In this study, the C-Dots were observed with no significant shift in the emission peak which centred at around 400 nm (excited at 345 nm), which implies that the most possible mechanism is attributed to electron transfer. The Pb (II) ions effectively quench the fluorescence via facilitating the recombinant of excited electrons in the conduction band to the hole in the valence band. This effect is seen predominant since lead is considered a heavy element that encounters the effect of relativistic contraction. Such effect will promote the tendency of drawing electron towards the nucleus of the elements. Once this occurs near the effective surface of the C-Dots, the initial fluorescent process will be disturbed leaving the C-Dots with low or no fluorescence.

4. Conclusion

This work has demonstrated the success in converting BSA into C-Dots via a simple and effective carbonisation method using strong acid. The C-Dots were found to be highly fluorescence with good stability under different pH conditions. The fluorescence of the C-Dots has been found to be effectively quenched by particularly Pb (II) ions. With this finding, the C-Dots were further characterised analytically as probe for Pb (II) detection in aqueous solution. A dynamic detection range up to 6 mM has been obtained with an evaluated limit of detection of 5.05 μM. This study serves as a novel demonstration of an ease and economical method to produce C-Dots that can lead to other potential for optical sensing applications. This is especially with further modifications such as surface capping of the C-Dots or doping the C-Dots with various dopants are performed that might improve the sensitivity of the detection.

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