5 Determination of histamine in fresh and canned tuna fish by photoluminescence sensing using TGA-CdTe nanoparticles and cationic solid phase extraction

5.1 The photoluminescence quenching of the TGA-CdTe nanoparticles by histamine

The photoluminescence intensity of quantum dots is sensitive to surface changes caused by interactions with chemical species in the dispersed system. In the present work, it is demonstrated that the photoluminescence of TGA-modified CdTe nanoparticles is effectively quenched by histamine. The change in the photoluminescence intensity followed a Stern-Volmer model expressed as the ratio of F_0/F, where F_0 is the photoluminescence of the nanoparticle dispersion in the absence of histamine and F is the photoluminescence measured from the nanoparticle system in the presence of histamine. The quenching was strongly dependent on the pH of solution, amount of buffer where quantum dots are dispersed, concentration and average size of TGA-modified CdTe nanoparticles. These experimental conditions were optimized aiming to achieve robust conditions for histamine determination in tuna fish.

5.2 Adjustment of the composition of the TGA-CdTe nanoparticle dispersion

5.2.1 Concentration of quantum dots in the dispersion

The quenching effect caused by the presence of histamine (fixed at 1.7 x 10^{-4} mol L^{-1} final concentration) was investigated in dispersions containing different amounts of TGA-CdTe nanoparticles. The amount of nanoparticles was varied by introducing different volumes of the synthesized nanoparticle stock

dispersion (from 4 to 20 µL), which enabled a range between 3.8 x 10^{-8} and 1.9 x 10^{-7} mol of quantum dots (2.1 nm of average diameter) dispersed in 1 L of aqueous solution. A robust and sensitive photoluminescence quenching (measured at 515/350 nm) was observed, as indicated in Figure 44, in the system containing lower amounts of quantum dots (3.7 to 7.5 mol in 1 L of aqueous solution) as indicated by the higher F_0/F ratio (about 1.30). Higher amounts of nanoparticles significantly decreased the sensitivity of the quenching response due to the high photoluminescence measured from the dispersion. The chosen volume of the quantum dots stock dispersion was 8 µ L (7.5 x 10^{-8} mol of nanoparticles in 1 L of aqueous solution).

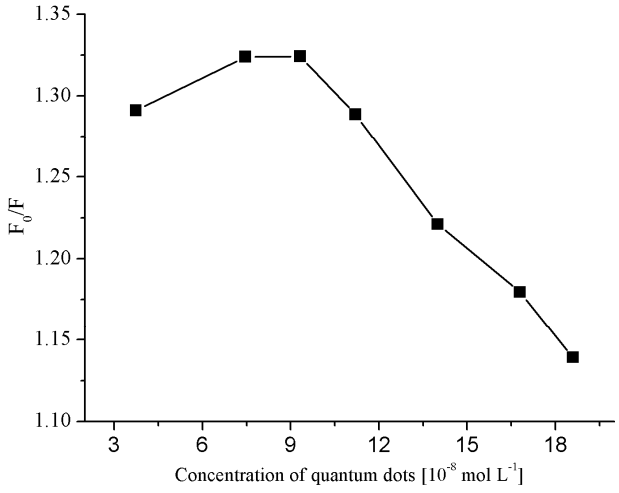

Figure 44- Effect of the amount of the synthesized quantum dots on the photoluminescence intensity measured from the TGA-CdTe quantum dots aqueous dispersion. Signal variation expressed as F_0/ F (where F_0 and F are respectively the photoluminescence of the quantum dots dispersion before and after the addition of 1.7 x10^{-4} mol L^{-1} of histamine).

5.2.2 pH value and amount of Buffer

The pH of the aqueous solution used to disperse the quantum dots was varied from 6.0 to 10.0. Phosphate buffer 0.01 mol L^{-1} (final concentration) was used to set the pH in range from 6.0 to 9.0. For the pH values of 9.5 and 10, the adjustment was made by the addition of a 0.01 mol L^{-1} NaOH solution into the

aqueous dispersion. The experiment was made in the dispersions with and without the presence of histamine (2 x 10^{-4} mol L^{-1}). The signal profile shown in Figure 45 indicated low photoluminescence at the acid range because of the removal of the stabilizing TGA capping from the surface of the quantum dots (dissociation of Cd^{2+}-TGA) as the binding thiolates are protonated [145]. Moreover, in the basic range, the photoluminescence intensity is enhanced resulting in a fairly robust response from pH values between 8.0 and 9.0. In this basic pH range, the carboxylic group of TGA is deprotonated resulting in a homogenous density of negative charges that enabled a better dispersion of the quantum dots. At more basic media, the photoluminescence intensity sharply decreases. As a robust photoluminescence quenching is observed in pH range from 8.0 to 9.5 in the presence 2 x 10^{-4} mol L^{-1} of histamine, the selected pH value for all further experiments was 8.0.

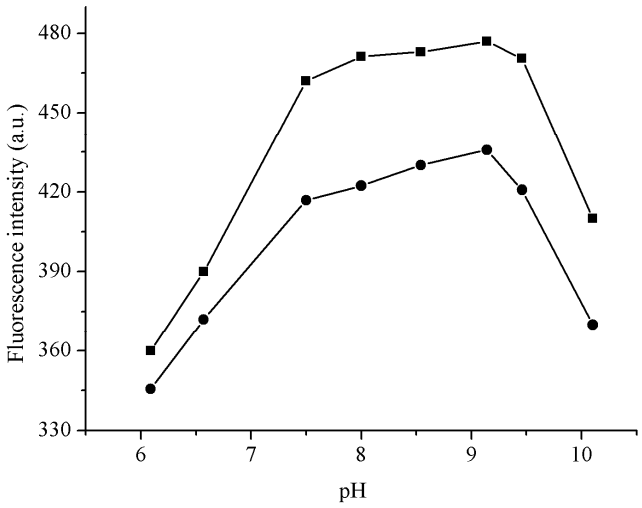

Figure 45- Effect of pH value on the photoluminescence measured from the TGA-CdTe quantum dots dispersion: (■) The Quantum dispersion without addition of histamine. (●) in the presence of 2 x 10^{-4} mol L^{-1} of histamine.

5.2.3 Stability of photoluminescence intensity and reaction time

Under the selected pH condition, the stability of the analytical response was studied by monitoring the photoluminescence measured from the

nanoparticles dispersion without of histamine (control). The photoluminescence was monitored every 10 min up to 120 min and during this interval, it was observed that the measured photoluminescence was quite stable (random fluctuation with standard deviation of the intensity measurements no higher than 5%). Then, experiments with the addition of histamine were made. In this case, the first measurement was made after 1 min stirring and 1 min system equilibration time. After that, other measurements were made every 5 min up to 60 min total monitoring time. It was observed that right after addition of the histamine; the photoluminescence decreases immediately and remains constant for at least 60 min (random fluctuation standard deviation of the intensity measurements no higher than 5%). This result also indicated that the final 0.01 mol L^{-1} concentration of the phosphate buffer (pH 8.0) was enough to grant stability of the system. For the analytical method, it was established all measurements to be made after 2 min (to perform stirring and equilibration of solution) of the addition of the sample into the cuvette.

5.2.4 Effect of the size and surface modifier on the quenching of TGA-CdTe quantum dots

The quenching effect of histamine was evaluated using dispersions of nanoparticles with three different sizes. This study indicated a size dependency of the effect caused by the analyte with a more effective quenching achieved in dispersions containing nanoparticles of the smaller size (2.1 nm of diameter) as indicated by the Stern-Volmer plots (Figure 46) made at 25 ^{o}C. Stern-Volmer constants (K_{sv}) for systems containing nanoparticles with average diameter of 2.1, 2.6 and 3.2 nm were respectively 1.4 x 10^3, 7.9 x 10^2 and 4.5 x 10^2 L mol^{-1} for different linear concentration ranges of histamine (Table 9).

Table 9- Variation in quenching of TGA- CdTe quantum dots in function of the particle size in the linear response range between 3.3 and 55 x 10^{-5} mol L^{-1}.

TGA-CdTe Size (nm)	Stern-Vomer Constant ($L\ mol^{-1}$)
2.1	1.4×10^3
2.6	7.9×10^2
3.2	4.5×10^2

At the pH 8, the carboxylic group on the surface of TGA-CdTe would be fully ionized and appear with negative charge (carboxyl group in TGA has pK_a 3.53.) and the protonated amino group could scavenge electrons from the exciton (electron-hole pair) as result of excitation of the TGA-CdTe nanoparticles. As the size of quantum dots decreases, the energy of conduction band increases due to the quantum confinement effect. Redox potentials of the conduction band become more negative thereby enhancing the reducing power with a decrease in particle size [157]. Due to higher surface to volume ratio in smaller nanoparticles, most of the constituent atoms reside on the surface of the particles being more efficiently transfered to the chemical species adsorbed on the surface of the nanoparticle, reducing the chances of radiative recombination of electron-hole pair [158]. Therefore, a more efficient quenching with the decreasing in particle size can be accounted as consequence of the enhanced reducing power and the higher ratio of surface-to-core atoms.

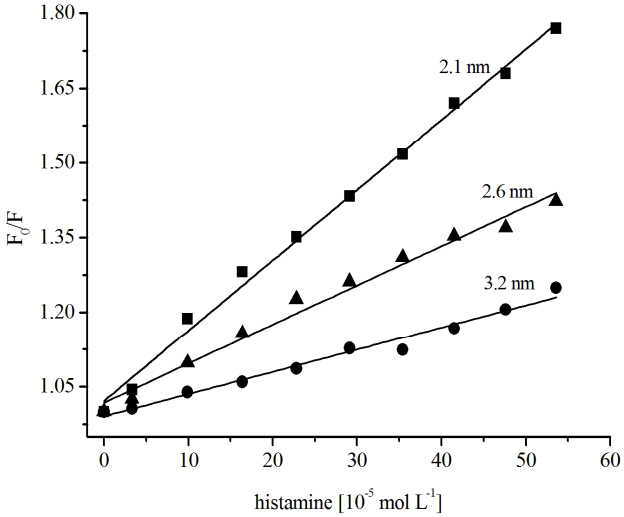

Figure 46 – Stern-Volmer plots for the binding of histamine in aqueous dispersion of TGA-CdTe quantum dots with sizes of 2.1nm (■) 2.6 nm (▲) and 3.2 nm (●). Excitation at 350 nm and emission measured at 515, 545 and 580 nm.

In order to further investigate the role of the surface modifier (TGA) in the photoluminescence quenching of quantum dots by histamine, another ligand modified CdTe quantum dots was used to establish grounds for comparison. The chosen ligand was cysteamine and, cysteamine-CdTe quantum dots were synthesized. Cysteamine contain primary amine as terminal stabilizing group when bound to the quantum dots.

In Figure 47, the photoluminescence profiles from dispersions of cysteamine-CdTe quantum dots are compared to the ones obtained from TGA-CdTe quantum dots. It is observed that the presence of histamine (at a 1.6 x 10^{-4} mol L^{-1}) induce a much less effective photoluminescence quenching in the cysteamine-CdTe quantum dots (F_0/F = 1.04) when compared to the effect achieved in the TGA-CdTe quantum dots dispersion (F_0/F = 1.32). These results showed that histamine induced quenching in negatively charged nanoparticles (TGA-CdTe) due to electrostatic interaction between surface of nanoparticles (negative charge due carboxylic group) and the protonated amino group of histamine. On the other hand, no quenching took palce in positively charged nanoparticles (cysteamine-CdTe). These results further confirm the role of surface modfier on photoluminescence quenching of TGA-CdTe

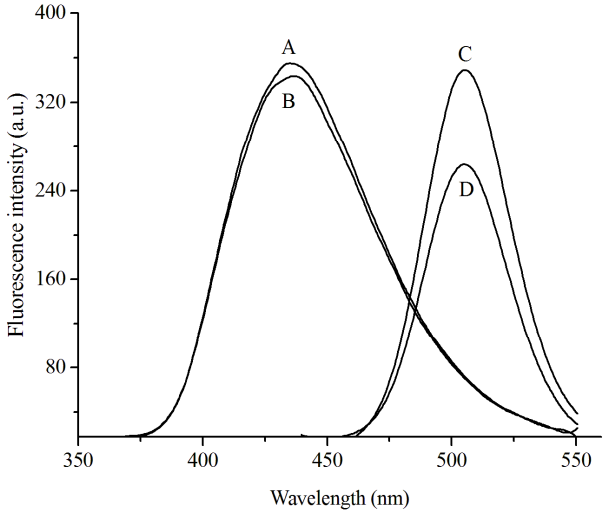

Figure 47 - Photoluminescence measured from quantum dots dispersions of: (A) Cysteamine-CdTe and (B) Cystamine-CdTe in the presence of 2.3 ×10^{-4} mol L^{-1}, (C) TGA-CdTe, (D) TGA-CdTe in the presence 2.3 ×10^{-4} mol L^{-1} of histamine.

5.3 Mechanism of interaction between histamine and TGA-CdTe quantum dots

Any physical or chemical interactions between a chemical species and the surface of the quantum dots may induce changes in their photoluminescence characteristics (quenching, amplification, shifts, etc.). Photoluminescence quenching may occur due to a number of reasons such as electron transfer, surface adsorption, surface complexations, nanoparticle aggregation and electrostatic attractions [48]. The absorption UV-vis spectra of CdTe were obtained from the dispersions in the absence and in the presence of histamine (in the range from 1 x 10^{-5} and 1 x 10^{-3} mol L^{-1}) but no significant differences were observed (Figure 48). Since no changes in the absorption profile of TGA-CdTe dispersion was found, it is an indication that histamine did not cause the aggregation of quantum dots, which can lead to reduced photoluminescence intensity.

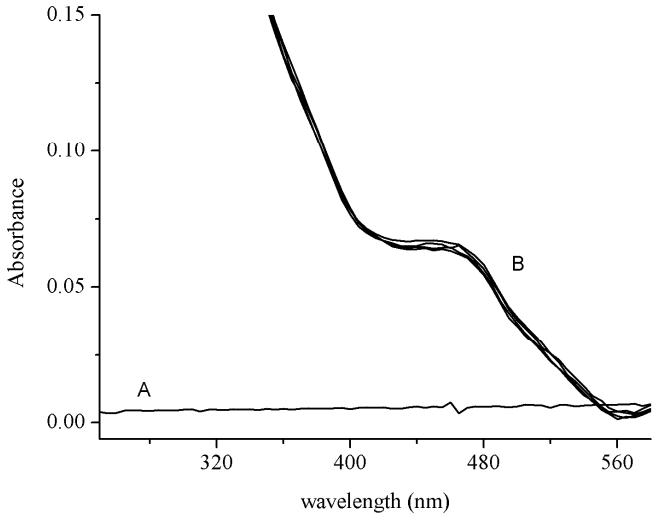

Figure 48 - Electronic absorption spectra: A. histamine (1x 10 $^{-4}$ mol L^{-1}) B. CdTe quantum dots in the presence of histamine present in the concentration range from 1 x 10^{-5} and 1 x 10^{-3} mol L^{-1}.

The mechanism upon the photoluminescence quenching occurs may be static or dynamic. The nature of the process can be distinguished by evaluating the dependence of the photoluminescence quenching in function of the temperature [53]. From the Stern-Volmer plots, constructed using TGA-CdTe quantum dots dispersions kept at three different temperatures (298, 303 and 308 K), it is observed that the sensitivity of the curve that relates F_0/F and concentration of the histamine decreases as the temperature was increased (Figure 49). Such behavior indicates that the interaction between histamine and TGA-CdTe is associative in nature (static quenching). The nature of the interaction was further investigated using time-resolved fluorescence. For the static quenching, the photoluminescence lifetime of the probe does not vary when a quencher is present. In contrast to what is expected in dynamic quenching, where changes in photoluminescence lifetime of the probe is affected by the presence of the quencher. The photoluminescence decay (Figure 50) recorded from TGA-CdTe quantum dots is similar when obtained from dispersions in the absence of histamine and in the presence of histamine (at two different concentrations: 6.6 x10^{-5} and 3.9 x10^{-4} mol L^{-1}) resulting in similar photoluminescence lifetimes. Therefore, the static nature of the quenching was confirmed.

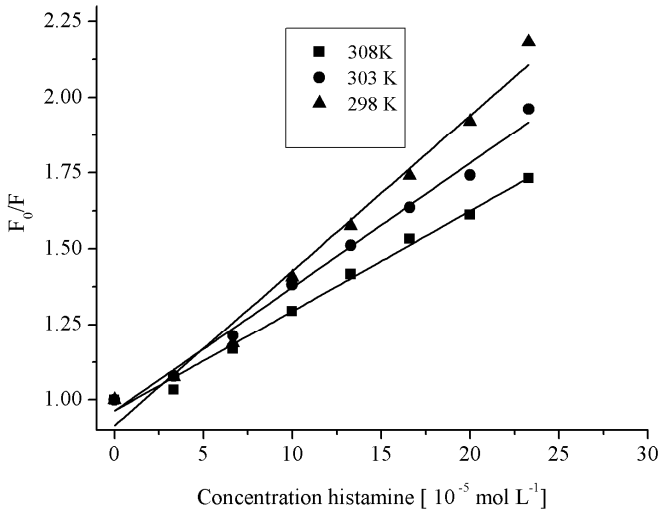

Figure 49- Stern–Volmer plots for the aqueous dispersions of TGA-CdTe quantum dots at 298 K (▲), 303 K (●) and 308 K (■)

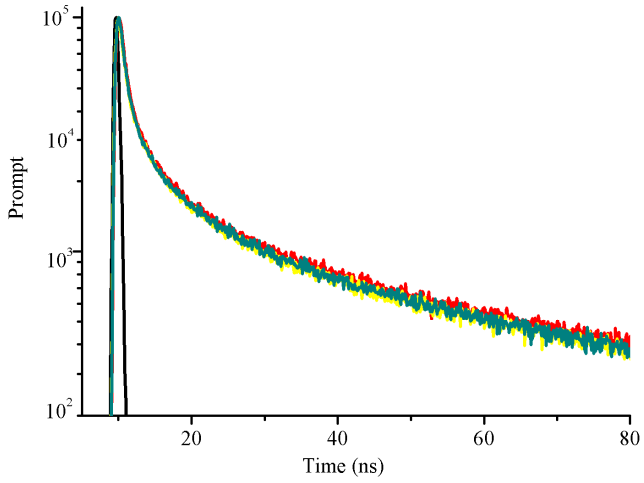

Figure 50- A typical photoluminescence decay profile of CdTe quantum dots (2.1 nm average size) in the absence and in the presence of histamine at 6.6 x10-5 and 3.9 x10^{-4} mol L-1 concentration levels.

Further experiments with the addition of Ni^{2+} were made in order to evaluate the behavior of the histamine/TGA-CdTe quantum dots system. The

addition of nickel into a TGA-CdTe dispersion whose photoluminescence was previously quenched by the presence of histamine induced the restoration of the photoluminescence (directly proportional to the amount of Ni^{2+} added) as indicated in Figure 51. Such signal restoration is due to the modulating effect of nickel on the interactions between quantum dots and histamine. The formation of a complex between the nickel ion and histamine is very favourable, which can be attributed to the fact that histamine contains both an imidazole group and an amino group that are in close proximity to each other, resulting in the formation of a stable Ni^{2+} complex and therefore withdrawing histamine from the nanoparticles surfaces. As a consequence photoluminescence from nanoparticles is restored.

It is important to point out that no variation of photoluminescence was observed in experiments made with the addition of Ni^{2+} in the working dispersion in absence of histamine. Tests with the addition of other ions like Cu^{2+}, Zn^{2+}, Ca^{2+} and Mg^{2+} showed a less effective signal restoration effect since the formation of a histamine complex with these ions are less favourable [159].

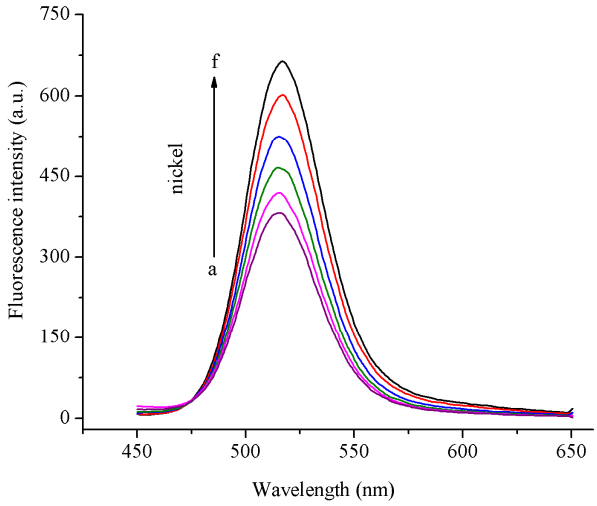

Figure 51- photoluminescence emission restoration of probe due to modulating effect of nickel on the interactions between quantum dots and histamine. (a) TGA-CdTe fuorescence quenched with histamine 5.1 x10^{-4} mol L^{-1}, (b) 3.3 x 10^{-8}, (c) 1.6 x10^{-7}, (d) 3.3 x 10^{-7}, (e) 5.1 x 10^{-7}, (f) 8.0 x 10^{-7} mol L^{-1}.

5.4 Analytical characteristics of observed photoluminescence quenching

A Stern-Volmer model (Equation 5.1) could be readily used to establish a relationship between the measured photoluminescence (F) and the concentration of histamine [histamine]. F_0 is the photoluminescence made from the nanoparticle dispersion before the addition of histamine. Since absorptivity of histamine above 300 nm is close to zero, even at the high concentration of histamine tested, no correction of the measured photoluminescence was needed to compensate attenuation of signal caused by inner-filter effect.

$$F_0/F = 1 + K_{sv}\,[\text{histamine}] \qquad (5.1)$$

The photoluminescence emission spectra measured from the quantum dots dispersions were recorded in function of the increasing concentrations of histamine (from 3.3 x 10^{-5} to 5.7 x10^{-4} mol L^{-1}) as indicated in Figure 52. Analytical curves were constructed by adding increasing concentrations of histamine in the quantum dots dispersion, prepared under the optimized conditions, followed by the measurent of the photoluminescence (Table 10) A typical analytical curve is shown in Figure 53 with a linear range of the analytical response up to 5.5 x 10^{-4} mol L^{-1} (61.1 µg mL^{-1}) of histamine (final concentration in the dispersion) with correlation and determination coefficients close to the unity (R^2 = 0.998 and r = 0.999 as indicated in the). The Stern-Volmer equation model of the analytical curve was $F_0/F = 1.4 \times 10^3\,[histamine] + 1.01$.

The limit of detection (LOD) was 9.6 x 10^{-6} mol L^{-1} (1.1 µg mL^{-1}) which means that using 50 µL of sample, the absolute limit of detection is 53 ng of histamine. The LOD was calculated as the concentration of histamine able to reduce the original photoluminescence of the dispersion of quatum dots (x_b), also called blank, to a value equals to $x_b - 3s_b$, where s_b is the standard deviation of 10 blank measurements.

The precision of the histamine measurement was calculated as the standard deviation of F_0/F, the $s_{(F0/F)}$, value taking into consideration ten independent solutions (in two different histamine concentrations). In order to do this, the Equation 5.1 was used.

$$s_{(F0/F)} = F_0/F \times [(s_F/F)^2 + s_{F0}/F_0)^2]^{1/2} \qquad (5.1)$$

The $s_{(F0/F)}$, in percentage values, was 2.3% and 3.1% at, respectively the 1.6 x 10^{-4} mol L^{-1} and 4.2 x 10^{-4} mol L^{-1} concentration levels.

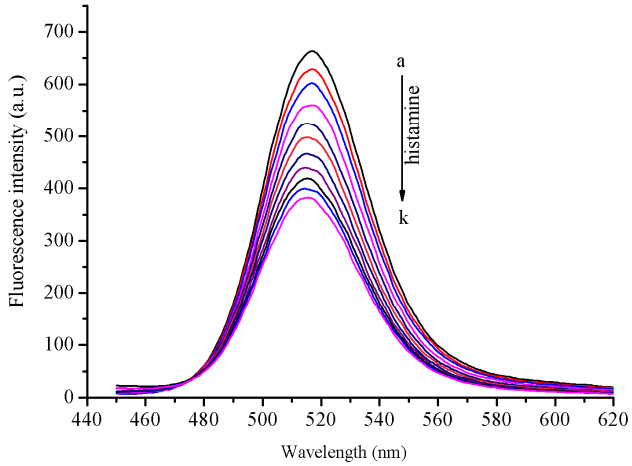

Figure 52 - Photoluminescence spectra from TGA-CdTe quantum dots dispersions in the presence of different concentrations of histamine (mol L^{-1}): (a) 0, (b) 3.3 x 10^{-5} , (c) 6.6 x10^{-5}, (d) 1.3x10^{-4}, (e) 2.0 x10^{-4}, (f) 2.6 x10^{-4}, (g) 3.2 x10^{-4}, (h) 3.9 x10^{-4}, (i) 4.5 x10^{-4}, (j) 5.1 x10^{-4}, (k) 5.7 x10^{-4} mol L^{-1}.

Table 10- Optimized experimental conditions for the determination of histamine using TGA-CdTe quantum dots aqueous dispersion.

Experimental parameters	Optimized Value
Type of quantum dots	TGA-CdTe
Phosphate buffer solution	0.01 mol L^{-1}
pH	8.0
Reaction time	2 min
Concentration of quantum dots	5 x 10^{-8} mol L^{-1}
Size of quantum dots	2.1 nm

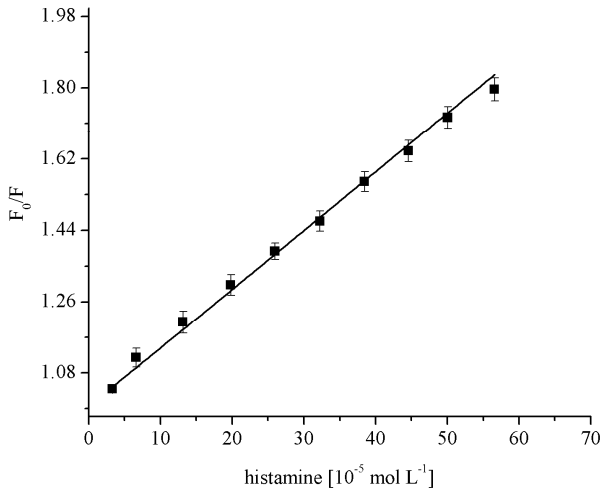

Figure 53 - Stern-Volmer-type calibration curve for the determination of histamine.

5.5 Selectivity studies

The selectivity of the TGA-CdTe nanoparticles in sensing histamine was studied by the evaluation of the effect of the presence of other chemical species like amino acids (histidine, valine, tyrosine, lysine, phenylalanine, threonine, methionine, tryphtophan and cysteine) and metal ions ($Mg^{2+,}$ Na^{+} K^{+}, Zn^{2+} ,Cl^{-}). Changes in photoluminescence intensity due to the presence of these chemical

species were expressed in percent values (see Table 11.). It was found that TGA-CdTe nanoparticles are farily insensitive to the presence of most of the tested amino acids. However, a less pronounced quenching was observed for tryptophan and phenylalanine and tyrosine.

The selectivity was further improved by applying a simple extraction procedure using a weak acidic cationic exchange resin (Amberlite - CG 50) as sorbent. This cationic resin is widely used for the selective separation of histamine from other biogenic amines and amino acids [160]. Histamine has two basic centers, the primary amine group (aliphatic group), and nitrogen atom at the imidazole ring. At physiological conditions, the aliphatic amino group (pK_a around 9.4) will be protonated (histamine becomes a monovalent cation due to the protonated primary amine) while the nitrogen at imidazole ring (pK_a about 5.8) will not be protonated. The solution containing histamine, after the addition of a 10% trichloroacetic acid solution, was passed through the cationic solid phase cartridge previously conditioned with about 5 mL of a 0.2 mol L^{-1} acetate buffer (pH 4.6). After the application of sample, the cartridge was thoroughly washed with 10 mL of acetate buffer (0.2 mol L^{-1}, pH 4.6). The washing resulted in the removal of traces of remaining amino acids and biogenic amines while the histamine is still retained on the resin. Histamine was then eluted with 0.5 mL of HCl aqueous solution (0.2 mol L^{-1}). After elution, the histamine solution was neutralized with 0.01 mol L^{-1} NaOH solution before added to the TGA-CdTe quantum dot dispersion.

The effectiveness of the extraction and the recovery (97%) was checked by passing standard solution of histamine and comparing the recovery value achieved in the TGA-CdTe calibration curve with the signal observed from a standard not passed through the SPE cartridge.

Table 11- Effect of potential interfering substances on the photoluminescence emission of CdTe quantum dots.

Coexisting substances	Concentration (μ mol L^{-1})	Photoluminescence variations (%)
Phenylalanine	500	+ 5.5
Valine	500	+0.5
Cysteine	500	+2.0
Tyrosine	500	-5.0
Lysine	500	-1.5
Methionine	500	-1.0
Threonine	500	- 0.5
Histidine	300	-4.0
Tryptophan	300	- 6.0
NO_3^-	20	-0.9
Mg^{2+}	20	+0.8
Ca^{2+}	500	-1.5
K^+	500	0.6
Zn^{2+}	500	+1.5
Na^+	1000	-0.2
Cl^-	1000	-0.2

5.6 Determination of histamine in fish sample

The easy handling of cation exchange resins and its combination with the sensitive TGA-CdTe sensing provides a very simple and easy approach for the determination of histamine in complex samples. The proposed photoluminescence sensing approach has been tested in the determination of histamine in the flesh of fresh and canned tuna fish samples. Sample treatment was described in a previous chapter. Tuna fish was homogeneized and spiked with histamine at concentration level of 1.3 x 10^{-4} and 4.0 x 10^{-4} mol L^{-1}. Samples were submitted to the extraction in the cationic solid phase cartridge. The potential loss of the analyte during sample preparation was checked by submitting a standard solution 4.2 x 10^{-4} mol L^{-1} to the same extraction procedure proposed for the fish flesh. After passing throuh the cationic solid phase cartidge, the recoveries calculated from Stern-Vomer model were 96.5 %. The recoveries close to 100 % confirms that the losses of histamine during extraction were insignificant.

Table 12- Determination of histamine in fresh and canned tuna fish spiked with different amount of histamine (n = 3)

Spiked sample	Concentration of histamine (10^{-4} mol L^{-1})			Recoveries (%)	
	Added	Founded by probe method	Founded by reference. method	Probe method	Reference . method
Tuna fresh	1.33	1.41 ± 0.10	1.26	106.2	94.5
	4.0	4.15 ± 0.27	3.64	103.8	91.1
Canned tuna	1.33	1.35 ± 0.13	-	108.2	-
	4.0	3.99 ± 0.23	-	99.9	-

The histamine recovery results using the proposed approach were satisfactory (close to 100%), which indicated the feasibility of the sensing using TGA-CdTe quantum dots (Table 12). The results obtained in flesh of fresh tuna fish were compared with the results achieved a reference method using the colorimetric determination that is based on the reaction between the imidazole ring of histamine and p-phenyldiazonium sulfonate (complex absorbs at 496 nm). The results achieved using both methods were in closed agreement as indicated by Student't-test (The t_{calc} = 0.36 for 1.33 x 10^{-4} mol L^{-1} a and 0.2 for 4.0 x 10^{-4} mol L^{-1} with $t_{critical}$ = 4.3 for 9% confidence level for n =3).