7 Development of cysteine-ZnS photoluminescent probe for determination of thyroxine in saliva and pharmaceutical formulations

7.1 The photoluminescence quenching of the cysteine-ZnS quantum dots by thyroxine

The photoluminescence intensity from the cysteine-ZnS quantum dots dispersion is sensitive to surface changes caused by interactions with chemical species in the dispersed system. The present chapter describes the efficient photoluminescence quenching from the cysteine-ZnS quantum dots dispersion in the presence of thyroxine. The quenching of the photoluminescence intensity followed a Stern-Volmer model and it was strongly dependent on the pH of solution, concentration of CTAB, temperature and amount of cysteine modified ZnS quantum dots. Conditions were optimized to enable de determination of thyroxine in different samples.

7.2 Optimization of the system for analytical measurements

7.2.1 Amount of cysteine-ZnS quantum dots in the dispersion

The quenching effect of cysteine-ZnS quantum dots caused by the presence of thyroxine (fixed at 9.5 $x10^{-7}$ mol L^{-1} final concentration) was investigated in dispersions containing different amounts of nanoparticles. The amount of nanoparticles was varied by introducing different volumes of the synthesized quantum dots stock dispersion (from 0.250 to 2 mL) which enabled a range between 1.6 $x10^{-4}$ to 1.3 x 10^{-3} mol of quantum dots dispersed in 1 L of aqueous solution. Higher amount of aqueous dispersion decreased the sensitivity of the response due to the high photoluminescence measured from the dispersion of the quantum dots. In contrast, lower concentration of nanoparticles results to weak

photoluminescence intensity that can result narrow linear range of concentration. A robust and sensitive photoluminescence quenching (Figure 64) was observed in the system containing lower amounts of quantum dots as indicated by the higher F_0/F ratio. To avoid self quenching and achieve high sensitivity, the chosen volume of the quantum dots stock dispersion was 0.8 mL (4.5×10^{-4} mol of nanoparticles in 1 L of aqueous solution).

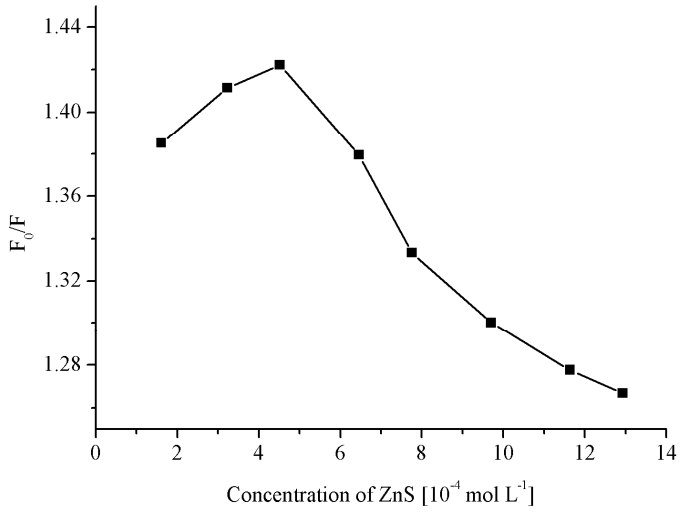

Figure 64 - Effect of the amount of the synthesized nanoparticles on the photoluminescence quenching of the cysteine-ZnS quantum dots aqueous dispersion. Signal variation expressed as F_0/F (where F_0 and F are respectively the photoluminescence of the quantum dots dispersion before and after the addition of 9.5×10^{-7} mol L^{-1} of thyroxine).

7.2.2 Influence of pH on the photoluminescence quenching of quantum dots

The effect of pH of the aqueous dispersion on the interaction between thyroxine and quantum dots was studied in order to find the appropriate pH for sensitive probing of tyroxine. The pH was varied in the range of 6.5 to 9.0 (phosphate buffer at 0.01 mol L^{-1} final concentration). The experiments were performed in dispersion with different pH values with and without the prescence of thyroxine (3.8×10^{-7} mol L^{-1}). The signal profile (Figure 65) indicated large

photoluminescence quenching in pH range from 8 to 8.5. Therefore, optimum pH value selected for all experiments was 8.

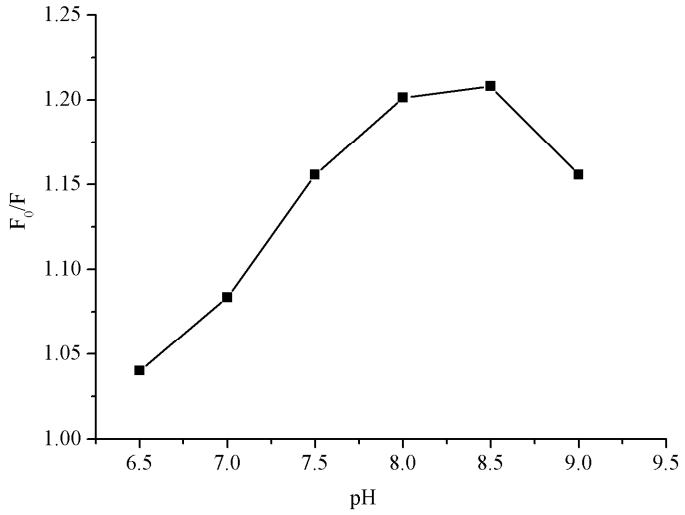

Figure 65 - Influence of pH value of the photoluminescence quenching measured as F_0/ F (F_0 is quantum dispersion in the absence of thyroxine and F in the presence of thyroxine) versus pH of quantum dots dispersion measured at fixed concentration 3.8 x10^{-7} mol $L^{-1}$of thyroxine.

7.2.3 Effect of surfactants on the photoluminescence quenching

The effect of different surfactant such acetyltrimethyl ammonium bromide (CTAB), sodium dodecyl sulphate (SDS) and Triton X-100 on thyroxine induced photoluminescence quenching of cysteine-ZnS was investigated in concentration 8 x 10^{-6} to 1 x 10^{-3} mol L^{-1}. The improvement in the photoluminescence quenching efficiency was obtained in the presence of CTAB at concentration of 5 x 10^{-5} mol L^{-1}, which is below the critical micelle concentration (CMC) as indicated in Figure 66. In contrast, anionic and neutral surfactants had no effect on photoluminescence quenching of the cysteine-ZnS caused by thyroxine. As the surface of ZnS capped with cysteine is negatively charged due to the deprotonation of the carboxylic group at pH 8.0, the CTAB (a cationic surfactant) is to interact to nanoparticles surface, via electrostatic interaction. Better photoluminescence quenching observed below the CMC is probably due to the

slight posivite surface changes, which facilitates interactions between nanoparticles and thyroxine. At higher concentration of CTAB, the surfactant may block the acess to thyroxine to the nanopartciles and the resulting smaller photoluminescence quenching.

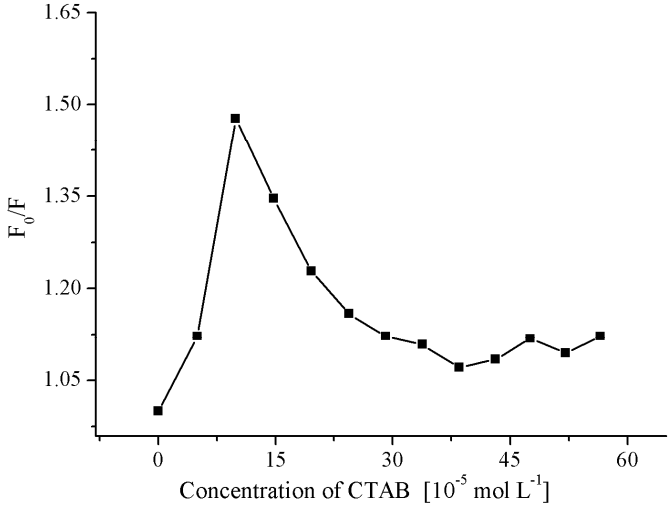

Figure 66 - Effect of CTAB on the photoluminescence quenching of cysteine-ZnS quantum dots at fixed concentration of thyroxine (final concentration in aqueous dispersion, 1.2 x 10^{-6} mol L^{-1}).

7.2.4 Effect of temperature

The effect of temperature on the photoluminescence intensity of cystein-ZnS nanopartilces probe was studied at temperature ranging from 18 to 45 ^{o}C. The photoluminescence measured from quantum dot was found to be inversely dependent on temperature with a fairly constant signal intensity found between 18 and 25 ^{o}C (Figure 67). The decrease in photoluminescence intensity measured from the quantum dots dispersion is due to the fact that the increased kinetic energy of medium removes the cysteine capping from ZnS surfaces that leads to decrease photoluminescence intensity. In addition, with the increasing of the temperature, the quenching caused by the increase of the rate of collisions between chemical species in the dispersion may be playing an important role in the decrease of the photoluminescence intensity. Therefore, room-temperature

(about 25 ^{o}C) was selected in all photoluminescence measurement for sensing of thyroxine.

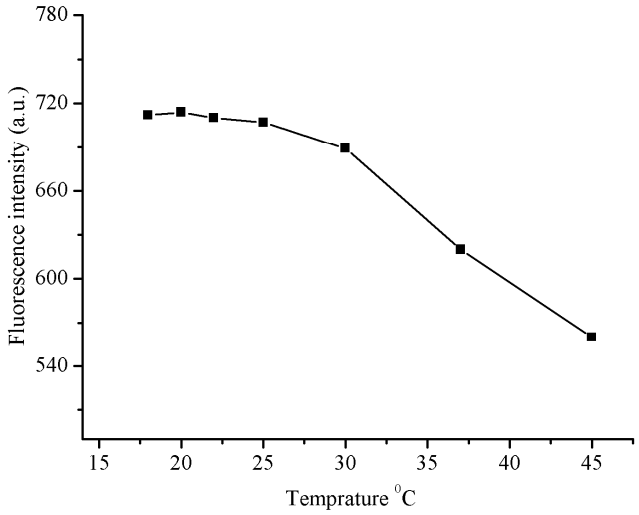

Figure 67 - Photoluminescence measured from cysteine -ZnS dispersion (4.5 x10^{-4} mol of nanoparticles in 1 L of aqueous solution) at different temperatures.

7.2.5 Stability of photoluminescence intensity and reaction time

Under the selected pH, temperature and amount of quantum dots in the dispersion, a study of the stability of photoluminescence intensity measured from the control dispersion of quantum dots (cysteine-ZnS without addition of thyroxine) was studied in function of the time (measured every 10 min up to 120 min). The photoluminescence was found to be stable up to 120 min (less than 3% random variation of signal) as indicated in Figure 68. The photoluminescence from the quantum dots dispersion was also monitored in function of time after the addition of a fixed amount of thyroxine. Measurements were made every 2 min up to 30 min starting 2 min (1 min of mixing and 1 min of equilibration of the solution) after the addition of thyroxine. The photoluminescence quenching occured immediatelly and after 5 min, the signal was stable up for more than 30 min (Figure 69). For the analytical method, it was established all measurements to be made after 5 min of the addition of thyroxine into the quantum dots dispersion.

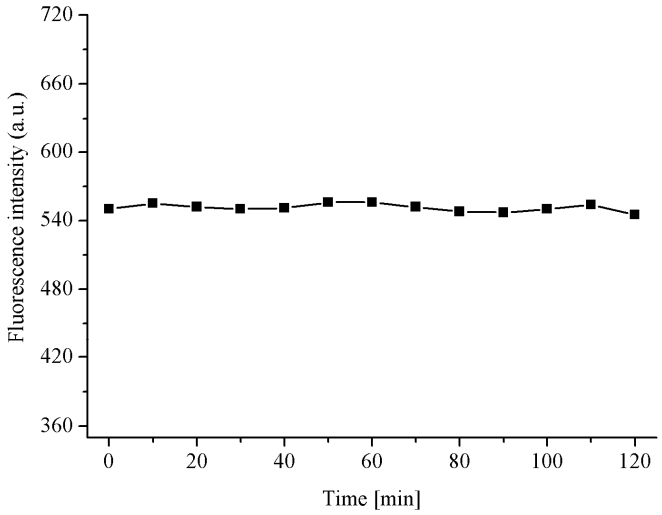

Figure 68 - Stability study of the photoluminescence intensity of the cystein-ZnS nanoparticles dispersion.

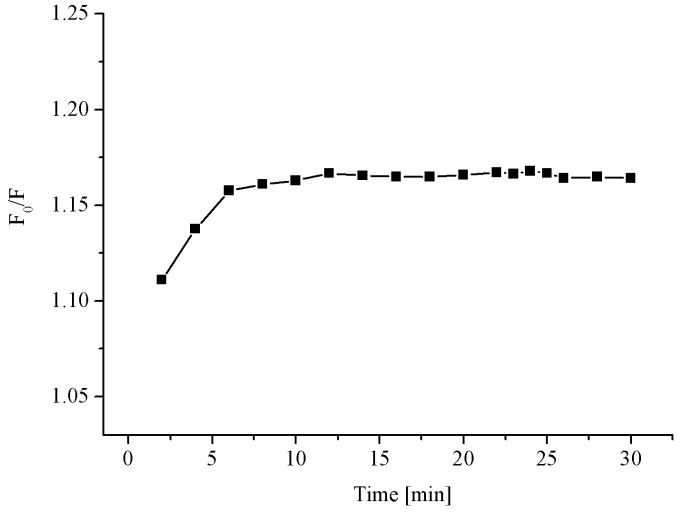

Figure 69- Stability of the photoluminescence of the cysteine-ZnS nanoparticles probe quantum dots after mixing thyroxine (final concentration in solution 4.0 x 10^{-7} mol L^{-1}).

7.3 Mechanism of interaction

Several mechanisms have been proposed for the photoluminescence quenching in quantum dots, including inner filter effect, non-radiative pathways, electron transfer, surface adsorption, surface complexations and equilibrium attractions [163]. Thyroxine has a wide UV-vis absorption profile with maximum absorption at 235 nm and 330 nm. Taking into consideration a 6×10^{-5} mol L^{-1} of thyroxine the absorbance is 0.87 at 235 nm and 0.09 at 330 nm and 0.008 at 312 nm. For higher concentrations of thyroxine, inner filter effect may affect the response at the excitation wavelength of 312 nm, which was chosen for excitation of cysteine-ZnS quantum dots. Such interference would require a mathematical correction for the inner-filter effect. However, the quantum dots probe response to thyroxine is in the 1×10^{-7} to 1×10^{-6} mol L^{-1} concentration level, a range significantly lower than the range of thyroxine that contribute with significative absorbance. Therefore, the inner-filter effect can be neglected. In Figure 70 the low contribution of absorption profile from thyroxine at 312 nm is shown at the concentration range from 3.9×10^{-7} and 4.0×10^{-6} mol L^{-1}.

Moreover, since no changes in the absorption spectral chemical profile of the cysteine-ZnS takes places in the presence of thyroxine, it is concluded that no aggregation (chemical degradation that can lead to the reducing of fluorescence) of quantum dots are taking place (Figure 71).

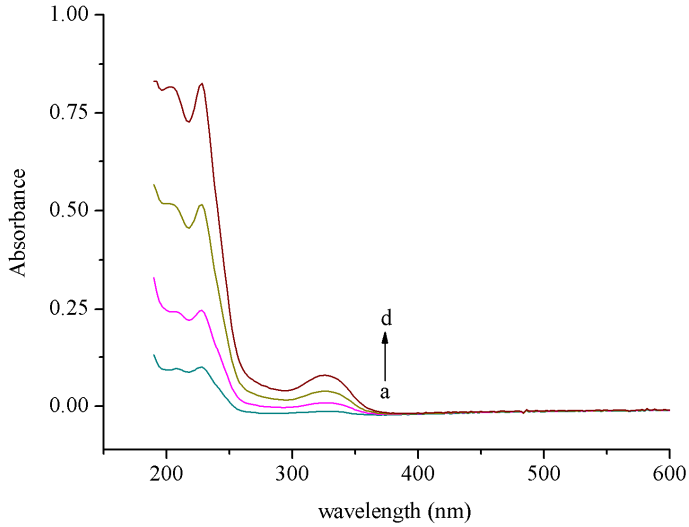

Figure 70 - UV-Visible absorption spectra of thyroxine; (a) 3.92 x 10^{-7} (b) 9.8 x 10^{-7}, (c) 3.8 x10^{-6}, (d) 6 x 10^{-5} mol L^{-1}.

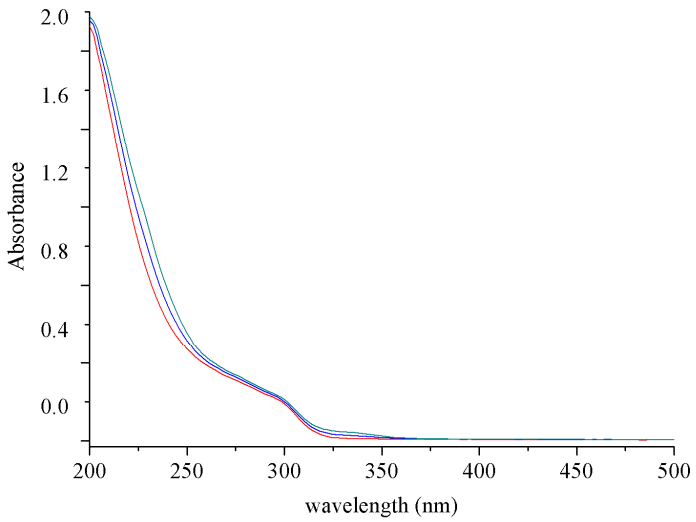

Figure 71- UV-visible absorption spectra of cysteine-ZnS nanoparticles in the prescence of thyroxine; (a) 0, (b) 3.92 x 10^{-7}, (c) 4 x 10^{-6} mol L^{-1}

In order to find if the mechanism of photoluminescence quenching is wherter static or dynamic, a study of the dependence of photoluminescence upon temperature was made [52]. From the Stern-volmer plots constructed at two

different temperatures (298 and 308 K) it is observed that the sensitivity decreases as the temperature increased (Figure 72). These results indicate the thyroxine and cysteine-ZnS interactions associative in nature (static quenching)

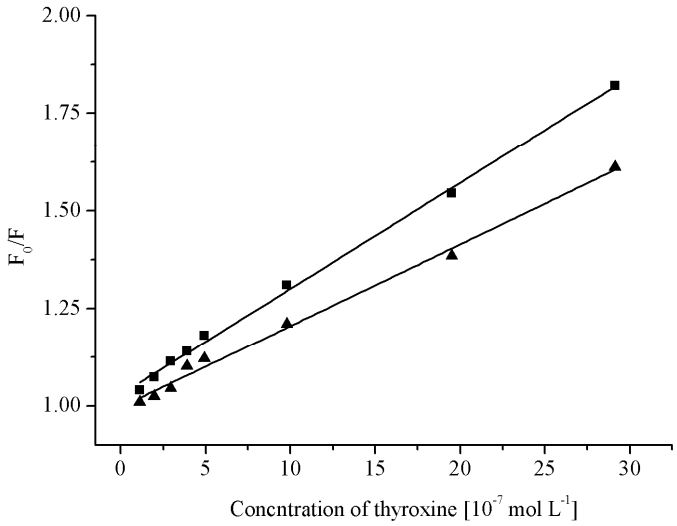

Figure 72 - The Stern–Volmer curves for the quenching of cysteine-ZnS in the presence of thyroxine at temperatures of 298 K (■) and 308 K (▲)

7.4 Analytical characteristics of photoluminescence quenching

Under the optimized experimental conditions (Table 17) a Stern-Volmer model (Equation 7.1) could be readily used to establish a relationship between measured photoluminescence (F) at concentration of thyroxine [thyroxine]. F_0 is the photoluminescence made from the probe dispersion before the addition of thyroxine.

$$F_0/F = 1 + K_{sv}\,[\text{thyroxine}] \qquad (7.1)$$

The results show that thyroxine quenches the photoluminescence intensity of the cysteine-ZnS quantum dots in concentration dependent pattern from 1.1 x 10^{-7} to 4.0 x 10^{-6} mol L^{-1} (Figure 73).

Table- 17 -Optimized experimental conditions for the thyroxine determination using the cysteine-ZnS optical probe.

Experimental parameter	Optimized Value
Type of quantum dots	cysteine-ZnS probe
Phosphatebuffer solution	0.01 mol L^{-1}
pH	8.0
Time required to perform measurement	5 min
Amount of quantum dots	4.5 x10^{-4} mol per 1 L
Temperature	25 ^{o}C
CTAB	1.2 x 10^{-6} mol L^{-1})
Eluent	1 mL of methanol

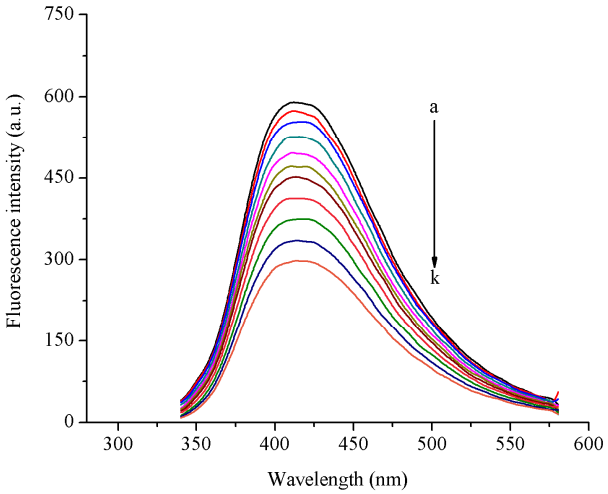

Figure 73 - Photoluminescence emission spectra of L-cysteine-ZnS nanoparticles in the presence of different concentrations of thyroxine: (a) 0, (b) 1.12 x 10^{-7} , ' (c) 1.98 x 10^{-7}, (d) 2.95 x 10^{-7}, (e) 3.92 x 10^{-7}, (f) 4.91 x 10^{-7}, (g) 9.8 x 10^{-7}, (h) 1.95 x10^{-6}, (i) 2.91 x10^{-6}, (j) 3.8 x10^{-6}, (k) 4.0 x10^{-6} mol L^{-1}.

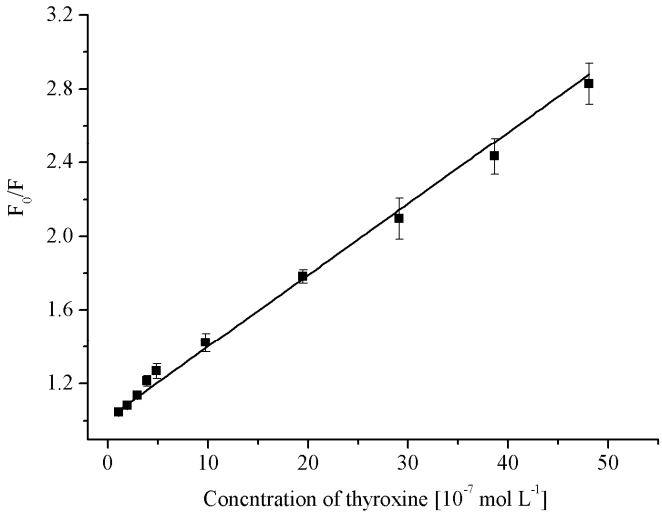

Figure 74 - Stern-Volmer-type calibration curve for the determination of thyroxine using cysteine-ZnS nanoparticles as optical probe.

Analytical curves were constructed by adding increasing concentrations of thyroxine on the quantum dots dispersion and measuring the photoluminescence decrease. A typical analytical curve (F_0/F versus concentration of thyroxine) is

shown in Figure 74, showing a linear range of the analytical response in the concentration range from 1.1 x 10^{-7} to 4.0 x 10^{-6} mol L^{-1} of thyroxine (final concentration in the dispersion) with correlation coefficient of 0.9926. The equation model of the analytical curve was $F_0/F = 3.3 \times 10^5$ [thyroxine] + 1.01.

The LOD value was calculated as the concentration of thyroxine that changes the photoluminescence signal by $F_0 - 3s_{F0}$, where s_{F0} is the standard deviation of ten replicate measurements of the photoluminescence intensity of the cysteine-ZnS dispersion before the addition of thyroxine. Similarly, the LOQ value was calculated as the $F_0 - 10s_{F0}$. The LOD and the LOQ were respectively 6.2 x 10^{-8} mol L^{-1} (48.3 ng mL^{-1}) and 2.0 x 10^{-8} mol L^{-1} (15.4 ng mL^{-1}) respectively. The precision of the thyroxine measurement from proposed probe was calculated as the variation of the F_0/F value taking into consideration ten independent solutions (in two different thyroxine concentrations). In order to do this, the following equation was used: $s_{(F0/F)} = F_0/F \times [(s_F/F)^2 + s_{F0}/F_0)^2]^{1/2}$. The $s_{(F0/F)}$, in percentage values, was 2.8% and 4.2% at, respectively the 3.9 x 10^{-7} mol L^{-1} and 2.9 x 10^{-6} mol L^{-1} concentration levels.

7.5 Selectivity studies

For practical applications of the proposed cysteine-ZnS quantum dots based method for the determination of thyroxine in biological samples (saliva) and in pharmaceutical formulations, the effect of some possible relevant interfering substances was evaluated. The chosen substances were the ones commonly found in pharmaceutical formulations and in biological fluids (including several amino acids). Changes in photoluminescence intensity due to the presence of these chemical species were expressed in percent values (Table 18).

All the tested substances imposed signal variations (at the specified concentrations) under the 4% in photoluminescence of nanoparticles dispersion. The quenching effect of mixture of few amino acids and mixture of lactose, silicon dioxide, citric acid, calcium, magnesium, potassium, sodium is also investigated in the absence and in the presence of thyroxine. From the Figure 75, it can be concluded that their prescence is not interfering the quenching effect produced by thyroxine on the quantum dispersion.

Table 18- Effect of some potential interfering substances on the photoluminescence of the cysteine-ZnS quantum dots aqueous dispersion.

Potential interferent	Concentration μ mol L^{-1}	Change of fluorescence (%)
cysteine	150	+ 4.0
histidine	150	+3.1
tyrosine	150	+1.4
phenylalanine	150	+0.55
valine	150	+0.55
methionine	150	-0.55
lysine	150	+1.13
threonine	150	-1.42
lactose	100	- 2.6
silicon dioxide	100	+1.8
citric acid	100	+ 2.0
calcium	100	+ 4.1
magnesium	100	+3.8
potassium	100	+ 2.1
sodium	100	+ 3.1

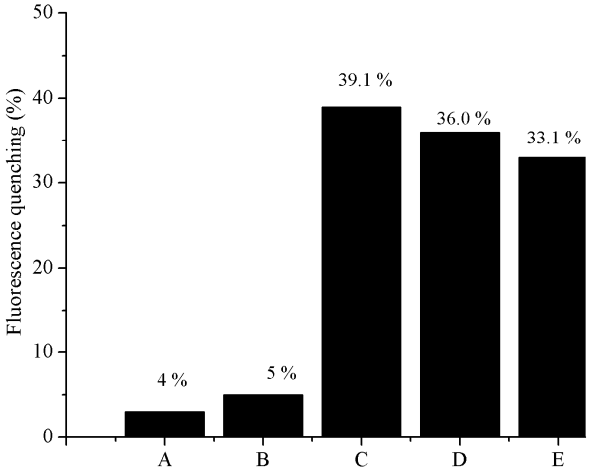

Figure 75 – Effect on photoluminescence of cysteine-ZnS quantum dots dispersion. A) mixture of amino acids; B) mixture of common pharmaceutical excipients C) thyroxine with a mixture of amino acids (1:5) D) thyroxine with a mixture of common pharamaceutical excipients (1:20) E) thyroxine.

7.6 Application of the cysteine-ZnS quantum dots dispersion on the determination of thyroxine

The proposed photoluminescence cysteine-ZnS probe has been applied for the determination of thyroxine in pharmaceutical formulation (containing artificial thyroxine hormone as active component) and in human saliva. Three different portions from ten grinded tablets were diluted to give a final concentration of 9.8 x 10^{-7} mol L^{-1} in the quantum dot aqueous dispersion. The recoveries achieved using the proposed probe method at a concentration level of 9.8 x 10^{-7} mol L^{-1} was 97.0 ± 5.2% taking into consideration the value of thyroxine indicated in the pharmaceutical formulation instructions.

Table 19- Applications of the cysteine- ZnS probe method for determination of thyroxine in pharmaceutical formulation and saliva.

Sample	Thyroxine level	Portion 1	Portion 2	Portion3	Average result (%)
Levotiroxina sódica	200 μg per tablet	93.3	94.8	103.0	97.0 ± 5.2
Saliva spiked	2.9×10^{-7} mol L^{-1}	88.9	90.1	86.5	86.0 ± 5.1

In order to evaluate the applicability of photoluminescence probe in clinical assays, the analysis of saliva samples (fortified with thyroxine at concentration of 2.9×10^{-7} mol L^{-1}) were performed. The saliva fortified with known amount of thyroxine was mixed with 5 mL of ethanol then immediately centrifuged for 15 min at 3000 rpm. After centrifugation, the supernant was passed through C18 SPE column and washed with deionzed water. After elution with 1 mL methanol, the eluate was evaporated and the residue re-suspended with the nanoparticle dispersion (made in phosphate buffer pH 8.5). From the photoluminescence quenching the concentration of thyroxine was find out and the recoveries were 86.0 ± 5.1 %. The relatively low recoveries may be due to loss of thyroxine in pretreatment of saliva Table 19.