6 TGA-CdTe quantum dots sensing and molecularly imprinted polymer based solid phase extraction for the determination of kanamycin.

6.1 Enhancement of the TGA-CdTe photoluminescence caused by aminoglycosides

In the present chapter, it is shown that the photoluminescence intensity of TGA-CdTe quantum dots was drastically enhanced by the interactions with kanamycin and other aminoglycosides. The enhancement in photoluminescence intensity was modelled using a Langmuir model. This effect was analytically usefull for the determination of kanamycin since the signal enhancement was immediate and remained stable during the time required to perform measurements. There was a proportional relationship between the increased photoluminescence with the amount of kanamycin enabling application of the approach in differente samples. Selectivity was improved by solid phase extraction procedure using a molecularly imprinted polymer.

6.2 Factors affecting the CdTe-TGA quantum dots photoluminescence enhancement

6.2.1 Influence of the pH of the aqueous dispersion on the photoluminescence enhancement caused by kanamycin

Generally, the hydrogenionic activity of the aqueous solution plays a key role in the interactions between the analyte and the quantum dots, also affecting the degree of aggregation of quantum dots and, therefore, the stability of the dispersions [152]. In this work, aqueous quantum dots dispersion with pH values adjusted to between 6.5 and 9.0 were used to evaluate the effect of the presence of kanamycin (fixed concentration of 1.3×10^{-7} mol L^{-1}). The photoluminescence

profile shown in Figure 54 indicates that at pH 8, the maximum photoluminescence enhancement factor (measured as F/F_0) was observed in the presence of kanamycin.

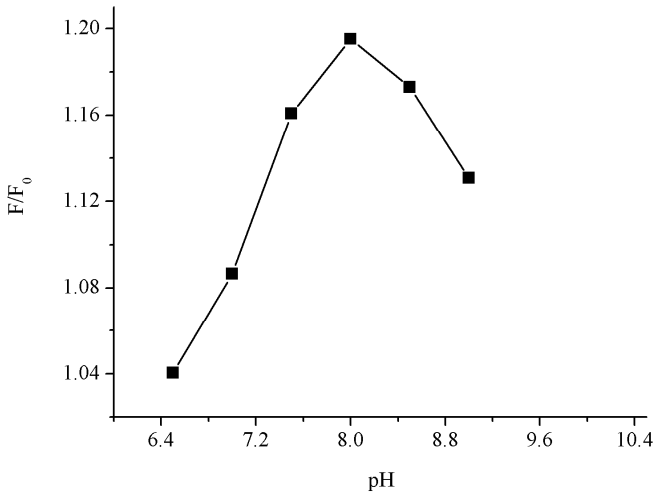

Figure 54 - Effect of pH value of the aqueous dispersion on the enhancement of the photoluminescence of TGA-CdTe quantum dots measured as F/F_0, where F_0 and F are respectively the photoluminescence of the quantum dots dispersion after and before the addition of kanamycin (1.3×10^{-7} mol L^{-1} final concentration).

6.2.2 Effect of temperature and reaction time on the photoluminescence enhancement

The photoluminescence of the nanoparticles was monitored in function of time after the addition of a fixed amount of kanamycin. Measurements were made every 2 min up to 60 min starting after 2 min of the addition of kanamycin (1 min of mixing and 1 min of equilibration of the solution). The enhancement in photoluminescence was immediate and, after reaching its maximum, the signal was found to be stable up to 60 min. The effect of the temperature of the dispersion on the magnitude of the photoluminescence enhancement produced after the interaction with kanamycin was studied at the temperature range from 22 to 36 ^{o}C (with incremental temperatures of 2 ^{o}C). A fairly constant F/F_0 ratio was found between 22 to 26 ^{o}C (Figure 55), therefore temperature was found to be a fairly robust parameter at this range. For higher temperatures, a decrease of

photoluminescence was observed, which is an indication that the bonding between kanamycin and quantum dots is disrupted. In the analytical point of view, which aims reliable and sensitive measurements, photoluminescence was measured at room temperature (about 25 °C) and after 2 min of equilibration time.

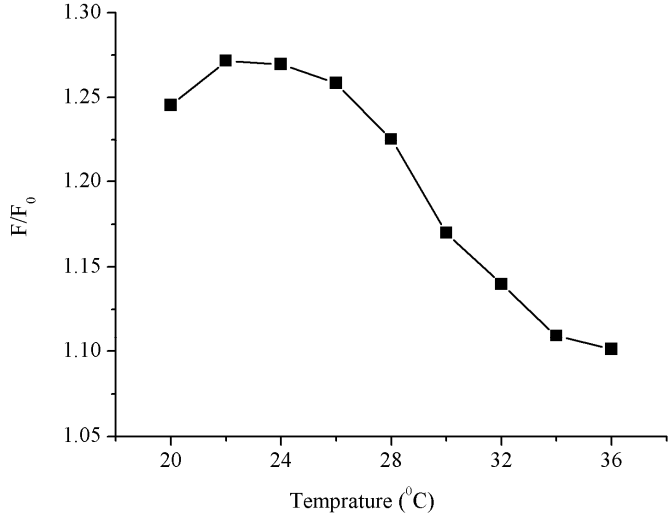

Figure 55 - Effect of the temperature on the photoluminescence enhancement of the aqueous quantum dots dispersion after addition of kanamycin (1.2 x10^{-7} mol L^{-1} final concentration).

6.2.3 Concentration of quantum dots dispersion

The kanamycin induced photoluminescence was also investigated using dispersion containing different amounts of the TGA-CdTe nanoparticles. For this purpose, different volumes of nanoparticles stock solution (from 5 to 45 μL) were dispersed deionized water resulting in amounts between 8.8 x 10^{-8} and 7.1 x 10^{-7} mol of nanoparticles 1 L of aqueous dispersant. The most pronounced photoluminescence enhancement (F/F_0 =1.18) was observed in the dispersion system containing amounts of nanoparticles from 2.4 x 10^{-7} to 4 x10^{-7} mol as shown in Figure 56. This signal ratio value decrease at higher amount (4.7 x10^{-7} to 7.1 x 10^{-7} mol dispersed in 1 L) of quantum dots tested.

As the amounts of quantum dots dispersed the kanamycin available to interact per nanoparticle decreases (molecules available to occupy the number of

binding sites required to get an effective interaction), making the photoluminescence enhancement effect less effective. The chosen amount of quantum dots to be used in the working dispersion was 3 x 10^{-7} mol per 1 L of aqueous continuous phase.

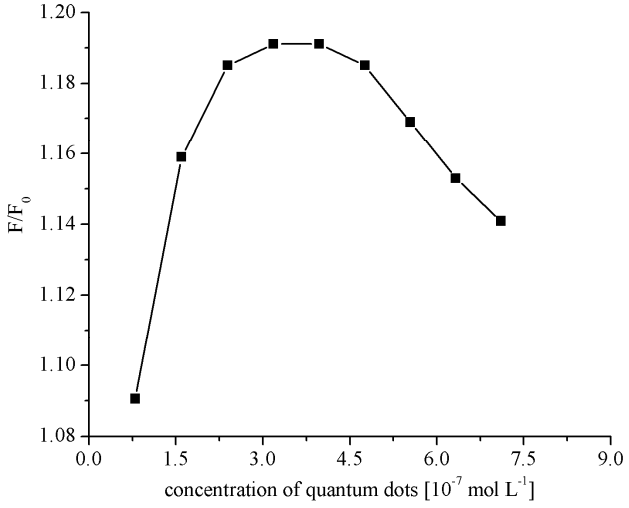

Figure 56 - Effect of the amount of the synthesized quantum dots on the photoluminescence of the aqueous quantum dots dispersion. Dispersions containing kanamycin at a fixed concentration of 6.7 x 10^{-8} mol L^{-1}.

6.2.4 Size dependence of TGA-CdTe photoluminescence enchancement

Quantum dots with different sizes were used to evaluate the size dependency of the photoluminescence enhancement. Dispersions prepared with quantum dots with average sizes of 2.2, 2.8 and 3.5 nm were used. It can be observed in Figure 57 that interactions of the nanoparticles with kanamycin showed strong size-dependency. The greater enhancement observed in the dispersion containing smaller particles covering a concentration range of kanamycin up to 10 x 10^{-7} mol L^{-1}. Due to higher surface to volume ratio in smaller nanoparticles, better enhancement in photoluminescence is observed in comparison to what was measured from larger particles.

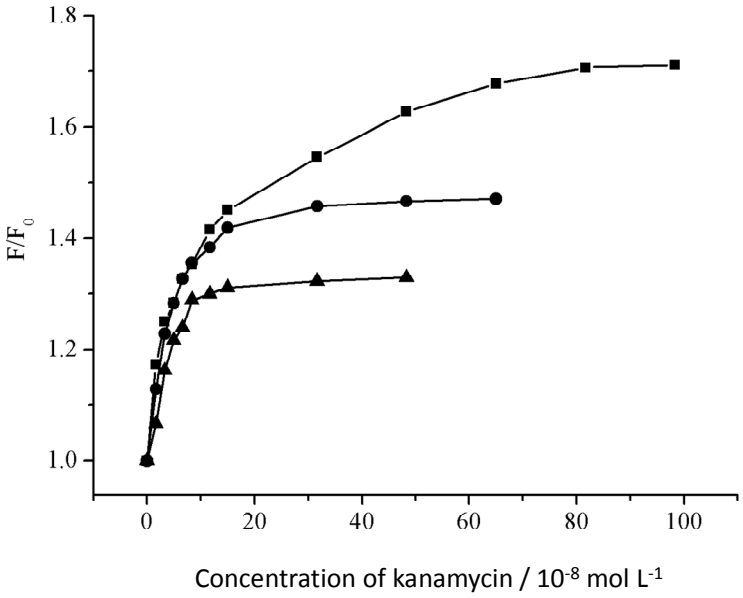

Figure 57- Photoluminescence enhancement curves induced by kanamycin in TGA-CdTe quantum dots aqueous dispersions: Quantum dots average sizes: 2.2 nm (■) 2.8 nm (▲) and 3.5 nm (●). Signal evaluated as F/ F_0, where F_0 and F are respectively the photoluminescence of the quantum dots dispersion before and after the addition of kanamycin.

6.3 Modeling the photoluminescence sensing of kanamycin with TGA-CdTe nanoprobe

Under the optimized conditions (Table 13), the photoluminescence intensity measured from the quantum dots dispersion increases as the concentration of kanamycin is increased from 1.6 x 10^{-8} to 6.5 x 10^{-7} mol L^{-1} (Figure 58). However, concentrations of kanamycin higher than 8.7 x 10^{-8} mol L^{-1} did not cause any changes in photoluminescence intensity due to the saturation of the surface of the nanocrystals that decreases the availability of binding sites. As expected, the relationship between the increasing of photoluminescence and the concentration of the analyte was not linear (Figure 59).

Table 13- Optimized experimental conditions for the kanamycin determination using the TGA-CdTe probe.

Experimental parameter	Optimized Value
Type of quantum dots	TGA-CdTe probe
Phosphatebuffer solution	0.01 mol L^{-1}
pH	8.0
Time required to perform measurement	2 min
Amount of quantum dots	3x 10^{-7} mol per 1 L
Temperature	25 ^{o}C
Washing solution	Methanol/water (80/20% v/v)
Eluent	Acidic water (pH ~ 3.5)

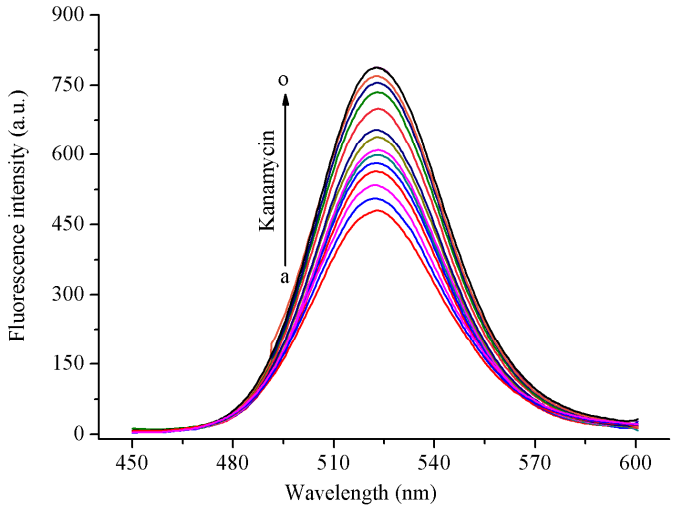

Figure 58 - Photoluminescence spectra of TGA-CdTe quantum dots in the presence of different concentrations of kanamycin: (a) 0, (b) 1.66, (c) 3.33, (d) 5, (e) 6.66, (f) 8.33, (g) 11.66, (h) 15, (i) 31.66, (j) 48.33, (k) 65, (l) 81.6, (m) 98.33, (n) 131.6, (o) 165 x 10^{-8} mol L^{-1}

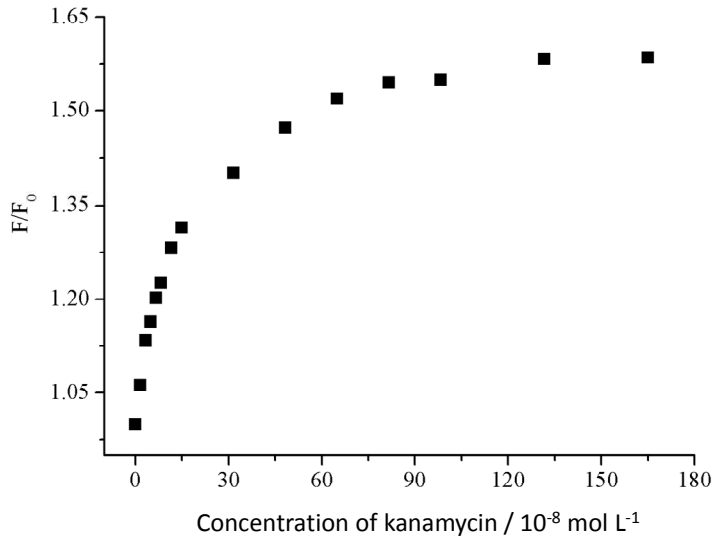

Figure 59- Photoluminescence enhancement of TGA-CdTe quantum dots (expressed as F/F_o in function of the concentration of kanamycin.

6.4 Effect of other aminoglycosides and macrolide antibiotics on the photoluminescence measured from the TGA-CdTe quantum dots dispersion

In order to study the effect caused by other aminoglycosides like neomycin, streptomycin, gentamycin, amikacin and tobramycin on the photoluminescence of quantum dots dispersion, experiments with these aminoglycosides were performed under the same optimized experimental condition of kanamycin (Table 13). It was found that the tested aminoglycosides also induced enhancement in the photoluminescence due to the interaction with quantum dots. This behavior, in fact, was expected as these aminoglcosides have the same basic structure of kanamycin, consisting of one or more amino sugars linked by glycoside bondings to an aminocyclitol component. However, the efficiency of the photoluminescence enhancement varied depending upon the aminoglycoside. These efficiencies were evaluated in terms of the equilibrium binding constants, which were calculated from Langmuir bonding isotherms. It is likely that photoluminescence enhancement is closely related to the multiple primary amines distributed across the individual aminosugars and aminocyclitol moieties in aminoglycoside molecules. The calculated equilibrium binding constant (Table 14) and hence the resultant photoluminescence enhancement of quantum dots dispersion was greater for neomycin with binding constant of 2.7×10^{8} L mol^{-1} (six primary amines groups) and lower for streptomycin with binding constant of 6×10^{5} L mol^{-1} (two primary amines groups). To further understand the structural factors that are responsible for the photoluminescence enhancement of the CdTe dispersion, tests were carried out using the erythromycin (Figure 60), an antibiotic characterized by a macrocyclic lactone ring containing 14, 15 or 16 atoms with the sugars linked via glycosidic bonds. Like aminoglycosides, erythromycin contain hydroxyl group however, unlike aminoglycosides, it does not present any primary amines groups in its structure. The addition of erythromycin (up to 1×10^{-5} mol L^{-1}) caused slight quenching (4 %) in the photoluminescence measured from quantum dots dispersion.

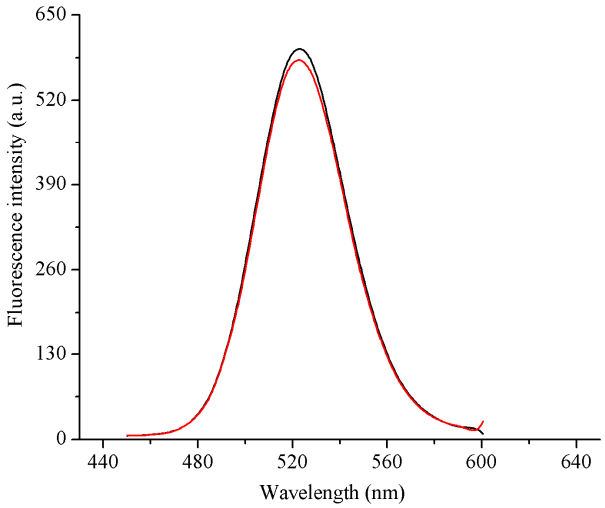

Figure 60 - Effect of erythromycin to (in final solution1 x 10^{-5} mol L^{-1}) on photoluminescence of TGA-CdTe nanoparticles dispersion.

Table 14 - Effect of different amioglycosides and erythromycin on photoluminescence enhancement of TGA-CdTe optical probe.

Aminoglycosides & Macrolides	Normalized Linear range x 10^{-7} mol L^{-1}	Binding Constant (B) x 10^{7} L mol^{-1}
Kanamycin	0.28 - 8.2	5.65
Neomycin	0.16 – 1.6	27.8
Streptomycin	0.33 – 9.8	0.06
Gentamycin	0.31 – 9.1	5.8
Amikacin	0.30 – 9.8	5.45
Tobramycin	0.16 – 2.3	13.3
Erythromycin	0.33 -100	-

6.5 Mechanism of interaction

The observed photoluminescence enhancement caused by the presence of kanamycin may result from the generation of new and efficient radiative pathway due to adsorption of kanamycin or due to the passivation of surface that minimizes the non-radiative processes. The passivation suppresses the motion of surface constituents enhancing the efficiency of the emission from the cluster.

As seen previously, the maximum quantum dots photoluminescence enhancement observed in the presence of kanamycin was observed at pH about 8. Such pH dependency demonstrates the importance of the electrostatic interaction between nanoparticles and kanamycin. In the more alkaline environment, the carboxylic group of the ligands bound on the surface of nanoparticles is deprotonated and below pH about 9 (pK_a = 9.4), the primary amine group of kanamycin is protonated. Therefore, maximum adsorption will be achieved when a relevant fraction of both, nanoparticles and kanamycin are charged with the -COO^- group covering the surface of quantum dots and the -NH_3^+ group of the kanamycin in solution. The strong electrostatic attraction between these groups result in a strong adsorption and consequently a stronger photoluminescence enhancement takes place. The less electrostatic attraction between kanamycin and the TGA-CdTe nanoparticles occurs when the no charged carboxylic acid groups exists at lower pH and when the no charged amine groups occurs at higher pH conditions resulting in comparatively in the less increase in photoluminescence.

Another important consideration is that despite the CdTe nanoparticles are modified with TGA, there may be still some traps on their surface. Thus, the adsorption of kanamycin may result due to the binding of primary amine group (a fraction of non-protonated amine groups of kanamycin may exist at pH about 8) to the surface of the CdTe through N-Cd bonding, which reduces the surface defects, minimizing non-radiative processes and increasing the luminescence.

6.6 Analytical characteristics of photoluminescent probe for the determination of kanamycin

For sensing purposes, analytical curves were constructed under the optimized conditions by adding increasing concentrations of kanamycin (C) on the nanoparticles dispersions. A typical plot of of C/F versus C is linear throughout the tested kanamycin concentration range from 2.8 x 10^{-8} to 8.2 x 10^{-7} mol L^{-1} (final concentration in the working solution), which confirms that binding of kanamycin on the surface of CdTe quantum dots does follow a Langmuir type binding isotherm (Figure 61) [156]. The remarkable Langmuirian fit suggests the probability of binding more than one kanamycin molecules to the surface of an individual nanoparticle. The regression equation was Y = 0.0013 [kanamycin] + 2.3 x 10^{-11} with correlation coefficient of 0.9996. The adsorption equilibrium binding constant (B) was calculated from the ratio slope/intercept value of the linearized plot (C/F versus C). The equilibrium binding constant represents the surface affinity of ligand (analyte) to the nanoparticles, which means that if the value of binding constant is high the corresponding surface affinity is strong. From the higher value of binding constant (5.65 x 10^{7} L mol^{-1}), it can be assumed that the surface affinity and binding capacity between kanamycin and surface of TGA-CdTe nanoparticles is very strong.

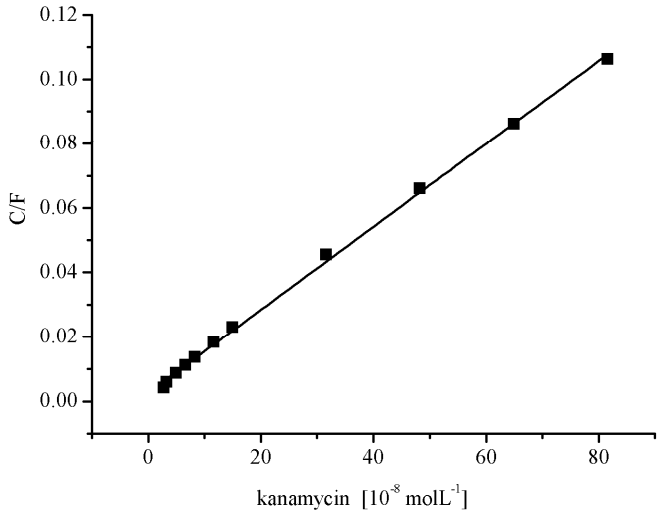

Figure 61 - Analytical curve for kanamycin using a Langmuir isotherm model.

The limit of detection (LOD) was calculated as the concentration of kanamycin that enables a photoluminescence signal equivalent to $F_0 + 3s_{F0}$, where s_{F0} is the standard deviation of ten replicate measurements of the photoluminescence intensity of the working quantum dots dispersion (without kanamycin). Similarly, the limit of quantification (LOQ) was calculated as the $F_0 + 3s_{F0}$. The LOD and the LOQ were respectively 2.5 x 10^{-8} mol L^{-1} (14.2 ng mL^{-1}) and 2.7 x 10^{-8} mol L^{-1} (16 ng mL^{-1}. Precision was calculated based on the propagated standard deviation of C/F (the $s_{C/F}$ value) as mentioned in section 4.2. For 1 x 10^{-7} mol L^{-1} of kanamycin the $s_{C/F}$ in percentage value was 3.5% .

6.7 Optimization of extraction conditions for kanamycin using a molecularly imprinted polymer

A molecularly imprinted polymer (MIP) developed in the laboratory [161] was used to selectively retain kanamycin. Although not selective for other aminoglycosides, the MIP may be used to eliminate the presence of other substances that may interfere in the signal of the optical probe. The selective retention of the MIP, used for solid phase extraction (SPE), is due to the existence of template cavities (due to inclusion of kanamycin as a template during the preparation) on the polymer. In order to confirm the selectivity of MIP towards kanamycin, the cartridges (1 mL syringe) packed with 100 mg of MIP and NIP (non-imprinted polymer prepared without the use of kanamycin as template) were first conditioned with 5 mL of methanol and 10 mL of deionized water.

Then both MIP and NIP were loaded with 200 μL of aliquot of standards solution of (1 x 10^{-5} mol L^{-1}) kanamycin. Mixtures with different proportions of water and methanol were tested as the solvent system to wash the column as kanamycin is not soluble in methanol (kanamycin is also insoluble in ethanol and acetone). Higher proportions of water (higher than 20% in the mixture with methanol) resulted in poorer recoveries due to the increase of polarity (increasing water content) that elutes part of the retained kanamycin (Figure 62). Better results for kanamycin were obtained using 5 mL of methanol/water (80/20% v/v) as solvent system to wash the column containing the retained kanamycin. Elution of kanamycin was made using 0.5 mL of acidic water solution (pH about 3.5). The eluate was neutralized with 0.01 mol L^{-1} of NaOH (as photoluminescence from

TGA-CdTe is pH sensitive) and the concentration of kanamycin was calculated by the linearized Langmuir model (Table 15)

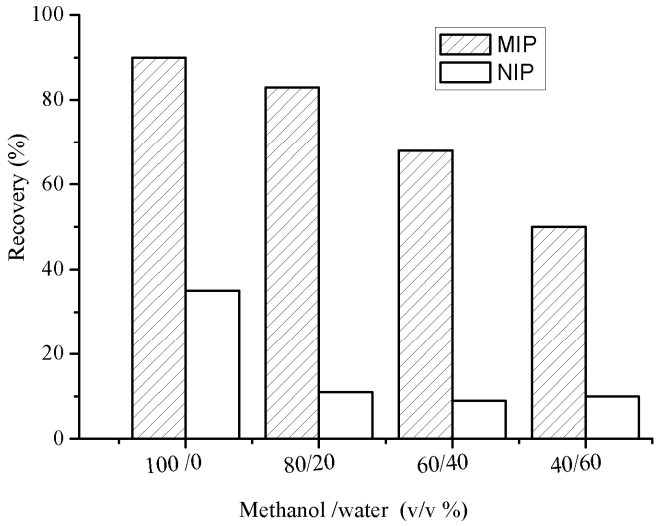

Figure 62 - Recoveries of kanamycin obtained in solid phase extraction using MIP and NIP after washing with solvent systems containing different methanol/water proportions. Analyte elution using acidified aqueous solution (pH about 3.5).

Table 15- Recoveries of kanamycin obtained in solid phase extraction using MIP and NIP after washing with solvent systems containing different methanol/water proportions

Methanol/ water (V/V) (%)	recoveries with MIP (%)	recoveries with NIP (%)
100/0	93	35
80/ 20	83	11
60 /40	68	9
40/60	50	10

In order to confirm the selectivity of the MIP towards kanamycin and other aminoglycosides, tests with the SPE cartridges were made. The SPE cartridges (1 mL syringe packed with 100 mg of either MIP or NIP) were previously conditioned with 5 mL of methanol and 10 mL of deionized water. The cartridges were loaded with a 200 µL aliquot of a 1 x 10^{-5} mol L^{-1} aminoglycoside standards solution of (kanamycin, neomycin, streptomycin, gentamycin, amikacin or tobramycin). A washing step was performed using 5 mL of methanol/water (80/20% v/v) and the elution was performed by using 0.5 mL of an acetic acid aqueous solution (pH about 3.5). The determination of these aminoglycosides were made by adding a small volume of the eluted and further neutralized solution into the TGA-CdTe quantum dost dispersions. The results showed that MIP retained high quantities of all of the tested aminoglycisides than NIP, showing the selectivity of MIP solid phase extraction towards the aminoglycosides (Figure 63).

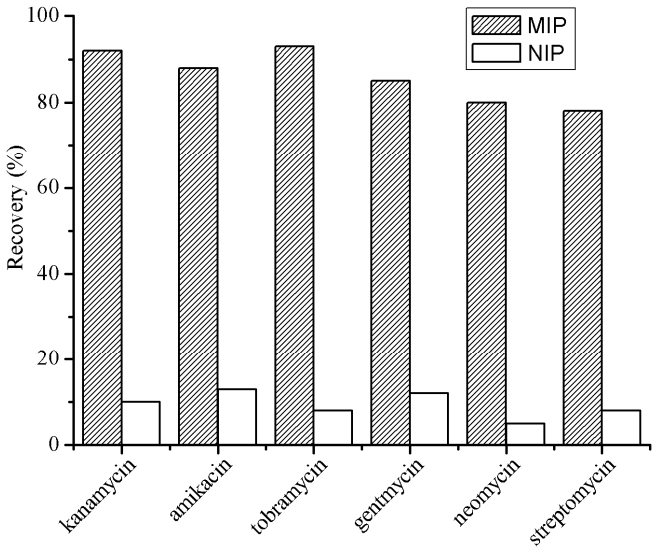

Figure 63 - Recoveries of kanamycin, amikacin, tobramycin, gentamycin, neomycin, streptomycin obtained by solid phase extraxtion using MIP and NIP: Aliquots of 200 µL of 1 x 10^{-5} mol L^{-1} aminoglycosides standard solutions.

6.8 Application of MIP solid phase extraction and TGA-CdTe probe

MIP solid phase extraction was used to selectively retain kanamycin from milk and water samples. Milk is a complex aqueous matrix in which proteins are the main constituents. Proteins do not only affect the interactions between analyte molecules (kanamycin) and MIP but the photoluminescence emission from quantum dots is susceptible to many biomolecules including proteins [162]. In order to minimize the chance of a small fraction of proteins being carried out to the eluate during the SPE procedure, a pre-clean up of milk samples using trichloroacetic acid (10% m/v) and n-hexane was carried out before performing MIP solid phase extraction.

The milk samples (whole and skimmed milk.) were fortified at concentration levels of 3.0×10^{-7} and 8×10^{-7} mol L^{-1} before performing any sample treatments. After elution and neutralization with NaOH to about pH 7, the solution was added to the TGA-CdTe quantum dots dispersion, The relative enhanced photoluminescence was measured and compared to the Langmuir model constructed with kanamycin standards. The average recoveries were in the range from 86.6 to 93.7% as indicated in Table 16. Although recoveries were satisfactory they may be improved by submitting the calibration standards to the SPE procedure.

Table 16 - Application of photoluminescent optical probe for determination of kanamycin in milk and water samples (n = 3).

Samples	Concentration of kanamycin ($\times 10^{-7}$ mol L^{-1})		Recoveries (%)
	Amount added	Amount found	
Whole milk	3.0	2.59 ± 0.05	86.6
	8.0	7.39 ± 0.31	92.6
Skimmed milk	3.0	2.72 ± 0.13	90.7
	8.0	7.49 ± 0.16	93.7
Stream water	0.25	0.20 ± 0.01	81.0

In order to evaluate the applicability of the proposed method in environmental samples, the analysis of stream water fortified with kanamycin at a concentration of 2.5 x 10^{-8} mol L^{-1} was carried out. The use of MIP solid phase extraction not only allowed the separation of kanamycin from water matrix but also allowed pre-concentration of analyte. The pre-concentration of kanamycin was performed by passing diluted solution (25 mL of 2.5 x 10^{-8} mol L^{-1}) through the MIP solid phase extraction cartridge. The kanamycin was diluted with 3 mL of deionized water contanting 3 x 10^{-7} mol per 1 L of nanoparticles in a cuvette (using mircopipette). The proceedure enabled an 8.4 fold analyte pre-concentration. The sensing with probe method resulted in recoveries (n = 3) of 81.0 % for the concentration of 2.5 x 10^{-8} mol L^{-1} after pre-concentration.