2 Experimental

2.1 Reagents and Materials

Ultrapure water (18.2 MΩ cm) was used for the preparation of all aqueous solutions and it was obtained from the Milli-Q gradient A10 ultra-purifier system (Milipore, USA). Captopril, histamine, L-thyroxine, kanamycin, tobramycin, gentamycin, amikacyn, streptomycin, neomycin, sodium borohydride (98% purity), tellurium powder (200 mesh, 99% purity), cadmium chloride pentahydrated (99% purity), mercaptopropinoic acid, 2-mercaptopropinonic acid, thioglycolic acid, cysteamine hydrochloride, 5, 5'-dithio (bis -2-nitrobenzoic acid), triethyl amine, histidine, tyrosine, phenylalanine, β-cyclodextrin, thyroxine, L-cysteine hydrochloride monohydrate, zinc acetate dihydrated were all purchased from Sigma-Aldrich (USA).

Sodium acetate, acetic acid, sodium hydroxide, anhydrous disodium phosphate, anhydrous sodium phosphate, citric acid, trichloroacetic acid, ascorbic acid, trisodium phosphate monohydrate, sodium carbonate, sulfanilic acid, sodium nitrite and hydrochloric acid were obtained from Merck (Germany). Hydrochlorothiazide was kindly donated by the Universidade de Brasília Pharmacology Department (Brazil) while lactose and silicon dioxide were provided by the Technology Institute of Fundação Oswaldo Cruz (Farmanguinhos, Rio de Janeiro).

Captopril and thyroxine pharmaceutical tablets were purchased in local drugstores: *Captopril EMS* - 25 mg of captopril per tablet; *Captopril Eurofarma* - 25 mg of captopril per tablet and *Levotiroxina sódica* - 200 μg of thyroxine per tablet. For histamine determination Fresh and canned tuna were purchased in local supermarkets and stored under refrigeration at 4 ^{o}C. Whatman quantitative

ashless filter paper grade No 41 and Syringe filters PURADISC 0.45 μm were purchased fromWhatman Ltd, UK. Nitrogen gas (99.99% purity) was from Lynde, Brazil.

2.2 Instrumentation

Photoluminescence measurements were made on a Perkin-Elmer LS-55 luminescence spectrophotometer (Perkin-Elmer, UK) with solutions placed in 1 cm optical pathlength quartz cuvettes. Photoluminescence spectra were acquired using the FL Winlab ® software and measurements were performed with 10 nm excitation and emission spectral bandpasses and 1000 nm/min scan rate. Emission spectra were made using excitation at a wavelength according to the Table 2.

Table 2 – Excitation and emission wavelengths according to each analyte.

Analytes	Excitation (nm)	Emission (nm)
Captopril	350	512
Histamine	350	515/545/580
Aminoglycosides	350	518/ 550/580
Thyroxine	312	424

A thermostatic system with stirring (PTP-1 Photoluminescence Peltier system with a PCB1500 water Peltier system, Perkin Elmer) was used to keep the solutions in the cuvette at specific constant temperatures. Photoluminescence lifetimes measurements were performed using an IBH-Horiba-Jobin Yvon TCSPC system. The light source used for excitation was a 365 nm NanoLED N-16 with 1.0 ns nominal pulse duration, 1 MHz repetition rate. UV-vis absorption spectra were made on Perkin-Elmer Lambda 15 spectrophotometer using 15 nm spectral bandpass.

Dynamic light scattering (DLS) measurements of the quantum dots were recorded in a Zetasizer Nano ZS (Malvern, UK), operating at 25°C using a He-Ne laser (633 nm) with measurement range from 0.6 nm to 6 mm and the analyzed range from 2 to 500 nm. Transmission electron microscopy (TEM) was made on JEOL 2010 Transmission Electron Microscope under 200 kV accelerating voltage (JEOL, Japan). Thermo Sorvall Biofuge Stratos centrifuge (Thermo Scientific, Germany) was used. For pH measurements, the pH-meter (MS Tecnopon, model MPA-210, Brazil) was employed. Raman spectra were obtained using a Perkin Elmer Raman Station 400 Raman Spectrometer.

2.3 Preparation of molecular imprinted polymer for group-selective recognition of aminoglycosides

Group selective molecularly imprinted polymer (MIP) for aminoglycosides like kanamycin, tobramycin, gentamycin, amikacin, streptomycin and neomycine were prepared by sol-gel process using kanamycin as the template molecule. The sol-gel MIP preparation procedure is simple and employs mild reaction conditions. In a typical synthesis, a 1 mL aqueous saturated solution of kanamycin is mixed with 320.0 μL of 3-aminopropyl trimethoxysilane (APTMS) and 265.0 μL of tetraethyl orthosilicate (TEOS) followed by the addition of 200.0 μL of 5% trifluoroacetic acid (as catalyst). The mixture was heated under magnetic stirring at 40 ^{o}C until the appearance of turbidity. The gel obtained was cooled to room temperature and to ensure dryness, these materials were kept for 12 h in an oven at 120 ^{o}C. The polymer was broken and washed with 200.0 mL of deionized water to remove the template molecule. The extraction of analyte (kanamycin sulfate) was monitored by CdTe-TGA photoluminescent probe (excitation at 350 nm). Finally, the polymer was dried at 120 ^{o}C and sieved to obtain regular size distribution of particles. The MIP was then stored at room temperature. The corresponding non-imprinting polymer (NIP) was prepared in the same manner except for the addition of kanamycin sulfate.

2.3.1 Evaluation of the molecular imprinted polymer for solid phase extraction of aminoglycosides

In order to achieve high extraction efficiency, the loading, the washing and the elution of samples should be carefully optimized. For the preparation of MIP solid phase extraction (SPE) column, 100 mg of MIP or NIP was packed into a 1 mL syringe that was coupled with syringe polyethylene filter (0.45 µm porous size). Before loading the samples, the column was conditioned with 5 mL of methanol and 10 mL of deionized water. A 200 µL of aliquot of each of the aminoglycoside solutions (1×10^{-5} mol L^{-1}) was loaded separately in SPE column. The conditions for washing and eluting were established based on the results obtained by measurements using the photoluminescent nanoprobe sensing. Approximately, 5 mL water/methanol (20/80% v/v) solution was selected as the washing solution and the retained analyte was eluted with 0.5 mL of acidic water (pH about 3.5).

2.4 Aqueous synthesis of quantum dots

2.4.1 Synthesis of CdTe modified with different ligands

The thyoglicolic acid modified CdTe nanoparticles (TGA-CdTe) were synthesized according to methods previously reported in the literature with minor modifications [145]. Briefly, 3.5 mmol of $CdCl_2 \cdot 2.5\ H_2O$ and 0.40 mmol of TGA were mixed in 50 mL of deionized followed by a pH adjustment to about 10.5 (using small volumes of 1 mol L^{-1} NaOH solution). Then the solution was placed in a three necked 100 ml round bottom flask and purged with nitrogen gas for 10 min. Then 2 mL of a freshly prepared NaHTe aqueous solution was injected into the reaction system under stirring and under nitrogen protection. Quantum dots with different sizes were prepared by varying the reflux time under heating at about 100 ^{o}C. The resultant dispersion was called stock dispersion of nanoparticles. The 2-mercaptopropionic modified CdTe nanoparticles (2MPA-CdTe) were synthesized using a similar procedure but employing 0.30 mmol of 2MPA instead of TGA. In order to investigate the mechanism of interaction and

role of TGA as the surface modifier, cysteamine modified CdTe nanoparticles were also synthesized according to literature procedure [146].

2.4.2 Synthesis of L-cysteine modified ZnS nanoparticles

The ZnS nanoparticles modified with cysteine were synthesized as reported in literature with slight modification [147]. In typical synthesis, 0.0214 g (1 x 10^{-4} mol) of zinc acetate dihyrdrated and 0.0172 g (1 x 10^{-4} mol) of L-cysteine hydrochloride monohydrated were dissolved in 100 mL of deionized water. Then, the solution was stirred for 15 min and the pH of the system was adjusted round to 9.0 by the addition of small volumes of 0.1 mol L^{-1} of NaOH solution. The resultant solution was poured to 150 mL three necked round bottom flask (reactor). A solution containing 2 x 10^{-4} mol of Na_2S in 10 mL deionized water was slowly injected through the syringe to the reactor under nitrogen protection. The Zn: S: cysteine molar ratio employed was 1:1:2. Then the mixture was refluxed (about 100 ^{0}C) for specific period of time. Aliquots of the reaction mixture were taken out at different intervals of time, through the syringe, to check the absorption and photoluminescence characteristics. The transparent and colorless colloidal dispersion of ZnS was treated with ethanol to precipitate the nanoparticles, then, the solid mass was dissolved in phosphate buffer solution (0.01 molL^{-1} and pH 8.5).

2.4.3 Procedure of quantum yield determination

Quantum yields (ϕ) were determined by preparing a quantum dot dispersion and a reference standard solution (rhodamine B). Photoluminescence measurements were recorded by exciting both (CdTe and rhodamine B) at 355 nm. The integrated photoluminescence intensity was calculated by measuring the entire area under the emission spectrum. In order to minimize the self-absorption, the absorbance was kept below 0.12. The measurement procedure involves the comparison of ϕ of the standard solution with the one of the dispersions, therefore, from the plot of absorbance in function of the integrated photoluminescence of the

standard and dispersions, the ϕ value could be calculated as indicated in Equation 2.1.

$$\phi_x = \phi_{ST} \left[\frac{Grad_x}{Grad_{ST}} \right] \left[\frac{\eta_x^2}{\eta^2_{ST}} \right] \quad (2.1)$$

where ϕ_x (ϕ_{ST}), $Grad_X$ ($Grad_{ST}$) and η_X (η_{ST}) represent respectively the quantum yield, the slope of the integrated photoluminescence intensity versus absorbance, and the solvent refractive index of the quantum dots dispersion and standard solutions (rhodamine B). In this experiment, the diluted modified CdTe with 2MPA and TGA nanocrystals (approximately 2 x 10^{-5} mol L^{-1}) were dispersed in deionized water (η_{water} = 1.33) and the diluted solution of rhodamine B (1x 10^{-4} mol L^{-1}) was dissolved in ethanol ($\eta_{ethanol}$ = 1.36). Under such conditions, the standard has a QY of 89% [148].

2.5 Photoluminescence measurements and samples preparation for determination of captopril, histamine, kanamycin and thyroxine

2.5.1 Photoluminescence measurements for sensing histamine

For the determination of histamine, 10 μL volume of the TGA-CdTe nanoparticles aqueous stock dispersion (estimated 2.8 x 10^{-5} mol of nanoparticle dispersed in 1 L of solution) was added into 3 mL aqueous solution containing 0.2 mL of pH 8.5 phosphate buffer. This dispersion was titrated by successive additions of 10 μL of stock solution of histamine (1 x 10^{-2} mol L^{-1}). The titration was performed manually by using a micropipette and the addition was made into the cuvette in which the content was kept under constant stirring. After the addition of histamine the system was allowed to react for 2 min. The stirring was tuned off and the solution allowed to achieve equilibrium before performing the photoluminescence measurements (excitation at 350 nm). The wavelength chosen for the photoluminescence detection depended upon the average size of the nanoparticles in the dispersion. For final analysis, nanoparticles with average

diameter of 2.1 nm were used, which resulted in photoluminescence measurements at 515 nm. All results were corrected for dilution

2.5.2 Procedure for extraction of histamine from tuna fish

Histamine was extracted from the flesh of either fresh tuna or canned tuna according to a procedure reported in the literature with minor modifications [149]. About 5.0 g of tuna flesh, taken from the skinless dorsal part of the fish, was transferred to a 50 mL Teflon tube and mixed with trichloroacetic acid (2.5% in volume) aqueous solution. The sample was grinded and homogenized for 2 min using a blender. Then, the mixture was centrifuged at 23,000 rpm for 15 min at 15 ^{0}C. A specific volume of extract was neutralized and filtered through a filter paper. The extract was then passed through a cationic solid phase cartridge (Amberlite CG-50). The cartridge was then washed several times with acetate buffer (pH 4.6) before the histamine, retained in the column, is recovered with 0.5 mL of HCl solution (0.2 mol L^{-1}). The pH of the eluted solution containing histamine was adjusted to about 7 before the addition of an aliquot of the sample into the nanoparticle dispersion. A trichloroacetic acid (2.5%) solution was used as blank solutions and submitted to the same procedure of solid phase extraction.

2.5.3 Colorimetric method for determination of histamine

For colorimetric determination of histamine (reference method), p-phenyldiazonium sulfonate was employed to enable chemical derivatization. The derivatization solution was prepared by mixing 1.5 mL of a 0.9% (w/v) sulfanilic acid solution with a 4% hydrochloric acid aqueous solution and 1.5 mL 5% (w/v) sodium nitrite aqueous solution in a 50.00 mL volumetric flask and kept in ice bath for 5 min. A further addition of 6 mL more of 5% sodium nitrite solution was added and after 5 min, the volume was adjusted with distilled water. The reagent was stored in ice bath and used after 15 min of preparation. In a clean tube containing 5 mL of 1.1% sodium carbonate aqueous solution was mixed (in a slowly fashion) with 2 mL of the prepared reagent (chilled solution). Then, different concentrations of the standard solution of histamine and samples (after extraction through procedure in 2.5.2) were added. The absorbance was measured after 5 min at 496 nm [111]

2.5.4 Photoluminescence measurements for determination of kanamycin

The photoluminescent nanoprobe dispersions used for the sensing of kanamycin and others aminoglycosides were prepared by mixing 15 μL of TGA-CdTe nanoparticles in 5 mL aqueous solution containing 0.5 mL of phosphate buffer solution (0.01 mol L^{-1}, pH around 8). In order, to construct analytical curves, different volumes of a stock solution (either 5 x 10^{-5} or 1 x 10^{-4} molL^{-1}) of kanamycin, neomycin, streptomycin, gentamycin, amycacin and tobramycin were added to nanoparticles dispersion inside the cuvette. These additions (titration) were made manually using micropipette with the content inside the cuvette under constant stirring. After the addition of aminoglycosides, the system was allowed to react for 30 s. The stirring was turned off to equilibrate the solution, then, photoluminescence measurements were made with excitation at 350 nm. The quantum dots dispersion prepared in the same manner but without addition of aminoglycosides served as control (blank) and the photoluminescence measurement of control guarantee a reliable value for photoluminescence emission intensity.

2.5.5 SPE for milk and water samples

For the determination of kanamycin in whole and skin milk samples, 5,00 mL of the sample was diluted to 10,00 mL with deionized water and fortified with kanamycin at two concentration levels (2.5 x 10^{-7} and 8 x 10^{-7} mol L^{-1}). Then, 5.00 mL of the sample was transferred to a tube and centrifuged, at 4 $^{\circ}$C, using 3100 x g for 30 min. The solid fat that was at the top of the tube was removed with a spatula. The liquid part was transferred to another centrifuge tube and mixed with 0.5 mL of an aqueous solution of a trichloroacetic acid (10 % m/v). The mixture was vortex mixed for 30 s and again centrifuged (4 $^{\circ}$C and 3100 x g) for 30 min. The supernatant was separated and mixed with 1 mL of n-hexane. After vigorous shaking, followed by centrifugation, the organic portion was discarded and different volumes of aqueous portion containing analyte were

loaded into the SPE cartridge loaded with the MIP. After loading with the sample, the SPE cartridge was thoroughly washed with 5 mL of a water/methanol (20/80% v/v) solution to ensure the removal of interfering substances. Finally the sample was eluted with 0.5 mL acidic water (pH about 3.5). For the sensing of kanamycin, volumes of the eluate were added to the quantum dots dispersion and from the relative enhanced photoluminescence the concentration was measured. For environmental samples (stream water), the analyte fortified sample was passed through a 0.45 µm Teflon filter and then subjected to MIP solid phase extraction.

2.5.6 Photoluminescence measurements for determination of captopril

The stock solution of captopril (1.0×10^{-2} mol L^{-1}) was prepared by dissolving 22 mg of the analyte standard in deionized water and adjusting final volume to 10.00 mL in a volumetric flask. Standard solutions of lower concentration were prepared by further dilution of the stock solution with deionized water. 2MPA-CdTe quantum dots aqueous dispersions were prepared by mixing 100 µL of the synthetized quantum dots in a 5 mL volumetric flask containing 0.5 mL of the pH 9 phosphate buffer solution (0.01 mol L^{-1}). Then, volumes (varying from 10 to 100 µL) of sample or captopril standards were added before the volume of the mixture was adjusted with deionized water. These dispersions were transferred to quartz cuvettes in order to measure their photoluminescence at 510 nm upon excitation at 350 nm. Control measurements were made using quantum dots dispersions prepared in the same manner but without the additions of captopril.

2.5.7 Determination of captopril in human serum and tablets

For pharmaceutical analysis, a pool of 10 tablets of captopril medicine were grinded inside an agate mortar and then, an aliquot of the resultant powder was placed in water and vigorously shaken and then placed inside an ultrasonic bath for 20 min. Insoluble excipients and concomitants were separated by passing the solution through 0.45 µm pore size PVDS syringe filter. For determination of

captopril in human serum, a 1 mL aliquot of serum sample (from a healthy female volunteer) was diluted with deionized water and fortified with the captopril aqueous standard. The mixture was vigorously shaken and its final volume of solution was adjusted to 5.00 mL using deionized water. This solution was passed through C_{18} cartridge (500 mg packed in a 5 mL syringe). The cartridge was washed with water then all captopril was eluted 1 ml methanol and the collected solution was analysed using the proposed approach.

2.5.8 The Ellman´s method for determination of captopril

The Ellman´s method was adapted and used as reference method for the determination of captopril. The stock solution (2×10^{-3} mol L^{-1}) of 5, 5'-dithio (bis -2-nitrobenzoic acid) (DTNB) was prepared in phosphate buffer solution (0.1 mol L^{-1}, pH 7). For the calibration curve, 3 mL deionized water, 200 μL of DTNB and 400 μL of phosphate buffer solution were mixed in a cuvette. Different amount of captopril standard solution were added. The solution was allowed to stand for 5 min and then absorbance was measured at 412 nm. The blank was prepared with same procedure but without addition of captopril**.**

2.5.9 Photoluminescence measurements for determination of thyroxine

The stock solution of L-thyroxine (1.0×10^{-2} mol L^{-1}) was prepared by dissolving specific amounts of the drug standard in sodium hydroxide solution (5×10^{-4} mol L^{-1}). Standard solutions of lower concentration were prepared by further dilution of this stock solution. For determination of L-thyroxine, cysteine-ZnS nanoparticle dispersion, prepared in phosphate buffer (0.01 molL^{-1}, pH, 8.5) was transferred to a quartz cuvette. Photoluminescence measurements were made at 424 nm upon excitation at 312 nm. Volumes of sample or thyroxine standards were added to the nanoparticles dispersion (titration) at room temperature and under constant stirring. Measurements were made 5 min after the stirring was stopped. In order to obtain reliable quantitative results, measurements were also taken from cysteine-ZnS dispersion without the addition of thyroxine (control).

2.5.10 Sample preparation for analysis of thyroxine in pharmaceutical formulation and human saliva

For the determination of thyroxine in pharmaceutical formulation, pulverized commercial tablets were dissolved in water. The sample was then passed through C18 SPE column to retain thyroxine. The cartridge was washed with deionized water (to remove water soluble tablets recipients) and the thyroxine was eluted with 1 mL of methanol. The methanol was evaporated and the remaining residue was dissolved in ZnS nanoparticles buffered solution. The saliva sample (10 mL in graduated cylinder) was collected from a human female euthyroid volunteer that did not receive any other medical treatment nor ingested food or beverages prior the sample collection. The volunteer rinsed his mouth for 5 min with deionized water. The saliva fortified with known amount of thyroxine was mixed with 5 mL of ethanol then mixture was vortex mixed for 30 s and immediately centrifuged for 15 min at 3000 rpm. After centrifugation, the supernant was passed through C18 SPE column and washed with deionized water. After elution with 1 mL of methanol, the eluate was evaporated and the residue re-suspended with the nanoparticle dispersion (made in phosphate buffer pH 8.5). The same procedure was repeated for blank measurements.