



## Detecting Ni(II) in aqueous solution by 3-(2-pyridyl)-[1,2,3]triazolo[1,5-*a*]pyridine and dimethyl- $\beta$ -cyclodextrin



Carolina Jullian<sup>a,\*</sup>, Samuel Fernández-Sandoval<sup>a</sup>, Max Rojas-Aranguiz<sup>a</sup>, Horacio Gómez-Machuca<sup>a</sup>, Paola Salgado-Figueroa<sup>b</sup>, Cristián Celis-Barros<sup>c</sup>, Gerald Zapata-Torres<sup>c</sup>, Rosa Adam<sup>d</sup>, Belén Abarca<sup>d</sup>

<sup>a</sup> Departamento Química Orgánica y Físicoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Chile

<sup>b</sup> Centro de Estudios para el desarrollo de la Química, Cepedeq, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Chile

<sup>c</sup> Departamento Química Analítica e Inorgánica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Chile

<sup>d</sup> Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Valencia, Spain

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### ABSTRACT

A new supramolecular sensitizer for nickel(II) ion in aqueous solution based on a pyridyltriazolopyridine-cyclodextrin inclusion complex is proposed. The inclusion complexation behavior, characterization and binding ability of pyridyltriazolopyridine (PTP) with dimethyl- $\beta$ -cyclodextrin (DM $\beta$ CD) has been investigated both in solution and solid state by means of absorption, fluorescence, <sup>1</sup>H NMR, DSC, and molecular modeling methods. The stoichiometry of the inclusion complex is 1:1, and the thermodynamic studies indicate that the inclusion of PTP is mainly an entropic driven process. The 2D NMR studies revealed that the pyridyl-triazolopyridine is included by both sides of cyclodextrin which are in good agreement with the docking results. The fluorescence changes upon addition of divalent cations to the inclusion complex indicate a high selectivity and sensitivity for Ni<sup>2+</sup> by fluorescence quenching in neutral aqueous solution.

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## 1. Introduction

The active development of new fluorescent chemosensors for sensing metal ions has become an important goal in chemistry and biology. Specially, selective chemosensors for ions of transition metals in aqueous environments are relatively rare due to they are rather difficult to recognize directly in these environments (de Silva et al., 1997; Valeur & Leray, 2000), even though transition metal ions are fairly easy to chelate and detect in organic solvents.

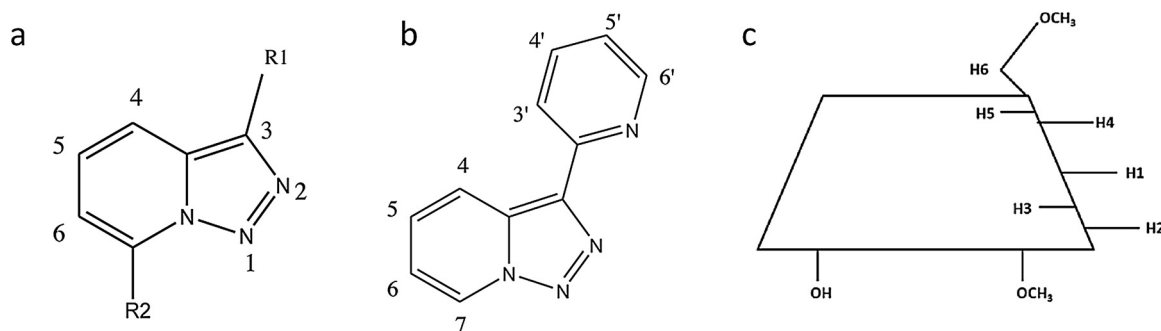
Nickel is an important element among the various heavy metals; it is used in many industries, catalytic processes, and it is present in various effluents. Nickel is a moderately toxic element compared to other transition metals, but its accumulation in the human body by chronic exposure can lead to lung fibrosis, cardiovascular and kidney diseases, the most serious concerns relate to nickel's carcinogenic activity (Denkhaus & Salnikow, 2002). Thus, selective

monitoring of Ni<sup>2+</sup> in industrial, environmental and food samples is needed.

The fusion of an electron donating group such as a triazole ring and a pyridine moiety as an electron acceptor group forms a triazolopyridine which conjugated with aromatic substituents on the 3- and/or 7-positions leads to a molecule endowed with useful fluorescent properties (Abarca, Aucejo, Ballestros, Blanco, & García-España, 2006), Fig. 1a. Interestingly, the metal ion coordination to the donor groups changes the efficiency of the intramolecular charge transfer changing the fluorescence spectra, so using this feature, the triazolopyridine system has been used as metal sensor. Most of the metal recognitions reported by this system has been carried out in non-aqueous media or in water-organic solvents mixtures (Ballesteros-Garrido et al., 2009; Chadlaoui et al., 2006; Reddington et al., 1998). The disadvantage of these compounds is their low aqueous solubility to be used as chemosensors under physiological condition.

On the other hand, cyclodextrins (CDs) are nontoxic macrocyclic oligosaccharides which are provided with a hollow hydrophobic interior and a hydrophilic outer surface (Connors, 1997). Due to these characteristics, CDs form inclusion complexes with a wide

\* Corresponding author. Tel.: +56 2 29782859; fax: +56 2 29782868.  
E-mail address: [cjullian@uchile.cl](mailto:cjullian@uchile.cl) (C. Jullian).



**Fig. 1.** Structures of 3- and/or 7-substituted-[1,2,3]triazolo[1,5-*a*]pyridine (a), 3-(2-pyridyl)-[1,2,3]triazolo[1,5-*a*]pyridine (PTP) (b), and 2,6-*O*-di-methyl- $\beta$ -cyclodextrin (DM $\beta$ CD) (c).

variety of organic compounds, where the inclusion of the less polar portion of the guest within the cavity and the polar or hydrophilic part of the guest remains exposed to the solvent is the most likely mode of binding. Thus, the guest induces the removal of water molecules from the interior cavity, changing its physicochemical properties which in turn increase drug solubility. Nevertheless, natural CDs have limited water solubility, to deal with this limitation, alkyl moieties such as hydroxyalkyl or methyl on free hydroxyl groups of CD has been introduced enhancing considerably the cyclodextrin solubility. The complexing ability of cyclodextrin derivatives compared to their natural counterparts is significantly modified. For example, 2,6-*O*-di-methyl- $\beta$ -cyclodextrin, DM $\beta$ CD, shows higher affinity for different host as well as higher solubilizing ability compared with natural cyclodextrins (Folch-Cano et al., 2011; Jullian, 2009; Jullian et al., 2008; Wang, Ouyang, Hao-Liang Yuan, & Liu, 2013).

This work was undertaken in order to improve the water solubility of 3-(2-pyridyl)-[1,2,3]triazolo[1,5-*a*]pyridine (PTP), Fig. 1b, through complexation with DM $\beta$ CD, Fig. 1c. The association constants, estimated from fluorescence studies at different temperatures were analyzed and used in order to get information on the plausible thermodynamic mechanisms involved in the association process. In addition, the results of 2D-NMR studies of the inclusion complex provided useful insights regarding the orientation of PTP in the DM $\beta$ CD nano-cavity which was later rationalized and contrasted with docking studies. Finally, this binary complex was proved as a chemosensor upon the presence of different bivalent cations in aqueous solutions, indicating that the inclusion complex displays a highly selective and sensitive response of fluorescent signal toward nickel. According to these results and based on this phenomenon, an accurate and rapid spectrofluorimetric detection method for monitoring the level of nickel(II) in environmental and biological samples is proposed.

## 2. Material and methods

### 2.1. General experimental information

The UV absorption measurements were carried out on an Agilent 8453 spectrophotometer and fluorescence studies of complexes were performed on a LS 55 Perkin-Elmer spectrofluorometer equipped with a xenon lamp source and thermostated bath. Fluorescence spectra were made with excitation and emission slits of 15 and 10 nm respectively. All the samples have an absorbance less than 0.1 to avoid inner filter effect.

Differential scanning calorimetry (DSC) measurements of the pure materials and binary system were carried out using a FP900 DTA DSC. The thermal behavior was studied by heating 3–5 mg of samples in flat-bottomed aluminum crucible in the range of 40–220 °C, at a rate of 10 °C/min.

NMR spectra were recorded on a Bruker Avance DRX-300 operating at 300.13 MHz for <sup>1</sup>H. Rotating-frame overhauser effect spectroscopy (ROESY) spectra were acquired in the phase sensitive mode with the same spectrometer and Bruker standard parameters.

The 3-(2-pyridyl)-[1,2,3]triazolo[1,5-*a*]pyridine was synthesized according to method described earlier (Abarca, Ballesteros, & Elmaznaouy, 1998). DM $\beta$ CD (Heptakis-2,6-*O*-dimethyl- $\beta$ -cyclodextrin) and metal perchlorate salts (Zn<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Mg<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Cd<sup>2+</sup>, Mn<sup>2+</sup>, Ca<sup>2+</sup>) were purchased from Sigma–Aldrich, Inc., St. Louis, MO. All solvents employed in the spectrophotometric analyses were of spectroscopic reagent grade. Deionised water from Milli-Q system apparatus (Millipore Corp., Billerica, MA) was used throughout the experiments.

Inclusion complexes were obtained by mixing an appropriate amount of stock solution of PTP dissolved in methanol with a buffered solution of DM $\beta$ CD where the final solution contains less than 1% of methanol. For the determination of association constants the concentration of PTP was  $1 \times 10^{-5}$  M and increased buffered solution of DM $\beta$ CD was added. The resulting mixture was equilibrated in a Precision thermostatic shaking water bath for 24 h after which the equilibrium was reached.

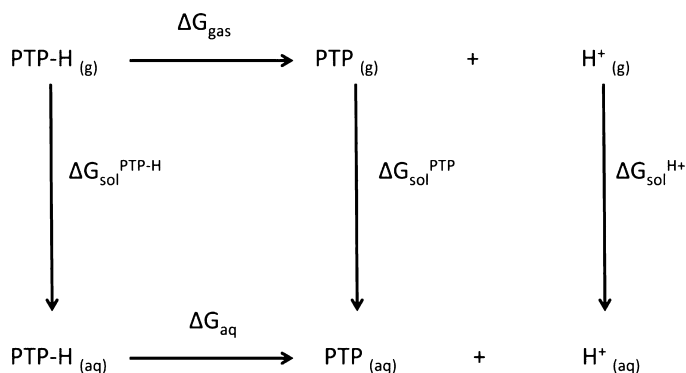
The stoichiometry of inclusion was determined by the method developed by Job (1928). Equimolar solutions of PTP and CD were mixed to a standard volume, varying the molar ratio but keeping the total concentration of the species constant. After stirring for 24 h, the absorbance at 310 nm was measured for all solutions and  $\Delta A = A - A_0$ , the difference in absorbance in the presence and in the absence of CD, was plotted against *R*;  $R = [\text{PTP} - \text{DM}\beta\text{CD}] / ([\text{PTP}] + [\text{DM}\beta\text{CD}])$ .

Solid-state PTP complex with DM $\beta$ CD in 1:1 molar ratios were prepared. Methanol solution of PTP was added drop wise to an aqueous solution of DM $\beta$ CD, and the reaction mixture was stirred at 50 °C for 12 h. After cooling to room temperature, the solution was frozen overnight and then lyophilized over period of 24 h using freeze-drier, Labconco freeze dry system.

The effect of various metal ions on the determination of Ni<sup>2+</sup> was investigated by analyzing the sample solutions containing  $1 \times 10^{-5}$  M of PTP in ethanol-phosphate buffer pH 7.4 or lyophilized complex, and  $1 \times 10^{-4}$  M interfering metal ions. Ni<sup>2+</sup> was added at a concentration of  $1 \times 10^{-4}$  M into above solutions. Before fluorimetric detection, the mixed solution was allowed to stand for 5 min to allow complete formation of stable solution.

### 2.2. Computational details

All pK<sub>a</sub> values were calculated through a thermodynamic cycle (Scheme 1) and the values obtained were put on Eqs. (1) and (2). All structures were optimized at B3LYP/6-31G(d,p) level of theory in order to find the lower energy geometry. Then, with



**Scheme 1.** Thermodynamic cycle used to calculate as  $pK_a$ . PTP-H refers to the protonated structure and PTP refers to the deprotonated structure.

the aim of obtaining the gas-phase free energy it was carried out a frequency single point calculation at a higher basis set B3LYP/6-311++G(2d,2p). In the solvation free energy calculation, SMD solvation model was used and the same level of theory and basis set was used as in the gas-phase case.

$$\Delta G_{aq} = \Delta G_{gas} + \Delta G_{sol}^{PTP} + \Delta G_{sol}^{H^+} - \Delta G_{sol}^{PTP-H} \quad (1)$$

$$pK_a = \frac{-\Delta G_{aq}}{2.303kT} \quad (2)$$

Autodock Vina program was used to carry out docking studies. This software handle docking as a stochastic global optimization of the scoring function, precalculating grid maps and the interactions between every atom type pair at every distance. In order to carry out docking studies it was necessary the 2,6-methoxy- $\beta$ CD modeling. Therefore, it was extracted the  $\beta$ CD ( $\beta$ cyclodextrin) from the crystallized structure of Cyclodextrin Glycosyltransferase (PDB code: 3CGT). Then, through XLeap implemented in AMBER program the methyl groups were added. Next, the new structure was optimized in order to obtain the right position of the methoxyl groups. Finally the PTP ligand was modeled and optimized using Gaussian98 at B3LYP/6-31G(d,p) level of theory.

### 3. Results

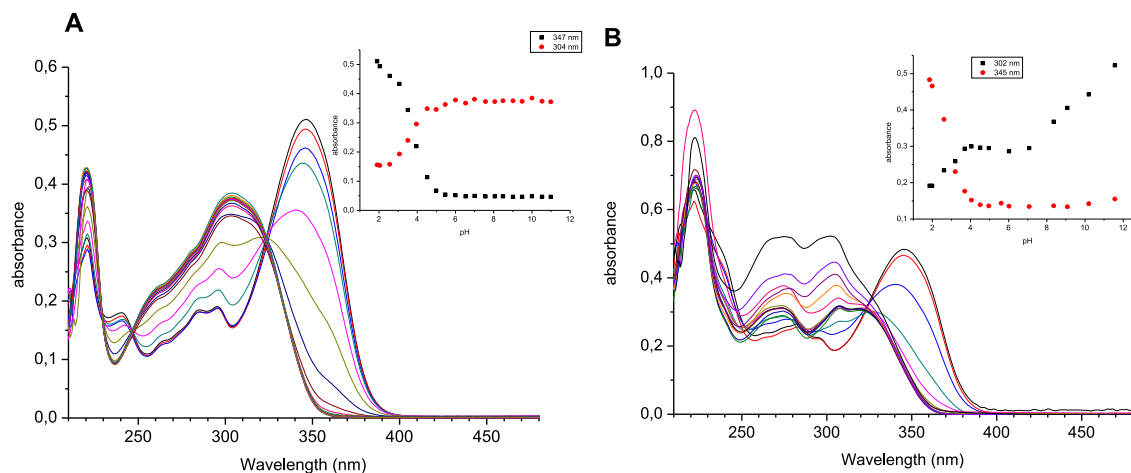
Since the use of fluorescent probes based on electron donor/acceptor moieties in the detection of metal ions are very sensitive to the media pH value, it is necessary to consider the pH

effects and to determine an optimal sensing condition. Thus, the influence of pH in the inclusion complex was investigated recording absorption spectra of PTP at various pH values. From Fig. 2A, it can be observed a maximum at 347 nm at low pH values and at higher pH (over pH 5) a new peak at 304 nm appeared whose intensity increased with an increase in pH. With the increment of pH a decrease in the intensity of the 347 nm peak and a concomitant increase in the intensity of 304 nm band is observed. This observed isobestic point indicates the presence of two species, namely the neutral and protonated. From the plot of absorbance versus pH it was possible to obtain only one  $pK_a$  value for PTP of 3.7 indicating that only one nitrogen atom of the two possible could be protonated, the pyridyl nitrogen ( $N_p$ ) and nitrogen 2 of the triazolopyridine ( $N_{TP}$ ). In order to ascertain which one becomes protonated, high accuracy  $pK_a$  calculations were carried out using quantum chemical calculations. The theoretical results indicated that the  $pK_a$  values for  $N_{TP}$  and  $N_p$  were  $-1.4$  and  $4.0$ , respectively. According to these values, it is clear that the protonation of the nitrogen of the pyridine ring correspond to the protonation observed in the above described pH study.

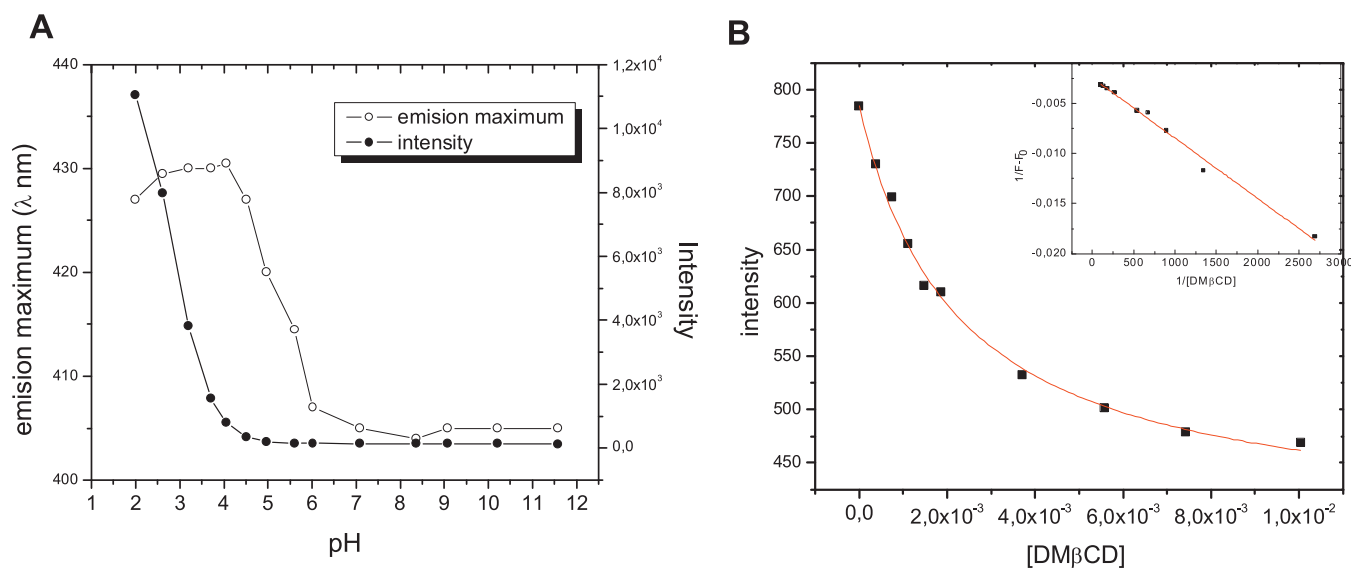
The absorption spectra of the inclusion complex PTP-DM $\beta$ CD of the different prototropic species are shown in Fig. 2B. It can be observed a maximum at 345 nm at low pH values for the cationic form and over pH 3 a new peak at 302 nm appeared which correspond to the neutral form. From the plot of absorbance versus pH values, the  $pK_a$  value of the inclusion complex PTP-DM $\beta$ CD was calculated to be  $pK_a = 2.9$ . This value is lower than the free ligand, which indicates that once the ligand is inside the cyclodextrin became more acidic than compared to the free ligand.

Once determined the optimal condition for the fluorescence study, we excited at the isobestic point, and the emission peak appears at 430 nm at pH 2.0. When the pH is increased the fluorescence band is blue shifted (405 nm) and the intensity of the newly fluorescent band decreases dramatically, suggesting the deprotonation of the pyridine nitrogen. Fig. 3A shows the pH-dependence of PTP fluorescence in the inclusion system, PTP-DM $\beta$ CD. Also, it can be noticed that the fluorescence intensity of the PTP-DM $\beta$ CD complex is constant at pH 6.0–12.0. At lower pH, the results indicate a bathochromic shift of the maximum emission of PTP, the fluorescence intensity increase progressively up to pH 4 to reach a constant emission.

The effect of DM $\beta$ CD on the fluorescence spectra of PTP decrease the emission intensity indicating that the triazolopyridine is entrapped into the cyclodextrin cavity forming an inclusion complex. Owing to the intensity and maxima of the emission band



**Fig. 2.** (A) Absorption spectra of PTP as a function of pH, *Inset*: variation of the absorbance at 347 (■) and 304 (●) at different pH. (B) Absorption spectra of PTP-DM $\beta$ CD as a function of pH, *Inset*: variation of the absorbance at 345 (■) and 302 (●) at different pH.



**Fig. 3.** (A) Effect of pH on the fluorescence emission intensity (●) and emission maximum (○) of pyridyltriazolopyridine. (B) Nonlinear curve fitting of PTP upon addition of increasing concentration of DMβCD. *Inset:* Linear curve fitting according to Benesi–Hildebrand equation.

is constant at pH 6.0–10.0, a final pH 7.4 was chosen as the ideal experimental conditions to determine the association constants to further thermodynamic studies.

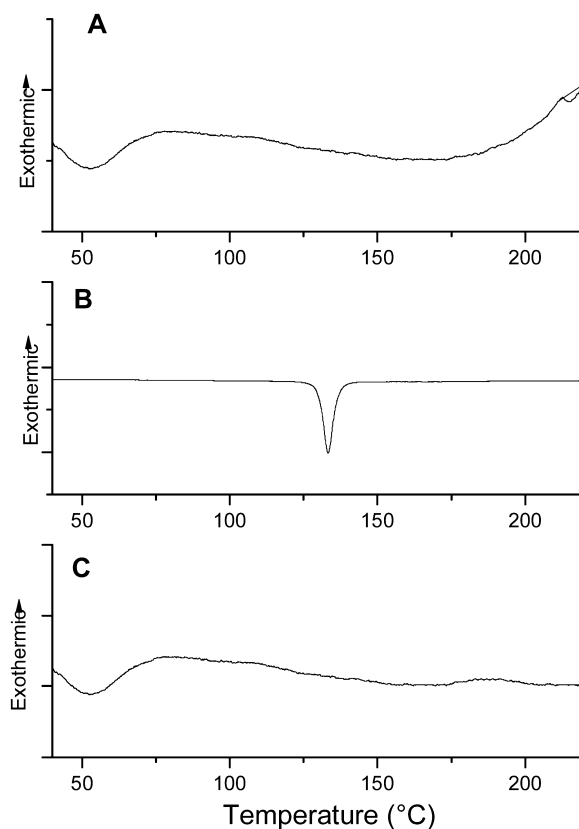
In order to understand the binding stoichiometry of PTP-DMβCD complex, we carried out the Job plot experiments. The maximum absorbance occurred at 0.5 mole fraction, which indicates a 1:1 ratio for PTP-DMβCD complex (data not shown).

When increasing concentrations of DMβCD are added to the reaction medium, the fluorescence intensity decreased until a minimum was reached (Fig. 3B). The representation of  $1/(F - F_0)$  versus  $1/[DM\beta CD]$  (double reciprocal plot) (Benesi & Hildebrand, 1949), known as a Benesi–Hildebrand plot (inset Fig. 3B), leads to a straight line corroborating the 1:1 stoichiometry obtained by the continuous variation method.

Nonlinear least-squares regression analysis (NLR) is an alternative approach to the graphical method. It requires preliminary parameter estimates which are determined from a linear regression approach. By use of a nonlinear regression program, the data are directly fitted into Eq. (3) (Connors, 1987).

$$F = F_0 + \frac{(F_\infty - F_0)K[DM\beta CD]}{1 + K[DM\beta CD]} \quad (3)$$

where  $F_\infty$  is the fluorescence intensity when total PTP has been complexed in CDs,  $F_0$  is the fluorescence of PTP in the absence of CDs, and  $F$  is the observed fluorescence at each CDs concentration tested. The NLR program estimates  $K$  by fitting the data through iteration and these representations showed a good correlation with the experimental comportment observed when DMβCD concentration was increased. The association constants,  $K_a$ , of PTP-DMβCD at different temperatures were determined and the results are summarized in Table 1, which shows that the association constant for the PTP-DMβCD complexes increase with increasing temperature,



**Fig. 4.** Differential scanning calorimetric (DSC) thermograms of: (A) DMβCD, (B) PTP and (C) inclusion complex, PTP-DMβCD.

**Table 1**  
The association constant of PTP-DMβCD complex at different temperatures and thermodynamic parameters.

	$K_a$ (M <sup>-1</sup> ), 303 K	$R$	$K_a$ (M <sup>-1</sup> ), 308 K	$R$	$K_a$ (M <sup>-1</sup> ), 312 K	$R$	$\Delta S$ , kJ mol <sup>-1</sup> K <sup>-1</sup>	$\Delta H$ , kJ mol <sup>-1</sup>	$\Delta G$ , kJ mol <sup>-1</sup>
TP-DMβCD	431	0.987	444	0.995	512	0.998	0.094	13.529	-14.483

**Table 2**  
 $^1\text{H}$  NMR shifts,  $\delta$  (ppm) for PTP in the absence and in the presence of DM $\beta$ CD in  $\text{D}_2\text{O}$ .

	PTP protons $\delta$ (ppm)								DM $\beta$ CD protons $\delta$ (ppm)			
	H4	H5	H6	H7	H6'	H5'	H4'	H3'	H3	H5	H6a	H6b
Free	8.21	7.46	7.18	8.76	8.53	7.33	nd	nd	3.90	3.81	3.65	3.65
Complexed	8.35	7.54	7.27	8.85	8.59	7.32	7.86	8.06	3.73	3.61	3.49	3.15

nd: not detected.

as expected for an endothermic process. The integrated form of the van't Hoff equation (Eq. (4))

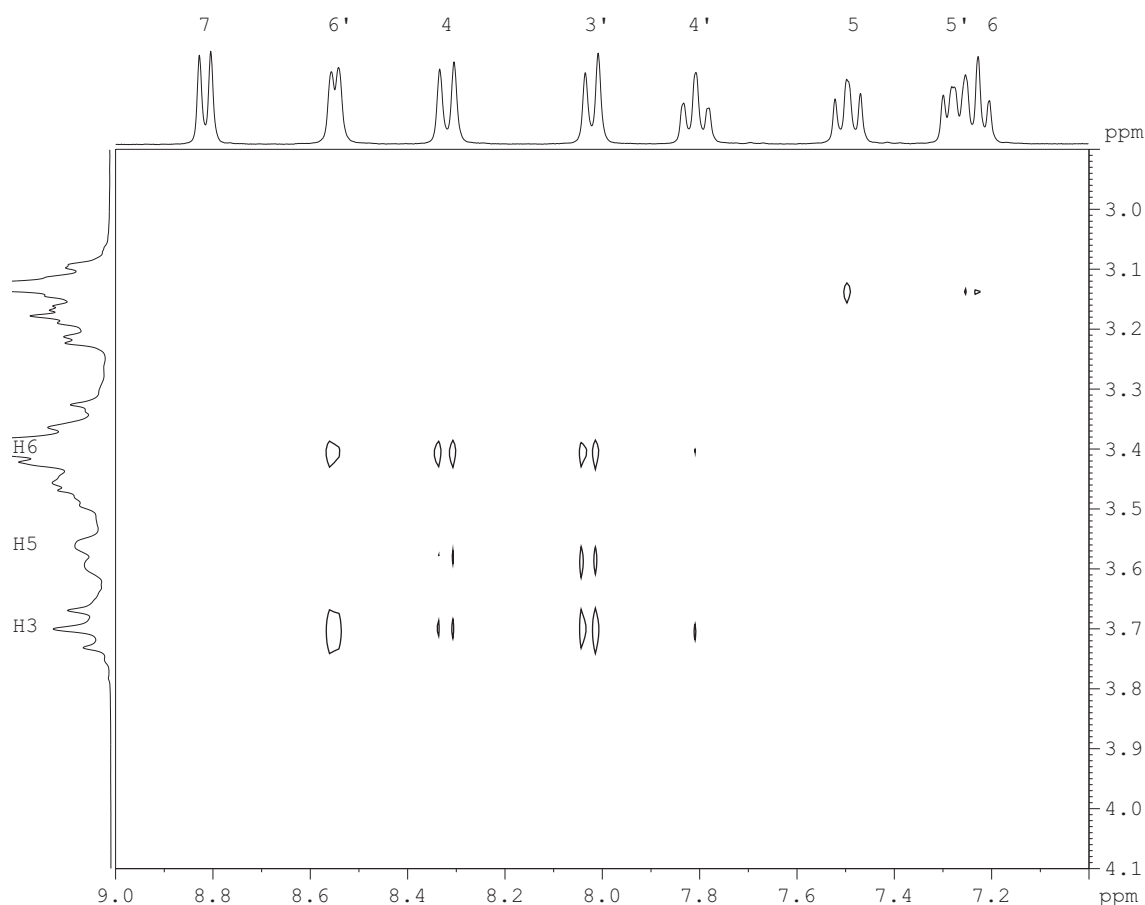
$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (4)$$

allows for calculations of enthalpy and entropy changes from variations of the stability constants with temperature. The negative value for the Gibbs energy means that the binding process is a spontaneous process and thermodynamically favored.  $\Delta H$  and  $\Delta S$  are positive in the experimental range, which indicates that this inclusion is entropically driven. Apparently, when pyridyl-triazolopyridine is free in solution, it seems to have a strong interaction with its solvent shell. Upon binding, this solvent shell is broken up, leading to the partly unfavorable enthalpic change.

The thermal properties of PTP, DM $\beta$ CD, and the inclusion complex were investigated using DSC experiments; the results indicated a broad endothermic peak at approximately  $50^\circ\text{C}$  for DM $\beta$ CD as a result of the amorphous nature. PTP exhibited one sharp endothermic peak at  $130^\circ\text{C}$ . However, this peak was not found in the thermogram of the inclusion complex PTP-DM $\beta$ CD.

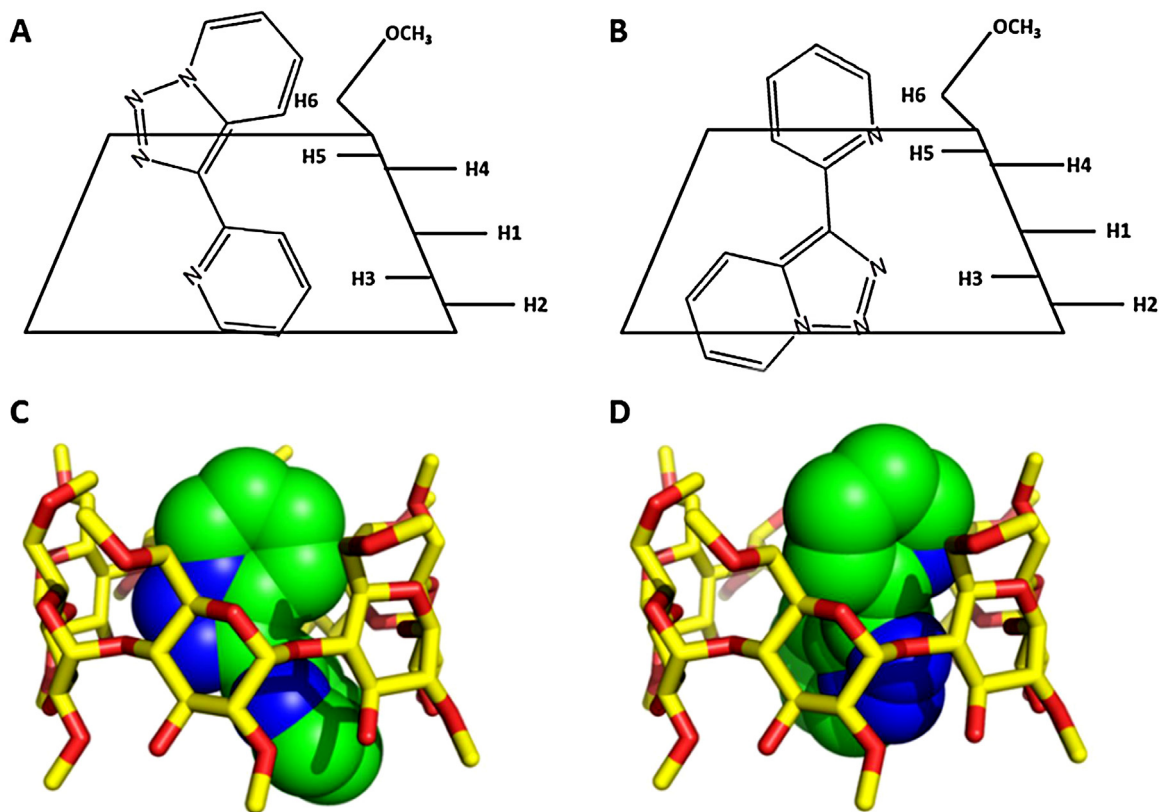
The disappearance of the melting peak of PTP at  $130^\circ\text{C}$  is indicative of the formation of the inclusion complexes (Fig. 4).

NMR is the most powerful technique used to determine the inclusion of a guest molecule into the hydrophobic CD cavity in solution. The information gained from NMR spectroscopy relies on the observation of selective line broadening and/or chemical shift displacements of the  $^1\text{H}$  NMR spectral signals of the guest and host protons. The formation of inclusion complexes can be proved from the changes of chemical shifts of PTP or DM $\beta$ CD in  $^1\text{H}$  NMR spectra. Due to the poor solubility of PTP toward  $\text{D}_2\text{O}$ , we were forced to employ at least 5% volume of MeOD- $d_4$  as a cosolvent to prepare the sample to make the NMR spectrum, and then compared it with the spectrum of lyophilized inclusion complex formed dissolved in  $\text{D}_2\text{O}$ . Table 2 lists the detailed chemical shifts variations of PTP and the internal protons of DM $\beta$ CD before and after forming inclusion complex. Downfield shifts are observed for most of the PTP protons, making it difficult to suggest which part of the PTP is actually penetrating the cyclodextrin cavity. The H-3 and H-5 protons of the glucose units are facing to the interior of the CD cavity, whereas H-6 protons are located at the rim with the primary alcohols. Upfield shifts of the interior proton signals of the cyclodextrin



**Fig. 5.** Partial contour plot of the two dimensional ROESY spectrum of pyridyltriazolopyridine in the presence of DM $\beta$ CD in  $\text{D}_2\text{O}$ .





**Fig. 6.** A, B possible PTP-DM $\beta$ CD complex molecular structures based on the ROESY spectra. C, D, molecular structures of the most stable PTP-DM $\beta$ CD calculated by docking studies.

are indicative that aromatic guest molecule are located close to the protons for which a shift is observed. This displacement is due to the anisotropic magnetic effect induced by the presence of the pyridyltriazolopyridine, corroborating the formation of the inclusion complex.

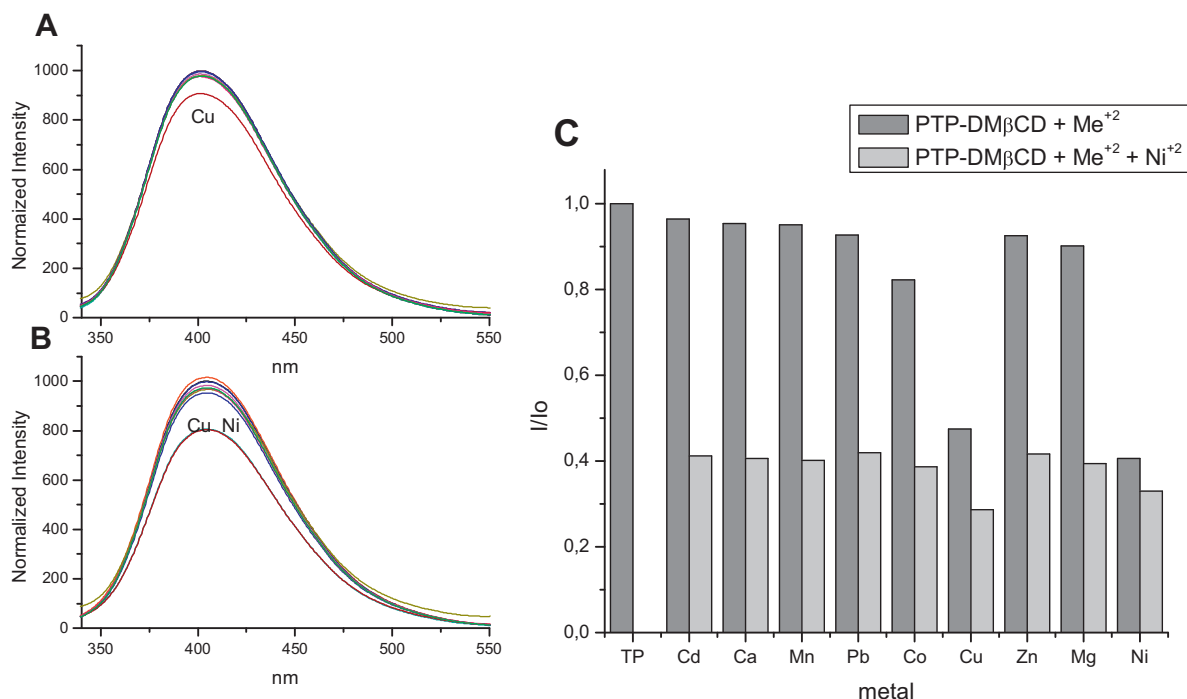
Thus, for studying spatial conformations of cyclodextrin inclusions, two dimensional NMR (2D-NMR) was used to investigate inter- and intra-molecular interactions arising upon complex formation. The presence of NOE cross-peaks between protons from two species indicates spatial contacts within 0.4 nm. To gain more conformational information, we used 2D ROESY and analyzed it qualitatively. HSQC spectrum was acquired in order to unambiguously determine DM $\beta$ CD chemical shifts of H-3, H-5 and H-6, spectra not shown. According to the ROESY spectrum of PTP-DM $\beta$ CD, Fig. 5, H-3' and H-6' protons of PTP correlate with H-3 and H-6 DM $\beta$ CD protons. It is very unlikely that only one geometric orientation may explain the resulting cross peaks, because H-3 and H-6 of DM $\beta$ CD are in opposite side. Therefore, two inclusion geometries are proposed for PTP-DM $\beta$ CD complex where pyridyltriazolopyridine could be included by both sides of cyclodextrin, in one case pyridine is protruding from the secondary rim, Fig. 6A, and in the other case pyridine is protruding from the primary rim of cyclodextrin, Fig. 6B.

In order to rationalize the NMR experimental results described above, we carried out molecular modeling studies of the complex. These studies revealed that two preferred final relative orientation for the complex study occurs in spite of the different initial conformation and orientation arbitrarily imposed. The best docking poses obtained are shown in Fig. 6. In one case Fig. 6C, the conformation obtained has the triazolopyridine-ring oriented toward the primary rim, while pyridine-ring remains exposed to the external surface

by the secondary rim. The other conformation is in the opposite direction with respect to the previous complex, Fig. 6D, therefore these results are in good agreement with the inclusion geometries obtained by the ROESY experiments.

To get insight into the binding properties as a sensitizer of pyridyltriazolopyridine-DM $\beta$ CD toward different metal ions, we investigated the fluorescence changes upon addition of divalent cations. The fluorescence intensity changes upon the addition of the metal cationic ions are shown in Fig. 7. We showed that the intensity of pyridyltriazolopyridine is slightly influenced by the presence of the metallic cation, Fig. 7A, except for copper where a quenching is observed. For the inclusion complex (Fig. 7B) the fluorescence intensity of PTP-DM $\beta$ CD-Ni system decreases more remarkably than PTP-Ni, which could be attributed to supramolecular assembly after binding with metal ions in the presence of DM $\beta$ CD solution because, it is expected that produce novel functions that are different from those found in free molecules (Yamauchi, Hayashita, Nishizawa, Watanabe, & Teramae, 1999). The stoichiometry of the ternary complex PTP-DM $\beta$ CD-Ni was confirmed by Job's plot method which exhibits a maximum at mole ratio 0.5 reflecting the formation of the complex by one mole of PTP-DM $\beta$ CD with one mole of Ni<sup>2+</sup> (data not shown).

The selectivity is one of the most important properties of the response of a sensor. This property represents the preference of a sensor response to the primary ion with respect to the potentially interfering ions. The selectivity toward Ni<sup>2+</sup> alone was further ascertained by the competition experiment by adding 10 equiv. Ni<sup>2+</sup> ion to the competing metal ion ligand mixtures, Fig. 7C, where the emission was quenched as in the presence of Ni<sup>2+</sup> alone. These results suggest that PTP-DM $\beta$ CD can be used as a potential selective fluorescent chemosensor for Ni<sup>2+</sup>.



**Fig. 7.** (A) Fluorescence intensity of PTP ( $1 \times 10^{-5}$  M) in ethanol: buffer pH 7.4, 1:1, in the presence of 10 equiv. of  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Ca}^{2+}$  ions. (B) Fluorescence intensity of PTP-DM $\beta$ CD ( $1 \times 10^{-5}$  M) in phosphate buffer pH 7.4, in the presence of 10 equiv. of  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Ca}^{2+}$  ions. (C) Changes of fluorescence intensity ( $I/I_0$ ) of PTP-DM $\beta$ CD ( $1 \times 10^{-5}$  M) in the presence of 10 equiv. of  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Ca}^{2+}$  ions and upon addition of 10 equiv. of  $\text{Ni}^{2+}$ .

A quantitative analysis of the fluorescence data was made as a function of the  $\text{Ni}^{2+}$  ion concentration. The data were plotted according to the Stern–Volmer equation (Eq. (5))

$$\frac{F_0}{F} = 1 + K_{\text{SV}}[\text{Ni}^{2+}] \quad (5)$$

where  $F_0$  and  $F$  are fluorescence intensities in the absence and in the presence of  $\text{Ni}^{2+}$ , respectively. The calibration plot of  $F_0/F$  versus  $[\text{Ni}^{2+}]$  shows a good linear relationship ( $R = 0.9998$ ) for nickel ion in the concentration range from 0 to  $1.5 \times 10^{-5}$  M. The Stern–Volmer quenching constant ( $K_{\text{SV}}$ ) was estimated as  $1.582 \times 10^5 \text{ M}^{-1}$ , data not shown.

The Limit of Detection (LOD) for the analysis for nickel is determined from the plot of fluorescence intensity (Quiroga-Campano et al., 2013) as a function of the concentration of  $\text{Ni}^{2+}$  in the lineal range from  $0.5 \times 10^{-6}$  to  $5 \times 10^{-6} \text{ mol L}^{-1}$ , with a slope of  $-4.723 \times 10^7$  and a correlation coefficient of 0.9819 ( $n = 8$ ). The detection limit is  $0.065 \text{ mg L}^{-1}$ , indicating that this result is acceptable for the recognition of nickel in aqueous solution (Ensafi & Bakhshi, 2003).

#### 4. Conclusions

The inclusion complex of PTP with DM $\beta$ CD was prepared in solution and solid state. The stoichiometry was confirmed by Job plot indicating that the inclusion complex of PTP with DM $\beta$ CD has a 1:1 molar ratio. Thermodynamic studies of the PTP-DM $\beta$ CD complex indicate that inclusion of PTP is mainly an entropic driven process. Complex formation was monitored by 2D dimensional ROESY experiments through the detection of intramolecular dipolar interaction between hydrogens 3' and 6' of PTP and the interior hydrogen H-3 and H-6 of DM $\beta$ CD. Molecular modeling studies performed to construct a three-dimensional model of the complex verify the experimental results, confirmed the experimental inclusion geometry proposed.

Finally the investigated fluorescence changes upon addition of divalent cations to the inclusion complex indicates a high selectivity and sensitivity for  $\text{Ni}^{2+}$  by fluorescence quenching in neutral aqueous solution. We have developed a new supramolecular sensitizer for nickel(II) in water by using pyridyltriazolopyridine as fluoroionophore included in DM $\beta$ CD.

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