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Electronic Supplementary Information

Thermodynamics of the inclusion within cyclodextrins and structural evidences of Cu(Indomethacin)$_2$ and Zn(Indomethacin)$_2$ complexes in aqueous solution.

Adrián Ricardo Hipólito-Nájera[a], Norma Rodríguez-Laguna[a], Luis I. Reyes-García[a], Alberto Rojas-Hernández[b], Rodolfo Gómez-Balderas*[a] and Rosario Moya-Hernández*[a]

[a.] Laboratorio de Fisicoquímica Analítica, Unidad de Investigación Multidisciplinaria, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, 54700, Cuautitlán Izcalli, Edo. Méx., México.

[b.] Departamento de Química, Área de Química Analítica, UAM-Iztapalapa, Apdo. Postal 55−534, 09340 Iztapalapa, Cd. Méx., México

[c.] Instituto de Química, Universidad Nacional Autónoma de México, Circuito exterior, Ciudad Universitaria, 04510 Coyoacán, Cd. Méx., México.

*To whom correspondence should be addressed

email: rodolfo.gomez@unam.mx, rosariomoya@gmail.com.

Tel. (+5255) 5623 1999, ext. 39420

Methodology for the determination of the soluble $Cu(IMC)_2(H_2O)_2$ by UV-Vis

Calibration Standards.

In 5 volumetric flasks of 10 mL, were added 0, 0.18, 0.88, 1.75 and 3.50 mL of Cu(II) 0.01 M and adding deionized water to the fill level. From these solutions 3 mL were poured in 5 different volumetric flasks of 5 mL each. Adding 0.5 mL of $CuCl_2$ 0.01 to reach at least 1.20×10^{-3} M of Cu(II); 0.5 mL of NH_3/NH_4^+ 1 M at pH 8.4 buffer were added; 0.7 mL of EDTA 0.04 M prepared in NH_3/NH_4^+ 1 M at pH 8.4 buffer were poured to the 5 mL flask. Finally, deionized water was added to the fill level at each calibration standard. Each calibration standard was prepared for triplicate.

Calibration curve.

The UV-Vis spectra of the previous calibrations standards were obtained. Fig S1 shows an example of the average spectra of each calibrations standards obtained for calibration curve. Fig S2 shows the calibration curve created from points at 730 nm from Fig. S1, fitting to a linear least squares showing slope and y-intercept with the determination coefficient (R^2) (Fig. S1 and S2 was used for LOD, LOQ, Table S1; and HP-β-CD experiments, Fig. S6, Table S5. For the rest of CDs experiments an independent calibration curve was constructed).[1]

LOD and LOQ of Cu(II)-EDTA method.

To determine limit of detection (LOD) and limit of quantification (LOQ) 10 blank samples (n=10) were prepared with 0.5 mL of $CuCl_2$ 0.01 M, to reach at least 1.20×10^{-3} M of Cu(II) in final 5 mL; then was added 0.7 mL of EDTA 0.04M prepared in NH_3/NH_4^+ 1 M at pH 8.4 buffer, finally was added NH_3/NH_4^+ 1 M at pH 8.4 buffer to the to 5 mL volumetric flask fill level.

Table S1.Values obtained for the determination of LOD and LOQ of Cu(II)-EDTA (n=10).

Average of blanks (M)	σ_0	σ'_0	**LOD (M)**	**LOQ (M)**
2.88×10^{-6}	5.79×10^{-6}	1.83×10^{-6}	5.49×10^{-6}	1.83×10^{-5}

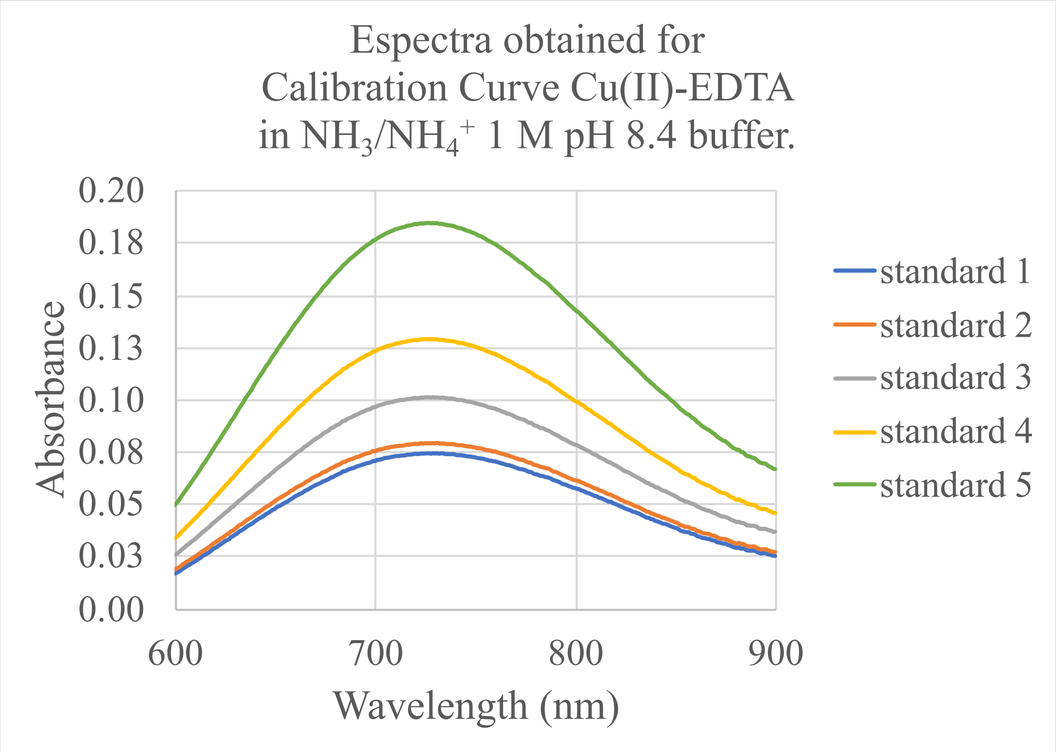

Fig. S1. Spectra obtained of calibration standards for determination of Cu(II)-EDTA.

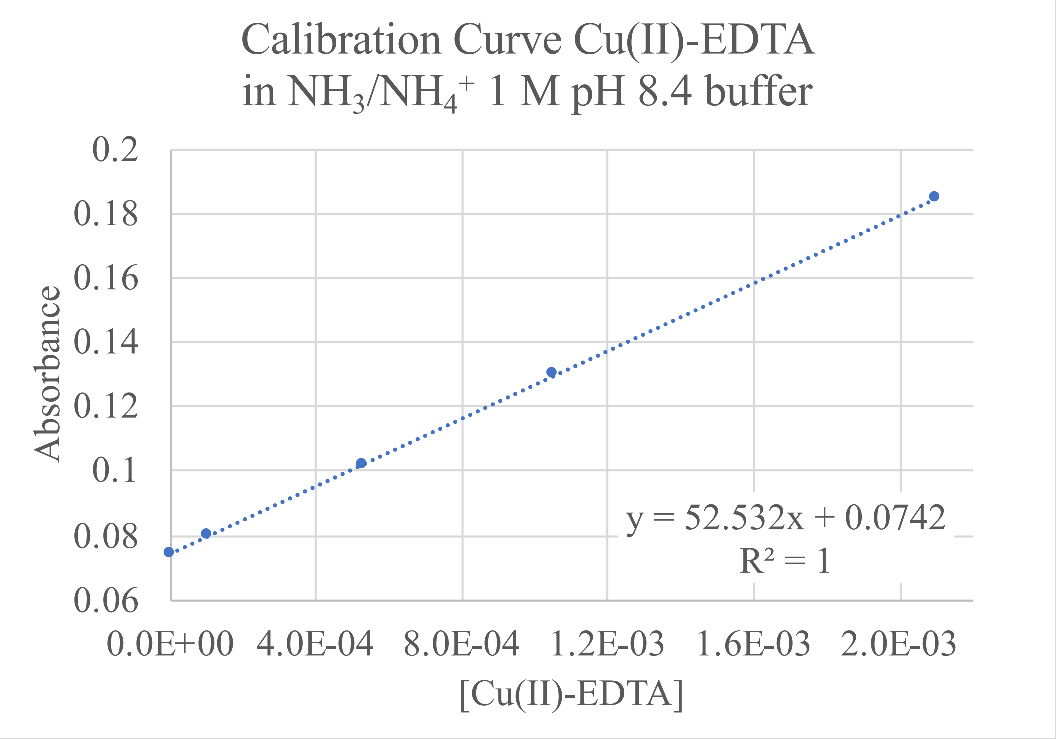

Fig. S2. Calibration curve for determination of Cu(II)-EDTA in 1 M NH_3/NH_4^+ buffer pH 8.4. The data were selected at 730 nm (n=3).

The UV-Vis spectra of the blanks were obtained. The concentrations of the blanks was determined using the equation of calibration curve Fig. S2, the average concentration in molarity (M) of blanks are shown in Table S1 with the results of standard deviation σ_0; calculated standard deviation: $\sigma'_0 = \sigma_0/\sqrt{n}$; n = number of blank samples, LOD= 3 × σ'_0 and LOQ = 10 × σ'_0.[2]

Experimental samples.

The different CDs (α-, γ-, HP-β- o HP-γ-CD) were prepared around 0.08 M (β-CD was prepared at 0.017 M) at 25 mL volumetric flasks in deionized water.

Different volumetric flasks of 5 mL were prepared adding a volume of solution of CD. In each volumetric flask, 3 mg of insoluble $Cu(IMC)_2(H_2O)_2$ were added. The CD solutions with insoluble $Cu(IMC)_2(H_2O)_2$ were left in agitation for 3 days. After this time, the solutions were filtrated (filtrate), 3 mL of the filtrate were taken and poured in a volumetric flask of 5 mL, then it was poured 0.5 mL of $CuCl_2$ 0.01 M to reach at least 1.20×10^{-3} M of Cu(II); 0.5 mL of NH_3/NH_4^+ 1 M at pH 8.4 buffer were added then 0.7 mL of EDTA 0.04M prepared in NH_3/NH_4^+ 1 M at pH 8.4 buffer were poured to the 5 mL flask. Finally, NH_3/NH_4^+ 1 M at pH 8.4 buffer was added to the fill level at each experimental sample.

The UV-Vis spectra of the experimental samples were obtained. Fig S3-7 shows the spectra obtained following the method. With the points at 730 nm of each curve of Fig. S3-7, the soluble amount of Cu(II)-EDTA was determined with a calibration curve like Fig. S2, the concentration of Cu(II)-EDTA obtained with calibration curve for each experiment with different CDs, are presented in Tables S2-5. The amount of Cu(II)-EDTA in solution was related to the $Cu(IMC)_2(H_2O)_2$ solubility and is presented in Tables S2-5. The relation of $Cu(IMC)_2(H_2O)_2$ solubility with the amount of CD, this diagrams are called phase solubility diagrams showed in Fig. 9a) of the main article. Finally, Table S6 shows the percentage of increment of $Cu(IMC)_2(H_2O)_2$ relating the highest level concentration obtained with CDs of the phase solubility diagrams with the average of the lower concentration or intrinsic solubility of $Cu(IMC)_2(H_2O)_2$ (S_0).

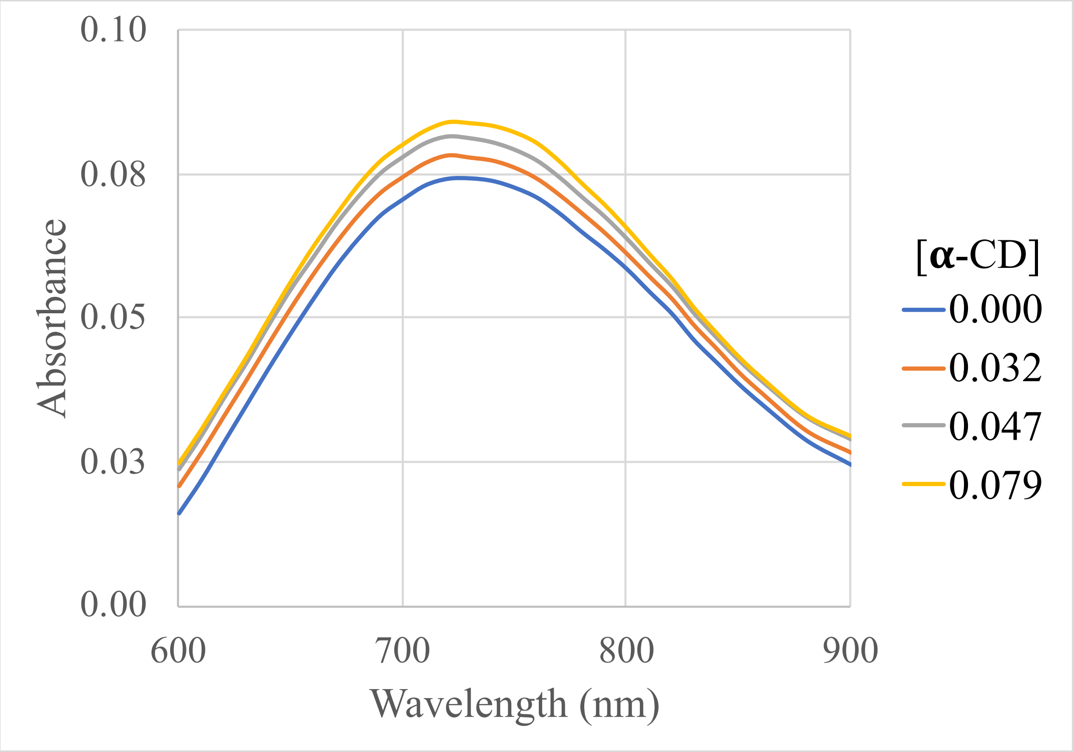

Fig. S3. Spectra obtained of Cu(II)-EDTA from experimental samples for the experiment with α-CD.

Table S2. Concentration of Cu(II)-EDTA from calibration curve for the α-CD experiment (Fig. S3) and concentration of $Cu(IMC)_2(H_2O)_2$ in filtrate..

[α-CD]	**Experimental sample* [Cu(II)-EDTA]**	**Filtrate [$Cu(IMC)_2(H_2O)_2$]**
0.000	$(9.44 \pm 22.50)\times10^{-6}$	$(1.57 \pm 3.75)\times10^{-5}$
0.032	$(9.18 \pm 1.78)\times10^{-5}$	$(1.53 \pm 0.30)\times10^{-4}$
0.047	$(1.61 \pm 0.25)\times10^{-4}$	$(2.69 \pm 0.41)\times10^{-4}$
0.079	$(2.16 \pm 0.34)\times10^{-4}$	$(3.60 \pm 0.56)\times10^{-4}$

*Obtained by calibration curve (n=3).

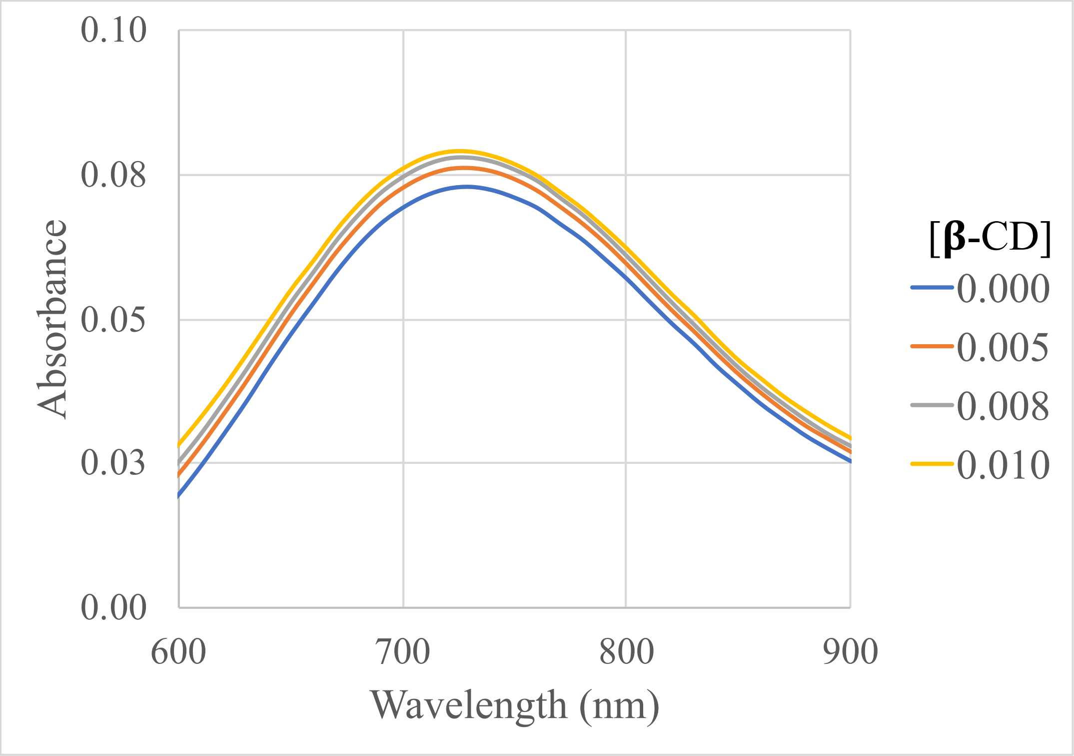

Fig. S4. Spectra obtained of Cu(II)-EDTA from experimental samples for the experiment with β-CD.

Table S3. Concentration of Cu(II)-EDTA from calibration curve for the β-CD experiment (Fig. S4) and concentration of $Cu(IMC)_2(H_2O)_2$ in filtrate.

[β-CD]	**Experimental sample* [Cu(II)-EDTA]**	**Filtrate $[Cu(IMC)_2(H_2O)_2]$**
0.000	$(5.32 \pm 2.10)\times10^{-5}$	$(8.87 \pm 3.51)\times10^{-5}$
0.005	$(1.06 \pm 0.26)\times10^{-4}$	$(1.77 \pm 0.44)\times10^{-4}$
0.008	$(1.30 \pm 0.32)\times10^{-4}$	$(2.17 \pm 0.54)\times10^{-4}$
0.010	$(1.47 \pm 0.36)\times10^{-4}$	$(2.45 \pm 0.61)\times10^{-4}$

∗Obtained by calibration curve.

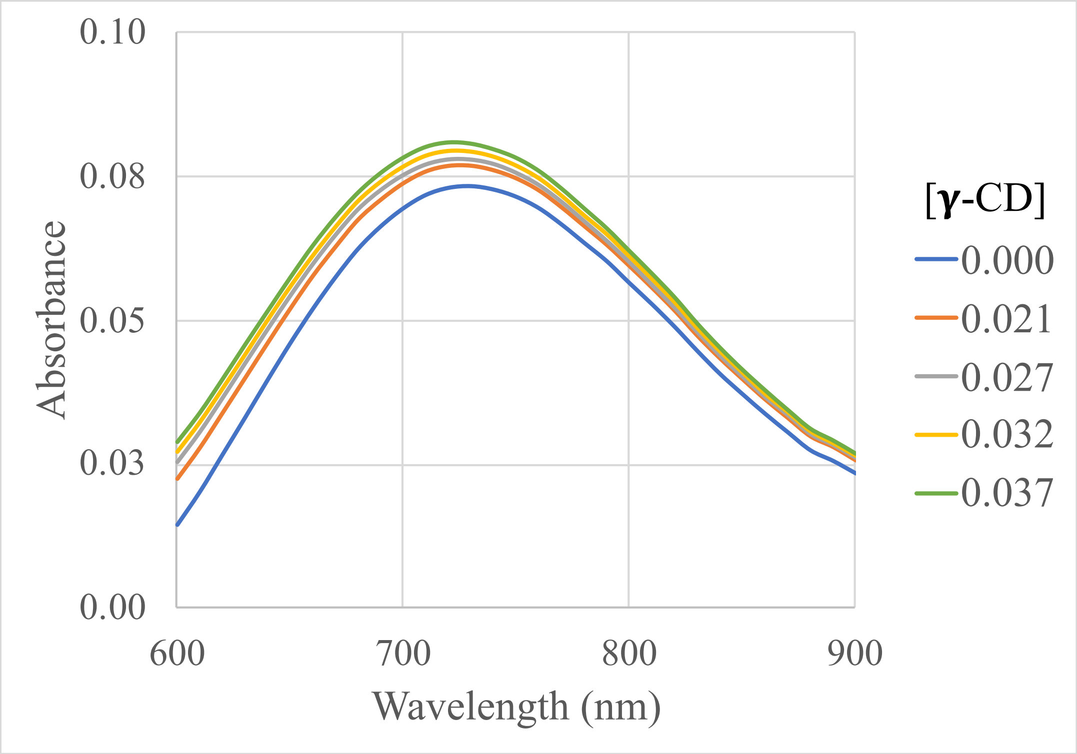

Fig. S5. Spectra obtained of Cu(II)-EDTA from experimental samples for the experiment with γ-CD.

Table S4. Concentration of Cu(II)-EDTA from calibration curve for the γ-CD experiment (Fig. S5) and concentration of $Cu(IMC)_2(H_2O)_2$ in filtrate

[γ-CD]	**Experimental sample* [Cu(II)-EDTA]**	**Filtrate [$Cu(IMC)_2(H_2O)_2$]**
0.000	$(4.70 \pm 8.10)\times10^{-7}$	$(7.84 \pm 1.35)\times10^{-7}$
0.021	$(5.90 \pm 0.60)\times10^{-5}$	$(9.83 \pm 1.01)\times10^{-5}$
0.027	$(7.89 \pm 0.61)\times10^{-5}$	$(1.32 \pm 0.10)\times10^{-4}$
0.032	$(9.82 \pm 0.65)\times10^{-5}$	$(1.64 \pm 0.11)\times10^{-4}$
0.037	$(1.20 \pm 0.74)\times10^{-5}$	$(1.99 \pm 0.12)\times10^{-4}$

∗Obtained by calibration curve.

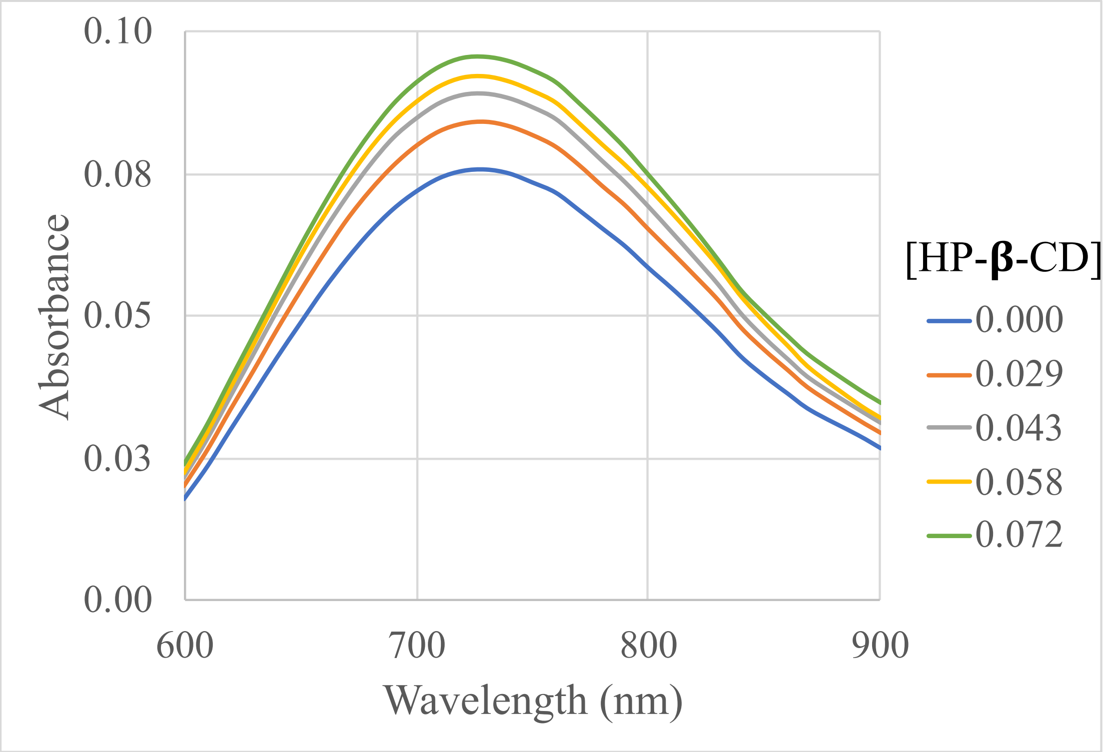

Fig. S6. Spectra obtained of Cu(II)-EDTA from experimental samples for the experiment with HP-β-CD.

Table S5. Concentration of Cu(II)-EDTA from calibration curve for the HP-β-CD experiment (Fig. S6) and concentration of $Cu(IMC)_2(H_2O)_2$ in filtrate

[HP-β-CD]	**Experimental sample* [Cu(II)-EDTA]**	**Filtrate [$Cu(IMC)_2(H_2O)_2$]**
0.000	$(3.15 \pm 1.49)\times10^{-5}$	$(3.88 \pm 1.84)\times10^{-5}$
0.029	$(1.91 \pm 0.15)\times10^{-4}$	$(2.35 \pm 0.18)\times10^{-4}$
0.043	$(2.88 \pm 0.14)\times10^{-4}$	$(3.55 \pm 0.18)\times10^{-4}$
0.058	$(3.43 \pm 0.14)\times10^{-4}$	$(4.24 \pm 0.18)\times10^{-4}$
0.072	$(4.12 \pm 0.14)\times10^{-5}$	$(5.08 \pm 0.17)\times10^{-4}$

∗Obtained by calibration curve.

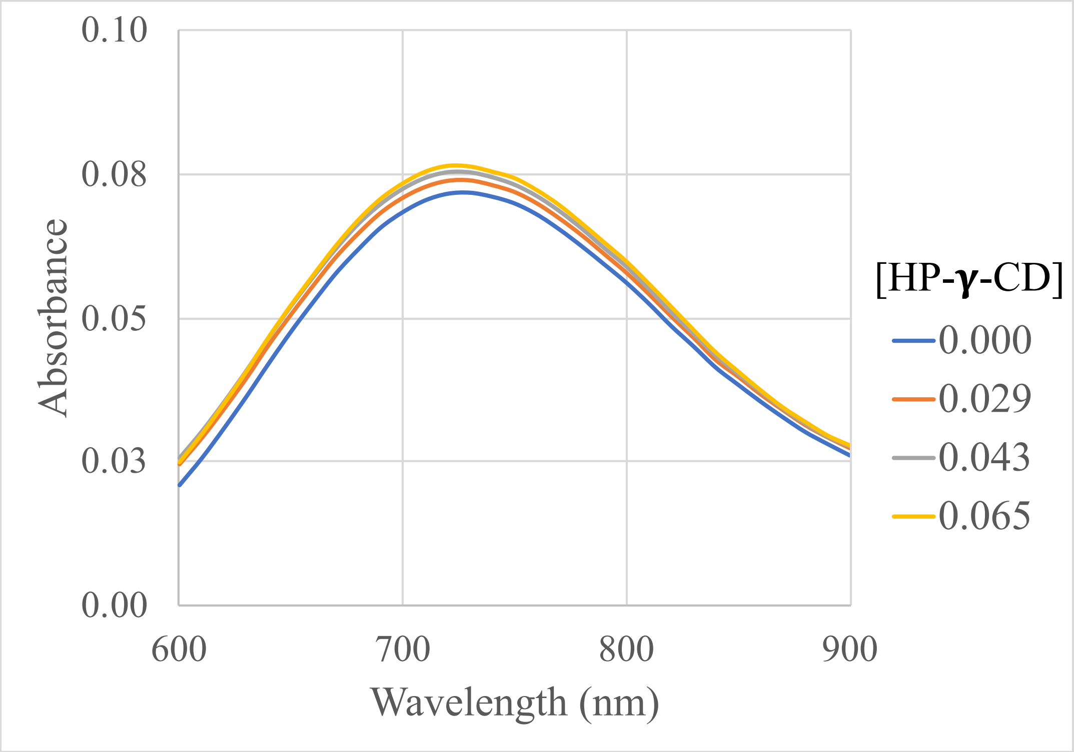

Fig. S7. Spectra obtained of Cu(II)-EDTA from experimental samples for the experiment with HP-γ-CD.

Table S6. Concentration of Cu(II)-EDTA from calibration curve for the HP-γ-CD experiment (Fig. S7) and concentration of $Cu(IMC)_2(H_2O)_2$ in filtrate

[HP-γ-CD]	**Experimental sample∗ [Cu(II)-EDTA]**	**Filtrate $[Cu(IMC)_2(H_2O)_2]$**
0.000	$(8.27 \pm 11.60)\times10^{-6}$	$(1.38 \pm 1.95)\times10^{-5}$
0.029	$(4.01 \pm 1.09)\times10^{-5}$	$(6.69 \pm 0.18)\times10^{-4}$
0.043	$(6.11 \pm 1.07)\times10^{-5}$	$(1.02 \pm 0.18)\times10^{-4}$
0.058	$(7.98 \pm 1.05)\times10^{-5}$	$(1.33 \pm 0.17)\times10^{-4}$

∗Obtained by calibration curve.

Table S7. Intrinsic solubility (S_0), the highest concentration obtained in phase solubility diagrams and the percentage of increased solubility of Cu(IMC)$_2$(H2O)$_2$.

[Cu(IMC)$_2$(H$_2$O)$_2$]	**S_0 (M)**	**Highest concentration (M)**	**Increased solubility (%)**
α-CD	$(1.57 \pm 3.75)\times10^{-5}$	$(3.60 \pm 0.56)\times10^{-4}$	1140.80 ± 69.12
β-CD	$(8.87 \pm 3.51)\times10^{-5}$	$(2.45 \pm 0.61)\times10^{-4}$	776.38 ± 71.77
γ-CD	$(7.84 \pm 1.35)\times10^{-7}$	$(1.99 \pm 0.12)\times10^{-4}$	630.61 ± 67.33
HP-β-CD	$(3.88 \pm 1.84)\times10^{-5}$	$(5.08 \pm 0.17)\times10^{-4}$	1609.80 ± 67.38
HP-γ-CD	$(1.38 \pm 1.95)\times10^{-5}$	$(1.33 \pm 0.17)\times10^{-4}$	421.46 ± 68.61
Average	$(3.16 \pm 2.12)\times10^{-5}$		

Methodology for the determination of the soluble Zn(IMC)$_2$(H$_2$O)$_2$ by UV-Vis

Calibration Standards.

A total of 5 calibration standard were prepared adding 0, 3, 6, 9 and 12 μL of $ZnCl_2$ 0.01M in volumetric flasks of 5 mL, an amount of 0.5 mL of 4-(2pyridylazo) resorcinol (PAR) 1.00×10^{-3} M was added and a solution of phosphate 0.1 M pH 7 was added to the fill level in each calibration standard. All standards were prepared in triplicate.

Calibration Curve.

Calibration standards were measured by UV-Vis. The mean of the three spectra obtained for calibration curve are shown in Fig. S8 and the calibration curve constructed from points at 490 nm, from spectra of Fig. S8, against Zn(II)-PAR concentration is shown in Fig. S9, slope, y-intercept and the determination coefficient (R^2) were obtained from a linear least squares fit (1)(Fig. S9, was used for LOD and LOQ, Table S8; and HP-γ-CD experiments, Fig. S14 and Table S13. For the rest of CDs experiments an independent calibration curve was constructed).

LOD and LOQ of Zn(II)-PAR method.

To determine limit of detection (LOD) and limit of quantification (LOQ) 10 blank samples (n = 10) were prepared with an amount of 0.5 mL of PAR 1.00×10^{-3} M and a solution of phosphate 0.1 M pH 7 was added to the fill level in each blank samples volumetric flask of 5 mL.

The UV-Vis spectra of the blanks were obtained. The concentrations of the blanks was determined using the equation of calibration curve Fig. S9, the average concentration in molarity (M) of blanks are shown in Table S8 with the results of standard deviation σ_0; calculated standard deviation: $\sigma'_0 = \sigma_0/\sqrt{n}$; n = number of blank samples, LOD= 3 × σ'_0 and LOQ = 10 × σ'_0.[2]

Table S8. Table of the values to obtain LOD and LOQ of Cu(II)-EDTA (n=10).

Average of blanks (M)	σ_0	σ'_0	**LOD (M)**	**LOQ (M)**
3.60×10^{-7}	1.24×10^{-7}	3.91×10^{-8}	1.17×10^{-7}	3.91×10^{-7}

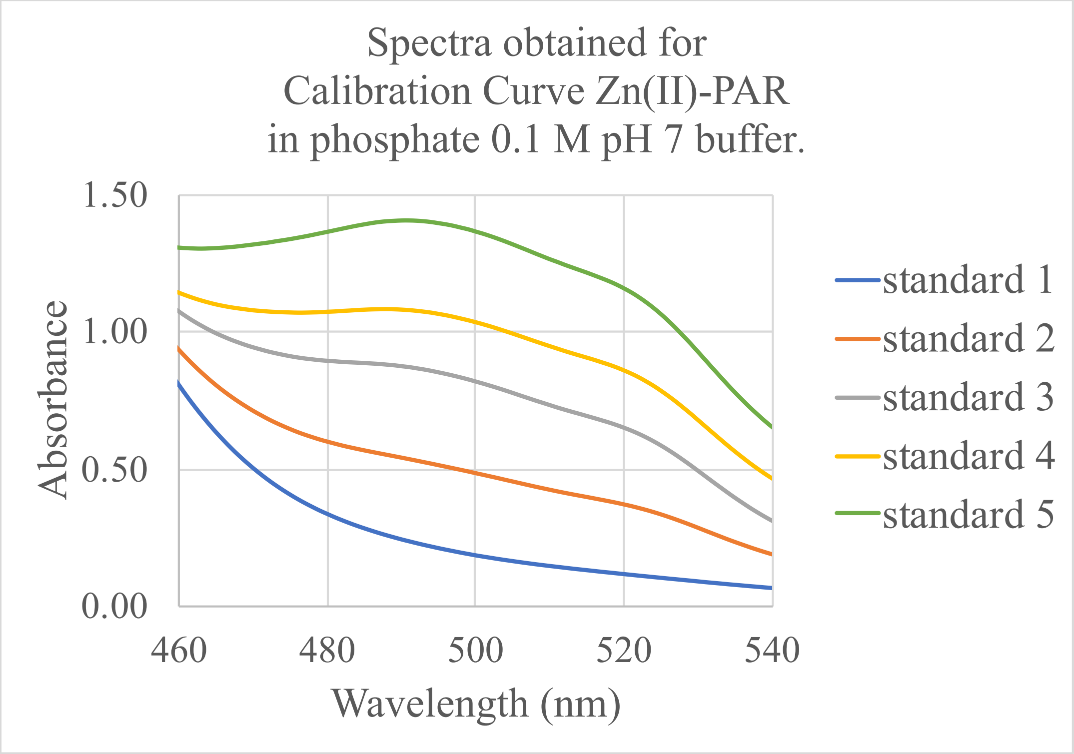

Fig. S8. Spectra obtained of calibration standards for determination of Zn(II)

Experimental samples.

The different CDs (α-, β-, γ-, HP-β- o HP-γ-CD) were prepared around 0.08 M (β-CD was prepared at 0.017M) at 25 mL volumetric flasks in deionized water.

In different volumetric flasks of 5 mL were added different amounts of CD 0.08 M adding deionized water to the fill level, then 3 mg of $Zn(IMC)_2(H_2O)_2$ were added on each volumetric flask. These volumetric flasks were left in agitation by 3 days. After this time the solutions were filtrated (filtrate).

In another 5 mL volumetric flask, 0.1 mL (0.3 mL in case of α- and β-CD) of filtrate, 0.5 mL of PAR 1.00×10^{-3} M and a solution of phosphate 0.1 M pH 7 was added to the fill level for each experimental sample.

The experimental samples were measured by UV-Vis for triplicated. The mean of the three spectra obtained for experimental samples are shown in Fig. S10-S14. The amount of Zn(II)-PAR in solution was related to the soluble amount of $Zn(IMC)_2$ with the amount of CD $_2$ (Table S9-S13), this diagrams are called phase solubility diagrams showed in Fig. 9b) of the main article. Finally, Table S14 shows the percentage of increment of $Zn(IMC)_2(H_2O)_2$ relating the highest level concentration obtained with CDs of the phase solubility diagrams with the average of the lower concentration or intrinsic solubility of $Zn(IMC)_2(H_2O)_2$ (S_0).

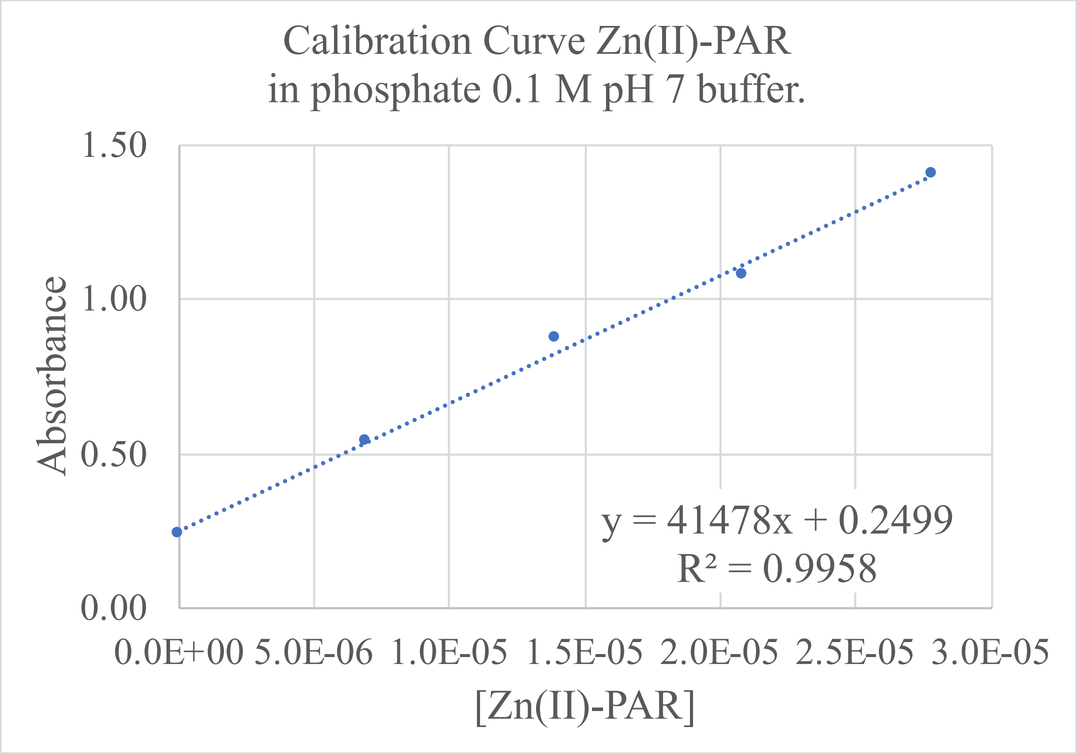

Fig. S9. Calibration curve for determination of Zn(II) with PAR in 0.1 M phosphate buffer pH 7. The data were selected at 490 nm.

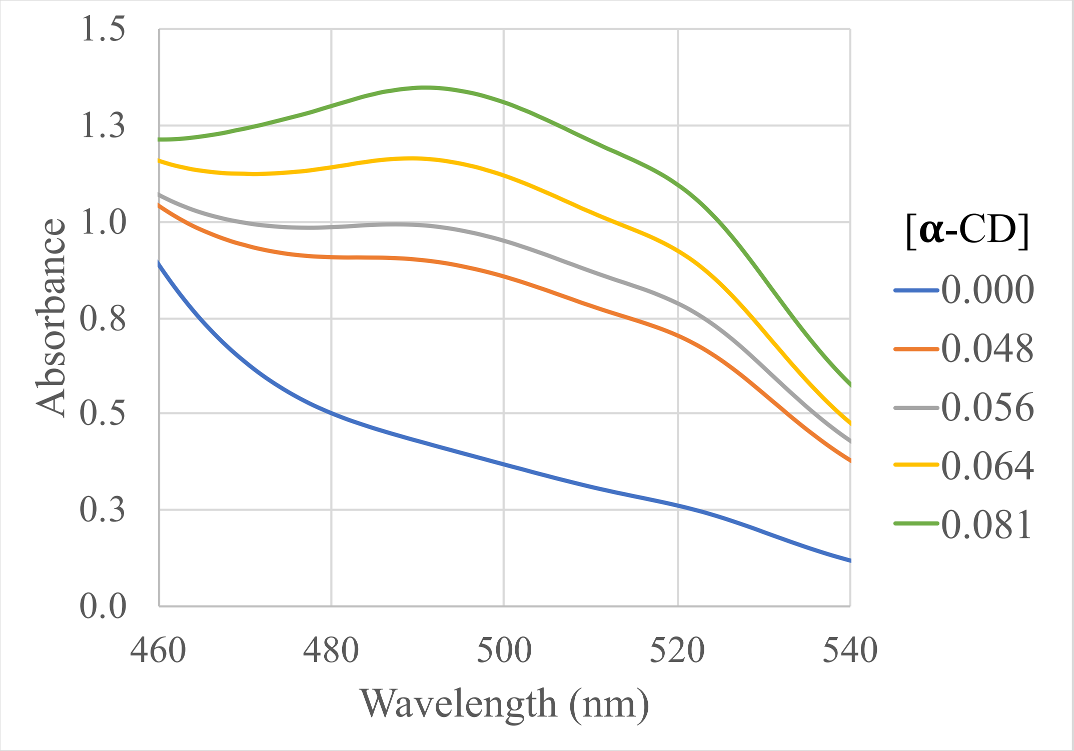

Fig. S10. Spectra obtained of Zn(II)-PAR from experimental samples for the experiment with α-CD.

Table S9. Concentration of Zn(II)-PAR from calibration curve for the α-CD experiment (Fig. S10) and concentration of $Zn(IMC)_2(H_2O)_2$ in filtrate.

[α-CD]	**Experimental sample* [Zn(II)-PAR]**	**Filtrate $[Zn(IMC)_2(H_2O)_2]$**
0.000	$(3.56 \pm 2.23)\times10^{-6}$	$(1.78 \pm 1.12)\times10^{-5}$
0.048	$(1.42 \pm 0.21)\times10^{-5}$	$(2.37 \pm 0.36)\times10^{-4}$
0.056	$(1.62 \pm 0.37)\times10^{-5}$	$(2.70 \pm 0.37)\times10^{-4}$
0.064	$(1.98 \pm 0.24)\times10^{-5}$	$(3.30 \pm 0.40)\times10^{-4}$
0.081	$(2.40 \pm 0.27)\times10^{-5}$	$(4.00 \pm 0.45)\times10^{-4}$

∗Obtained by calibration curve (n=3).

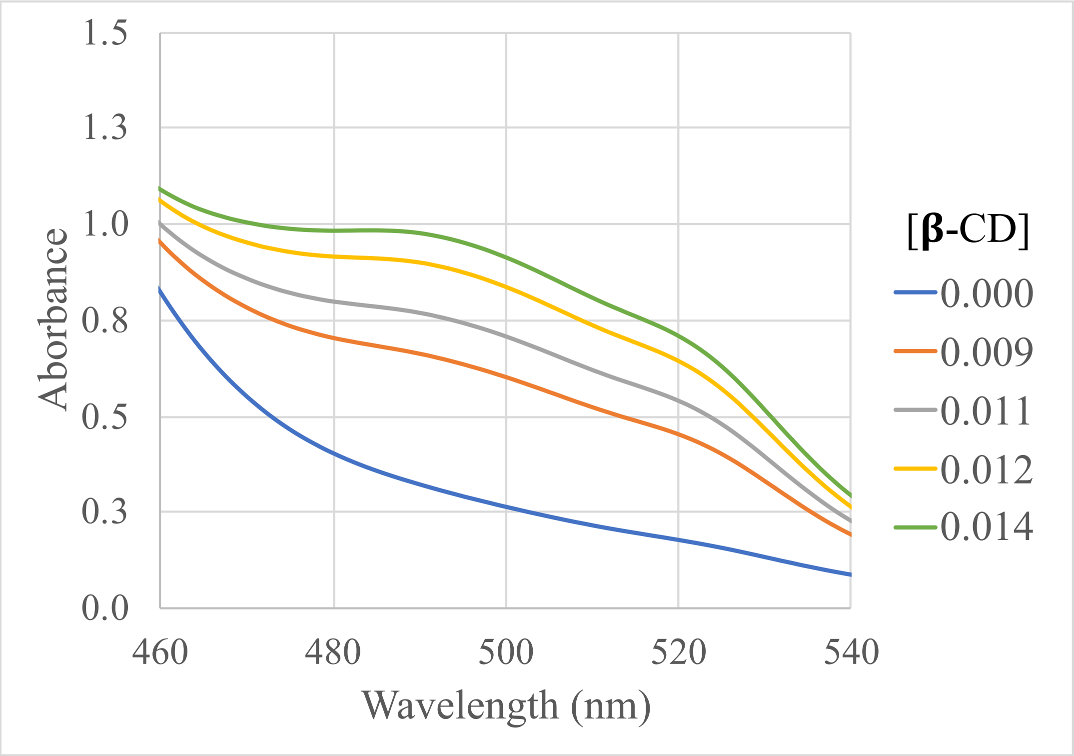

Fig. S11. Spectra obtained of Zn(II)-PAR from experimental samples for the experiment with β-CD.

Table S10. Concentration of Zn(II)-PAR from calibration curve for the β-CD experiment (Fig. S11) and concentration of Zn(IMC)$_2$(H$_2$O)$_2$ in filtrate.

[β-CD]	**Experimental sample* [Zn(II)-PAR]**	**Filtrate [Zn(IMC)$_2$(H$_2$O)$_2$]**
0.000	$(3.08 \pm 2.37)\times10^{-6}$	$(1.54 \pm 1.35)\times10^{-5}$
0.005	$(1.07 \pm 0.22)\times10^{-5}$	$(1.79 \pm 0.11)\times10^{-4}$
0.008	$(1.31 \pm 0.21)\times10^{-5}$	$(2.19 \pm 0.11)\times10^{-4}$
0.010	$(1.78 \pm 0.22)\times10^{-5}$	$(2.96 \pm 0.11)\times10^{-4}$

∗Obtained by calibration curve.

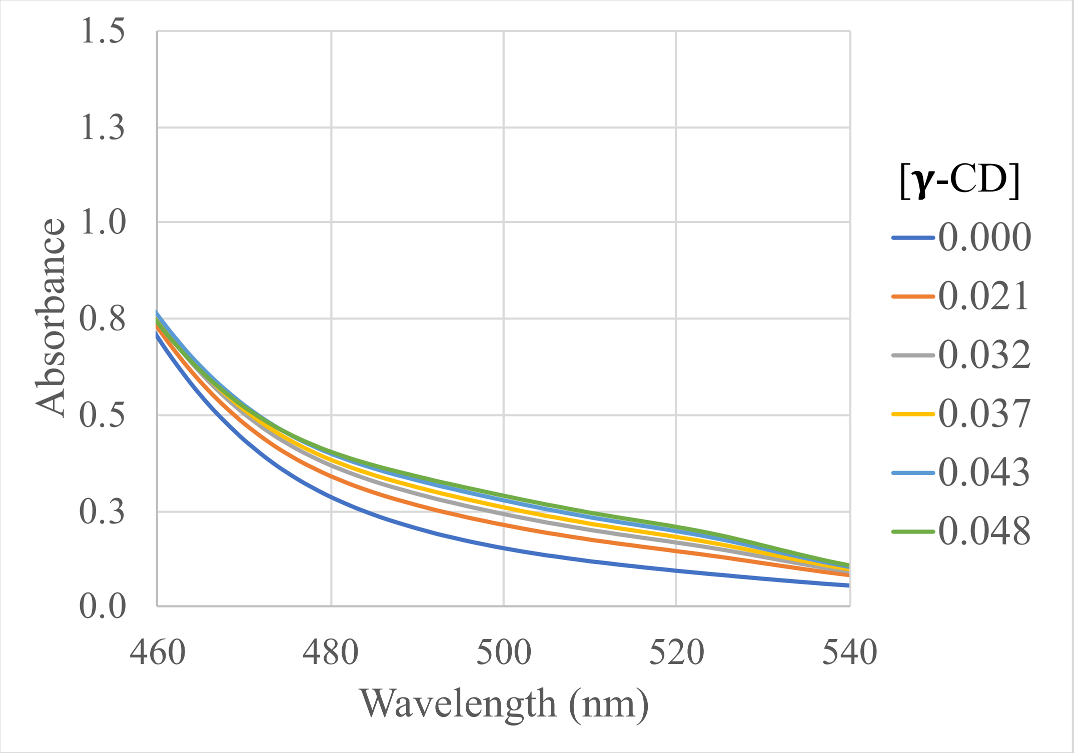

Fig. S12. Spectra obtained of Zn(II)-PAR from experimental samples for the experiment with γ-CD.

Table S11. Concentration of Zn(II)-PAR from calibration curve for the γ-CD experiment (Fig. S12) and concentration of $Zn(IMC)_2(H_2O)_2$ in filtrate.

[γ-CD]	Experimental sample* [Cu(II)-EDTA]	Filtrate $[Cu(IMC)_2(H_2O)_2]$
0.000	$(9.13 \pm 10.70)\times10^{-7}$	$(4.56 \pm 5.35)\times10^{-5}$
0.021	$(1.95 \pm 1.03)\times10^{-6}$	$(9.77 \pm 5.15)\times10^{-5}$
0.032	$(2.44 \pm 1.01)\times10^{-6}$	$(1.22 \pm 0.51)\times10^{-4}$
0.037	$(2.75 \pm 1.00)\times10^{-6}$	$(1.38 \pm 0.50)\times10^{-4}$
0.043	$(3.08 \pm 0.99)\times10^{-6}$	$(1.54 \pm 0.50)\times10^{-4}$
0.048	$(3.27 \pm 0.98)\times10^{-6}$	$(1.63 \pm 0.49)\times10^{-4}$

∗Obtained by calibration curve.

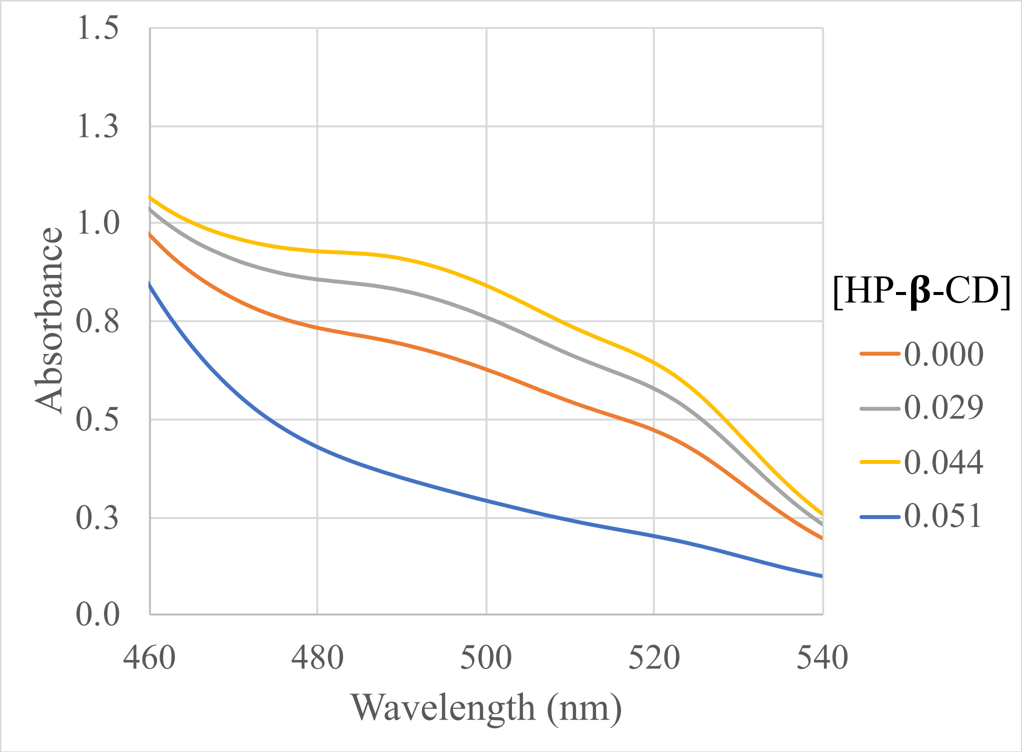

Fig. S13. Spectra obtained of Zn(II)-PAR from experimental samples for the experiment with HP-β-CD.

Table S12. Concentration of Zn(II)-PAR from calibration curve for the HP-β-CD experiment (Fig. S13) and concentration of $Zn(IMC)_2(H_2O)_2$ in filtrate.

[HP-β-CD]	**Experimental sample* [Zn(II)-PAR]**	**Filtrate $[Zn(IMC)_2(H_2O)_2]$**
0.000	$(3.90 \pm 3.22)\times10^{-6}$	$(9.75 \pm 8.05)\times10^{-6}$
0.029	$(1.16 \pm 0.28)\times10^{-5}$	$(5.82 \pm 1.38)\times10^{-4}$
0.044	$(1.52 \pm 0.27)\times10^{-5}$	$(7.59 \pm 1.37)\times10^{-4}$
0.051	$(1.73 \pm 0.28)\times10^{-5}$	$(8.64 \pm 1.40)\times10^{-4}$

*Obtained by calibration curve.

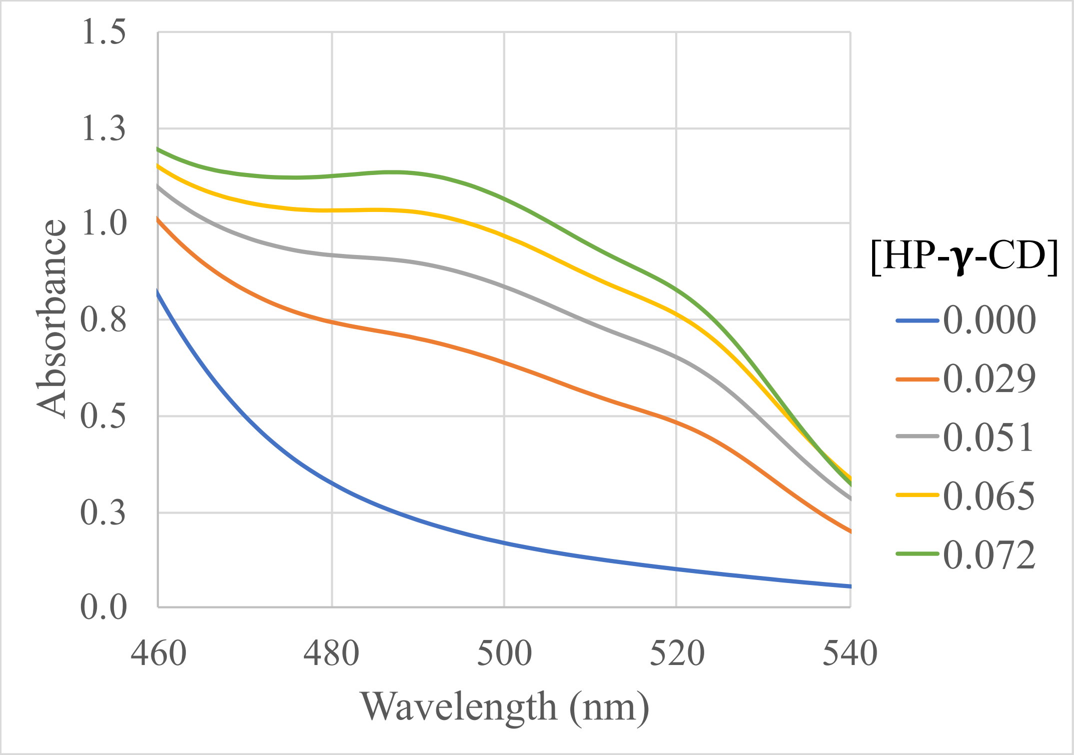

Fig. S14. Spectra obtained of Zn(II)-PAR from experimental samples for the experiment with HP-γ-CD.

Table S13. Concentration of Zn(II)-PAR from calibration curve for the HP-γ-CD experiment (Fig. S14) and concentration of $Zn(IMC)_2(H_2O)_2$ in filtrate.

[HP-γ-CD]	**Experimental sample* [Zn(II)-PAR]**	**Filtrate [$Zn(IMC)_2(H_2O)_2$]**
0.000	$(1.43 \pm 0.29)\times10^{-5}$	$(3.58 \pm 1.45)\times10^{-5}$
0.029	$(1.07 \pm 0.29)\times10^{-5}$	$(1.79 \pm 0.15)\times10^{-4}$
0.051	$(1.55 \pm 0.29)\times10^{-5}$	$(2.59 \pm 0.14)\times10^{-4}$
0.065	$(1.87 \pm 0.30)\times10^{-5}$	$(3.12 \pm 0.15)\times10^{-4}$
0.072	$(2.12 \pm 0.30)\times10^{-5}$	$(3.53 \pm 0.15)\times10^{-4}$

∗Obtained by calibration curve.

Table S14. Intrinsic solubility (S_0), the highest concentration obtained in phase solubility diagrams and the percentage of increased solubility of $Cu(IMC)_2(H_2O)_2$.

[Zn(IMC)$_2$(H$_2$O)$_2$]	**S_0 (M)**	**Highest concentration (M)**	**Increased solubility (%)**
α-CD	$(1.78 \pm 1.12)\times10^{-5}$	$(4.00 \pm 0.45)\times10^{-4}$	1608.36 ± 78.58
β-CD	$(1.54 \pm 1.35)\times10^{-5}$	$(2.96 \pm 0.11)\times10^{-4}$	1190.19 ± 77.88
γ-CD	$(4.56 \pm 5.35)\times10^{-5}$	$(1.63 \pm 0.49)\times10^{-4}$	655.41 ± 83.44
HP-β-CD	$(9.75 \pm 8.05)\times10^{-6}$	$(8.64 \pm 1.40)\times10^{-4}$	3474.07 ± 78.44
HP-γ-CD	$(3.58 \pm 1.45)\times10^{-5}$	$(3.53 \pm 0.15)\times10^{-4}$	1419.38 ± 77.90
Average	$(2.49 \pm 1.93)\times10^{-5}$		

∗Obtained by calibration curve.

Ca-EDTA standard reaction

The results of the Ca-EDTA to check reliability of the equipment is reported in Fig. S15 and Table S15.

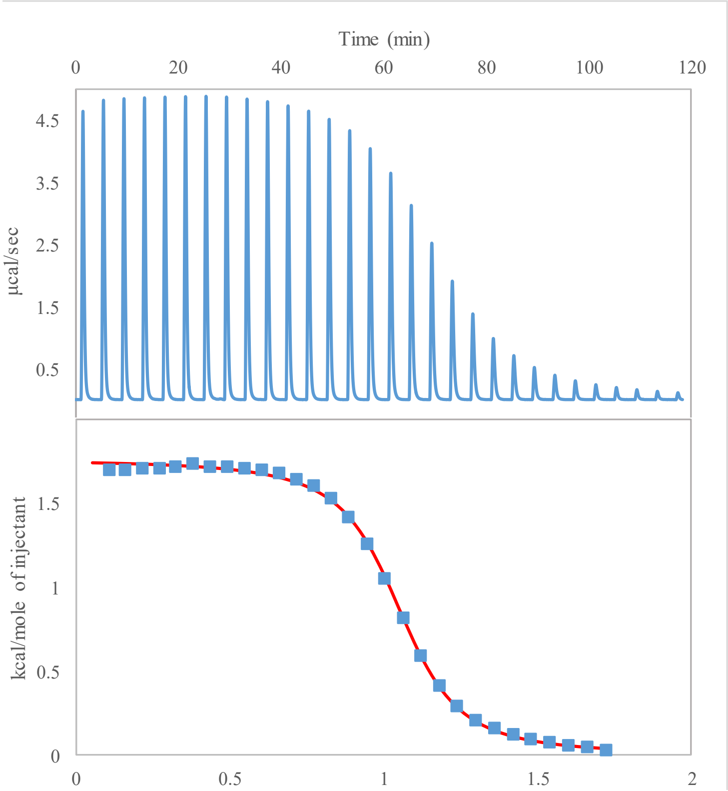

Fig. S15. ITC titration of EDTA with Ca^{2+} at pH 5.5. 1.06mM EDTA and 10.1 mM Ca^{2+}in acetate buffer at 298.15 K.

Table S13. Experimental parameters of the Ca-EDTA, compared with the reference values.

Parameter	**Experimental values**	**Reference values**[3]
N	1.030 ± 0.002	1.07 ± 0.05
$\log K_b$	5.01 ± 0.02	5.08 ± 0.02
ΔH_b (kcal/mol)	1.76 ± 0.01	1.80 ± 0.07

References.

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