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α-Cyclodextrin and methylmercury chloride: a new strategy to recover organomercurials

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Methylmercury (MeHg) is one of the most toxic forms of mercury in the environment. It can be accumulated in fish through the food chain; after, consumption the fish is then dangerous to fetuses and younger children, causing abnormal brain development and nervous system disorders. Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six, seven or eight units of glucose. In accord with the dimensions and hydrophilic–lipophilic properties, one can obtain inclusion of hydrophobic guests in a CD cavity. In the present work we used this characteristic of CD to obtain an inclusion compound between MeHgCl and the α-cyclodextrin, looking for a new method to reduce MeHgCl toxicity and pre-concentration. The inclusion compound was characterized through IR, ^{1}H, ^{13}C NMR and Raman spectroscopy. X-ray diffraction and thermal analysis (TG, DTG and DSC) methods were also used. Finally, biological tests were carried out and the minimum inhibitory concentrations (MICs) for MeHgCl, α-cyclodextrin, the MeHgCl–CD complex and a physical mixture were determined. This host–guest strategy using cyclodextrins offers an alternative and powerful method for mercury remediation.

Keywords: methylmercury chloride; cyclodextrins; inclusion compounds; mercury remediation

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INTRODUCTION

Mercury (Hg) is widely spread in the environment, by natural and anthropogenic sources. Its high volatility, easy transformation in natural compartments and biomagnification characteristics contribute to its classification as a toxic heavy metal. Regarding its toxicity, three major chemical forms of the metal must be distinguished: elemental mercury, and inorganic and organic mercurials. Mercury participates in a cycle in the environment through a series of complex chemical and physical transformations that occur in air, soils and water compartments, where it changes its oxidation states readily.[1]

Transformation of mercury in the environment can occur as biomagnification and bioaccumulation processes. In aquatic environments, microorganisms can convert mercury into methylmercury (MeHg). This product is one of the most toxic forms of mercury. MeHg is taken up by plankton algae and is concentrated in fish via the food chain, reaching much higher concentrations than originally found in the environment.[2] As a result, the

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consumption of fish becomes the primary pathway of human exposure to MeHg. Poisoning can occur and fetuses are particularly at risk, suffering central nervous system damage, mental retardation and a lack of physical development, which becomes evident two to five years after birth. Adults can suffer sensory and motor skills damage.[3]

Methylmercury poisoning in humans is well known as 'Minamata disease', based on the occurrence of this toxicity in people living along the western coastal areas of southern Japan in the 1950, after ingestion of MeHg-contaminated fish.[4]

Up to 95% of ingested MeHg is absorbed in the gut. It readily penetrates the blood–brain and placental barriers,[1] due to its high lipoaffinity and liposolubility. An erythrocyte/plasma concentration ratio of around 20:1 for MeHg has been reported. Its concentration in blood correlates well with MeHg intake. However, total mercury in hair is more frequently reported in the literature (since it has a good correlation with mercury in blood levels) due to its lower fluctuations and easier sampling. It can also help to recapitulate previous exposure.

As previously reported,[5–7] contributions to mercury exposure by contaminated fish is more relevant than occupational exposure in gold-mining activities in the Amazon Basin, Brazil. Rivers emerging from gold mining activities may be shown to have fish contaminated by MeHg (W. Jardim, personal communication).

According to Barbosa,[5,6] and based on the work of several authors who analyzed Brazilian fish samples, the mean concentration of mercury in piscivorous species was 662 ng g^{-1} (n = 1152), and the average for herbivorous fish was 65 ng g^{-1} (n = 71). The World Health Organization (WHO) regards 50 ng g^{-1} as a critical value for mercury in fish. On the other hand, Limaverde Filho and Campos[8] pointed out that around 90–100% of mercury content in fish is in the methylmercury form.

CDs are cyclic (α-1,4)-linked oligosaccharides consisting of six, seven or eight units of glucose, known as $\alpha-$, $\beta-$and $\gamma-$cyclodextrins respectively. According to the dimensions and the hydrophilic–lipophilic properties, several chemical species can be included totally or partially in the CD cavity. Host–guest interactions can change the physico-chemical properties of guests.[9–14] Toxicological studies of CDs were carried out by Irie and Uekama,[15] who found that α-CD is excreted almost completely in an intact form into the urine of rats, after parenteral administration. Otherwise, by the oral route, α-CD is practically resistant to stomach acids or salivary and pancreatic amylases and is extensively hydrolyzed in the colon.

In this paper we report the preparation of an inclusion compound of methylmercury chloride and α-cyclodextrin and its characterization through thermal analysis (TG, DTG and DSC), Xray powder pattern diffraction (XRD), IR, Raman and ^{1}H, ^{13}C NMR spectroscopy, and biological tests. Our approach was through the modification of methylmercury liposolubility using the host–guest strategy.

EXPERIMENTAL

Reagents

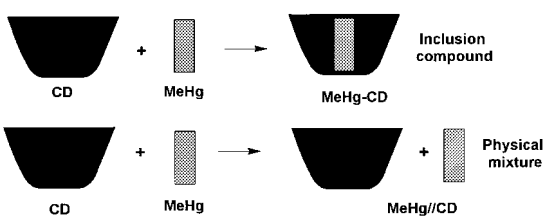
Analytical grade chemicals and Milli-Q water was used throughout. α-Cyclodextrin hydrate (CD) was purchased from the Aldrich Chemical Company Inc. (85 609-6). Methylmercury chloride was from Stream Chemical.

Preparation of the inclusion product

The inclusion compound of methylmercury chloride and α-cyclodextrin (MeHgCl–CD) was prepared in a molar ratio of 1:1 in an aqueous medium, by a co-precipitation method as related in the literature.[16,17] A physical mixture (MeHgCl//CD) was prepared in the same molar ratio, for comparison purposes (Scheme 1).

Physical measurements

Infrared (IR) spectra, were recorded using a Perkin-Elmer spectrometer model 283B, with Csl pellets, in the range 4000–200 cm^{-1}. ^{1}H and ^{13}C NMR spectra in dimethyl sulfoxide (DMSO) solution



Scheme 1 Formation of MeHgCl–CD (inclusion compound) and MeHgCl//CD (physical mixture), represented schematically.

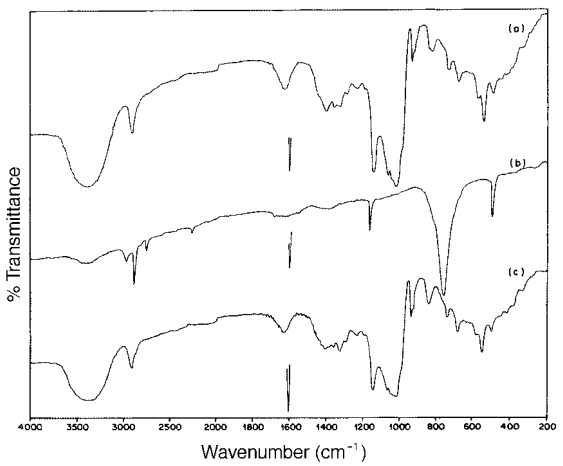


Figure 1 IR spectra of (a) α-CD; (b) MeHgCl; (c) MeHgCl–CD inclusion compound.

were recorded on a Bruker Avance DPX 200 FT NMR spectrometer. Chemical shifts are referenced downfield relative to external $(Me)_4Si$. TG and DTG curves were registered on a TGA-50 Shimadzu thermogravimetric analyzer and DSC curves on a DSC-50 Shimadzu differential scanning calorimeter, respectively. A heating rate of 10 °C min^{-1} under a dynamic N_2 atmosphere was used. Raman spectra were obtained with the samples in the solid state using a Renishaw MicroRaman Imaging System, excited with the line at 632.8 nm of a He–Ne laser of Spectra-Physics. Details of the Raman apparatus were described in a recent publication.[18] XRD was performed using an X-ray Rigaku spectrometer, Geigerflex model, fitted with a LiF monochromator and employing Cu K_α radiation. Mercury cold-vapor analysis of the inclusion compound was carried out on a Perkin-Elmer atomic absorption spectrometer, Analyst 300 model, having a FIAS 400/AS-90 device.

Biological tests

Susceptibility testing was performed by a tube dilution method[19] with a final bacterial inoculum of 10^5 CFU ml^{-1}. Nutrient broth was employed as the basal medium. The *Escherichia coli* reference strain used was ATCC 25922. Methylmercury chloride or MeHgCl–CD was added to 3 ml of basal medium in a concentration range from 1–15 μM. A tube containing an identical amount of the basal medium was supplemented with α-CD and included in each assay as a growth control. After 24 h of incubation at 37 °C the minimum inhibitory concentration (MIC) was defined as the lowest concentration that completely inhibited growth.

RESULTS AND DISCUSSION

Initially it was verified that the stoichiometry of the inclusion compound was a 1:1 molar ratio for MeHgCl–CD.

IR spectra

Figure 1 shows the IR spectra of α-CD (a), MeHgCl (b) and the MeHgCl–CD (c) inclusion compound. Table 1 lists the frequencies of bands for each compound with assignments.[20] The IR spectrum of MeHgCl showed a strong band at 760 cm^{-1} assigned to a CH_3 rocking movement which is absent in the IR spectrum of the inclusion compound. It seems that MeHgCl has lost this rocking movement on interaction with the CD cavity. Also the $\nu(CH_3)$ asymmetric and symmetric stretching modes at 2980 and 2770 cm^{-1} respectively in the inclusion IR spectrum were not observed in contract to the IR of MeHgCl alone. Finally, a broadening of the CD (ν(C–O)) band at 1140 cm^{-1} in the MeHgCl–CD spectrum was observed, in contrast to α-CD. These results suggest interaction between the methyl group and the CD cavity.

^{13}C, ^{1}H NMR

The ^{13}C NMR data of α-CD, MeHgCl and MeHgCl–CD are shown in Table 2. The numbering

Table 1 IR frequencies (cm^{-1})

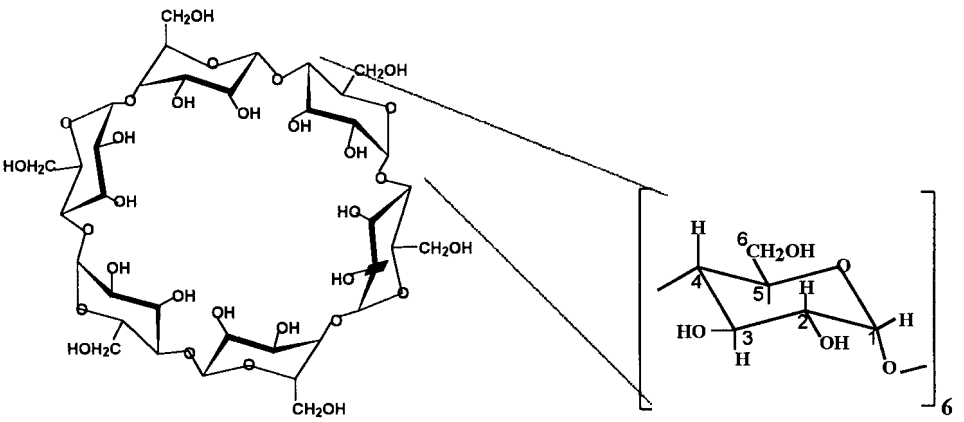
CD	MeHgCl	MeHg–CD	Assignment[a]
~3500			ν(OH)
	2980	—	$\nu(CH_3)_a$
2910	2900	2910	$\nu(CH_3)_a$; (CH)
	2770	—	$\nu(CH_3)_s$
1630			
1400			
1140	1160	1140 (broad)	$\delta(CH_3)_s$
1020			
540			
	760 (strong)	—	CH_3 rock
	500	500	$\nu(MC)_a$

[a] Subscripts a, s denote asymmetric and symmetric modes.

Table 2 ^{13}C NMR chemical shifts[a]

^{13}C	CH_3M	C1	C2	C3	C4	C5	C6
α-CD	—	101.95	72.11	73.50	81.98	73.25	60.01
MeHgCl	5.81	—	—	—	—	—	—
MeHgCl-CD	5.82	101.98	71.53	73.28	82.09	72.12	60.01
[(MeHgCl–CD) − δ(CD)]		0.03	−0.58	−0.22	0.11	−1.13	0

[a] Reference: tetramethylsilane (TMS).



Scheme 2 Carbon numbering in CD.

of the assigned carbon atoms is indicated in Scheme 2. Analysis of the ^{13}C NMR MeHgCl–CD spectra showed chemical shifts downfield (C5 > C2 and C3) in relation to α-CD alone. This result reflects changes in electrical effects in the cavity caused by the guest upon inclusion. On the other hand deshielding, changes were observed upfield for C1 and C4. These results suggest host–guest interaction.[21] Changes in the chemical shifts were not observed for C6. The ^{1}H NMR data are presented on Table 3. Analysis of the ^{1}H NMR inclusion compound spectrum showed some shielding chemical shift changes for all signals. These results indicate interaction of MeHgCl with the α-CD cavity.

Raman spectra

In Fig. 2 the Raman spectra of pure MeHgCl (B), the inclusion compound (C), and for comparison purposes pure α-CD (A) and a physical mixture (D), are presented. It is clear (Fig. 2) that the Raman bands of the Hg–Cl vibrational mode[20] observed for MeHgCl at 293 cm^{-1} are shifted to 329 cm^{-1} in the inclusion compound spectrum. The other two

Table 3 ^{1}H NMR chemical shifts[a]

^{1}H	H1	H2	H3	H4	H5	H6	CH_3
α-CD	4.80	3.29	3.77	3.40	3.59	3.65	—
MeHg	—	—	—	—	—	—	0.75
MeHg–CD	4.78	3.27	3.75	3.37	3.55	3.61	0.74
Δδ[MeHg–CD] – [CD]	−0.02	−0.02	−0.02	−0.03	−0.04	−0.04	

[a] Reference: TMS.

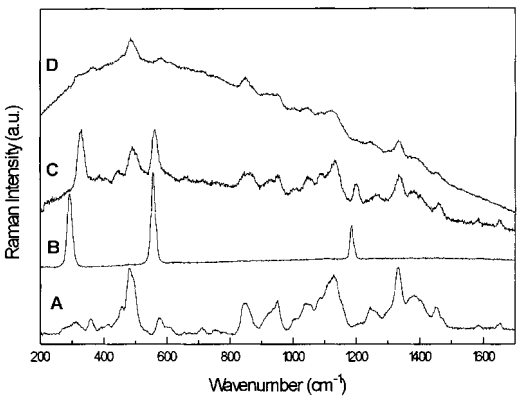


Figure 2 Raman spectra of (A) pure α-CD; (B) pure MeHgCl; (C) MeHgCl–CD inclusion compound; (D) MeHgCl//CD physical mixture.

Raman bands observed in the spectrum of MeHgCl, at 555 and 1189 cm^{-1}, and assigned to Hg–C and CH_3 symmetric deformation modes, are also shifted to 563 and 1201 cm^{-1}. This observation is unusual in CD inclusion compounds, since the interactions are not strong enough to promote a substantial shift of the vibrational modes of the guest. In this case these results might be due to a specific MeHgCl interaction within the cavity. The Raman spectrum of the mechanical mixture is entirely dominated by vibrational modes due to CD and must be compared with spectrum A. These facts are a strong indication that MeHgCl is encapsulated into a CD cavity, and that the interaction is through the methyl moiety of the molecule.

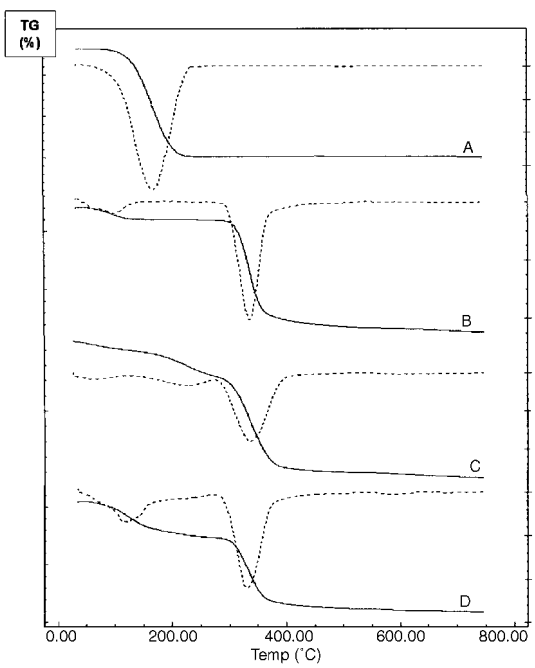


Figure 3 TG (-) and DTG (---) curves of (A) MeHgCl; (B) α-CD; (C) MeHgCl–CD inclusion compound; (D) MeHgCl//CD physical mixture.

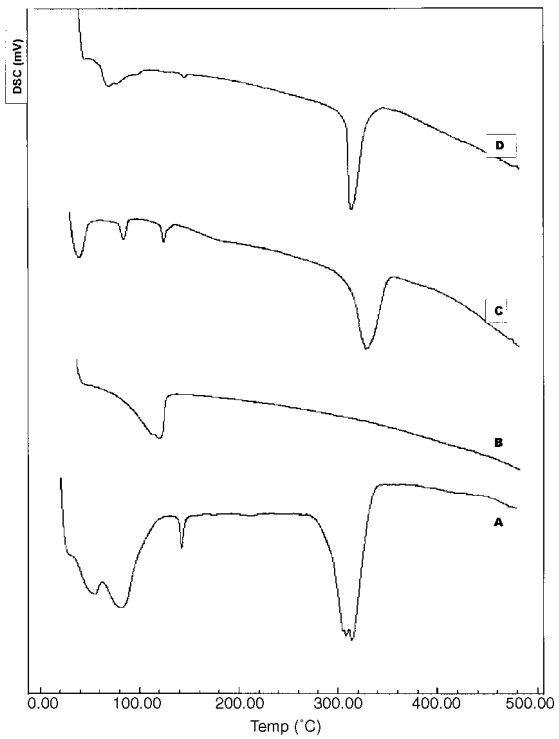


Figure 4 DSC curves (A–D as in Fig. 3).

Thermal analysis

Thermogravimetry – differential thermogravimetry (TG–DTG) and differential scanning calorimetry (DSC) curves of MeHgCl, inclusion compound MeHgCl–CD and the physical mixture MeHgCl// CD are shown in Figs 3 and 4 respectively.

The TG and DTG curves of MeHgCl (Fig. 3A), show thermal stability between 25 and 167 °C, after which was observed a strong weight loss indicating complete thermal decomposition of MeHgCl. This result fits with the melting point of MeHgCl (170 °C).[23] The DSC curve of MeHgCl (Fig. 4B) exhibited a broad endothermic peak around 120 °C due to a possible change of phase of the compound. The TG and DTG curves for α-CD (Fig. 3B)

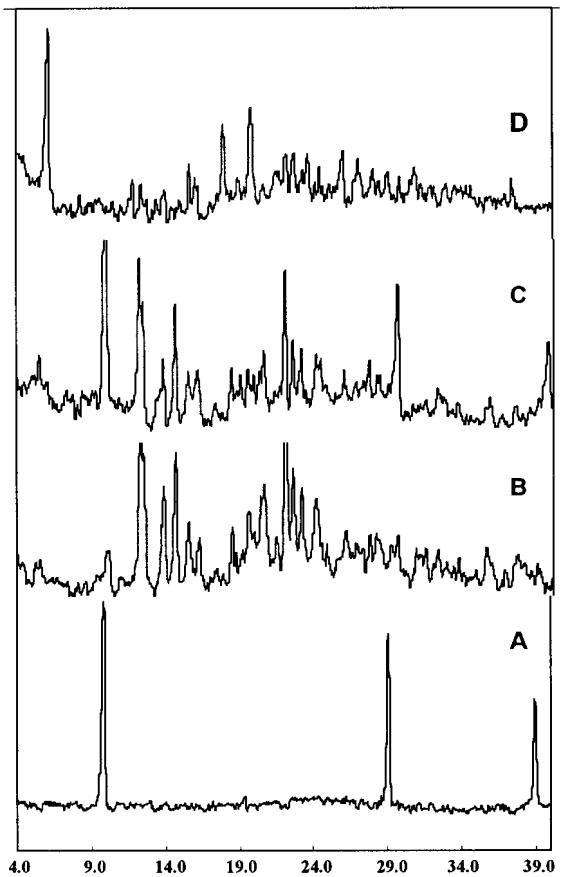


Figure 5 XRD patterns (A–D as in Fig. 3).

presented two decay steps. The first one, around 100 °C, is due to loss of water molecules inside the cavity, and the second, around 340 °C, is associated with CD melting and decomposition. These results are reinforced by the DSC curve of α-CD (Fig. 4A), which exhibited the same thermal phenomena. TG–DTG curves of the inclusion compound (Fig. 3C) showed a different thermal behavior when compared to MeHgCl, α-CD and the physical mixture (Fig. 3D) curves. It was observed (Fig. 3C) that a first step of thermodecomposition (around 75 °C) is associated with loss of water molecules in the complex; this phenomenon was verified in the DSC curve (Fig. 4C) at the same temperature. On the other hand, a new endothermic peak at 121 °C, was observed in the DSC curve perhaps due to a new phase transition of the inclusion compound different from the peak observed in free MeHgCl. Nonetheless, a second thermal weight loss event was observed around 230 °C in the inclusion compound TG curve, due to decomposition of the MeHgCl moiety in the host–guest compound. Finally total thermal decomposition of the compound around 340 °C was observed, which was verified also in the respective DSC curve. The TG, DTG and DSC curves of the physical mixture showed a superposition of the thermal events of MeHgCl and α-CD in the same range of temperatures discussed for both free components.

Comparing the calculated heat in the fusion region in the DSC curves, we found ΔH values of -382.7 and -210 kJ mol^{-1} for CD and MeHgCl–CD respectively. In accord with the interpretation of Gelb *et al.*,[23,24] one can infer that more negative enthalpy values are characteristic of stronger dipolar interactions, which were broken after inclusion.

X-ray analysis

The XRD diffraction patterns of the MeHgCl, α-CD, MeHgCl//CD physical mixture and MeHgCl–CD inclusion compound are presented on Fig. 5. The XRD pattern of MeHgCl (Fig. 5A), showed a polycrystalline system and exhibited three main peaks at $2\theta = 9.9$ °, 29 ° and 39 °. The XRD pattern diffraction of α-CD (Fig. 5B) showed a polycrystalline system, as reported in the literature.[11] The XRD pattern diffraction of the physical mixture (Fig. 5C) showed the typical diffraction peaks, 2θ, observed in MeHgCl and α-CD respectively. However some minor changes were observed in the $2\theta = 29$ ° and 39 ° peaks from MeHg, shifting to $2\theta = 29.6$ ° and 39.7 ° respectively in the physical mixture. Analysis of the XRD pattern diffraction of MeHgCl–CD (Fig. 5D) showed a more amorphous profile when compared with the XRD pattern of MeHgCl α-CD and the physical mixture. Those peaks at $2\theta = 29.6$ and 39.7 ° observed in the physical mixture were absent and new ones were observed at $2\theta = 6.2$, 18.0 and 19.9 °. On the other hand, the characteristic peaks of MeHgCl and α-CD were not observed. These results suggest formation of a new crystalline phase upon host–guest interaction.

Biological tests

To test the efficacy of formation of the inclusion compound MeHgCl–CD, the MIC for free MeHgCl, α-CD (not shown), MeHgCl–CD and MeHgCl//CD was determined. The results are shown in Fig. 6. It was found that at a concentration of 4 μM for free MeHgCl and MeHgCl–CD,

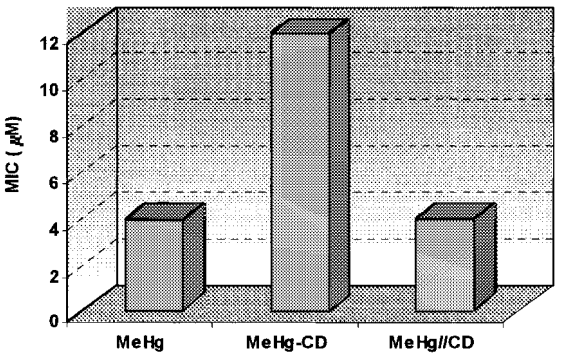


Figure 6 MICs of MeHgCl, MeHgCl–CD and MeHgCl//CD.

bacterial inhibition was observed. On the other hand, toxic activity by the MeHgCl–CD inclusion compound was not observed up to 12 μM, in contrast to free MeHgCl. These results show a three-fold higher MIC for the inclusion compound when compared with free MeHgCl.

Also, we observed that α-CD did not inhibit bacterial growth, even at 50-fold higher concentrations. However, a higher bacterial growth was observed, possibly due to an additional energy source for the bacteria.

Finally, the culture that contained the inclusion compound kept at the same turbidity as the control tube for a one-week period. This result suggests the stability of the inclusion compound in a water medium.

CONCLUSIONS

The inclusion compound of methylmercury chloride–α-cyclodextrin was obtained and characterized by thermal and spectroscopic analysis. Increase in water solubility of the included MeHgCl was observed, when compared with free MeHgCl. One can infer a decrease in toxicity of MeHgCl on increasing its hydrophilic character after inclusion. This host–guest strategy suggests a new method of recovering toxic hydrophobic organomercurial substances from the environment and biological systems. Studies are being developed in terms of testing viability *in vivo*.

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