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Chlorambucil inclusion complexes study with β-cyclodextrins through NMR, IR spectroscopy, x-ray crystallography and capillary electrophoresis

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ABSTRACT

The capacity of cyclodextrins to house molecules such as chlorambucil was studied by ^{1}H and ^{13}C NMR through inclusion complexes formed from chlorambucil with three β-cyclodextrins; in addition, infrared spectroscopy, X-ray crystallography and capillary electrophoresis studies were conducted. Solid complexes were obtained by co-precipitation method, with ^{1}H and ^{13}C homonuclear studies, 2D techniques (COSY, HETCOR and ROESY) of ^{1}H-^{13}C heteronuclear correlation of liquid-phase nuclear magnetic resonance and solid-phase for ^{13}C. The results demonstrate the formation of inclusion complexes, their stability in aqueous solution, and provide evidence to identify the interactions between chlorambucil and the studied cyclodextrins.

KEYWORDS

Chlorambucil;
NMR;
X-ray;
Inclusion complexes;
β-cyclodextrins;
Capillary electrophoresis.

INTRODUCTION

Cyclodextrins are polysaccharides containing 6, 7 and 8 monomers of D-glucose joined by α 1-4 linkages. They have the ability to form inclusion complexes with molecules with a determined polarity and dimension, because they have a hydrophobic cavity and an outer hydrophilic surface[1-5]; based on that property, extensive applications have been found in many fields, including pharmaceutical technology, agriculture, forensic sciences, chemical synthesis, analytical chemistry, in order to improve aqueous solubility, dissolution, bioavailability, stability, reduce toxicity and control drug delivery[6-8].

β-CD is the most used cyclodextrin in pharmaceutical application, this is because its cavity has a good affinity with the hydrophobic fractions of many compounds; however, β-CD has low solubility (1.8% to 25°C)[9], and so recently some methylated, sulphated, hydroxylated and acetylated β-CD[10] have been prepared with the aim of improving the physicochemical and inclusion properties of native β-CD, leading to a great number of studies on inclusion complexes with different drugs[11-15].

Chlorambucil is a crystalline drug that is slightly soluble in water and soluble in ethanol, chloroform, acetone, diethyl ether and alkaline solutions, and is easily decomposed. It is a drug used in the treatment of chronic lymphocytic leukaemia, giant follicular lymphoma, Hodgkin's disease and it has also been tested for the treatment of Lupus erythematosus, nephritic syndrome, chronic hepatitis, psoriasis, and rheumatoid arthritis, and when having problems of low solubility and stability, inclusion complexes have been formed with cyclodextrins, noticeably improving these problems[16-18].

Moreover, inclusion complexes have been studied through different HPLC techniques, Fluorescence, CE, DSC and IR. In the last years, nuclear magnetic resonance (NMR) spectroscopy has played a very important role in the analysis of different macromolecules such as proteins and carbohydrates, since it provides information related to the interaction between molecules, and great acceptance has been observed toward the studying of interactions between drugs and cyclodextrin, both in liquid as in solid phase[19-23]. Recently, we have described studies on capillary electrophoresis of the chlorambucil-β-cyclodextrin complex[24].

In the present study, Chlorambucil inclusion complexes with dimethyl-β-cyclodextrin, trimethyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin were prepared through the co-precipitation method, likewise were characterized by liquid-phase nuclear magnetic resonance spectroscopy, through ^{1}H and ^{13}C studies, and 2-D homonuclear (^{1}H,^{1}H) and heteronuclear (^{1}H,^{13}C) experiments, ^{13}C solid state, IR spectroscopy, and X-ray crystallography and capillary electrophoresis.

EXPERIMENTAL

Material and reagents

Chlorambucil and trimethyl-β-cyclodextrin were acquired from Sigma-Aldrich. Dimethyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin were acquired from Beckman Coulter (Mexico). $CDCl_3$, deuterated trimethylsilyl (TMS) and D_2O were acquired from Sigma-Aldrich. Absolute ethanol, sodium borate decahydrate ($Na_2B_4O_7.10H_2O$), as well as the rest of the chemical compounds that were used, were analytical grade reagent.

Preparation of inclusion complexes by co-precipitation

The co-precipitation method has been reported as an efficient method for obtaining inclusion complexes with cyclodextrins[30,31]. The chlorambucil and each of the cyclodextrins were weighted at a 3:1 proportion, respectively; the cyclodextrin was dissolved in 4 ml absolute ethanol, with magnetic stirring and heating at 40°C until total dissolution, subsequently, the chlorambucil was added slowly maintaining constant stirring. The solution was heated to boil, removed, and kept at 4°C. The formed precipitate was filtered by 0.45μm membranes, washed with absolute ethanol, and kept in vacuum desiccators for its study.

It must be stressed that in previous studies the stechiometry of complexes were demonstrated is 1:1 ratio[31].

NMR experiments

^{1}H and ^{13}C experiments were obtained at 37°C with a Varian system operating at 200 MHz. The chemical shifts were given in ppm, using trimethylsilyl (TMS) as reference at 0ppm. The samples were prepared dissolving 20-25 mg of each dry powder; chlorambucil (CHL), dimethyl-β-cyclodextrin (DM-β-CD), trimethyl-β-cyclodextrin (TM-β-CD), chlorambucil-dimethyl-β-cyclodextrin (CHL-DM-β-CD) and chlorambucil-trimethyl-β-cyclodextrin (CHL-TM-β-CD) complexes were dissolved in $CDCl_3$ (99.8%), while 2-hydroxypropyl-β-Cyclodextrin (2-HP-β-CD) and the chlorambucil-hydroxypropyl-β-Cyclodextrin (CLH-2-HP-β-CD) inclusion complex were dissolved in D_2O (99.8%); in this experiment, D_2O at 4.8ppm was considered a marker. The samples were dissolved at least 20 minutes before each NMR experiment. Chlorambucil, DM-β-CD, TM-β-CD, HP-β-CD, Chlorambucil- DM-β-CD, Chlorambucil-TM-β-CD and Chlorambucil- HP-β-CD were characterized by homonuclear correlation (COSY) and heteronuclear correlation studies (HETCOR). ROESY experiments were performed in at 37°C in a JEOL system at 500MHz. Using D_2O as solvent for CHL-DM-β-CD complex, CD_3OD for CLH-2-HP-β-CD complex, and DMSO for CHL-TM-β-CD complex.

^{1}H ^{13}C

Figure 1 : Numerated structures of chlorambucil

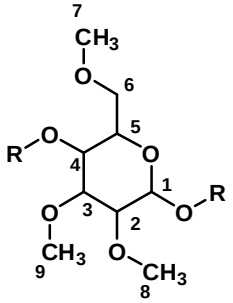
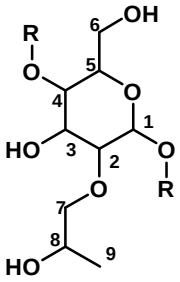
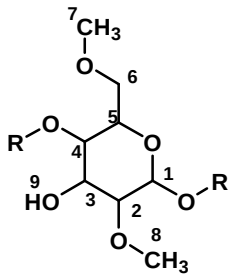
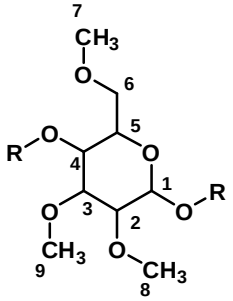
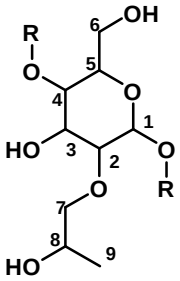
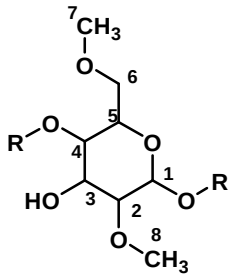
	TMβCD	2HPβCD	DMβCD
^{1}H			
^{13}C			

Figure 2 : Numerated structures for every one cyclodextrins used to complex chlorambucil. All hydroxyl groups were not numerated for ^{1}H chemical shifts, because they are marked and discussed as OH in the present work

Fourier transform infrared spectroscopy (FT-IR) method

The spectra were obtained using Fourier Transform Infrared Spectrometer Nicolet Magna 560. The samples were previously mixed with anhydrous KBr, and a disk was prepared by compression of that mixture. The scans were executed with spectra ranging from 4000 cm^{-1} to 400cm^{-1}.

Capillary electrophoresis studies

In previous studies we obtained the effective mobilities of CHL and CHL-TM-β-CD complex and was demonstrated the presence in solution of the complex[24]. In this study we carried out experiments in order to obtain the mobilities of the CHL-DM-β-CD and CHL-DM-CD complexes to prove the real existence of the complexes in solution.

The methodology used to carry out the experiments was described by Hernandez, et al.[24]. In general different concentrations (from 5 to 2000μg/mL) of CHL and CHL-β-cyclodextrins complexes were analyzed by capillary electrophoresis, using a PACE/MDQ Beckman- Coulter capillary electrophoresis system (Palo Alto, California) equipped with a diode array detector, all the experiments were carried out using a new fused silica capillary (Polymicro Technologies, Phoenix, Az.) of 75μm of internal diameter, 60 cm total length and 50 cm to the detector. Previous to use the capillary was rinsed whit 1.0 M NaOH and deionized water for 30 and 10 minutes respectively. The background electrolyte used to carry out the experiments was 20mM sodium tetraborate, hidrodynamical injection was used to introduce the samples into the capillary pressure applied was 0.5 psi during 5 seconds, the temperature was 20ºC and the voltage 20kV. The capillary was rinsed between each experiment whit 0.1 M sodium hydroxide, deionized water and background electrolyte for 10, 5, and 5 minutes respectively.

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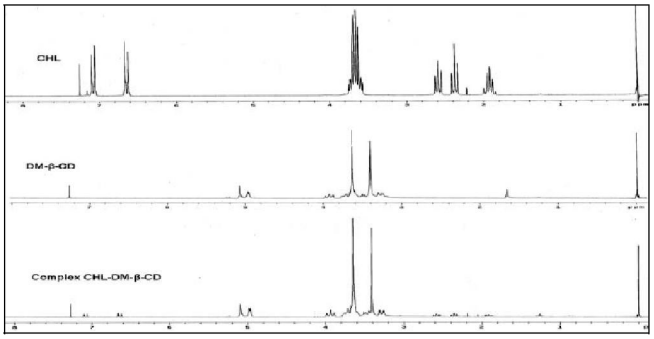

Figure 3 : ^{1}H NMR spectra CHL, DM-β-CD and CHL-DM-β-CD complex inclusion

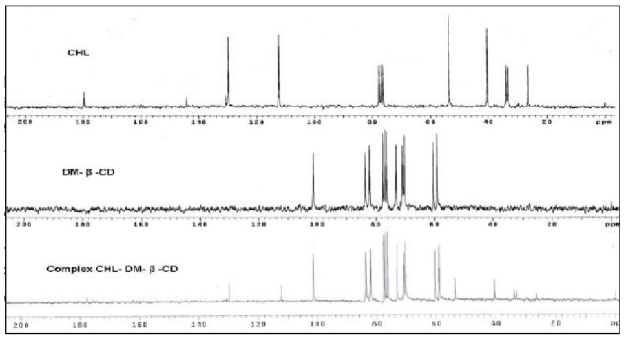

Figure 4 : ^{13}C NM Fig. 4 R spectra of CHL, DM-β-CD and CHL-DM-β-CD complex inclusion

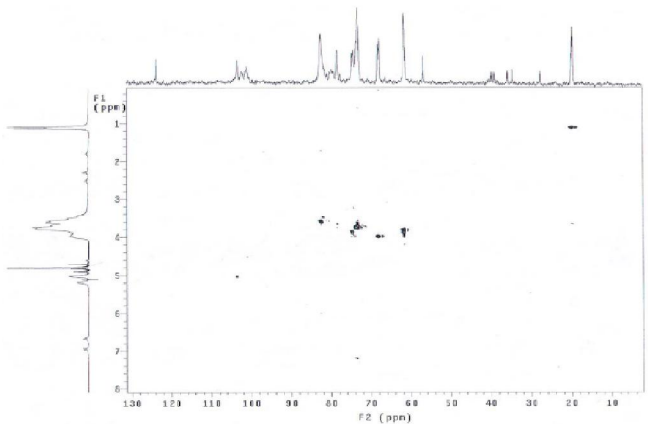

Figure 5 : HETCOR experiments of CHL-DM-β-CD

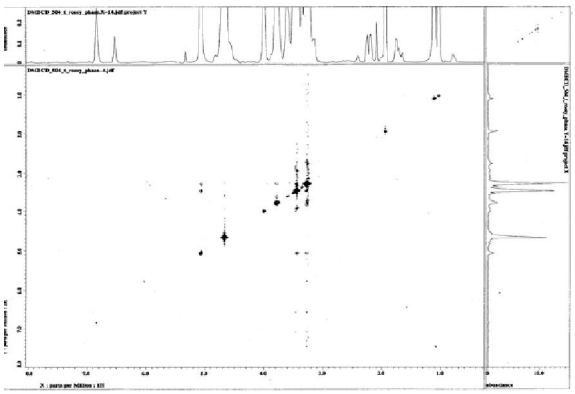

Figure 6 : ROESY experiment of CHL-DM- β-CD inclusion complex in D_2O

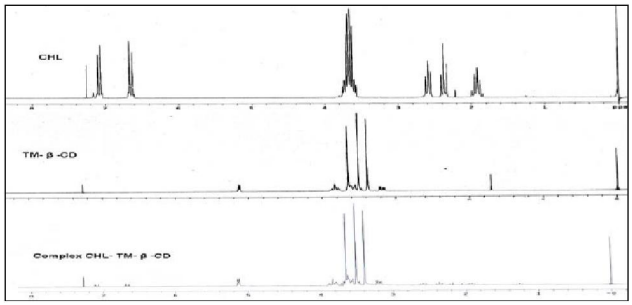

Figure 7 : ^{1}H NMR spectra CHL, TM-β-CD and CHL-TM-β-CD complex of inclusion

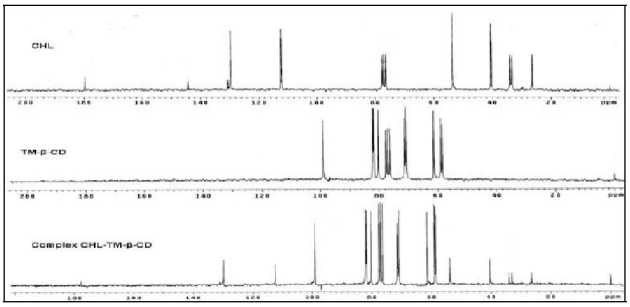

Figure 8 : ^{13}C NMR spectra CHL, TM-β-CD and CHL-TM-β-CD complex of inclusion

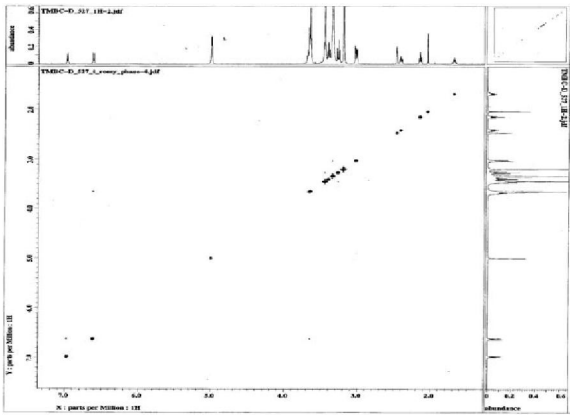

Figure 9 : ROESY experiment for CHL-TM-β-CD complex of inclusion in DMSO d_6

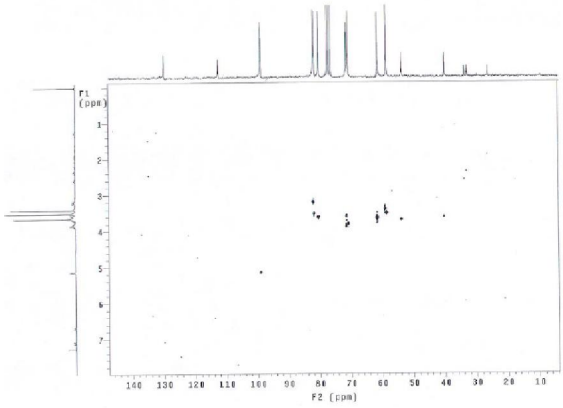

Figure 10 : HETCOR experiment of CHL-2-HP-β-CD of inclusion complex

RESULTS AND DISCUSSION

NMR studies

In order to make clear the results we present the numerated carbon and hydrogen structures of chlorambucil figure 1, and cyclodextrines figure 2 used in this study.

TABLE 1 presents the data obtained of ^{1}H and TABLE 2 for ^{13}C for free chlorambucil, DM-β-CD and the formed complex. It is observed that the H-7 of CHL is exhibiting a shield shift at 0.138ppm (Δδ with negative values) when the complex is formed; the rest of the hydrogen do not exhibit important changes. According with the results obtained for the DM-β-CD hydrogen, we observed that H-4, H-5 and H-6 undergo a shield effect at 0.062, 0.033 and 0.027ppm respectively, whereas H-1, H-2 and H-5 had been unshielded due to their movement to lower field; Gibaud et al.[25] and Ventura et al.[26] conducted NMR studies on DM-β-CD inclusion complexes with melarsoprol and papaverine hydrochloride, respectively, finding that H-5 of DM-β-CD is the principal cyclodextrin hydrogen interacting with the included molecules. Whereas this hydrogen is present into the cyclodextrin cavity, the studies conducted in the present work agree and corroborate results previously obtained by those scientists.

According to the results of C atoms of CHL, (C = O) shifts to 2.061ppm, C-3 to 0.242ppm, both with shield effects, whereas C-2, C-5 and C-9 display vulnerability effects between 0.030 and 0.060ppm, C-4 and C-6 were not detected in the experiment for the inclusion complex. It must be stressed that the carbons of DM-β-CD exhibited slight changes from 0.000 to 0.030ppm, C-1 and C-2 had suffered slightly shield effects. Figure 3 shows the ^{1}H spectra of the CHL, DMβCD and the inclusion complex obtained, and figure 4 shows the ^{13}C spectra for CHL, DMβCD and the complex.

DEPT, COSY and HETCOR figure 5 studies of 2D-NMR allowed us to conduct the correlations and assignations of ^{1}H and ^{13}C atoms; in the COSY experiment, it is possible to observe that the H-7 of chlorambucil correlate with H-5 of the DM-β-CD when the complex is formed, those results are corroborated with ROESY experiments (Figure 6).

The CHL-TMβCD complex experiment of ^{1}H showed that H-6 of chlorambucil was displaced at low fields in approximately 0.030ppm, exhibiting a shield effect, and H-7 was displaced 0.044ppm to up fields in comparison with free chlorambucil. Likewise in TM-β-CD it was found that H-5 is affected at 0.104ppm having a vulnerability effect (the results are shown in TABLE 3). The obtained data point the interaction of both molecules when the inclusion complex is formed. The observed shifts in the hydrogen experiment for TM-β-CD agree the data reported by Kong et al.[27] in a study with 5-(2-hydroxyphenyl)-10,15,20-tris (4-methoxyphenyl) porphyrin with β-cyclodextrins in which TM-β-CD H-5 is the atom that interacts with the guest molecule.

In the ^{13}C experiment, C-3 of CHL appears displaced in 0.242ppm and (C = O) 1.879ppm when the inclusion complex is formed as well as C9, having a shield effect, C-6 suffers a vulnerability effect of 0.212ppm; we also observed that C-2, C-1 and C-5 do not exhibit significant changes in their shifting. It should be stressed that the C atoms of TM-β-CD did not exhibit changes when the complex was formed. Figure 7 shows the obtained ^{1}H spectra of CHL, TM-β-CD and the inclusion complex, and figure 8 shows the ^{13}C spectra for CHL, TM-β-CD and the inclusion complex.

Data obtained showed that when the CHL-2HP-β-CD complex is formed the H-2 and H-4 have a displacement through up field. In the other hand H-5, H-6 and H-7 change by 0.111, 0.179 and 0.107ppm respectively through low fields, exhibiting vulnerability an unshielded effect.

In 2-HP-β-CD, we found that when the complex is formed, the H-5 of 2HP-β-CD exhibit a change by 0.093ppm, H-6 shifts 0.050ppm and H-7 has been displaced in 0.142ppm, the three hydrogen show up field effect, which indicates that these atoms participate in an important interaction with CHL in the complex formation, these changes in β-CD lead us to conclude that H-5 of 2-HP-β-CD interacts with H-6 and H-7 of CHL, whereas H-7 could interact with H-7 of CHL. In the ^{13}C experiment, we observed huge changes in all the carbons of CHL, and C-9 overlaps with the DMSO signal at 39.5ppm and with the carbon atoms of 2-HP-β-CD between 0.037 and 0.081ppm; these data indicate a more complex interaction than that observed

TABLE 1 : ^{1}H NMR chemicals shifts (ppm) of CHL, DM-β-CD (δ) and CHL-DMβCD complex

Proton	Signal for CHL			Proton	Signal for DMβCD		
	δ ^{1}H free CHL	δ ^{1}H DMβCD-CHL complex	Δδ		δ ^{1}H free DMβCD	δ ^{1}H DMβCD-CHL complex	Δδ
H1	2.372	2.361	-0.011	H1	4.961	4.974	0.013
H2	1.917	1.912	-0.005	H2	3.268	3.282	0.014
H3	2.587	2.584	-0.003	H3	3.920	3.933	0.013
H4	7.082	7.061	-0.001	H4	3.503	3.441	-0.062
H5	6.645	6.641	-0.004	H5	3.747	3.714	-0.033
H6	3.652	3.514	-0.135	H6	3.703	3.676	-0.027
H7	3.677	3.676	-0.001	H7 Me	3.396	3.406	0.012
				H8 Me	3.628	3.639	0.011
				OH	5.067	5.094	0.027

^{1}H Δδ = ^{1}H δ complex - ^{1}H δ free CHL or DMβCD

TABLE 2 : ^{13}C NMR chemicals shifts (ppm) of CHL, DM-β-CD (δ) and CHL-DMβCD complex. The last column on the right sides show the chemical shifts for solid ^{13}C NMR for CHL and cyclodextrine when CHL-DMβCD complex is formed

Carbon	Signal for CHL				Carbon	Signal for DMβCD			
	δ ^{13}C free CHL	δ ^{13}C DMβCD-CHL complex	Δδ	NMR for solids		δ ^{13}C free DMβCD	δ ^{13}C DMβCD-CHL complex	Δδ	NMR for solids
C1	33.812	33.812	0.000	32	C1	101.337	101.307	-0.030	99.105
C2	26.387	26.447	0.080	24	C2	83.546	83.516	-0.030	
C3	33.236	32.994	-0.242	32	C3	73.181	73.181	0.000	72.089
C4	130.584			127.87	C4	82.061	82.031	-0.030	
C5	129.705	129.735	0.030		C5	70.848	70.848	0.000	
C6	112.308	112.278	-0.030		C6	70.302	70.302	0.000	
C7	144.161			142.477	C7 Me	58.997	59.028	0.031	57.512
C8	53.663	53.633	-0.030		C8 Me	60.331	50.331	0.000	57.512
C9	40.369	40.419	0.030	39.5					
(C=O)	179.651	177.59	-2.061	172					

^{13}C Δδ = ^{13}C δ complex- ^{13}C δ free CHL or DMβCD

with the CHL-DM-β-CD and CHL-TM-β-CD complexes. The results of the hydrogen chemical shifts found in 2-HP-β-CD are similar to those found by Loukas et al.[11] between Riboflavin and 2-HP-β-CD and those obtained by Vianna et al.[21] with dexamethasone acetate inclusion in 2-HP-β-CD; Chao et al.[12] found that H-3 and H-5 of 2-HPβCD are the most affected in the forming of the inclusion complex with ciprofloxacin. Our results indicate that the aromatic region of CHL is introduced into the cavity of 2HP-β-CD. In the study, only H-5 was affected by the CHL inclusion, In a NMR study conducted by Masson et al.[2], it is proposed that H-1 H-2 and H-3 of CHL remain outside the Cyclodextrine cavity; our results show that H-1 H-2 H-3 of CHL do not undergo meaningful shifting, which indicates that they apparently are localized outside of the cyclodextrin cavity.

The NMR results for CHL-2-HP-β-CD complex show that CHL exhibits more changes and interactions between CHL and cyclodextrin, than observed in the two complexes previously described, those experimental results are summarized in TABLE 5 and TABLE 6, and are corroborated using HETCOR figure 10 and ROESY correlation figure 11.

Our results showed that H-1 H-2 H-3 of CHL do not undergo meaningful shifting, which indicates that they are outside the cyclodextrin cavity, the N-dichloroethyl fraction being the region that is oriented toward the interior of the cavities. The results of NMR solid phase were very similar to those found in liquid phase that is why are not showed in this paper. However those allowed us to confirm the formation of the complex

TABLE 3 : ^{1}HNMR chemicals shifts (ppm) of CHL, TM-β-CD (δ) and CHL-DM-β-CD complex

Proton	Signal for CHL			Proton	Signal for TMβCD		
	δ ^{1}H free CHL	δ ^{1}H TMβCD-CHL complex	Δδ		δ ^{1}H free TMβCD	δ ^{1}H TMβCD-CHL complex	Δδ
H1	2.372	2.364	-0.008	H1	5.121	5.135	0.014
H2	1.917	1.915	-0.002	H2	3.18	3.196	0.016
H3	2.587	2.588	0.001	H3	3.819	3.833	0.014
H4	7.082	7.095	0.013	H4	3.608	3.626	0.018
H5	6.645	6.669	0.024	H5	3.451	3.555	0.104
H6	3.677	3.707	0.030	H6	3.776	3.79	0.014
H7	3.652	3.608	-0.044	H7 Me	3.376	3.391	0.015
				H8 Me	3.636	3.652	0.016
				H8 Me	3.495	3.509	0.014

^{1}H Δδ = ^{1}H δ complex - δ free CHL or TM-β-CD

TABLE 4 : ^{13}C NMR Chemicals shifts (ppm) of CHL, TM-β-CD (δ) and CHL-DM-β-CD complex. The last column on the right shows the chemical shifts for solid ^{13}C NMR for CHL-DMβCD complex

Carbon	Signal for CHL				Carbon	Signal for DMβCD			
	δ ^{13}C free CHL	δ ^{13}C DMβCD-CHL complex	Δδ	NMR for solids		δ ^{13}C free DMβCD	δ ^{13}C DMβCD-CHL complex	Δδ	NMR for solids
C1	33.812	33.842	0.030	30.691	C1	96.943	98.943	0.000	97.939
C2	26.387	26.417	0.030		C2	82.001	82.001	0.000	81.03
C3	33.236	32.994	-0.242	30.691	C3	70.878	70.878	0.000	
C4	130.584			127.87	C4	80.273	80.273	0.000	
C5	129.705	129.735	0.030	127.87	C5	81.728	81.728	0.000	79.669
C6	112.308	112.520	0.212	109.8	C6	71.363	71.363	0.000	69.757
C7	144.161			142.252	C7 Me	58.937	58.937	0.000	58.095
C8	53.663	53.745	0.062	53.525	C8 Me	61.422	61.452	0.030	59.845
C9	40.389	40.267	-0.122	41.38	C9Me	68.482	58.512	-.030	55.374
C=O	179.651	177.772	-1.879	171.601					

^{13}C Δδ = ^{13}C δ complex- ^{13}C δ free CHL or TM-β-CD

studied.

IR-FT studies

The complexes were analyzed by FT-IR spectroscopy, with a NICOLET magna 560 spectrometer with a resolution of 1 and a sensitivity of 0.1 units, the FT-IR spectrum worked from 4000 to 400cm^{-1} and it was achieved by KBr pellet. Figure 12 shows infrared spectra of the studied systems, in which we can appreciate that CHL exhibits an absorption band of 3000 to 2000cm^{-1} corresponding to (OH) of (–COOH), a narrow and intense band at 1703cm^{-1} for the (C = O) group, and for the aromatic group (C = C), a band at 1614cm^{-1}. The bands are modified in the CHL-DM-β-CD, CHL-TM-β-CD and CHL-2-HP-β-CD complexes, the absorption band corresponding to -COOH group seems to disappear in the three complexes, while the band for -C = O group appears show less intensity at 1735, 1738 and 1710cm^{-1} for CHL-DM-β-CD, CHL-TM-β-CD and CHL-2-HP-β-CD respectively, and the (C = C) band appears at 1617, 1619 and 1618cm^{-1} for complexes CHL-DM-β-CD, CHL-TM-β-CD and CHL-2-HP-β-CD, being less narrow and lower intense than in free CHL; this change in the CHL bands indicates the presence of intramolecular interactions through hydrogen bridges between the aromatic ring of CHL when it enters into the hydrophobic cavity of β-CD when the inclusion complexes are formed, these changes are not observed in the study of physical mixture, similar changes were observed by Bayomi et al.[28] in the inclusion complex

TABLE 5 : ^{1}H NMR chemicals shifts (ppm) of CHL, 2HP-β-CD (δ) and CHL-2HP-β-CD complex

Proton	Signal for CHL			Proton	Signal for HPβCD		
	δ ^{1}H free CHL	δ ^{1}H HPβCD-CHL complex	Δδ		δ ^{1}H free HPβCD	δ ^{1}H HPβCD -CHL complex	Δδ
H1	2.503	2.519	0.016	H1	5.031	5.061	0.030
H2	1.835	1.793	-0.042	H2	3.563	3.578	0.015
H3	2.290	2.291	0.001	H3	3.919	3.931	0.012
H4	6.992	6.936	-0.056	H4	3.505	3.501	-0.004
H5	6.548	6.657	0.111	H5	3.829	3.738	-0.093
H6	3.594	3.773	0.179	H6	3.889	3.839	-0.060
H7	3.571	3.676	0.107	H7	3.756	3.614	-0.142
				H8	3.964	3.962	-0.002
				H9	1.100	1.104	0.004

^{1}H Δδ = ^{1}H δ complex- ^{1}H δ free CHL or 2HP-β-CD

TABLE 6 : ^{13}C NMR chemicals shifts (ppm) of CHL, 2HP-β-CD (δ) and CHL-2HP-β-CD complex. ^{13}C Δδ = ^{13}C δ complex-^{13}C δ free CHL or DMβCD

Carbon	Signal for CHL				Carbon	Signal for HPβCD			
	δ ^{13}C free CHL	δ ^{13}C HPβCD -CHL complex	Δδ	NMR for solids		δ ^{13}C free HPβCD	δ ^{13}C HPβCD -CHL complex	Δδ	NMR for solids
C1	33.099	34.560	1.481	23.111	C1	109.358	103.449	0.051	99.882
C2	26.637	27.619	0.982		C2	82.582	82.627	0.045	79.809
C3	33.371	35.742	2.371	127.481	C3	74.603	74.566	-0.037	
C4	129.69	136.090	6.400	127.481	C4	79.763	79.809	0.046	
C5	129.326	132.150	2.822		C5	73.444	73.444	0	70.534
C6	111.905	123.545	11.940	142.447	C6	51.552	61.506	-0.076	58.575
C7	144.455	145.728	1.273		C7 Me	78.460	78.506	0.048	
C8	52.242	56.957	4.715		C8 Me	88.145	68.064	-0.081	64.703
C9	41.161				C9Me	19.782	19.770	0.008	17.28
C = O	174.406	179.005	4.597	173.9333					

^{13}C Δδ = ^{13}C δ complex - ^{13}C δ free CHL or 2HP-β-CD

TABLE 7 : Electrophoretic migrations of free chlorambucil and chlorambucil complexes

Sample	Free CHL	CHL-DM-β-CD complex	CHL- 2HP-β-CD complex	CHL-TM-β-CD complex
μ_{eff} (m^2 V^{-1} s^{-1})	-1.56809E-07	-1.63857E-07	-1.61898E-07	-1.57896E-07
% SRD	1.929	1.846	1.869	1.916

of nifedipine with βCD and by Wen et al.[1] in the study of carvelidol-βCD complexes.

X-Ray crystallography analysis

Crystals were obtained for the CHL-TM-β-CD complex while for CHL-DM-β-CD and CHL-2-HP-β-CD this study cannot be applied because we obtained a powder instead crystals, therefore the conditions for the X-ray diffraction were not the optimal.

The study of the CHL-TM-β-CD complex by X-ray crystallography was conducted in the department of Chemistry of CINVESTAV. Figure 13 shows that the region of the molecule corresponding to the dichloroethyl-N-phenyl is introduced in the cavity of TM-β-CD, while the region of the butanoic acid remains outside the cyclodextrin. The images displayed were obtained with the program Mercury version 1.4.1[29]. The structure obtained from the X-ray crystallography study was deposited in the Cambridge Crystallographic database under registration number: CCDC 267560,

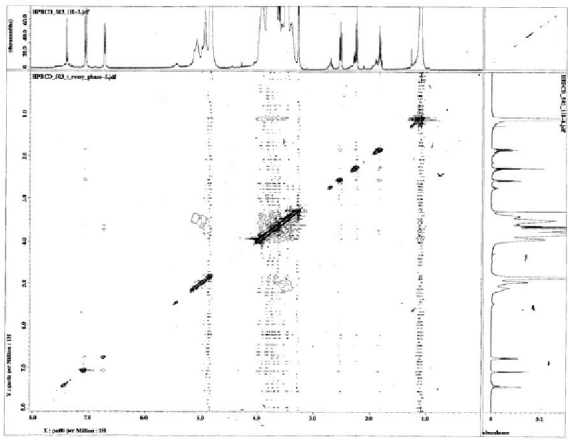

Figure 11 : ROESY experiments of CHL-2-HP-β-CD of inclusion complex in CD_3OD

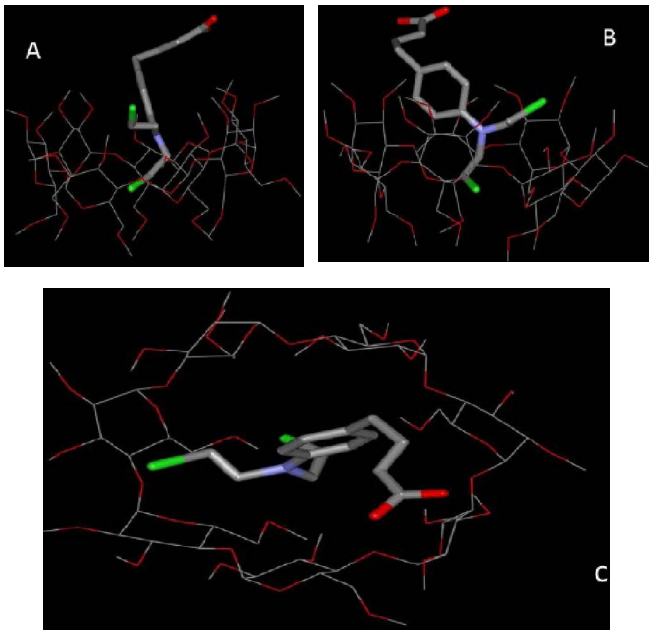

Figure 13 : Structure of the CHL-TMβCD complex inclusion obtained by X-ray crystallography: lateral view of inclusion complex in two perspectives respecting to chlorambucil (A, B); superior view of complex (C)

under the name of: 4-[4-(bis(2-chloroethylamino) phenyl] butanoic acid-2,3,6-tri-O-methylcyclohepta-amylose complex, the molecular formula of the reported structure is: $C_{77}H_{131}Cl_2N_1O_{37}$.

Capillary electrophoresis

The obtained results by the technique of capillary electrophoresis for the migration of the inclusion complexes and free chlorambucil are shown in TABLE 7. The results observed on the table, make evident the presence in solution of the inclusion complexes, since their effective mobilities are different that free

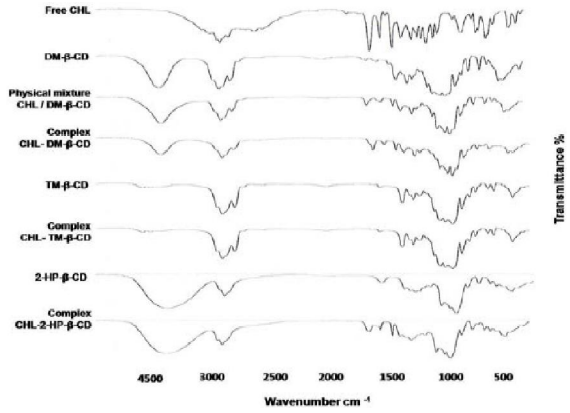

Figure 12 : FT-IR spectra of CHL, DMβCD, physical mixture CHL/DMβCD, complex CHL-DMβCD, TM-β-CD, complex CHL-TMβCD, 2-HP-βCD and complex CHL-2-HP-β-CD

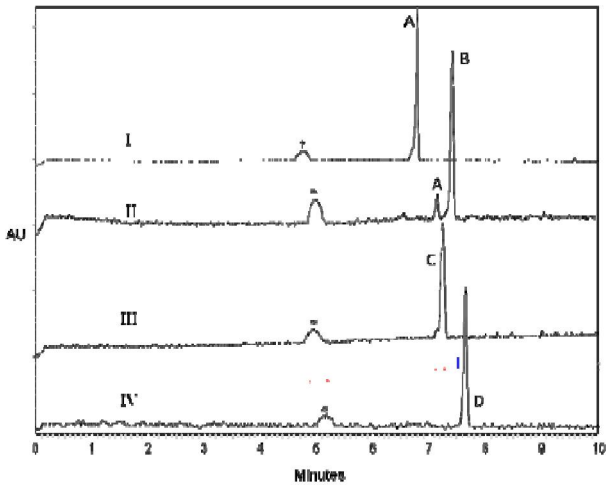

Figure 14 : Experimental single run electropherograms of I) Free chlorambucil (CHL), II) CHL-DM-β-CD complex, III) CHL-2-HP-β-CD complex and IV) CHL-TM-β-CD complex. The asterisk symbol (*) represents the electrosmotic flow (EOF) marker. Experimental conditions 20mM tetraborate buffer pH = 9.2, 20kV applied voltage, hydrodynamic injection 0.5 psi 5 seconds

chlorambucil (ANOVA test $\alpha = 0.05$). Besides, it is very know that Chlorambucil has not stability in aqueous solution due to it degradation by hydrolysis is to fast in practically all the pH range, but specially in high pH values[34]. In previous studies[33-35] it was demonstrated the monohydroxyl and dihydroxyl degradation products, considering those studies and data obtained by our group studying the degradation of chlorambucil by capillary electrophoresis[32], it is observed that this drug is very unstable and practically at time 0 it began to be hydrolysing. That is why we decided to achieve the capillary electrophoresis runs all the complexes but not the Chlorambucil after 12 hours to be dissolved in the

aqueous solution.

Specific peaks for every one of the molecules tested in this study are shown in figure 14. Showed peaks are Free Chlorambucil (Figure 7 peak A), and the formed complexes CHL-DM-β-CD, CHL-2-HP-β-CD and CHL-TM-β-CD (Figure 7 peak B, C and D respectively). Is necessary to point the presence of residual free Chlorambucil or hydrolysis product (considering that the experiments were achieved after 12 hours of the preparation of aqueous solutions) peak named A in the electropherogram II of the figure 14, this finding proves the co-existence of both free CHL or degradation product and CHL-DM-β-CD species in solution. In recent and preliminary studies we observed that the three complexes in study have an important stability in aqueous solutions in comparison whit aqueous solution of Chlorambucil which is unstable and form degradation compounds rapidly. Actually studies of stability of free CHL and CHL-Cyclodextrines in aqueous solutions were conducted in order to obtain solid evidence about the long term existence of those complexes, although the information obtained so far indicate that the stability in aqueous solutions of Chlorambucil inside the cyclodextins is bigger than free chlorambucil.

CONCLUSIONS

The NMR experiments allowed us to determine the interactions that occur when chlorambucil is introduced in the cyclodextrin cavity forming the inclusion complex, it was found that H-5 in the three β-CD interacts with H-6 and H-7 of CHL, these data were shown by the X-ray crystallography study where it can be observed that the dichloroethyl-N-phenyl region of CHL is introduced in the CD cavity, and the described information is confirmed. FT-IR studies demonstrate displacement and a change in the form of the absorption bands, which indicates interaction with cyclodextrin, is necessary to point that those remarkable changes were not observed in the physical mixture of CHL and Cyclodextrines. In the other hand the capillary electrophoresis experiments demonstrated the presence of complexes in aqueous systems, evidencing one of the most important advantages of inclusion complexes as alternative for those drugs with low solubility or stability in aqueous solutions.

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