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MASTER’S DEGREE THESIS

EFFECT OF BINDING CONSTANTS ON THE EXTRACTION OF HERBICIDES WITH CYCLODEXTRIN POLYMERS

Supervised by Dr. Alex Fragoso Sierra

MASTER’S DEGREE IN NANOSCIENCE, MATERIALS AND PROCESSES

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EFFECT OF BINDING CONSTANTS ON THE EXTRACTION OF HERBICIDES WITH CYCLODEXTRIN POLYMERS

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ABSTRACT

In this study, the binding constants of inclusion complexes between native cyclodextrins alpha, beta and gamma (α, β, γ) with 4-chlorophenoxyacetic acid (4-CPA), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), were determined using ultraviolet-visible spectroscopy (UV-vis). Both linear and non-linear regression methods were employed for this purpose. The relationship with the removal efficiencies of β and γ cyclodextrin-based polymers containing hexamethylene diisocyanate (HDI) and epichlorohydrin (EPI) was evaluated using maltodextrin polymers with HDI and EPI, as controls. The binding constants followed the order 2,4,5-T > 2,4-D > 4-CPA and α-CD > β-CD > γ-CD with 1:1 stoichiometries. This suggests a dependence on the cyclodextrin cavity size and the polarity of the herbicide molecules. Finally, the relationship with the removal efficiencies demonstrate that the primary adsorption mechanism involved could be the formation of the inclusion complex, although the effect of other adsorption mechanisms is not ruled out.

1 INTRODUCTION

As world population is expected to increase, food production requires an overall raising. Thus, an increase in the use of chemical control products is also expected to happen because these substances can help to obtain better farm revenues and increased yields.[1] In this regard, chlorophenoxy compounds are well known and widely used pesticides for the control of broadleaf weeds.[2] But they are also known as environmental pollutants and highly toxic substances for human beings. Chlorophenoxy herbicides are structurally stable, non-biodegradable, carcinogenic and potentially toxic at low concentrations.[3] As the US Environmental Protection Agency (EPA) says, in drinking and irrigations waters it is allowed up to 70 $\mu g\ L^{-1}$ of chlorophenoxyacetic acids. The European Union is stricter than the US EPA as they allow between 0.1 $\mu g\ L^{-1}$ to 0.5 $\mu g\ L^{-1}$.[4] So, efficient methods to remove pollutant herbicides are highly needed for the purposes of environmental remediation.

Cyclodextrins (CDs) are a good option to implement in the removal and control of pollutant herbicides.[5] They are a green alternative to other methods because of their biodegradable properties.[6,7]

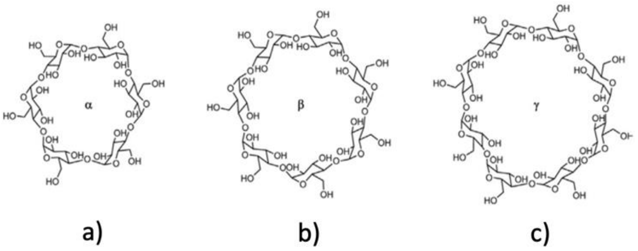

Figure 1: Schematic illustration of three different types of native cyclodextrins a) α-CD b) β- CD c) γ-CD.

Cyclodextrins have a toroidal structure with a hydrophilic exterior shell and a hydrophobic inner cavity, making them capable of hosting a wide range of organic guest molecules in host guest systems.[8] In aqueous solutions, cyclodextrins are able to react to form water-soluble inclusion complexes.[9] Even better, they are used to adsorb pesticides and other polar organic pollutants from contaminated water and soils.[10] Garrido et al., 2012 performed a complete study of the inclusion effect of cyclodextrins with methylchlorophenoxyacetic acid (MCPA). It was demonstrated that inclusion complexes of phenoxy alkyl acids could have specific applications for environmental protection due to the encapsulation of acids in the cavity of the cyclodextrins.[11]

As complex formation between chlorophenoxy herbicides and cyclodextrins is an equilibrium reaction (Eq. 1), one of the main parameters to determine is the binding constant (K_f).[12]

$$H + G \rightleftarrows HG \qquad K_f = \frac{[HG]}{[H][G]} \qquad (Eq.1)$$

Binding constants determination of the complexes is carried out using spectroscopic techniques like NMR (Nuclear Magnetic Resonance), fluorimetry and UV-visible spectroscopy (UV-vis).[13]

UV-vis is a method based in measuring the changes in absorbance at a defined wavelength for the herbicide solutions while adding cyclodextrin.[14] In practice, cyclodextrins does not absorb light in the UV region wavelengths while chlorophenoxy herbicides, in fact, do absorb. Stoichiometry of the reactions can also be defined by UV-vis methods. Although, cyclodextrins can be used in aqueous solutions to remediate polluted waters, it is more practical to use them in a solid-state water-insoluble phase. Actually, adsorption has been widely used to remove pollutants from water.[15] In this regard, cyclodextrin-based polymers are novel materials that seem promising in this area of research. They integrate high surface areas, interactions due to the polymeric nature (surface sorption, hydrogen bonding, hydrophobic interactions, etc.), the complexation between host and guest and attractive characteristics such as lower costs and biodegradability.[16] Cyclodextrin-based hexamethylene diisocyanate (HDI) and epichlorohydrin (EPI) polymers are among the most studied adsorbent materials, apart from being minimally toxic, exhibiting excellent bioavailability, biodegradability, and capability for inclusion complexation and chemical modification, these cyclodextrin polymers possess notable benefits (Fig 2).

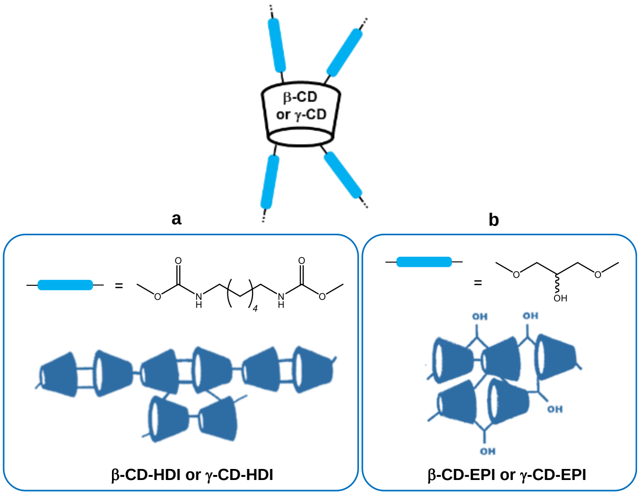

Figure 2: Structure of CD-based polymers used in this work a) CD-HDI b) CD-EPI. The schemes below the formulas of the spacers represent the proposed arrangements of the CD in both cases.

These advantages include their responsiveness to stimuli and their ability to form well-defined aggregated nanostructures, rendering them highly promising candidates for various applications. However, there is still a greater need of an integrated understanding of the sorption mechanisms regarding its interactions with organic pollutants.[17]

In this sense, the first part of this research presents the determination of binding constants for the inclusion complexes of alpha, beta and gamma cyclodextrins (α-CD, β-CD and γ-CD) with the chlorophenoxy herbicides 4-chlorophenoxyacetic acid (4-CPA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

Since there is no report in literature about the complexation of these herbicides with CDs, this part aims to understand the effect of the host cavity size and the number and position of chlorine atoms in the guests on the binding constants in aqueous solution.

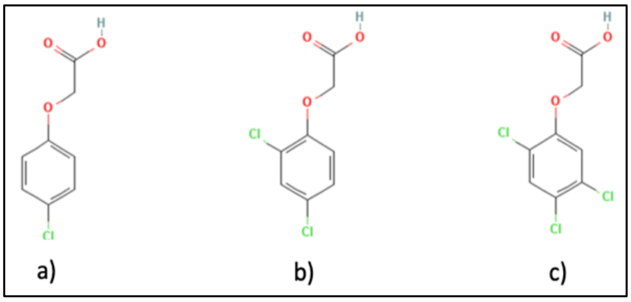

Figure 3: Structure of the chlorophenoxy herbicides a) 4-chlorophenoxyacetic acid, b) 2,4-dichlorophenoxycetic acid and c) 2,4,5-trichlorophenoxyacetic acid.

The second part is the evaluation of the herbicide removal efficiency for 4-CPA, 2,4-D and 2,4,5-T with six different types of polymers: β-CD-EPI, β-CD-HDI, γ-CD-EPI and γ-CD-HDI as well as two linear control polymers based on maltodextrin (MD-EPI and MD-HDI). Parameters such as polymer quantity, herbicide concentration and volume, temperature, time, polymer grain size and CD/crosslinker ratio will be constant. A selection of the best polymer for herbicide adsorption is expected. Finally, a comparison between the binding constants and the removal efficiencies will be performed. This research will show how binding constants and adsorption of cyclodextrins with chlorophenoxy herbicides are related.

2 EXPERIMENTAL SECTION

2.1 Materials and methods

Varian Cary 100 Bio UV-Visible spectrophotometer with Hellma 10 mm pathlength quartz cells were used to collect absorption data. Branson 2800 ultrasonic cleaner was used to mix the solutions properly. 400 μm sieve was used to filter cyclodextrin polymers particles with that diameter. α-CD>98% and γ-CD>96% were purchased from Wacker Cavatex WG. β-CD>96% was purchased from Roquette. 2,4-D>97% was purchased from TCI Chemicals Japan. 2,4,5-T>95% and 4-CPA>98% were acquired from Sigma Aldrich/Merck. To prepare tris buffer (20 mM), tris (hydroxymethyl) aminomethane > 99% was acquired from Acros Organics and adjusted to pH 7.8 using 1 M hydrochloric acid solution. Sodium hydroxide was acquired from Thermo Fisher Scientific. Milli-Q® water 18.2 MΩ/cm was used to prepare the solutions. β-CD-EPI, γ-CD-EPI, β-CD-HDI, γ-CD-HDI, MD-EPI and MD-HDI were kindly provided by Interfibio Research Group. EPI polymers CD/crosslinker ratio was 1:14 and 1:16 for β-CD and γ-CD. HDI polymers CD/crosslinker ratio was 1:7 and 1:8 for β-CD and γ-CD. Herbicide solutions (100 μM) were prepared adding the minimum amount of 0.1 M NaOH to the chlorophenoxy acetic acids to dissolve it, then volume was completed with tris buffer solution. Cyclodextrins stock solutions (10 mM) were prepared in Milli-Q® water and diluted when necessary.

2.2 Supramolecular titrations

One of the most common methods to quantify supramolecular interactions is a titration of guest (herbicide) to host (cyclodextrin) or vice versa, wherein changes in a physical property are recorded through techniques such as, UV-vis, NMR, fluorescence spectroscopy, etc. Figure 4 illustrates a typical schematic of the supramolecular titration process involving a guest and a host.

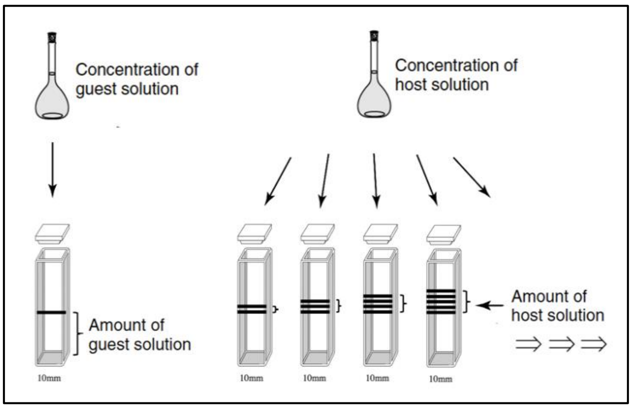

Figure 4: Illustration of a typical supramolecular titration experiment. Adapted from. [18]

2.2.1 UV-vis spectroscopy

UV-vis spectroscopy was employed owing to its versatility and practicability. Absorbances were chosen at the wavelength of 228 nm for 4-CPA, 228 nm for 2,4-D y 288 nm for 2,4,5-T. The highest and most defined absorbance peaks are found at the aforementioned wavelengths. Absorbance data was acquired by introducing a highly concentrated solution of the host (10 mM) to a diluted solution of the guest (100 μM). Hirose et.al [18] suggest that for constants ranging between 25 and 400 M^{-1} the ideal

$[H_0]/[G_0]$ ratio is 100. Binding constants have been reported for 2,4-D within this range.[8,19] There is no clear references for 4-CPA and 2,4,5-T; therefore, the same concentration ratio was chosen for these titrations. When constants were obtained below the 25-400 M^{-1} range, the $[H_0]/[G_0]$ ratio was adjusted to the corresponding value according to fig. 5.

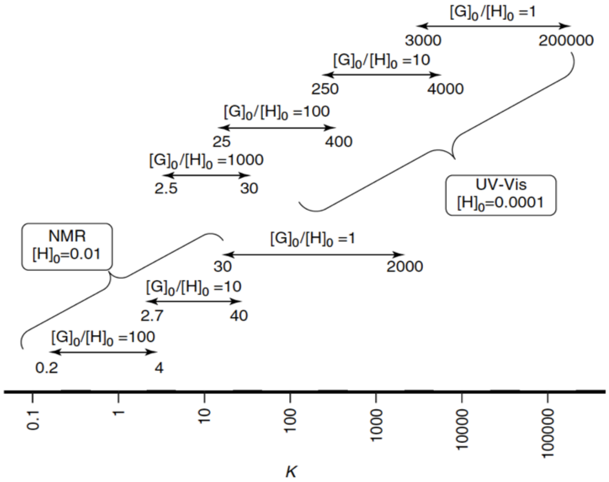

Figure 5: Suggested $[G_0]/[H_0]$ ratios for NMR and UV-vis at different binding constants by Hirose. et al. [18]

2.3 Binding models fitting: Linear and non-linear methods

To determine constants and stoichiometry, Thordason et. al [20] recommend fitting the data to various mathematical models. This is done in order to select the model that yields the best results in terms of the coefficient of linear regression and squared residuals. Indeed, the researcher suggests testing as many models as possible. Since Benesi-Hildebrand model (Eq.2) linearizes the data and assumes a 1:1 stoichiometry, it is one of the several models tested in this study. A nonlinear regression model proposed by Douglas Skoog[21] (Eq. 3) for 1:1 stoichiometry was also tested.

$$\frac{b}{\Delta A} = \frac{1}{\Delta \mathcal{E}(K_f)[H][G_O]} + \frac{1}{\Delta \mathcal{E}[G_O]} \qquad \text{Eq. 2}$$

$$\frac{\Delta A}{b} = \frac{\Delta \mathcal{E}(K_f)[H][G_O]}{(1 + K_f)[H]} \qquad \text{Eq. 3}$$

In Eq. 2 and Eq.3, A represents the absorbance, b is the path length of light through the cell, $\mathcal{E}$ is the molar absorptivity coefficient, K_f is the binding constant, $[G_O]$ initial guest concentration and $[H]$ is the host concentration after each addition. The derivation of these equation has been extensively discussed in other studies.[21,22] For the stoichiometries 1:2 and 2:1 BINDFIT software was used. It is an online tool used to fit absorbance data with non-linear regression models.[20] BINDFIT functionality and operational details can be found at supramolecular.org. (See Annex A.1 for the complete data).

Experimentally, 2,5 mL of herbicide solution (100 μM) was introduced into cells with a 10 mm pathlength, the initial absorbance of the guest was measured. Then, aliquots of the host solution (10 mM, containing 100 μM of herbicide) were added to the cell in the following order: 20 μL, 20 μL, 20 μL, 40 μL, 40 μL, 60 μL, 60 μL, 80 μL, 80 μL, 80 μL, 100 μL, 100 μL. A total volume of 700 μL of cyclodextrin was added to reach a concentration of 21,9 mM. After each addition, the mixture was stirred for 10 minutes, next, the absorbance of the system was measured. The obtained data were fitted to both linear and non-linear models to determine the stoichiometry and the binding constants. The experiments were carried out in triplicate. The choice of 12 additions is not arbitrary, as Hirose et.al[18] recommends 10 to 15 titrations points.

2.4 Determination of herbicides removal efficiency

Firstly, calibration curves for the herbicides in water were prepared in the concentration range of 0 to 100 μM at the wavelengths of 228 nm for 4-CPA, 228 nm for 2,4-D and 288 nm for 2,4,5-T. Herbicide stock solution (100 μM) was prepared and diluted to 5 μM, 10 μM, 25 μM, 50 μM, 75 μM and 90 μM and absorbance was measured to establish the regression curve.

For sorption experiments, Martel methodology[23] was adapted. For this, 100 mg of CD polymer and 2 mL of herbicide (100μM) were introduced into an Eppendorf tube, and stirred properly for 5 minutes. The percentage of removal efficiency was determined by UV-vis spectroscopy of the remained solution after being filtered. For each polymer, the experiment was carried out in 1-hour intervals for 5 hours and every 24 hours during 3 days. The removal efficiencies were calculated using the following equation:

$$\%P = \left(\frac{C_O - C_e}{C_O}\right) \times 100\% \qquad \text{Eq. 4}$$

Where, C_o is the initial herbicide concentration and C_e is the final herbicide concentration of the supernatant solution. To determine C_e, the absorbance was measured and interpolated in the corresponding calibration curve.

3 RESULTS AND DISCUSSIONS

3.1 Binding constants

Fig. 6 shows a typical UV-Vis titration experiment (see Annex A.1 for the complete data). In this case, the presence of isosbestic points at 245 nm, 270 nm and 295 nm in the titration of 2,4-D with α-CD is indicative of an equilibrium between complexed and free guest [21] and, therefore, of the formation of an inclusion complex.

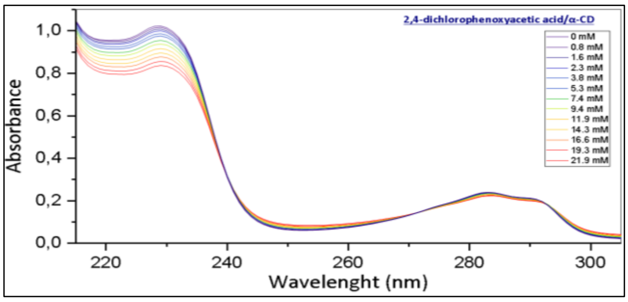

Figure 6: UV-vis spectra for the titration of 100 μM 2,4-D with α-CD

Table 1 collects the calculated binding constants of the studied systems. According to the calculations performed using linear and non-linear models, it is clear that the binding constants are higher as the number of chlorine atoms increase in the aromatic ring of the herbicides. It is also evident that the cyclodextrins with the highest constants follow the order α-CD > β-CD > γ-CD with the only exception of 4-CPA where the constants with α-CD and β-CD are essentially the same. It strongly suggests that the molecular structure affects the way these compounds included within the cyclodextrin cavity. The polarity of the herbicides changes as the aromatic ring is progressively substituted by chlorine atoms. This can be noticed in the water solubilities of each component as 4-CPA has the highest solubility followed by 2,4-D and 2,4,5-T (0.848, 0.682 and 0.268 g dm^{-3} at 20 °C respectively).[4] The position and quantity of chlorine atoms significantly changes the polarity of the phenoxy group. In the case of 4-CPA, the position of the chlorine at C_4 and the position of the acetate group at C_1 cause the appearance of a dipolar moment for the molecule due to the differences in electronegativity in the Cl-C bond. In the case of 2,4-D, the existence of Cl at the C_2 position alters the dipolar moment, decreasing it because the presence of an additional chlorine slightly equalizes the charge distribution (Fig. 3). For 2,4,5-T, the third chlorine at the C_5 position further equalizes the charge distribution, making it globally the least polar of the three herbicides. The decrease of the constants with the type of cyclodextrin suggests that the inclusion reaction of the herbicides is affected by the size of the hydrophobic cavity of the molecule. The lowest constants were obtained for γ-CD, which has the widest cavity (Fig. 1). It is important to note that these constants are particularly small compared to α-CD and β-CD constants. The formation of inclusion complexes is mainly driven by van der Waals forces and hydrophobic interactions.[24] In aqueous solutions where there is no guest molecule, the hydrophobic cyclodextrin cavity is filled by water molecules placed in energetically unfavored positions. When a guest is present in the medium, water is rapidly substituted in an enthalpically driven process. As the size of the cyclodextrin increases, the substitution of high enthalpy water molecules becomes weaker. This phenomenon is one of the main forces governing the formation of inclusion complexes, so the results in Table 1 are in good agreement with the literature. It was expected that the constants would decrease as the cavity size increases in the order α-CD > β-CD > γ-CD.[25]

Table 1 Binding constants of native cyclodextrins calculated through linear and non-linear methods.

	4-CPA		**2,4-D**		**2,4,5-T**	
Method	**Linear**	**Non-linear**	**Linear**	**Non-linear**	**Linear**	**Non-linear**
α-CD	22 ± 2	18 ± 1	105 ± 44	68 ± 12	203 ± 27	114 ± 17
β-CD	20 ± 4	22 ± 5	65 ± 23	43 ± 7	172 ± 27	97 ± 5
γ-CD	4 ± 2	≈ 0	22 ± 3	12 ± 3	26 ± 3	16 ± 3

3.2 Calibration curves for extraction experiments

For the extraction experiments, a concentration range of herbicide between 0 and 100 μM was selected. This upper value is ~400 times higher than the allowed concentration for these herbicides so it covers a wide range of possible situations of their presence in water environments.

Hence, calibration curves of the three studied herbicides in water were first obtained and used to determine their concentration after extraction by UV-Vis spectroscopy (Fig. 7, 8 and Annex A.2).

Table 2 contains the equation of the regression lines, coefficients of linear correlation (R^2) and the standard error for the three herbicides. Overall, the coefficients of linear correlation indicate a strong fit of the absorbance data to the regression curve.

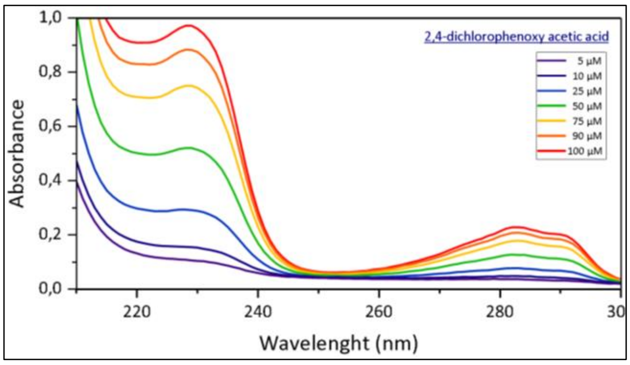

Figure 7: UV-vis spectra of 2,4-D in water at different concentrations.

Table 2: Parameters of the calibration curves of chlorophenoxy herbicides in water.

Herbicides	***Regression curve***	R^2	***SE***
4-CPA	0.0113[4-CPA] +0.1116	0.9978	0.006
2,4-D	0.0092[2,4-D] +0.0563	0.9992	0.006
2,4,5-T	0.0026[2,4,5-T]+0.0174	0.9996	0.001

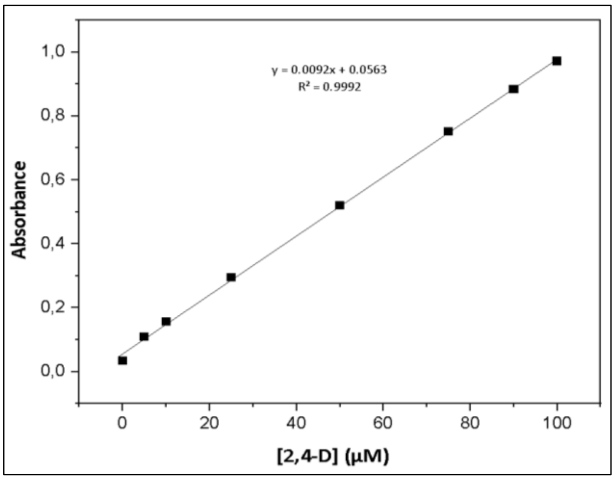

Figure 8: Calibration curve at 228 nm for 2,4-D in aqueous solution obtained from the spectra of Fig. 7.

3.3 Cyclodextrin polymers removal efficiency

Table 3 presents the calculated values for herbicide removal efficiencies (P) from aqueous solution using six types of cyclodextrin polymers after 72 hours at 25°C.

Table 3: Removal efficiency percentage of chlorophenoxy herbicides (100 μM in water) with cyclodextrin polymers.

% Removal Efficiency	***4-CPA***	***2,4-D***	***2,4,5-T***
β-CD-HDI	39±2	32±5	58±10
β-CD-EPI	66±3	68±7	82±9
γ-CD-HDI	16±3	27±5	45±4
γ-CD-EPI	18±6	37±6	46±3
MD-HDI	≈0	≈0	11±2
MD-EPI	≈0	≈0	≈0

In general, removal efficiencies tend to increase as the number of chlorine atoms in the herbicide molecule increases (2,4,5-T > 2,4-D > 4-CPA) with the exception of 4-CPA when interacting with β-CD-HDI. The removal efficiencies tend to increase as contact time between polymers and herbicide solutions is increased. For β-CD-HDI, β-CD-EPI and γ-CD EPI, the absorptions are almost instantaneous, except for γ-CD-HDI (Fig. 11). Both maltodextrin polymers produce negative removal efficiencies, meaning that these polymers do not adsorb the herbicide but rather they swell in water, concentrating the solution. No removal was obtained even after previously swelling the polymers. Maltodextrins are linear chains while cyclodextrins are cyclic chains of saccharides (Fig. 9), this fundamental difference in their structures could lead to the herbicides not including with the maltodextrin polymers.

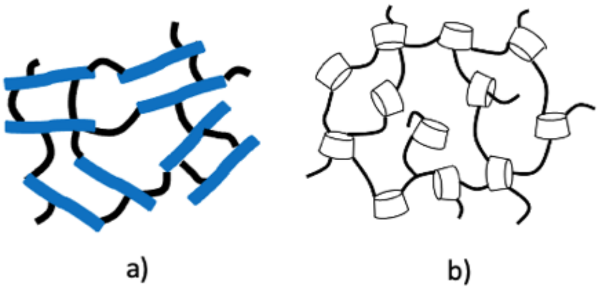

Figure 9: Cyclodextrin polymers structure a) Maltodextrin polymers b) Cyclodextrin polymers.

Fig. 10 shows that the removal efficiencies of polymers crosslinked with EPI are greater than those of HDI. In their review, Crini denoted that similar sorption results have been obtained, where EPI-crosslinked β-CD gels are more efficient than HDI-crosslinked β-CD gels.[17]

Wilson et al. [26] found that the sorption capacities of p-nitrophenol (PNP), expressed as milligrams of sorbate per gram of sorbent, are slightly higher for epichlorohydrin-based polymers (37 - 87 mg/g) compared to diisocyanate-based polymers (18.3 - 80.1 mg/g). They concluded that the former is notably valuable and efficient for treating solutions containing phenolic species, which is in accordance with the obtained results.

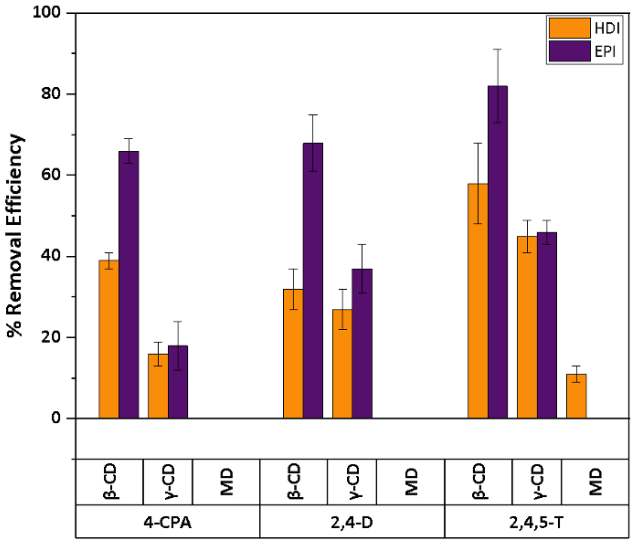

Figure 10: Comparison of removal efficiencies for all the experiments with HDI and EPI crosslinkers

Thus, the greater efficiency can be attributed to differences in the mobility of the polymer network and its hydrophobic properties, as related to the structures of the EPI and HDI polymers.[17]

HDI polymers are non-polar and highly hydrophobic, featuring a rigid structure that hinders the access of water and solutes into the pores. Conversely, EPI polymers are hydrophilic and facilitate water entry into the pores, allowing herbicides to access the cyclodextrin cavities within the polymer network and enabling greater accessibility to them.[27]

Additionally, it is crucial to mention the impact of the polymer network arrangement on the accessibility of pores for the herbicides present in solution. As depicted in Figure 2, polymers crosslinked with HDI exhibit a configuration of CDs within elongated tunnels, resulting in a lower quantity of open pores per unit of surface area of the sorbent. Conversely, polymers crosslinked with EPI display a random distribution of CDs in various potential positions, providing them with a greater number of open pores per unit of surface area. Consequently, the probability of herbicides encountering a cyclodextrin cavity within the polymer is higher in the case of EPI, which accounts for its superior removal efficiency.[27]

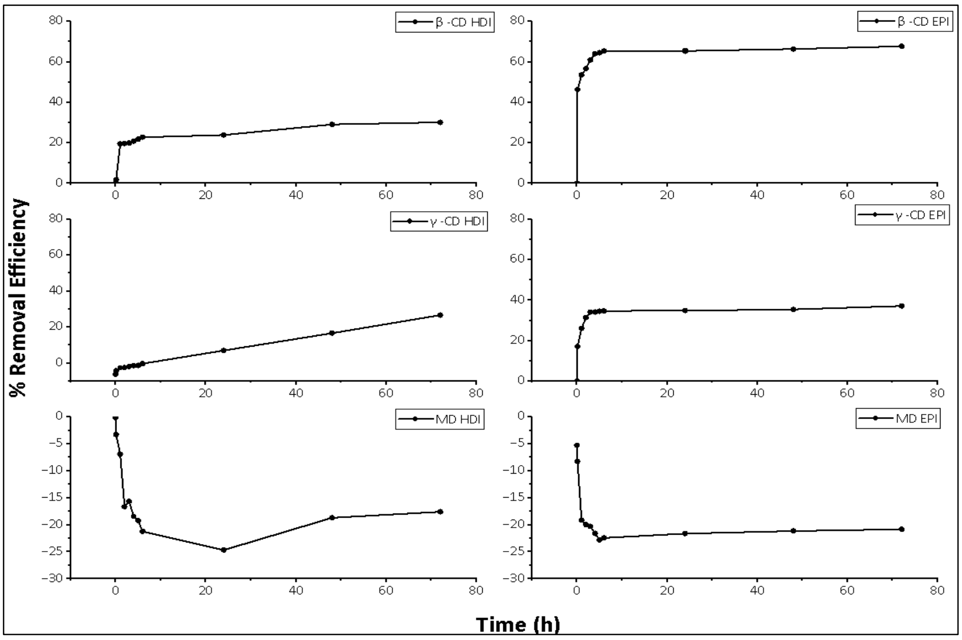

Figure 11: Removal efficiencies as a function of time for all the polymers and 2,4-D

It is evident that β-CD polymers remove more herbicide than the γ-CD polymers (Fig. 10). The removal efficiencies of these polymers follow the order of the previously determined constants. Furthermore, maltodextrin polymers used as control groups exhibit no significant removal, except for the HDI-crosslinked maltodextrin polymer, which shows a low efficiency (11%). The specific case of removal observed for MD-HDI with 2,4,5-T may be attributed to the interaction of additional adsorption mechanisms such as van der Waals forces, hydrophobic interactions, electrostatic interactions, physical adsorption and Yoshida interactions referred to the hydrogen bond formed between the OH groups of polymer and the π electron of the herbicides phenolic groups.[17,28]

In general, other studies have demonstrated that maltodextrins do not form inclusion complexes with fully ionized species.[29] Given that the $\mathrm{pK_a}$ of 2,4-D and 2,4,5-T is 2.80 and 2.56 respectively, at the pH of 7.8 of the employed buffer both herbicides are entirely ionized and would be incapable of complexing with the maltodextrins in the polymers. The results suggest that the removal efficiencies for the three herbicides are closely related to the binding constant of herbicide/cyclodextrin inclusion complexes, constituting the predominant adsorption mechanism. Finally, the comparison between β-CD and γ-CD polymers yields results that follow a trend. Polymers crosslinked with EPI exhibit higher removal efficiency than polymers crosslinked with HDI (Fig. 12 and Fig. 13). Additionally, polymers containing β-CD also show a tendency to remove more herbicide than γ-CD (Fig. 13). The upward trend in removal efficiency corresponds to the increase in the number of chlorine atoms in the aromatic ring of the herbicides, aligning with the binding constants regardless of the polymer and cyclodextrin type.

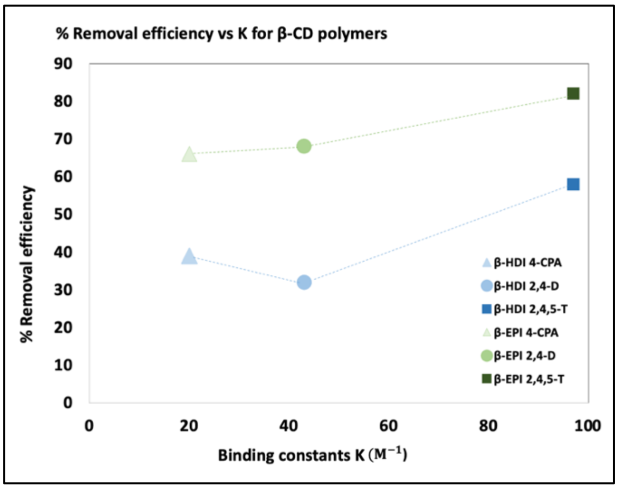

Figure 12: Comparison between removal efficiency of HDI and EPI polymers for β-CD.

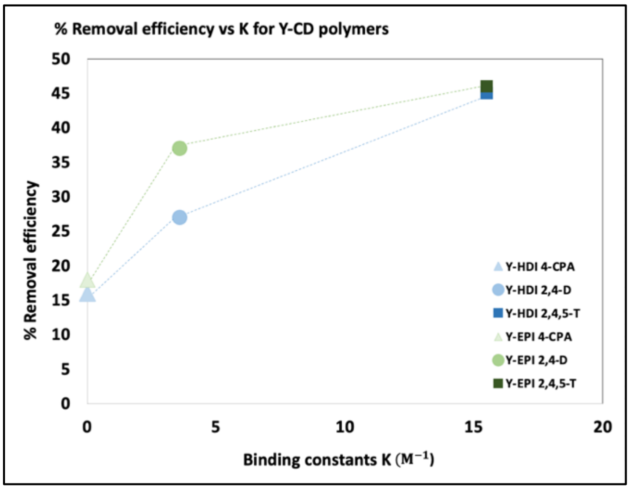

Figure 13: Comparison between removal efficiency of HDI and EPI polymers for γ-CD.

4 CONCLUSIONS

Binding constants of 4-CPA, 2,4-D, and 2,4,5-T with α-CD, β-CD and γ-CD were determined in this work. It was found that higher constant values are associated with the number of chlorine atoms in the herbicide structures and additionally correlated with the size of the cyclodextrin cavity. Higher number of chlorine atoms and smaller cavity size result in higher binding constant. This phenomenon could be related to the polarity of the herbicide molecules and the energetically frustrated water molecules in the cyclodextrin cavity. The relationship between the removal efficiency of some cyclodextrin-based polymers (β-CD-EPI, β-CD-HDI, γ-CD-EPI and γ-CD-HDI) with binding constants were also determined. It was found that removal efficiencies are directly influenced by the inclusion complexes formed between cyclodextrins and herbicides. Since the maltodextrin polymers (HDI, EPI) do not exhibit removal. Thus, higher binding constants are linked to higher removal efficiencies. However, the possibility of other adsorption mechanisms cannot be ruled out and therefore more research work is necessary to elucidate it.

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