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Dependence of Viscosity and Diffusion on β-Cyclodextrin and Chloroquine Diphosphate Interactions

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Abstract: Mutual diffusion coefficients of chloroquine diphosphate (CDP) in aqueous solutions both without and with β-cyclodextrin (β-CD) were measured at concentrations from (0.0000 to 0.0100) mol dm^{-3} and 298.15 K, using the Taylor dispersion technique. Ternary mutual diffusion coefficients (D_{ik}) measured by the same technique are reported for aqueous CDP + β-CD solutions at 298.15 K. The presence of β CD led to relevant changes in the diffusion process, as showed by nonzero values of the cross-diffusion coefficients, D_{12} and D_{21}. β-CD concentration gradients produced significant co-current coupled flows of CDP. In addition, the effects of β-CD on the transport of CDP are assessed by comparing the binary diffusion coefficient of aqueous CDP solutions with the main diffusion coefficient (D_{11}) measured for ternary {CDP(1) + β-CD(2)} solutions. These observations are supported by viscosity analysis. All data allow to have a better interpretation on the effect of cyclodextrin on the transport behavior of CDP.

Keywords: chloroquine diphosphate; diffusion; drugs; solutions; transport properties

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1. Introduction

Chloroquine diphosphate (CDP) is a 4-aminoquinoline-based drug with a broad spectrum of applications, including all types of malaria infections and averse to cell growth and/or inducing cell death in human leukemia K562 cell [1–3]. CDP is also indicated for the treatment of inflammatory diseases and rheumatoid arthritis [4,5]. More recently, CDP has been highly cited as a consequence of its potential, though not confirmed, to treat severe acute respiratory syndrome coronavirus 2 [6].

CDP is soluble in water and characterized by having a high bioavailability when administrated orally [4]. However, this drug also shows some significant side effects [7]. These can be reduced by forming host–guest supramolecular compounds with cyclodextrins [2]. It is known that due to the amphiphilic behavior of cyclodextrins, supramolecular interactions mainly occur inside the hydrophobic CD's cavity with hydrophobic guests [8,9]. However, interactions between guest molecules and cyclodextrins can also occur via, for example, H-bonding involving hydroxyl groups located outside the CD's cavity [10,11]. Despite its solubility in aqueous solutions, the molecular structure of the CDP suggests the ability of the quinolone group to form host–guest supramolecular compounds with β-cyclodextrin. For example, Fan et al. have reported the complexation of 8-nitro-quinoline (at solid state) with β-cyclodextrin [12], whilst the equilibrium constant between quinolone and β-CD has been computed as equal to ca. 380 L mol^{-1} [13]. These values are in line

with the binding constant of 1890 L mol^{-1} for a 1:1 CDP: β -CD complex obtained by Roy et al. [2]. Recently, it has been demonstrated by NMR and computational studies that chloroquine is able to protrude both α- and β–cyclodextrins, being that the stronger interaction occurs with the β-cyclodextrin [14].

Although much work has been done on the development of CDP-containing systems [1,15], its kinetics in aqueous solution are still poorly understood. In the present paper, transport properties (diffusion coefficients and viscosities) of CDP in water and in aqueous solutions containing β-cyclodextrin are reported.

Specifically, we have measured binary diffusion coefficients of this drug in water, and multicomponent chemical ternary diffusion coefficients (D_{11}, D_{22}, D_{12} and D_{21}) for CDP(1) + β-CD(2) aqueous solutions, using the Taylor dispersion technique. The behavior diffusion of these systems (binary and ternary) and the coupled flows occurring in the solution were studied in order to better understand if there are interactions between CD–CDP by estimating its association constant, leading to better insight of these systems' structure.

The comparison of Jones–Dole viscosity coefficients for CDP in water and in the {water + β-CD 0.0070 mol dm^{-3}} mixture allowed to evaluate the solute–solvent interactions occurring in these solutions.

Additionally, interdiffusion coefficients correspond to the maximum limit value for the release kinetics of drugs (or complexes), in these systems.

2. Materials and Methods

2.1. Materials

Chloroquine diphosphate (CDP) (Merck) an β-cyclodextrin (β-CD) (Sigma-Aldrich) were used as received, without further purification (Table 1). All solutions were freshly prepared and degassed by sonication before each experiment.

Table 1. Sample description.

Chemical Name	Source	CAS Number	Purity
Chloroquine diphosphate (CDP) ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$)	Merck	50-63-5	mass fraction $\geq$ 0.98
β-Cyclodextrin (β-CD)	Sigma-Aldrich (water mass fraction of 0.131)	7585-39-9	mass fraction $\geq$ 0.99
Water	Millipore-Q water (ρ = 1.82 $\times$ 10^5 Ω·m at 298.15 K)	7732-18-5	

2.2. Techniques

2.2.1. Viscosity Measurements

For viscosity measurements, a set of CDP aqueous solutions were prepared at concentrations 0.0010; 0.0020; 0.0050; 0.0070 and 0.0100 mol.dm^{-3} by dissolving the corresponding solid in pure water, under continuous stirring, at 298.15 K. Likewise, another set of CDP solutions were also prepared in a mixture {water + β-CD 0.0070 mol dm^{-3}} as solvent at the same concentrations above indicated and following the same procedure.

Viscosity measurements were performed using a microviscometer (Lovis 2000 ME Anton Paar) at 298.15 ± 0.01 K. The average value of the viscosity data at each concentration was obtained from five independent measurements. The viscometer was calibrated with Milli-Q water (from A10 Millipore) before every set of experiments. The uncertainty of the values for this parameter was calculated as equal to 0.04 mPa s based on calibration data. The repeatability of the experiments was ± 0.05%.

2.2.2. Diffusion Measurements

Taylor dispersion technique for measuring diffusion in solutions has gained increasing popularity due to its fast and reliable analysis of multicomponent systems.

The theory of the Taylor dispersion technique is well described in the literature [16–21] and, consequently, only a summarized description of both the apparatus and the procedure used in our study is presented here.

At the begin of each experience, a 6-port Teflon injection valve (Rheodyne, model 5020) was used to introduce 63 mm^3 of solution into a laminar carrier stream with a flow rate of 0.17 cm^3 min^{-1} leading retention times ca. 1.1 × 10^4 s. The dispersion tube and the injection valve was kept at 298.15 ± 0.01 K in an air thermostat. The radius of the tube is equal to 0.32200 ± 0.00003 mm. The monitoring of the injected samples dispersion, at the dispersion tube outlet, was done using a differential refractometer (Waters model 2410).

Detector voltages, $V(t)$, were measured by using a digital voltmeter (Agilent 34401 A).

The diffusion of CDP in aqueous solutions (binary system) is described by Fick's equation

$$J = -D\nabla C \tag{1}$$

where C is the molar concentration of the solute and D the binary diffusion coefficient.

At the tube outlet, the distribution of the dispersed solute is followed by passing the carrier through a differential refractometer which gives a linear response to changes in V(t) composition dependent property. Combining this detector output signal V(t) and the equation derived by Taylor [22–24], that accurately describes the dispersion of the solutes, and that considers that the flow is laminar, the binary diffusion coefficients were evaluated by fitting the dispersion Equation (2) to the detected voltages. That is,

$$V(t) = V_0 + V_1 t + V_{max}\,(t_R/t)^{1/2}\exp[-12D(t - t_R)^2/r^2 t] \tag{2}$$

V_1 the baseline slope, V_0 is the baseline voltage, V_{max} the peak height, t_R the mean sample retention time and r the inner radius of the dispersion tube.

Ternary mutual diffusion coefficients (D_{ik}) of aqueous {CDP(C_1) + β-CD(C_2)} solutions were computed by using coupled Fick equations (Equations (3) and (4)) [20,21].

$$J_1 = -D_{11}\nabla C_1 - D_{12}\nabla C_2 \tag{3}$$

$$J_2 = -D_{21}\nabla C_1 - D_{22}\nabla C_2 \tag{4}$$

J_1 and J_2 represent the molar fluxes of CDP (1) and β-CD (2), respectively, driven by the concentration gradients ∇C_1 and ∇C_2 of each solute 1 and 2. Cross-diffusion coefficients D_{12} and D_{21} give the coupled flux of each solute, driven by a concentration gradient in the other solute. While a negative D_{ij} coefficient indicates countercurrent coupled transport of solute i from regions of lower to higher concentration of solute j, a positive D_{ij} cross-coefficient ($i \neq j$) indicates co-current coupled transport of solute i from regions of higher to lower concentrations of solute j. Main diffusion coefficients D_{11} and D_{22} give the flux of each solute, driven by its own concentration gradient.

Ternary dispersion experiences were prepared by injecting {CDP(1) + β-CD(2)} solution samples of composition $C_1 + \Delta C_1$, $C_2 + \Delta C_2$ into carrier streams of composition C_1, C_2. In the tracer diffusion studies, the concentration of the component studied under trace conditions was zero; that is, in the carrier solutions, for tracer of CDP, = 0, $C_2 = C_2$, and for tracer of β-CD, $C_2 = 0$ and $C_1 = C_1$. Considering the equation that describes ternary dispersion profiles provided and that the flow is also laminar, whose development is well reported in references [20,25,26]), the ternary D_{ij} coefficients were calculated by fitting the Equation (5)) (Figure 1)

$$V = V_0 + V_1 + V_{max}\sqrt{\frac{t_R}{t}}\,\frac{(a+b\alpha_1)\sqrt{D_1}e^{-12D_1(t-t_R)^2/r^2t} + (1-a-b\alpha_1)\sqrt{D_2}e^{-12D_2(t-t_R)^2/r^2t}}{(a+b\alpha_1)\sqrt{D_1} + (1-a-b\alpha_1)\sqrt{D_2}} \tag{5}$$

being:

$$\alpha_1 = \frac{R_1\Delta C_1}{R_1\Delta C_1 + R_2\Delta C_2} \tag{6}$$

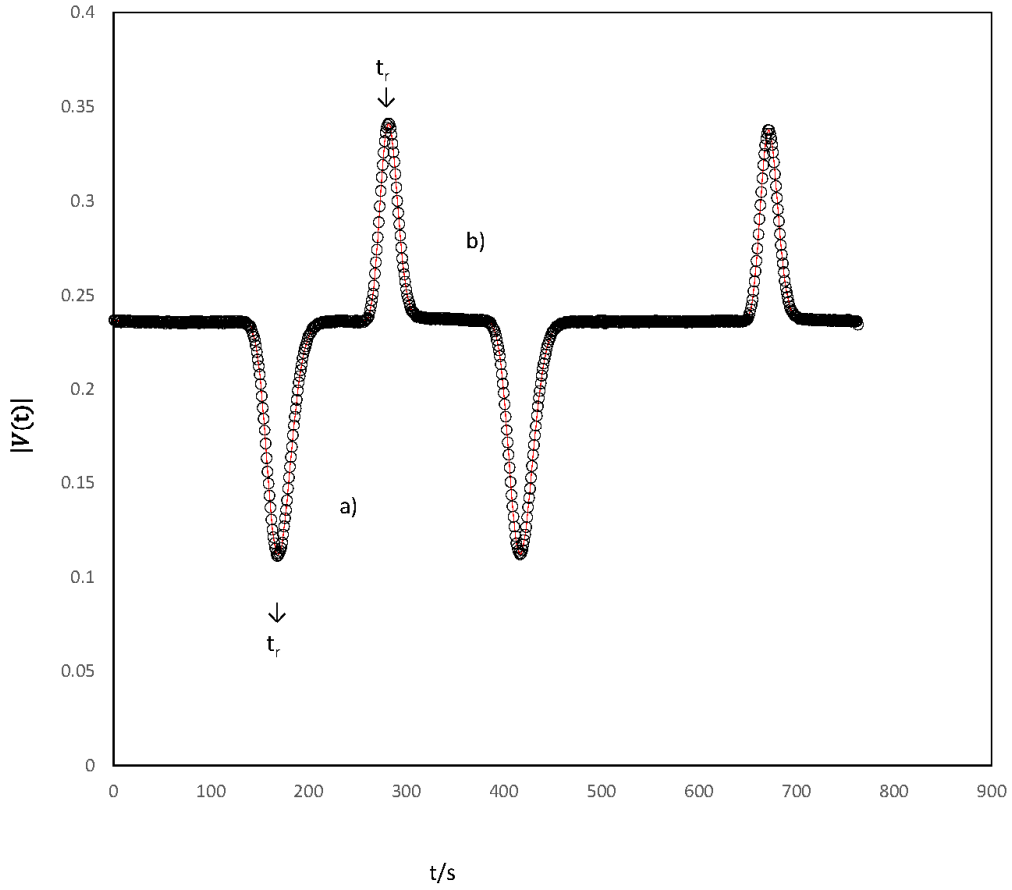

Figure 1. Ternary dispersion profiles relative to {chloroquine diphosphate (CDP) (C_1) + β-cyclodextrin (β-CD) (C_2)} solutions generated by injecting: (**a**) a 0.063 cm^3 sample of chloroquine diphosphate 0.005 mol dm^{-3} into 0.007 mol dm^{-3} β-cyclodextrin (profile ΔC_1 = 0.005 mol dm^{-3}, ΔC_2 = 0); (**b**) a 0.063 cm^3 sample of 0.002 mol dm^{-3} β-cyclodextrin into 0.007 mol dm^{-3} β-cyclodextrin (Profile ΔC_1 = 0 mol dm^{-3}, ΔC_2 =0.002 mol dm^{-3}). Measured (o) and fitted (−, Equation (5)). t_R represents the mean sample retention time.

D_1 and D_2 are the eigenvalues of the matrix of D_{ik} coefficients (Equations (7) and (8)) and α_1 is the fraction of the initial refractive index difference due to CDP. R_1 and R_2 are the detector sensitivities for CDP and β-CD, respectively: $R_1 = \partial V/\partial C_1$ and $R_2 = \partial V/\partial C_2$.

$$D_1 = \left\{ D_{11} + D_{22} + (D_{11}\, D_{22}) \sqrt{1 + \left[4\, D_{12}\, D_{21} / (D_{11}\, D_{22})^2 \right]} \right\} / 2 \tag{7}$$

$$D_2 = \left\{ D_{11} + D_{22}\, (D_{11}\, D_{22}) \sqrt{1 + \left[4\, D_{12}\, D_{21} / (D_{11}\, D_{22})^2 \right]} \right\} / 2 \tag{8}$$

Equation (5) was fitted to pairs of ternary profiles measured for $\alpha_1 \approx 0$ (initial CDP concentration difference) and $\alpha_1 \approx 1$ (initial β-CD concentration difference). D_{ik} were determined from the relative detector sensitivity R_2/R_1 and the D_1, D_2, a, b fitting parameters, using

$$D_{11} = D_1 + \frac{a(1-a-b)}{b}(D_1 - D_2) \tag{9}$$

$$D_{12} = \frac{R_2}{R_1}\, \frac{a(1-a)}{b}(D_1 - D_2) \tag{10}$$

$$D_{21} = \frac{R_1}{R_2}\, \frac{(a+b)(1-a-b)}{b}(D_2 - D_1) \tag{11}$$

$$D_{22} = D_2 + \frac{a(1-a-b)}{b}(D_2 - D_1) \quad (12)$$

The parameters a and b, in these Equations (9)–(12) are defined by

$$a = \frac{D_{11}\, D_1\ (R_1\ /R_2)D_{12}}{D_2\, D_1} \quad (13)$$

$$b = \frac{D_{22}\, D_{11} + \ (R_1\ /R_2)D_{12}(R_2\ /R_1)D_{21}}{D_2\, D_1} \quad (14)$$

3. Results

3.1. Viscosity Measurements

Viscosity values for CDP solutions both in pure water and in the mixture {water + β-CD 0.0070 mol dm^{-3}} are reported in Table 2.

Table 2. Viscosity values of aqueous solutions of chloroquine diphosphate (CDP) in pure water (η_w), and in a {water + β-CD 0.0070 mol dm^{-3}} mixture ($\eta_{(w+\ \beta\text{-CD})}$) as solvent, at P = 101.3 Pa and at T = 298.15 K.

C /(mol dm^{-3})	η_w [a] /(mPa s)	$\eta_{(w+\ \beta\text{-CD})}$ [b] /(mPa s)
0.0010	0.949	0.969
0.0020	0.948	0.970
0.0050	0.952	0.972
0.0070	0.950	0.978
0.0100	0.953	0.983

[a] viscosity values of chloroquine diphosphate (CDP) aqueous solutions; [b] viscosity values of chloroquine diphosphate (CDP) in {water + β-CD 0.0070 mol dm^{-3}} mixed solvent; standard uncertainties are: $u(C) = 5 \times 10^{-6}$ (mol dm^{-3}); $u(\eta) \cong$ 0,04 (mP.s); $u(T)$ = 0.01 K; $u(P)$ = 2.03 kPa.

3.2. Diffusion Measurements

Tables 3 and 4 show the mean values of the diffusion coefficients for binary systems (CDP/H_2O and β-CD/H_2O) and for the aqueous ternary system: CDP(1)/β-CD (2), at 298.15 K.

Table 3. Binary diffusion coefficients (D) measured in the present work of both aqueous solutions of chloroquine diphosphate(1) and of β-cyclodextrin(2), respectively, at P = 101.3 Pa and at T = 298.15 K.

C/(mol dm^{-3})	D_1/(10^{-9} m^2 s^{-1})	D_2/(10^{-9} m^2 s^{-1}) [a]
0.0000	0.710 [b]	
0.0020	0.690	
0.0040	0.684	
0.0050	0.678	0.322 [c]
0.0060	0.675	0.321 [c]
0.0070	0.672	0.319 [c]
0.0080	0.669	
0.0100	0.666	
0.0200	0.650	

[a] [27]. [b] This value represents the diffusion coefficient at infinitesimal concentration estimated by extrapolation of our experimental D values here presented, by applying the polynomial: $D = 0.715 - 0.5791\ C^{1/2} + 0.8374\ C$ (R^2 = 0.993), using a least squares procedure. [c] D values for these concentrations were measured in the present work. Standard uncertainties are: $u(C) = 5 \times 10^{-6}$ (mol dm^{-3}); $u(D) \cong 0.01 \times 10^{-9}$ (m^2 s^{-1}); $u(T)$ = 0.01 K; $u(P)$ = 2.03 kPa.

Table 4. Ternary mutual diffusion coefficients (D_{11}, D_{12}, D_{21}, D_{22}) of aqueous {chloroquine diphosphate (CDP) (C_1) + β-cyclodextrin (β-CD) (C_2)} solutions at P = 101.3 Pa and at T = 298.15 K.

C_1 [a]	C_2 [a]	X_1	$D_{11} \pm S_D$ [b]	$D_{12} \pm S_D$ [b]	$D_{21} \pm S_D$ [b]	$D_{22} \pm S_D$ [b]
0.0000	0.0070	0.000	0.689 ± 0.017	0.020 ± 0.020	−0.031 ± 0.015	0.383 ± 0.010
0.0010	0.0070	0.014	0.686 ± 0.012	0.004 ± 0.010	−0.010 ± 0.010	0.350 ± 0.020
0.0020	0.0050	0.250	0.602 ± 0.012	−0.030 ± 0.010	0.021 ± 0.020	0.330 ± 0.020
0.0035	0.0035	0.500	0.567 ± 0.010	−0.028 ± 0.011	0.020 ± 0.010	0.349 ± 0.015
0.0050	0.0020	0.750	0.594 ± 0.015	0.129 ± 0.012	0.018 ± 0.015	0.342 ± 0.010
0.0070	0.0000	1.000	0.670 ± 0.012	0.180 ± 0.012	0.010 ± 0.005	0.390 ± 0.011
0.0100	0.0000	1.000	0.667 ± 0.015	0.197 ± 0.060	−0.001 ± 0.001	0.395 ± 0.012

[a] C_1 and C_2 in units of (mol dm^{-3}); [b] ($D_{ik} \pm S_D$) in units of (10^{-9} m^2 s^{-1}). $u(C) = 5 \times 10^{-6}$ (mol dm^{-3}); $u(T)$ = 0.01 K; $u(P)$ = 2.03 kPa.

Mutual binary diffusion coefficients, D, of CDP in aqueous solutions, in Table 3, denote the average ones from, at least, four independent experiments. Good reproducibility (within ±1%) was obtained.

Table 4 show the average (D_{11}, D_{12}, D_{21}, D_{22}) values of aqueous {chloroquine diphosphate (CDP) (C_1) + β-cyclodextrin (β-CD) (C_2)} solutions determined for each carrier solution composition by fitting Equation (5) to five replicate pairs of dispersion profiles. Main diffusion coefficients D_{11} and D_{22} were generally reproducible within ± 0.015 × 10^{-9} m^2 s^{-1}. Cross-diffusion coefficients D_{12} and D_{21}, describing the coupled diffusion of CDP and β-CD; they were reproducible within ± 0.050 × 10^{-9} m^2 s^{-1}.

The main diffusion coefficients D_{11} and D_{22}, which give the molar fluxes of the CDP (1) and β-CD (2) components, respectively, driven by their own concentration gradients, are compared with those obtained for the binary systems (CDP/water and β-CD/water [27]—Table 3).

It should be noted that D_{11} values are higher than the D_{22} ones, and, in general, lower than the binary D of CDP in pure water (deviations between 0.1 and 17%; Table 3). However, the values found for D_{22} are similar to those of the binary diffusion coefficients reported for β-CD in aqueous solution [27]. These results indicate that while the addition of CDP produces relatively small changes in the diffusion coefficient of β-CD (D_{22}), the addition of β-CD leads to important changes in that of CDP (D_{11}) (up to 13%). This effect of decrease in the diffusion of CDP, due to the presence of β-CD, is also highlighted by the positive values of the D_{12} cross-diffusion coefficients, from which it can be concluded that in solutions containing CDP at concentrations 0.0050, 0.0070 and 0.0100 mol dm^{-3}, for which D_{12} >0 (Table 4), the gradient in the concentration of β-CD produces co-current coupled flows of CDP. Nevertheless, because the D_{21} values are almost zero, the CDP concentration gradient leads to weak countercurrent coupled flows of β-CD.

Considering that the D_{12}/D_{22} ratio gives the number of moles of CDP transported per mole of β-CD, we may state that one mole of diffusing β-CD co-transports as a maximum 0.5 moles of CDP. Through the D_{21}/D_{11} values, at the same concentrations, we can expect that one mole of diffusing CDP counter transports at most 0.04 moles of β-CD.

This behavior can be justified assuming the occurrence of CDP aggregates, with lower mobility and, consequently, being responsible for D_{11} decreasing. This effect is less relevant when we consider the effect of CDP on transport of β-CD, probably due to the resemblance of the mobilities of β-CD-free species and eventual aggregates of CDP and β-CD.

4. Discussion

The analysis of the dependence of the viscosity on the concentration was assessed by fitting the values of relative viscosity, η_r, to the Jones–Dole equation (Equation (15)) [28,29]

$$\eta_r = \frac{\eta}{\eta_0} = 1 + AC^{1/2} + BC \quad (15)$$

where A and B are empirical terms and C as the same meaning as before.

Being A and B coefficients related with the long-distance solute–solute interactions and to the solute–solvent interactions, respectively, through their values, it is possible

to analyze the structure-making or structure-breaking character of the electrolyte in the solution. That is, when coefficient B is positive, we can say that the solute has an organizing capacity of the solvent structure (structure-making character). Contrariwise, a negative value of the B coefficient is related to a solute with the ability to break the structure of water (structure-breaking character) [30].

From the fitting of the viscosity values of the CDP aqueous solutions (Table 2) to the Jones–Dole equation [28,29], we have obtained A = 1.75 $dm^{3/2}$ $mol^{-1/2}$ and B = −10.6 dm^3 mol^{-1}. The small positive A value and the negative B value suggest that weak interactions between CDP-CDP entities are present, and CDP is a structure-breaking solute (chaotropic solute) [31].

With the purpose of evaluating the changes occurring in the structure and behavior of this solute when β-CD is present in the aqueous medium, viscosity measurements in a {water + β-CD 0.007 mol dm^{-3}} mixture were carried out and the results also evaluated with the Jones–Dole equation. From this, slightly higher values for A and B parameters (that is, A = 2.39 $dm^{3/2}$ $mol^{-1/2}$ and B = −14.2 dm^3 mol^{-1}) were obtained, indicating that the situation is similar.

The interpretation of these data can be also achieved, by a more detailed treatment of the diffusion with supramolecular complexes, using some assumptions. That theory has been developed and well described in the literature [32–35]; In addition, the computation of ternary diffusion coefficients is also described in detail in the Supplementary Material; consequently, only some points are shown in this section.

Another approach to these data can be performed by assuming the occurrence of a 1:1 supramolecular complex between chloroquine diphosphate (CDP) and β-cyclodextrin (β-CD), (Equation (16))

$$\text{CDP} + \beta\text{-CD} \leftrightarrows \text{CDP-}\beta\text{-CD} \tag{16}$$

where the association constant, K, that describes the stability of these complexes, is given by Equation (17).

$$K = \frac{C_{\text{CDP}-\beta-\text{CD}}}{C_{\text{CDP}} \quad C_{\beta-\text{CD}}} \tag{17}$$

C_{CDP}, $C_{\beta\text{-CD}}$ and $C_{\text{CDP-}\beta\text{-CD}}$ represent the concentrations of free CDP and β-CD, and the concentration of the CDP–β-CD complex, respectively, which are correlated by the following mass balance equations,

$$\begin{aligned} C_1 &= C_{\text{CDP}} + C_{\text{CDP-}\beta\text{-CD}} \\ C_2 &= C_{\text{CD}} + C_{\text{CDP-}\beta\text{-CD}} \end{aligned} \tag{18}$$

Identifying these solute species as CDP = 1, β-CD = 2, and CDP–β-CD complexes = 3, respectively, which are in equilibrium according to the equation (Equation (16)), the Equations (3) and (4)), should be replaced by

$$J_1 = -D_{11}\nabla C_1 - D_{12}\nabla C_2 - D_{13}\nabla C_3 \tag{19}$$

$$J_2 = -D_{21}\nabla C_1 - D_{22}\nabla C_2 - D_{23}\nabla C_3 \tag{20}$$

$$J_3 = -D_{31}\nabla C_1 - D_{32}\nabla C_2 - D_{33}\nabla C_3 \tag{21}$$

However, assuming that in diluted solutions that all cross-diffusion terms are negligible (D_{12}, D_{13}, D_{21}, D_{23}, D_{31}, D_{32} = 0), and by noting that the total CDP flux (as well as β-CD flux) is the sum of the respective fluxes of free and CDP–β-CD complexes, by inserting this information in the Equations (3) and (4), after some mathematical rearrangement, it is possible to obtain the Equations (22)–(25). These equations supply the relations between the mutual diffusion coefficients D_{11}, D_{12}, D_{21}, D_{22} for the total CDP(1) + β-CD(2) solute components, and the diffusion coefficients D_{CDP}, $D_{\beta\text{-CD}}$, $D_{\text{CDP}-\beta\text{-CD}}$ which indicate the

diffusion coefficients of the free CDP, the free β-CD and the corresponding supramolecular complex, respectively.

$$D_{11} = \frac{1}{2}\{(D_{CDP} + (D_{CDP-\beta-CD})) + (D_{CDP} - (D_{CDP-\beta-CD}))[1 - K(c_2 - c_1)]R\} \quad (22)$$

$$D_{12} = \frac{1}{2}\{((D_{CDP-\beta-CD}) - D_{CDP}) + (D_{CDP} - (D_{CDP-\beta-CD}))[1 - K(c_2 - c_1)]R\} \quad (23)$$

$$D_{21} = \frac{1}{2}\{((D_{CDP-\beta-CD}) - D_{-CD}) + (D_{-CD} - (D_{CDP-\beta-CD}))[1 - K(c_2 - c_1)]R\} \quad (24)$$

$$D_{22} = \frac{1}{2}\{(D_{-CD} + (D_{CDP-\beta-CD})) + (D_{-CD} - (D_{CDP-\beta-CD}))[1 - K(c_2 - c_1)]R\} \quad (25)$$

where

$$R = \left\{[1 + K(c_2 - c_1)]^2 + 4Kc_1\right\}^{-1/2}$$

The computed values for the limiting diffusion coefficients, D_s, of species CDP, β-CD and CDP–β-CD are reported in Table 5.

Table 5. Diffusion coefficients, D_s, of species at infinitesimal concentration and T = 298.15 K and P = 101.3 kPa.

Species	$D_s/(10^{-9}$ m^2 s$^{-1})$
Chloroquine diphosphate (CDP)	0.670
β-cyclodextrin (β-CD)	0.380
CDP–β-CD	0.360

Standard uncertainties are: $u(T)$ = 0.01 K; $u(P)$ = 2.03 kPa.

From D_{11} at X_1 = 1 and from D_{22} at X_1 = 0, the diffusion coefficients of free CDP (D_{CDP} = 0.670 × 10^{-9} m^2 s^{-1}) and free β-cyclodextrin ($D_{\beta\text{-CD}}$ = 0.380 × 10^{-9} m^2 s^{-1}) are obtained, respectively.

By using the equation of Stokes–Einstein (Equations (26) and (27)):

$$D = \frac{k_B T}{6\pi\eta r_h} \quad (26)$$

$$D_{CDP-\beta-CD} = \left(D_{CDP}^3 + D_{\beta-CD}^3\right)^{1/3} \quad (27)$$

where k_B is the Boltzmann constant, T is the absolute temperature and η is the viscosity of the solvent, the diffusion coefficient of the CDP–β-CD complex can be estimated as equal to $D_{CDP\text{–}\beta\text{-CD}}$ = 0.360 ×10^{-9} m^2 s^{-1}.

K, was chosen in order to obtain the best agreement between these theoretical values (Equations (22)–(25)) and the experimental Dij data. In the present case, for CDP molar factions $X_1 \leq 0.5$, a complexation constant K equal to 30.0 ($\pm$0.8) mol^{-1} dm^3 was found.

This value demonstrates that the interaction between β-CD and CDP is weak, which can easily be justified by the high solubility of CDP in water, suggesting that the H-bonding plays an important role in the interactions between CDP and water and, probably, CDP and β-CD [9,36]. This is in line with similar systems involving other drugs such as, for example, L-dopa and paracetamol [37,38]. However, for $X_1 > 0.5$, the model is not applicable. In fact, for these concentrations, the gradient in the concentration of β-CD produces significant co-current coupled flows of CDP and, therefore, leads to disadvantageous conditions for the formation of supramolecular complexes with this sterically hindered cyclodextrin in solution. This fact is in complete agreement with the viscosity results. That is, CDP is a structure-breaking solute, suggesting that, indeed, there is no complexation between β-CD and CDP.

5. Conclusions

We can conclude that the diffusion of the CDP is influenced by the presence of this macromolecular cyclodextrin (β-CD), suggesting that at low concentrations of this drug there is a very weak interaction between these solutes (which is supported by the small value that can be estimated for the equilibrium constant of the complexation between both solutes, CDP and β-CD, K = (30 $\pm$ 0.8) mol^{-1} dm^3). This result is consistent with weak chloroquine binding to cyclodextrin, in contrast to a recent report of anomalously large-chloroquine binding constants. For more concentrated solutions, for which it is obtained that $D_{12} > 0$, a coflow of CDP is observed, showing thus, there is no predisposition of inclusion of CDP in the cavity of the sterically hindered β-CD. Support for this evidence is given by the viscosity data measured.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr9081433/s1, The theoretical computation of the ternary diffusion coefficients, contemplating the for-mation of supramolecular complexes, is described in detail in the Supplementary Material.

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