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Effect of Buffer Species on the Inclusion Complexation of Acidic Drug Celecoxib with Cyclodextrin in Solution

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Abstract

The interaction of celecoxib (Celox) with cyclodextrins (CDs) has been investigated by phase solubility techniques. In this study, the influences of CD type, pH, buffer type, buffer concentration and temperature on the tendency of Celox to form inclusion complexes with CDs were examined. The tendency of Celox to complex with CDs is in the order HP-β-CD > β-CD > γ-CD > α-CD, where the complex formation constants (K_{11}) were 1377, 693, 126 and 60 M^{-1}, respectively. Also ionization of the slightly acidic Celox ($pK_a = 9.7$) was found to reduce its tendency to complex (i.e., The K_{11} values of Celox/β-CD in 0.05 M phosphate buffer were 976 and 210 M^{-1} for neutral and ionized Celox, respectively). Increasing citrate and phosphate buffer concentration enhances the tendency of ionized Celox to complex with β-CD as a result of a corresponding decrease in the inherent solubility (S_0) of the Celox anion. On the other hand, these two buffers interact differently with neutral Celox and β-CD, where increasing phosphate buffer concentration at low pH enhances the complexation of neutral Celox by lowering S_0, while increasing citrate buffer concentration at low pH reduces complex formation as citrate buffer species, mainly citric acid, act as a solublizer and a competitor for Celox and β-CD. The contribution of Celox hydrophobicity for complex stability constitutes about 77% of the driving force for complex stability. The complex formation of neutral Celox with β-CD ($\Delta G^0 = -28.6$ kJ/mol) is driven by both enthalpy ($\Delta H^0 = -21.7$ kJ/mol) and entropy ($\Delta S^0 = 23.3$ J/mol K) changes.

Introduction

Cyclodextrins (CDs), as cyclic components, can form inclusion complexes with various compounds that fit partially or entirely inside the cavity. This can improve the physical, chemical and biological properties of the guest compounds [1]. The effect of various factors such as pH, buffer composition, and addition of different ionic strength adjusters on inclusion complex formation has been reported [2–8]. For example, the ionization of acidic compounds such as indomethacin and ibuprofen at pHs above their pK_a values reduces the tendency to complex with CDs [2, 3]. The interference of phosphate buffer and different ionic strength adjusters on the complexation of an azo dye, sodium *p*-(4-hydroxy-1-naphthylazo) benzenesulfonate with β-CD at pH 5.9 was studied by spectrophotometry [4]. The results showed that the complex formation constant was found to increase by increasing phosphate buffer concentration and is also enhanced in the presence of $H_2PO_4^-$, HPO_4^{-2}, SO_4^{-2}, IO_3^-, and F^- anions as a result of decreasing in the activity of water. In contrast, other ions including Cl^-, Br^-, I^-, NO_3^-, SCN^- and ClO_4^- appeared to reduce the complex formation constant, where these anions form inclusion complexes with β-CD, thus competing with the dye for the binding site of β-CD [4]. Another study using the conductance method showed that ClO_4^-, SCN^-, I^-, Br^- and NO_3^- did interact with α-CD while Cl^-, SO_4^{-2} and CH_3COO^- did not [5]. In general, the complex formation constants of some inorganic salts with β-CD were found to follow the order $ClO_4^- > I > SCN^- > Br^- > NO_3^- > Cl^-$ as obtained by a spectral competitive inhibition reaction of 4-nitrophenylazo-2́-hydroy-6́-sulfonaphthalene [6]. Phenolphthalein/β-CD complexation was found to be adversely affected by ClO_4^-, SCN^-, NO_3^- and Br^-, while Cl^- and SO_4^{-2} enhance complexation [7]. By using fluorescence method, buffer species were found to significantly reduce the complex formation constant (K_{11}) of 3-hydroxy-2-naphthoic acid/β-CD including $H_2C_6H_5O_7^-$,

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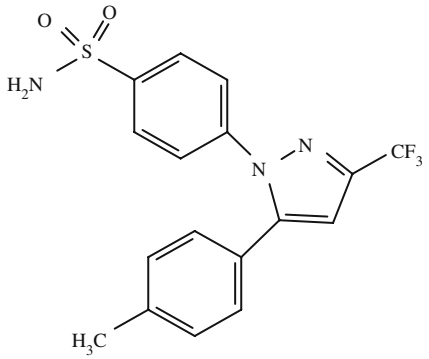
$HC_4H_4O_6^-$, $H_2C_8H_4O_4^-$ and $H_2PO_4^-$, while others (HCO_3^-, CH_3COO^-, HPO_4^{-2}, NH_4Cl and $HC_2O_4^-$) increase K_{11} by different extents [8]. Also addition of ionic strength adjuster ClO_4^- was found to reduce the complex formation constant by about 75%, while F^-, Cl^- and SO_4^{-2} enhance slightly the complexation (14%).

Celox is well known as a highly effective COX-2 inhibitory drug and is widely prescribed for the treatment of chronic inflammatory diseases such as rheumatoid arthritis and osteoarthritis. It is practically insoluble in water with a relatively low oral bioavailability (22–40%) [9]. Its bioavailability may be enhanced by using an ultra-fine and nanoparticulate Celox in suspension and solid formulations [10], a wetting agent [11], a solublizer [12, 13], a fast-melt formulation [14], by converting Celox into a glassy state by melt quenching [15], or by preparing inclusion complexes with β-CD and its dimethyl derivative by kneading, evaporation, freeze and spray drying methods [16–19]. Characterization of the prepared solid complexes was carried out by X-ray, DSC, H-NMR, IR, polarimetry and scanning electron microscopy.

In this work, the variation of complex formation constant (K_{11}) with CD type, pH, buffer type, buffer concentration and temperature was investigated. In addition, the drug hydrophobicity and the thermodynamic parameters were investigated to estimate their contribution to complex stability. The acidic drug Celox was selected as a case study.



Scheme 1. Chemical structure of celecoxib.

Materials and methods

Materials

Celox of 99.3% purity and CDs (α-, β-, HP-β- and γ-CDs) of more than 98.5% purity were provided by The Jordanian Pharmaceutical Manufacturing Company (JPM). All other chemicals were of analytical grade obtained from Merck/Germany and Surechem/UK.

Acid–base ionization constant determination

By UV/Visible spectrophotometry

Stock solution of Celox was prepared by dissolving predetermined amounts (50 mg) in 100 ml of methanol (≥99.9% purity) which were diluted further with 0.05 M citrate buffer of different pHs to obtain final solutions having fixed concentration of 0.026 mM. The absorbencies of these solutions were measured using first derivative UV/Visible spectrophotometry at 268 nm (Du-650i, Beckman/USA).

By pH solubility profile

Excess amounts of the drug (100 mg) were added to 50 ml of 0.05 M citrate buffer of different pHs ranging from 1 to 12. The pH of buffer solutions was adjusted by diluted sodium hydroxide solution for pHs above 2, while diluted hydrochloric acid was used to adjust the pH below 2. The samples were mechanically shaken in a thermostatic bath shaker at 30 °C (1086, GFL/Germany) for 2 days, which were found sufficient to establish equilibrium, an aliquot was filtered using a 0.45 μm filter (cellulose acetate, Advantec MFS Inc., Duplin, USA). The pH of the filtrate was measured by calibrated pH-meter (3030, Jenway/England). The concentration of Celox in each solution was determined by the HPLC method described below.

Rigorous nonlinear regression of experimental data corresponding to plot of ε (total molar absorptivity) or S_0 (inherent solubility of Celox) against pH was conducted using the Marquardt–Levenberg finite difference algorithm utilized by the SPSS statistical package (SPSS 10.0 for Windows Statistical Package, SPSS Inc., 233 S. Wacker Drive, Chicago, Illinois), and data plots were linked to Microsoft Excel for reproduction. To minimize errors resulting from sudden change of absorbance (A) and solubility (S_0) with pH near the pK_a value of Celox, a weighted nonlinear regression procedure was used in both cases where the weighing factor $w=(\Delta A)^2+[\Delta\text{pH}\ (\text{d}A/\text{dpH})]^2$ for the variation of absorbance (A) of Celox with pH, and $w=(\Delta S_0)^2+[\Delta\text{pH}\ (\text{d}S_0/\text{dpH})]^2$ for pH solubility profile, where $\Delta S_0=0.001$, $\Delta\text{pH}=0.01$.

Phase solubility studies

Solubility studies were performed as described earlier [20]. Excess amounts of the drug (200 mg) were added to 50 ml of the desired aqueous CDs solutions ranging in concentration from 0 to 18 mM. The solutions include water, citrate and phosphate buffers of different concentrations (0.05–1.0 M) and pHs (2.4, 6.0 and 12.3). The samples were mechanically shaken in a thermostatic bath shaker at 30 °C for 2 days, which were found sufficient to establish equilibrium, an aliquot was filtered using a 0.45 μm filter. The pH of the filtrate was measured by pH-meter. The concentration of Celox in each solution was determined by measuring the first derivative amplitude at 268 nm. The HPLC method was used for samples in the absence of CDs at pHs 2.4 and 6.0, where the inherent solubility (S_0) becomes too small to reliably estimate through first derivative spectrophotometry.

Phase solubility diagrams were analyzed to obtain estimates of the complex formation constants of soluble

complexes following rigorous procedures described earlier [21]. By assuming the formation of 1:1 (SL) and 1:2 (SL_2) soluble Celox/CD complexes, the individual formation constants of SL and SL_2 complexes defined as K_{11} and K_{12} are given by:

$$K_{11} = [SL]/[S][L] \quad (1)$$

$$K_{12} = [SL_2]/[SL][L] \quad (2)$$

The solubility (S_{eq}) of Celox in aqueous CD solutions of variable concentrations is given by:

$$\begin{aligned} S_{eq} &= [S] + [SL] + [SL_2] \\ &= [S] + K_{11}[S][L] + K_{11}K_{12}[S][L]^2 \end{aligned} \quad (3)$$

where [S] and [L] donate the concentrations of free Celox and CD, respectively. Since the solutions are saturated with Celox, $[S] = S_0$ which is the solubility of Celox at zero CD concentration, while [SL] and $[SL_2]$ represent the concentrations of 1:1 and 1:2 Celox/CD complexes, respectively.

$$\begin{aligned} S_{eq} &= S_0 + [SL] + [SL_2] \\ &= S_0 + K_{11}S_0[L] + K_{11}K_{12}S_0[L]^2 \end{aligned} \quad (4)$$

The total concentration of CD in solution (L_{eq}) is given by

$$\begin{aligned} L_{eq} &= [L] + [SL] + 2[SL_2] \\ &= [L] + K_{11}S_0[L] + 2K_{11}K_{12}S_0[L]^2 \end{aligned} \quad (5)$$

and thus

$$[L] = (-b \pm (b - aL_{eq})^{1/2})/(2a) \quad (6)$$

where

$$a = 2K_{11}K_{12}S_0 \text{ and } b = 1 + K_{11}S_0 \quad (7)$$

To compensate for citric acid/β-CD complex formation in citrate buffers at pH 2.4, the contribution of citric acid to complex formation, which was estimated at $K_c = 16.7\ M^{-1}$ from the variation of β-CD solubility with citric acid concentration at pH 2.4 was accounted for in the analysis of PSDs of Celox/β-CD. This compensation was carried out by adding the term "$K_c\ C_o$ [L]" to Equation (5), where K_c and C_o represent the complex formation constant of citric acid with β-CD ($16.7\ M^{-1}$) and the concentration of free citric acid in a particular citrate buffer solution, respectively.

Rigorous nonlinear regression of experimental data corresponding to each phase diagram was conducted and the results of rigorous analysis indicated only the formation of SL soluble complex.

pH Solubility profile of β-CD

Excess amounts of β-CD were added to 50 mL of citrate and phosphate buffers of different concentrations (0.05–1.0 M) and of different pHs ranging from pH 2 to 12. The samples were mechanically shaken in a thermostatic bath shaker at 30 °C for 2 days, which were found sufficient to establish equilibrium, an aliquot was filtered using a 0.45 μm filter. The β-CD concentration was determined by optical rotation (α) measurements on a Polarimeter (Polartronic D, Schmidt & Haensch/Germany) at 25 °C using a 1 dm cell.

High performance liquid chromatography

A Beckman Gold HPLC system (USA) with programmable detector 166 and programmable pump 116 was used. The system comprised acetonitrile, methanol and phosphate buffer (5:1:4 volume/volume) as the mobile phase. The buffer was prepared by dissolving 6.8 g of potassium dihydrogen phosphate in 1000 mL water and the pH adjusted to 3 with 10% H_3PO_4 solution. C18 column (Hypersil BDS, 250 × 4.6 mm dimension, Hypersil, UK) was used as the stationary phase. UV detection was conducted at 250 nm. A 100 μl injection loop and a flow rate of 2 ml/min were applied.

Quantitation of hydrophobic effect

A quantitative measure of the contribution of the hydrophobic effect (desolvation) to complex formation can be obtained from possible correlation of the free energy of complex formation ($\Delta G_{11} = -RT \ln K_{11}^x$) with the free energy of Celox solubility ($\Delta G_{S_0} = -RT \ln S_0^x$) obtained at different citrate and phosphate buffer concentrations (x depicts the mole fraction standard state, where the units of K_{11} (M^{-1}) and S_0 (M) transformed to mole fraction units by multiplying K_{11} with 55.5 and dividing S_0 by 55.5 (55.5 represents the number of moles of water in 1000 ml), respectively. For example, if ΔG_{11} varies linearly with ΔG_{S_0}, then the negative slope of the linear plot would indicate the contribution of the hydrophobic character of Celox towards complex formation. On the other hand, the intercept would provide a measure of the contribution of other factors, including specific interactions, to complex stability [22].

Estimation of thermodynamic parameters

By using Gibbs and Van't Hoff equations, ΔH^0, ΔS^0 and ΔG^0 are calculated as follows:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (1)$$

$$\ln(K) = \Delta S^0/R - \Delta H^0/RT \quad (2)$$

K represents $K_{11}{}^{x}$ or $S_0{}^{x}$ in mole fraction unit (x)
A plot of ln (K) versus $1/T$ produces:
Slope $= -\Delta H^0/R$ and Intercept $= \Delta S^0/R$

Results and discussion

Acid–base ionization constant (pK_a)

By using the spectrophotometric method, the 1st derivative absorbance of a fixed concentration of Celox (0.026 mM) at 268 nm was measured against pH in 0.05 M citrate buffer at 30 °C, and the results were fitted through nonlinear regression to obtain an estimate of the pK_a (the 1st derivative reading was divided by the drug concentration to obtain the 1st derivative molar absorptivity (ε) which was used in the fitting procedure yielding the best fits (ε^p) indicated by the solid lines in Figure 1a). While by using solubility method, nonlinear regression of solubility of Celox versus pH was used to obtain an estimate of pK_a (Figure 1b). Both methods gave a pK_a value of 9.7, which is in agreement with what was calculated by Advanced Chemistry Development (ACD) Software Solaris V4.67 (1994–2004 ACD). This value differs from what was reported earlier (p$K_a = 11$) [9, 17], however, the method of pK_a determination was not stated in these two studies.

Effect of CD type

Figure 2a depicts phase solubility diagrams (PSDs) obtained for Celox against each of α-, β-, γ- and HP-β-CD concentration in unbuffered water (pH = ~6) at 30 °C. At this pH, Celox exists as a neutral molecule (p$Ka = 9.7$) with an inherent solubility (S_0) of 0.004 mM. The K_{11} values were 1377, 693, 126 and 60 M^{-1} for HP-β-CD, β-CD, γ-CD and α-CD, respectively (Table 1). The low K_{11} values in case of γ-CD and α-CD are most likely due to two factors: (a) both α- and γ-CD are highly soluble in water thus lowering the driving force to complex with Celox, and (b) α-CD has a small cavity size that reduces the probability of including the bulky groups of Celox, while γ-CD has a large cavity size thus lowering effective interactions with Celox [23, 24]. The relatively high K_{11} values for HP-β-CD and β-CD indicate the existence of an effective geometric fit. The highest K_{11} value for HP-β-CD (1377 M^{-1}) indicates that the hydroxypropyl groups in HP-β-CD may facilitate inclusion of the *p*-tolyl group into the hydrophobic cavity [25], allowing its hydroxyls to form H-bonding with the sulphonamide and pyrazol groups [18].

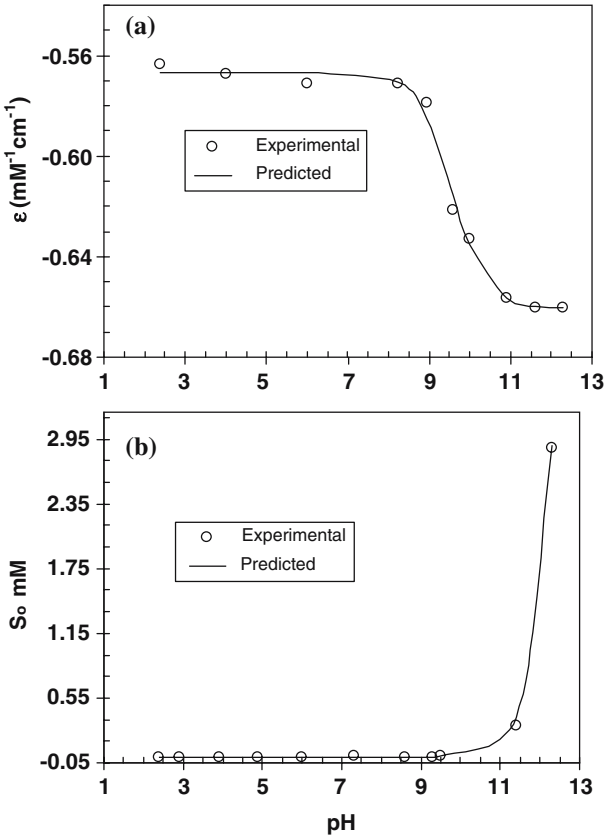


Figure 1. Plots of (a) the total molar absorptivity (ε) of a fix concentration of Celox (0.026 mM) at 268 nm, and (b) the variation of inherent solubility of Celox (S_0) against pH, both measured at 0.05 M citrate buffer at 30 °C.

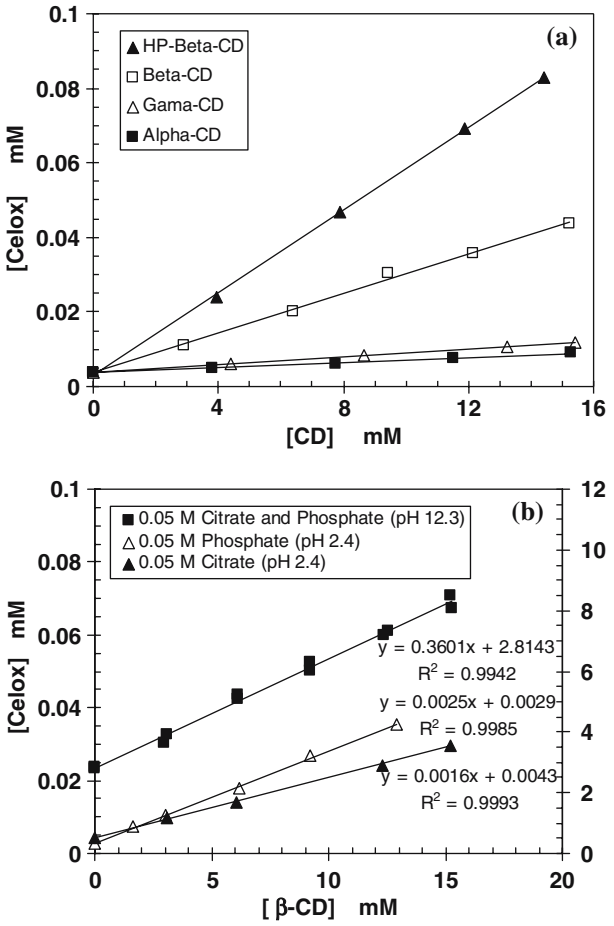


Figure 2. Phase solubility diagrams of (a) the Celox/CD systems in unbuffered water (pH 6) and (b) the Celox/β-CD system in 0.05 M citrate and phosphate buffers of pHs 2.4 and 12.3 at 30 °C, right *y*-axis corresponds to pH 12.3.

Table 1. Complex formation parameters for Celox/CDs systems in unbuffered water (pH 6) at 30 °C

CD	Phase solubility diagram type	K_{11} (M^{-1})	EF
α-CD	A_L	60	2.3
β-CD	A_L	693	11.0
γ-CD	A_L	126	2.9
HP-β-CD	A_L	1377	21.9

The enhancement factor (EF) = S_{eq}/S_0, where S_{eq} and S_0 are the solubilities of Celox in the presence (15 mM) and absence of cyclodextrin (CD), respectively.

The K_{11} value for Celox/β-CD system in water (693 M^{-1} at 30 °C) obtained is in agreement with those reported earlier (882 M^{-1} at 25 °C [16] and 592 M^{-1} at 37 °C [18]). The decrease in K_{11} values by increasing the temperature is expected as a result of an increase in S_0, which lowers the tendency of Celox to complex, but the significant difference from the other value reported by Reddy *et al.* [17] (215 M^{-1} at 20 °C) is most probable due to differences in the measured S_0.

Effect of pH

The PSDs of Celox/β-CD system obtained in 0.05 M citrate and phosphate buffers at pHs of 2.4 and 12.3 are shown in Figure 2b. The corresponding complex formation constants are listed in Table 2. The results indicate that the K_{11} value for neutral Celox in phosphate buffer at pH 2.4 is relatively high (976 M^{-1}), while it is lower for ionized Celox (210 M^{-1}) at pH 12.3. The same trend was reported earlier for acidic drugs (indomethacin and diclofenac) [2, 3] indicating that the ionization of the acid drug species at higher pH reduces its tendency to complex.

As shown in Figure 2b, the solubilizing efficiency, expressed by the slope, is higher for the ionized Celox (slope = 0.36) than for the neutral Celox (slope = 0.0016–0.0025). This indicates that a combination of drug ionization and cyclodextrin complexation for solubility enhancement is favorable from a formulation point of view [1].

Effect of buffer type and concentration

Figures 3 and 4 show the PSDs of neutral (at pH 2.4) and ionized Celox (at pH 12.3), respectively, obtained for different citrate and phosphate buffer concentrations. The corresponding values of S_0 and K_{11} are listed in Table 2. The results indicate that the tendency for neutral Celox to complex with β-CD decreases as S_0 increases This is most likely a reflection of the hydrophobic effect, which tends to squeeze Celox more into the hydrophobic β-CD cavity as S_0 for Celox decreases. It is interesting to note that K_{11} values for citrate buffer at pH 2.4 are lower than those of phosphate buffer at same pH (Table 2). This is evidently due to the fact that the solubilities for Celox and β-CD both increase with citrate buffer concentration [26], whereas the solubility of Celox actually decreases as phosphate buffer concentration increases at same pH (Table 2). The rise in the solubility of β-CD with citrate buffer concentration at pH 2.4 has been found due to citric acid/β-CD complexation, with a complex formation constant $K_c = 16.7$ M^{-1}. This leads to a competition between citric acid and Celox for the binding site of β-CD [4], which results in K_{11} values for Celox/β-CD much lower (364, 122, 61 and 33 M^{-1}) than when corrected for citric acid competition thus yielding K_{11} values of (560, 430, 370 and 340 M^{-1}) at citrate buffer concentrations of (0.05, 0.25, 0.50 and 1.0 M), respectively (Table 2). At pH 12.3, where Celox exists in the ionized form, increasing buffer concentration for both citrate and phosphate enhances the complexation by lowering the solubility of Celox (Table 2). This indicates that buffer type (organic or inorganic) may interfere differently with the inclusion complexation of drug with CDs [3, 27],

Table 2. Complex formation parameters for the neutral and ionized Celox/β-CD systems obtained at different concentrations of citrate and phosphate buffer at 30 °C

[Buffer] (M)	Neutral Celox (pH 2.4)			Ionized Celox (pH 12.3)	
	$S_0 \times 10^3$ (mM)	K_{11} (M^{-1})		S_0 (mM)	K_{11} (M^{-1})
		Uncorrected	Corrected*		
Citrate					
0.05	4.4	364	560	2.86	198
0.25	5.7	122	430	1.22	406
0.50	7.2	61	370	0.25	1566
1.00	9.8	33	340	0.11	2467
Phosphate					
0.05	2.6	976		2.80	210
0.25	2.1	1067		1.80	252
0.50	1.0	1895		0.35	936
1.00	0.45	3103		0.11	2230

*K_{11} values corrected for citric acid competing with Celox for β-CD at pH 2.4 (K_c for citric acid/β-CD complexation is 16.7 M^{-1}).

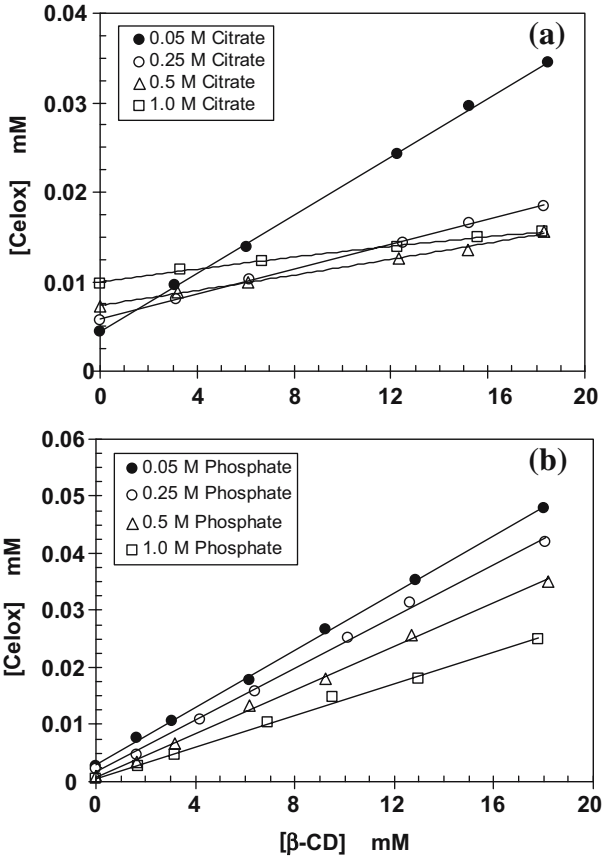


Figure 3. Phase solubility diagrams of the neutral Celox/β-CD system at pH 2.4 and 30 °C obtained at different (a) citrate and (b) phosphate buffer concentrations.

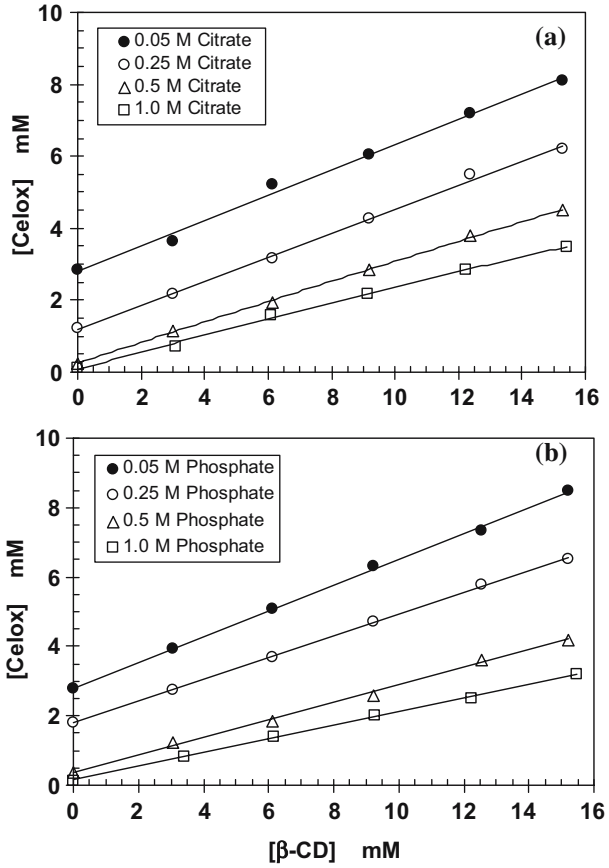


Figure 4. Phase solubility diagrams of the ionized Celox/β-CD system at pH 12.3 and 30 °C obtained at different (a) citrate and (b) phosphate buffer concentrations.

depending on the drug form (neutral or ionized). The variation of β-CD solubility with pH in different concentrations of citrate and phosphate buffers is shown in Figure 5 for reference.

The hydrophobic effect

To find the correlation between the strength of binding and the hydrophobic effect, different parameters may be involved, these parameters include partition coefficient, hydrophobic surface area, the number of carbon atoms of a homologous series of substrates and addition of organic cosolvent and salts to the media [28].

In the present work, the contribution of the hydrophobic interaction to complex formation was investigated by changing the buffer concentration (i.e. ionic strength) at same pH, which affects the S_0 and subsequently contributes differently to complex stability. The linear variation of $-RT \ln {K_{11}}^x$ against $-RT \ln {S_0}^x$ for all data listed in Table 2 indicates that almost 77.0% of the tendency for complex formation is driven by the hydrophobic character of Celox, while other factors including specific interactions constitute $+5.26$ (repulsive) and -3.98 (ion-induced dipole) kJ/mol, for the neutral and ionized Celox, respectively (Figure 6). In case of citrate buffer at pH 2.4, the hydrophobic effect is evident, where K_{11} value decreases by increasing buffer concentration (i.e. increasing S_0), but the contribution cannot be predicted without the compensation for citric acid/β-CD complex formation (slope higher than unity, -2.98), where citric acid competes with Celox to complex with β-CD ($K_c = 16.7\ M^{-1}$). The hydrophobic plot after reanalysis of PSDs of Celox/β-CD in citrate buffers at pH 2.4 to obtain corrected K_{11} values (Table 2), taking into consideration the contribution of citric acid to complex formation, yields a slope similar to that obtained in phosphate buffers at same pH.

Thermodynamic

The PSDs of neutral Celox obtained in 0.05 M citrate buffer (pH 6.0) at different temperatures are shown in Figure 7a. The complex formation parameters are listed in Table 3. From the corresponding van't Hoff plots of $\ln {K_{11}}^x$ and $\ln {S_0}^x$ (where x denotes the mole fraction standard state) against $1/T$, the thermodynamic parameters (ΔH^0, ΔS^0 and ΔG^0) were obtained for Celox solubility (S_0) and for the complex formation constant (K_{11}). Van't Hoff plots of $\ln {K_{11}}^x$ and $\ln {S_o}^x$ against $1/T$ are shown in Figure 7b, while the thermodynamic parameters are listed in Table 3. The solubility of Celox is an endothermic process ($\Delta H^0 = 49.4$ kJ/mol), where

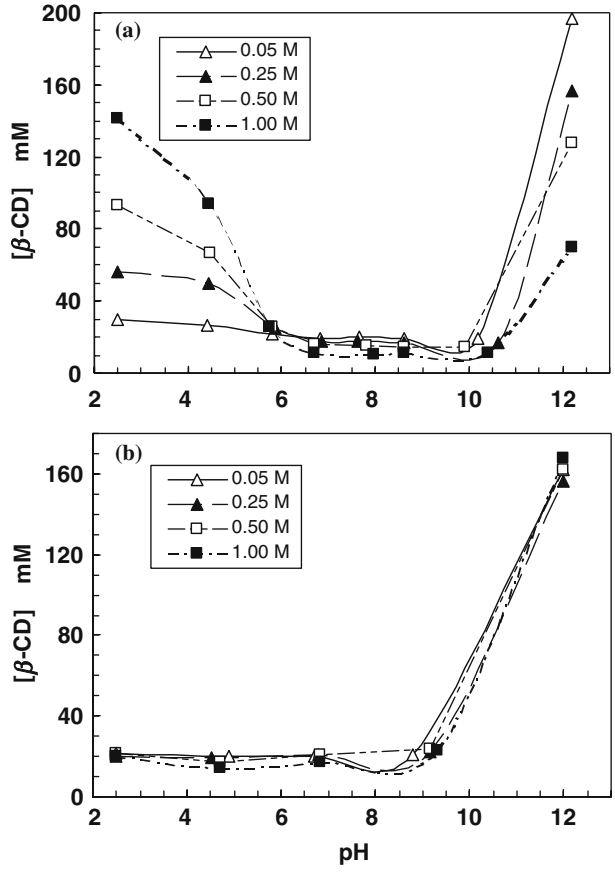


Figure 5. pH solubility profiles of β-CD in (a) citrate and (b) phosphate buffers of different concentrations (0.05–1.0 M) at 30 °C.

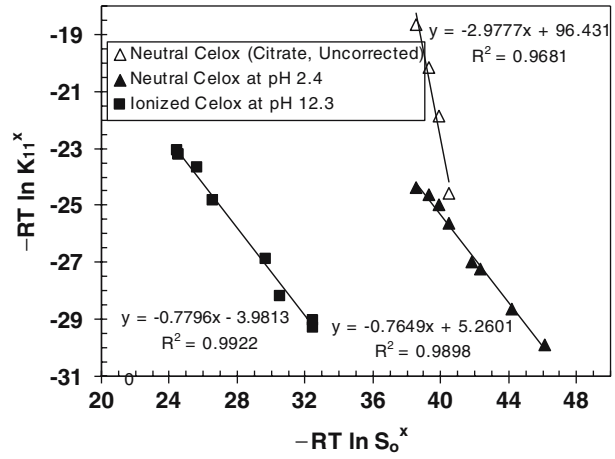


Figure 6. A plot of $-RT \ln K_{11}{}^{x}$ against $-RT \ln S_0{}^{x}$ for neutral and ionized Celox obtained in citrate and phosphate buffers of different concentrations (0.05–1.0 M) at pHs 2.4 and 12.3, x denotes the mole fraction standard state. The plot "Neutral Celox (Citrate, Uncorrected)" represents the data in citrate buffers before correction for citric acid competing with Celox for β-CD at pH 2.4.

S_0 increases as temperature increases. The positive entropy ($\Delta S^0 = 16.6$ kJ/mol K) indicates that the solubility is favored by entropy changes, suggesting that Celox becomes less ordered in solution. Complex formation for neutral Celox ($\Delta G^0 = -28.6$ kJ/mol) is driven by both enthalpy ($\Delta H^0 = -21.7$ kJ/mol) and entropy

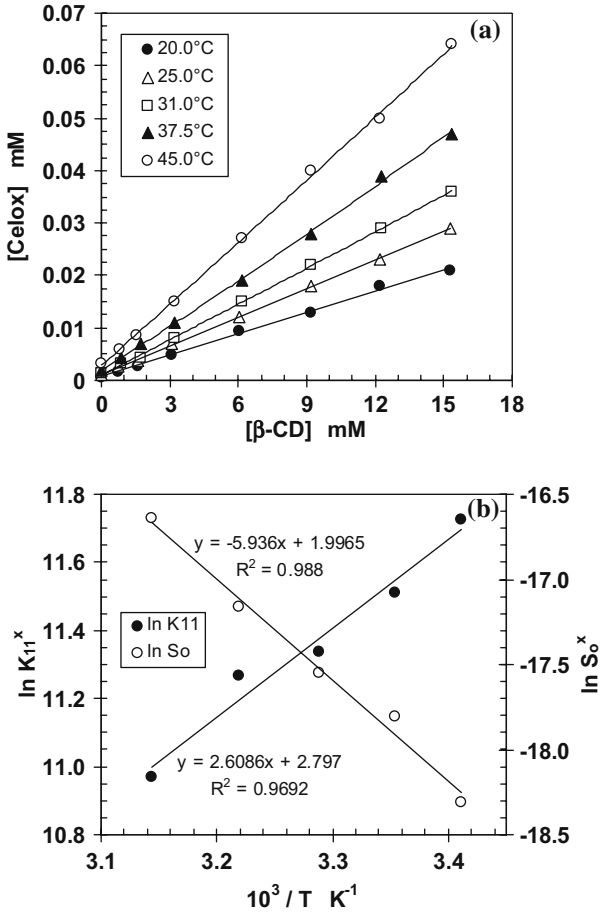


Figure 7. (a) Phase solubility diagrams of the Celox/β-CD system in 0.05 M citrate buffer at pH 6.0 and different temperatures; (b) Plots of ln $K_{11}{}^{x}$ and ln $S_0{}^{x}$ against $1/T$, x denotes the mole fraction standard state.

Table 3. Thermodynamic parameters of the Celox/β-CD system in 0.05 M citrate buffer (pH 6.0) obtained from van't Hoff plots

T (°C)	$S_0 \times 10^3$ (mM)	K_{11} (M^{-1})	
20.0	0.62	2224	
25.0	1.03	1795	
31.0	1.32	1510	
37.5	1.96	1410	
45.0	3.30	1045	
	ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (J/mol K)
Solubility of Celox	44.4	49.4	16.6
Celox/β-CD complex	−28.6	−21.7	23.3

($\Delta S^0 = 23.3$ J/mol K) changes. An exothermic enthalpy is attributed to van der Waals interactions between Celox and the β-CD cavity, while positive entropy indicated that the hydrophobic effect (desolvation) is a strong driving force for Celox/β-CD inclusion complexation [3]. It was reported that the interaction of Celox with dimethyl β-CD is unfavored by entropy changes, which was explained by the formation of a more rigid complex [19].

Conclusion

The results of this study on Celox/CD complexation in aqueous solution under different conditions of pH, buffer type, buffer concentration and temperature reveal the following: Celox forms 1:1 inclusion complexes with all the CDs investigated. The relatively high K_{11} values for HP-β-CD and β-CD indicate the existence of an effective geometric fit. Also the results indicate that drug ionization, media composition, and hydrophobic effects play an important role in the complex formation. The tendency of Celox to complex with β-CD in aqueous solution was mainly driven by the hydrophobic effect to within 77% of the complex stability. Neutral Celox/β-CD complex formation was found to be driven both by enthalpy ($\Delta H^0 = -21.7$ kJ/mol) and entropy ($\Delta S^0 = 23.3$ J/mol K) changes.

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