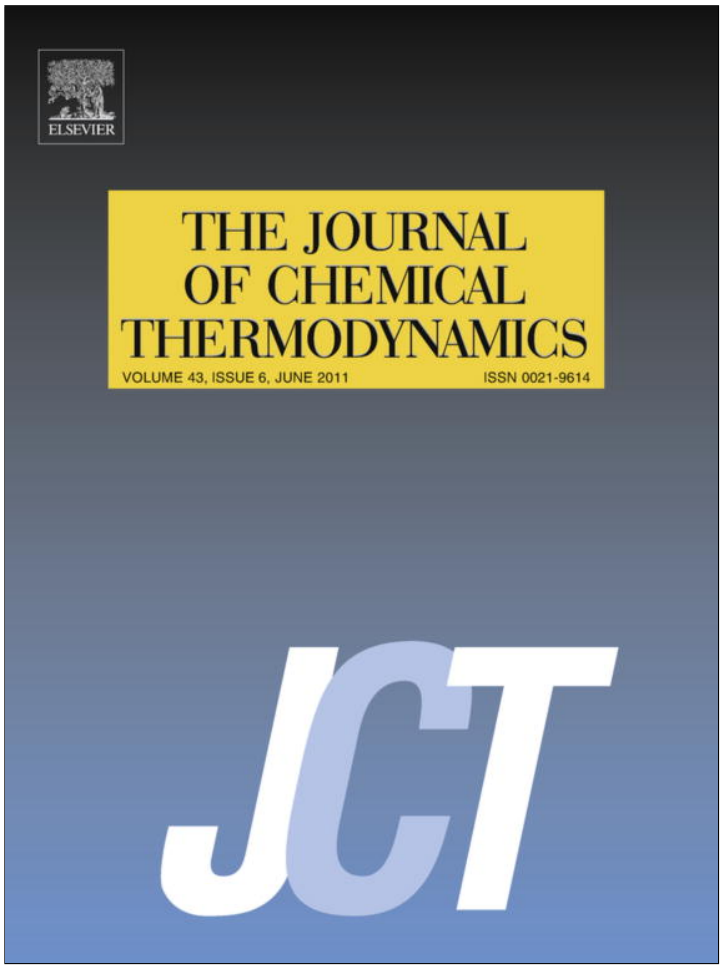

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Determination of the thermodynamic dissociation constant of capecitabine using spectrophotometric and potentiometric titration data

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ABSTRACT

Capecitabine has been studied by a combined potentiometric and spectrophotometric titration at $T = (298.15, 310.15)$ K at different ionic strengths. The thermodynamic dissociation constant was estimated by non-linear regression of {pK_a, I} data: $pK_a^T = 8.97(1)$ at $T = 298.15$ K and $pK_a^T = 8.74(0)$ at $T = 310.15$ K, where the figure in the brackets is the standard deviation in last significant digits. Thermodynamic parameters ΔH°/kJ · mol^{-1} and ΔS°/J · K^{-1} · mol^{-1} have been determined from the temperature variation of thermodynamic dissociation constants. The value of enthalpy $\Delta H^\circ = 33.9$ kJ · mol^{-1} shows the dissociation process is endothermic and the value of entropy $\Delta S^\circ = -51.9$ J · K^{-1} · mol^{-1} indicates that the dissociation is not a spontaneous process.

1. Introduction

Capecitabine, chemically 5′-deoxy-5-fluoro-N4-pentyloxycarbonyl-cytidine (figure 1) is the pro-drug for the anti-metabolite 5-fluorouracil (5-FU). It is a novel oral tumor-activated and tumor-elective fluoropyrimidine carbamate [1] and an oral chemotherapeutic agent used in the treatment of breast, esophageal and larynx, gastrointestinal and genitourinary tract cancers. Capecitabine represents the first-line treatment for metastatic colorectal cancer [2].

Capecitabine itself is inactive. Activation in the cytotoxin forms is a complex process. Capecitabine is metabolised in the liver first to 5′-deoxy-5-fluorocytidine and then to doxifluridine. The final step in the enzymatic pathway occurs at the tumor site where thymidine phosphorylase converts doxifluridine to the active metabolite 5-FU [3,4].

Capecitabine is a white to off-white crystalline powder with an aqueous solubility of 0.026 g · cm^{-3} at $T = 293.15$ K [5,6] and with p$K_a = 8.8$ [6].

Several HPLC methods have been developed over recent years to study capecitabine and its metabolites. The difference in polarity between capecitabine and the active metabolite 5-FU has so far prevented the simultaneous analysis of both compounds by HPLC. Reigner *et al.* [7] in the original method analyzed independently capecitabine using HPLC and UV detection and 5-FU using gas chromatography [8]. More recently, a LC–MS technique has been developed for capecitabine, but it is not suitable for 5-FU determination [9]. Zufia *et al.* [10] set up a method using UV detection that allows the simultaneous detection of capecitabine, 5-FU and dihydro-5-FU in plasma. Finally, Siethoff *et al.* [11] were able to determine the plasma concentration of both capecitabine and its different metabolites but by using column switching and MS–MS detection.

In this study, we have tried to complete the information on the protonation/dissociation constant for capecitabine by comparing two methods, spectrophotometry and potentiometry. Concurrently, the experimental determination of protonation constants was combined with their computational prediction based on knowledge of the chemical structures [12].

2. Theoretical

2.1. Procedure for the determination of the mixed protonation/dissociation constants

An acid–base equilibrium of the drug studied is described in terms of the thermodynamic protonation of the Brönstedt base

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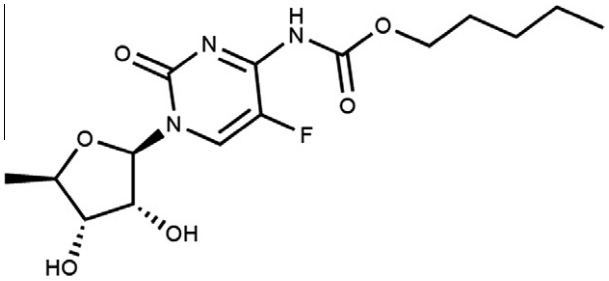

FIGURE 1. Chemical structure of capecitabine.

L^{z-1} according to the equation $L^{z-1} + H^+ + \leftrightarrows HL^z$ characterized by the protonation constant

$$K_H = a_{HL^z}/(a_{L^{z-1}}a_{H^+}) = \left([HL^z]/\left(\left[L^{z-1}\right][H^+]\right)\right)(y_{HL^z}/(y_{L^{z-1}}y_{H^+})) \quad (1)$$

The protonation equilibria between the anion L (the charges are omitted for the sake of simplicity) of a drug and a proton H are considered to form a set of variously protonated species L, HL, H_2L, H_3L, *etc.*, which have the general formula H_rL in a particular chemical model and which are represented by n_c the number of species, $r_i,\ i = 1,\ldots,n_c$ where index i labels their particular stoichiometry; the overall protonation (stability) constant of the protonated species, β_r, may then be expressed as

$$\beta_r = [H_rL]/([L][H]^r) = c/(lh^r) \quad (2)$$

where the free concentration [L] = l, [H] = h and [H_rL] = c. For dissociation reactions realized at constant ionic strength, the so-called "mixed dissociation constants" are defined as

$$K_{a,j} = a_{H^+}[H_{j-1}L]/[H_jL] \quad (3)$$

2.2. Determination of the thermodynamic protonation/dissociation constant

Let us consider the dependence of the mixed dissociation constant $K_a = a_{H+}[L^{z-1}]/[HL^z]$ on an ionic strength, when both ions HL^z and L^{z-1} have roughly the same ion-size parameter $\mathring{a}$ in the dissociation equilibrium $HL^z \rightleftarrows L^{z-1} + H^+$ with the thermodynamic dissociation constant $K_a^T = a_{H+}a_{L-}/a_{HL}$, and suppose that the overall salting-out coefficients is given by $C = C_{HL} - C_L$. This dependence is expressed by the Debye–Hückel equation

$$pK_a = pK_a^T - (A(1-2z)\sqrt{I})/(1 + B\mathring{a}\sqrt{I}) + CI \quad (4)$$

where $A = 0.5112\ \mathrm{mol}^{-1/2}\cdot\mathrm{dm}^{3/2}\cdot\mathrm{K}^{3/2}$ and $B = 0.3291\cdot 10^{10}\ \mathrm{mol}^{-1/2}\cdot\mathrm{m}^{-1}\cdot\mathrm{dm}^{3/2}\cdot\mathrm{K}^{1/2}$ for aqueous solutions at $T = 298.15$ K. The mixed dissociation constant pK_a represents a dependent variable while the ionic strength I represents the independent variable. Three unknown parameters $b = \{pK_a^{T,a;C}\}$, where pK_a^T is the thermodynamic dissociation constant, $\mathring{a}$ is the ion-size parameter and C is the salting-out coefficient, are to be estimated by minimizing the sum of the squared residuals [13]

$$U(\boldsymbol{b}) = \sum_{i=1}^{n} w_i\left[pK_{a,exp,i} - pK_{a,calc,i}\right]^2 = \sum_{i=1}^{n} w_i\left[pK_{a,exp,i} - f(I;pK_a^T,\mathring{a},C\right]^2 = \text{minimum} \quad (5)$$

The non-linear estimation problem is simply a problem of optimization in the parameter space, in which the pK_a and I are known and given values while the parameters pK_a^T, $\mathring{a}$, and C are unknown variables to be estimated. However, for small values of an ionic strength the pK_a can be estimated only.

2.3. Programing

To determine the dissociation constant of capecitabine, several programs were used, SQUAD(84) and SPECFIT for spectrophotometry pH titration data, ESAB2M and HYPERQUAD2008 for potentiometric titration data.

(1) The multi-component spectra analyzing program SQUAD(84) [14,15] may adjust the protonation constant β_{qr} and the molar absorption coefficient ε_{qr} for a given absorption spectra set by minimizing the residual-square sum function, U,

$$U = \sum_{i=1}^{n}\sum_{j=1}^{m}\left(A_{exp,i,j} - A_{calc,i,j}\right)^2 = \sum_{i=1}^{n}\sum_{j=1}^{m}\left(A_{exp,i,j} - \sum_{k=1}^{p}\varepsilon_{j,k}c_k\right)^2 = \text{minimum} \quad (6)$$

where $A_{i,j}$ represents the element of the experimental absorbance response-surface of size $n \times m$ and the independent variables c_k are the total concentrations of the basic components c_L and c_H being adjusted in n solutions. This means that the predicted absorbance-response surface is fitted to the given spectral data, with one dimension representing the dependent variable (absorbance), and the other two dimensions representing the independent variables, the total component concentrations (or pH) of n solutions, at m wavelengths. Minimization may be done algorithmically or heuristically. The algorithmic process usually finds a global minimum, whereas the heuristic process depends on human control. Unknown parameters to be determined may be divided into two equal groups: (1) a hypothetical chemical model which is supplied by the user and should contain an estimate of the number of light-absorbing species in solution, p and (2) the best estimates of the protonation constants, $\beta_{qr,i},\ i = 1,\ldots,p$, which are adjusted by SQUAD(84) regression Gauss–Newton and Newton–Raphson algorithms. Various hypotheses of chemical models with refined parameters have been proposed and tested and the statistical characteristics describing the degree-of-fit of regression spectra through experimental points have been calculated. The residuals are analyzed to test whether the refined parameters adequately represent the data, and should be randomly distributed about the predicted regression curve.

(2) Another popular program the SPECFIT/32 [16–18] is based on singular value decomposition and non-linear regression modeling using the Levenberg–Marquardt method to determine the stability constants from spectrophotometric titration data. The method referred to as "model-free" does not require any assumption as to the chemistry of the system other than the number of active complexes present, nor any assumptions as to the nature of absorbing complexes, their stoichiometry or a thermodynamic model. The solution is retrieved using constraints such as non-negativity for concentrations and absorptivities, closure (the sum of the concentrations of some species should be equal to a known quantity) and unimodality (no more than one in the concentration profiles). The latest version of SPECFIT/32 [17] makes use of multiwavelength and multivariate spectra treatment and enables a global analysis for equilibrium and kinetic systems with singular value decomposition and non-linear least-squares regression modeling using the Levenberg–Marquardt method. The method has proven to be superior for discriminating between chemical models. Factor analysis is used as a powerful tool to determinate of independent components in a given data matrix.

(3) The program ESAB [19,20] uses this strategy for treating *emf* or pa_{H+} data to find dissociation constants that give the "best" fit to experimental data. As primary data contain the total concentration H_T/mol · dm^{-3} of proton from burette and the measured pa_{H+}, one could trust pa_{H+} and minimize the residual sum of squares $(V_{exp} - V_{calc})^2$. The residual e is formulated with the volume of *added titrant* V/dm^3 from burette so that $e_i = (V_{exp,i} - V_{calc,i})$ and the resulting residual sum of squares $U(\mathbf{b})$ is defined

$$U(\mathbf{b}) = \sum_{i=1}^{n} w_i \left(V_{exp,i} - V_{calc,i}\right)^2 = \sum_{i=1}^{n} w_i . e_i^2 \tag{7}$$

where w_i is the statistical weight usually set equal to unity, while in ESAB it may be equal to

$$1/w_i = s_i^2 = s_E^2 + [dE_i/dV_i]^2 s_v^2 \tag{8}$$

and with good equipment, we have generally $s_E = 0.1$ mV or 0.01 pH units and $s_V = 0.0005$ to 0.0020 cm^3.

(4) In the program HYPERQUAD2008 [21] the objective function is given in the matrix notation $U = e^T \boldsymbol{W} \mathbf{e}$, where $\mathbf{e}$ is a vector of residuals representing a measurement in mV or pH and $\boldsymbol{W}$ is a matrix of weights. To minimize the objective function, the Gauss–Newton–Marquardt method is used. The SIGMA criterion of a goodness-of-fit is defined as SIGMA = $\sqrt{(\sum_{i=1}^{n} w_i e_i)/(n-m)}$ where the weights w are calculated from estimates of the error in emf or pa_{H+} and titer; the latter only being important in regions where the titration curve slopes more steeply. The Sigma squared may also be used as the chi-squared statistic criterion.

The adequacy of a proposed regression model with experimental data and the reliability of parameter estimates $pK_{a,i}$ found, being denoted for the sake of simplicity as unknown parameters b_j, and ε_{ij}, $j = 1, \ldots, m$, may be examined by the goodness-of-fit test, cf. a previous tutorial [22,23].

2.4. Determination of the number of light-absorbing species

A qualitative interpretation of the spectra aims to evaluate the quality of the dataset and remove spurious data, and to estimate the minimum number of *factors*, *i.e.*, contributing aqueous species, which are necessary to describe the experimental data. The INDICES program [24] determines the number of dominant light-absorbing species present in the equilibrium mixture. The various indicator function $PC(k)$ techniques in the INDICES program developed to deduce the exact size of the true component space can be classified into two general categories and were described in detail previously [25,26]: (a) precise methods based upon a knowledge of the experimental error of the absorbance data, $s_{inst}(A)$ (*e.g.*, Kankare's residual standard deviation $s_k(A)$, average error criterion $AE(k)$, Bartlett χ^2 criterion $\chi^2(k)$, standard deviation of eigenvalues $s(g)$), and (b) approximate methods requiring no knowledge of the experimental error (*e.g.*, Exner function $\psi(k)$, factor indicator function $IND(k)$, imbedded error function $IE(k)$). In general, most precise and approximate methods are based on the procedure of finding the point where the slope of the indicator function $PC(k) = f(k)$ changes.

2.5. Determination of enthalpy and entropy change

The enthalpy change (ΔH°/kJ · mol^{-1}) for the dissociation process was calculated using the Vaňt Hoff equation

$$d \ln K/dT = \Delta H^\circ/RT^2 \tag{9}$$

From the free-energy change ΔG°/kJ · mol^{-1} and ΔH°/kJ · mol^{-1} values, the entropy (ΔS°/J · K^{-1} · mol^{-1}) could be calculated:

$$\Delta G^\circ = -RT \ln K \tag{10}$$

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T, \tag{11}$$

where R (ideal gas constant) = 8.314 J · K^{-1} · mol^{-1}, K is the thermodynamic dissociation constant and T is the absolute temperature.

3. Experimental

3.1. Chemicals and solutions

Capecitabine were donated by TEVA Pharmaceuticals, Opava with declared purity 1.00 checked by a HPLC method. *Hydrochloric acid*, 1 mol · dm^{-3}, was prepared by diluting a concentrated HCl (p.a., Lachema Brno) with redistilled water and standardization against HgO and KI with a reproducibility better than 0.002 according to the equation $HgO + 4KI + H_2O \rightleftarrows 2KOH + K_2[HgI_4]$ and $KOH + HCl \rightleftarrows KCl + H_2O$. *Potassium hydroxide*, 1 mol · dm^{-3}, was prepared from the exact weight of pellets p.a., Aldrich Chemical Company with carbon-dioxide free redistilled water kept for 50 min prior to that in a sonographic bath. The solution was stored for several days in a polyethylene bottle in an argon atmosphere. This solution was standardized against a solution of potassium hydrogen-phthalate using the derivative method with reproducibility 0.001. *Mercury oxide, potassium iodide* and *potassium chloride*, p.a. Lachema Brno were not extra purified. *Twice-redistilled water* kept for 50 min prior to that in a sonographic bath was used in the preparation of solutions.

3.2. Apparatus and pH-spectrophotometric titration procedure

The apparatus used and the pH-spectrophotometric titration procedure have been described previously in detail [22,23,27]. The experimental and computation scheme to determine of the protonation constants of the multi-component system is taken from Meloun *et al., cf.* p. 226 in reference [28] and the five steps are described in detail elsewhere [22]:

1. Instrumental error of absorbance measurements, $s_{inst}(A)$
2. Experimental design
3. Number of light-absorbing species
4. Choice of computational strategy
5. Diagnostics indicating a correct chemical model.

When the minimization process terminates, some curve-fitting diagnostics are examined to determine whether the results should be accepted: the physical meaning of parametric estimates, the physical meaning of the species concentrations, the goodness-of-fit test and the deconvolution of spectra.

3.3. Apparatus and potentiometric titration procedure

The free hydrogen-ion concentration h was measured on the digital voltmeter Hanna HI 3220 with a precision of ±0.002pH with the use of a combined glass electrode Theta HC 103-VFR. Titrations were performed in a water-jacketed double-walled glass vessel of 100 cm^3, closed with a Teflon bung containing the electrodes, an argon inlet, a thermometer, a propeller stirrer and a capillary tip from a micro-burette. All pa_H measurements were carried out at $T = (298.15 \pm 0.1$ or $310.15 \pm 0.1)$ K. During the titrations, a stream of argon gas was bubbled through the solution both for stirring and for maintaining an inert atmosphere. The argon was passed through an aqueous ionic medium by prior passage through two

vessels also containing the titrand medium before entering the corresponding titrant solution. The gas is best introduced under and also above the surface of the titrand. Sometimes the flow under the surface has to be stopped while the pH is measured. If the gas-flow is too fast, solution might be lost spray on the walls.

The burettes used were syringe micro-burettes of 1.250 cm^3 capacity (META, Brno) with a 25.00 cm micrometer screw [29]. The polyethylene capillary tip of the micro-burette was immersed into a solution when adding the reagent, but pulled out after each addition in order to avoid leakage of reagent during the pH reading. The micro-burette was calibrated by weighing water on a Kern 770 balance with a precision of ±0.00015 in added volume over the whole volume range.

The potentiometric titrations of drugs with potassium hydroxide were performed using a hydrogen activity scale. Standardization of the pH meter was performed using WTW standard buffers values $pa_H = 4.006$ (4.024), $pa_H = 6.865$ (6.841) and $pa_H = 9.180$ (9.088) at $T = (298.15, 310.15)$ K respectively, in brackets.

When the ESAB program estimated the total proton concentration in a burette $H_T/\text{mol} \cdot \text{dm}^{-3}$ and the total concentration of the

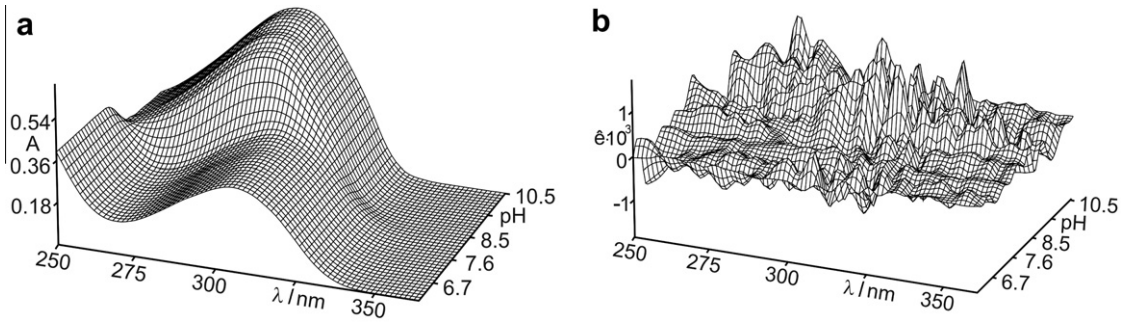

FIGURE 2. (a) Plot of absorbance against wavelength as the 3D-response representing the measured multiwavelength absorption spectra for capecitabine in dependence on pH at $T = 298.15$ K, (b) Plot of residuals against wavelength as the 3D map after a non-linear regression performed with SPECFIT and SQUAD programs (S-Plus).

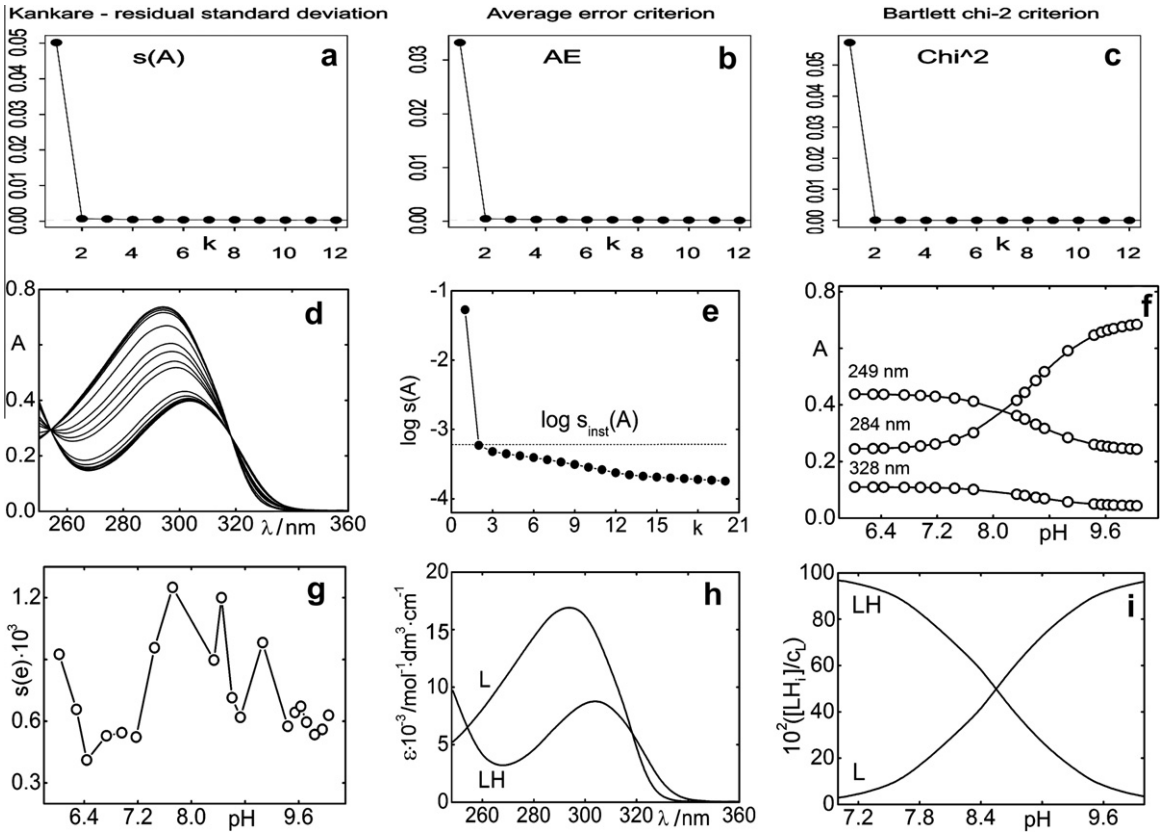

FIGURE 3. (a) Kankare's residual standard deviation $s_k(A)$ to estimate the minimum number of light-absorbing species, (b) average error criterion $AE(k)$ similar plot to the plot (a), (c) Bartlett χ^2 criterion $\chi^2(k)$ similar plot to the plot (a), (d) plot of absorbance against wavelength as the 2D-absorption spectra of $4 \cdot 10^{-5}$ M capecitabine in dependence on pH at $T = 298.15$ K, (e) Cattel's screen plot of the Wernimont–Kankare procedure for the determination of the number of light-absorbing species in the mixture $k^* = 2$ leads to $n_c = 2$ and the actual instrumental error of the spectrophotometer used $s_{inst}(A) = 0.00058$ (INDICES in S-Plus), (f) plot of the absorbance *vs.* pH curves for $\lambda = (249, 284, 328)$ nm in dependence on pH at $T = 298.15$ K, (g) plot of detecting influential outlying spectra with the use of the goodness-of-fit test and the plot of the residual standard deviation $s(e)$ *vs.* pH for 20 spectra in dependence on pH at $T = 298.15$ K, (h) pure spectra profiles of molar absorptivities *vs.* wavelengths for the variously protonated species L and HL. (i) Distribution diagram of the relative concentrations of both variously protonated species L and HL of capecitabine depending on pH at $T = 298.15$ K. The charges of species are omitted for the sake of simplicity (SPECFIT, ORIGIN).

drug in the titration vessel L_0/mol · dm^{-3} from the actual titration of a mixture of the drug and hydrochloric acid with potassium hydroxide, some group parameters are given in the input data for ESAB [19,20] such as the Nernstian slope and pK_w, which are both accessible from the literature [30]. Group parameters can be estimated by a regression analysis of both segments of a titration curve or from the acid segment only if the basic one might be affected by some carbonate or silicate in the alkali. With ESAB two group parameters, L_0/mol · dm^{-3} and H_T/mol · dm^{-3} were refined to give the best fit, while the fitness may be examined by the goodness-of-fit criteria.

3.4. Software used

Computation relating to the determination of dissociation constants were performed by regression analysis of the UV/VIS spectra using the SQUAD(84) [14,15] and SPECFIT/32 [18] programs. Computation relating to determining the dissociation constants was performed by regression analysis of titration curve using the ESAB program, version ESAB2M [19,20] and HYPERQUAD2008 program [21]. Most graphs were plotted using ORIGIN 8 [31] and S-Plus [24]. The thermodynamic dissociation constant pK_a^T was estimated with the MINOPT non-linear regression program in the ADSTAT statistical system (TriloByte Statistical Software, Ltd., Czech Republic), [32]. PALLAS [12] is a program for making predictions based on the structural formulae of drug compounds. Entering the compound topological structure descriptors graphically, pK_a values of organic compound are predicted using approximately hundreds of Hammett and Taft equations and quantum chemistry calculus.

3.5. Supporting information available

Complete experimental and computational procedures, input data specimens and corresponding output in numerical and

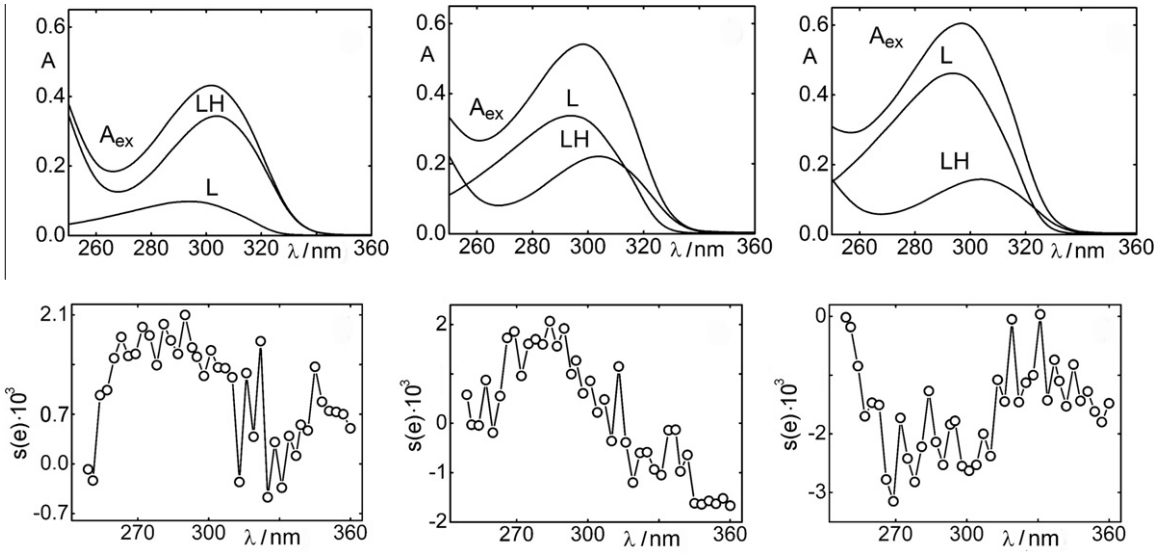

FIGURE 4. Deconvolution of the experimental absorption spectrum of capecitabine for 30 wavelengths into spectra of the individual variously protonated species L and HL, in solution of each particular absorption spectrum for a selected value of pH equal to 7.72, 8.45, and 8.73. The charges of species are omitted for the sake of simplicity (SQUAD, ORIGIN).

TABLE 1
Mixed dissociation pK_a constant of capecitabine acid at 298.15 K and various values of an ionic strength I/mol · dm^{-3} estimated by non-linear regression programs SPECFIT and SQUAD.

	I/(mol.dm^{-3})	0.0020	0.0029	0.0252	0.0474	0.0690	0.0880
SPECFIT	pK_a	8.720(3)	8.715(2)	8.701(1)	8.633(1)	8.696(2)	8.697(3)
	$s(A) \cdot 10^3$	0.63	0.38	0.37	0.24	0.41	0.61
SQUAD	pK_a	8.719(2)	8.718(1)	8.702(0)	8.633(0)	8.696(1)	8.697(1)
	$s(A) \cdot 10^3$	1.38	1.14	0.48	0.38	0.51	0.72

Standard deviation of parameter estimates in last valid digits are in brackets.

TABLE 2
Mixed dissociation pK_a constant of capecitabine acid at 310.15 K and various values of an ionic strength I/mol · dm^{-3} estimated by non-linear regression programs SPECFIT and SQUAD.

	I/(mol · dm^{-3})	0.0068	0.0290	0.0512	0.0734	0.0956	0.1178
SPECFIT	pK_a	8.550(2)	8.515(3)	8.505(5)	8.486(2)	8.483(2)	8.479(2)
	$s(A) \cdot 10^3$	0.73	0.71	0.73	0.50	0.54	0.65
SQUAD	pK_a	8.549(1)	8.515(1)	8.505(1)	8.486(1)	8.484(1)	8.479(0)
	$s(A) \cdot 10^3$	0.88	0.75	0.92	0.57	0.61	0.73

Standard deviation of parameter estimates in last valid digits are in brackets.

graphical form for the programs, INDICES, SQUAD(84), SPECFIT/32, ESAB2M and HYPERQUAD are available free of charge on line at http://meloun.upce.cz and in the menu DOWNLOAD and block DATA.

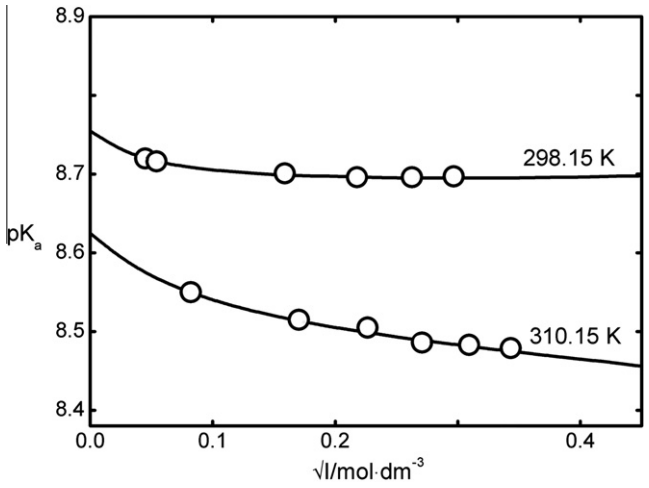

FIGURE 5. Plot of the dependence of the mixed dissociation constant pK_a of capecitabine on the square root of ionic strength, which lead to the parameter estimates of $pK_a^T = 8.76(1)$ at $T = 298.15$ K and $pK_a^T = 8.62(1)$ at $T = 310.15$ K.

4. Results and discussion

4.1. Spectrophotometry

The deprotonation of the capecitabine LH form indicates one simple equilibrium. The pH-spectrophotometric titration enables absorbance-response data (figure 2a) to be obtained for analysis by non-linear regression using the SPECFIT and SQUAD (84) programs. The reliability of estimated parameters (pK and ε's) can be evaluated on the basis of the goodness-of-fit test of residuals (figure 2b).

In the first step of the regression spectra analysis, the number of light-absorbing species n_c is estimated by the INDICES algorithm (figure 3a–c). The position of the break point on the $s_k(A) = f(k)$ curve in the factor analysis scree plot is calculated and gives $k^* = 2$ and so $n_c = 2$ with corresponding co-ordinate $\log s_k^*(A) = -3.23$, *i.e.* $s_k^*(A) = 0.00058$, which also represents the actual instrumental error $s_{inst}(A)$ of the spectrophotometer used. Due to the large variations in the indicator values, these latter are plotted on a logarithmic scale. All other selected methods of the modified factor analysis in the INDICES algorithm estimate the two light-absorbing components L^- and LH of the protonation equilibrium. The A–pH curves at $\lambda = (249, 284, 328)$ nm (figure 3f) show that a dissociation constant may be indicated.

The dissociation constant and the two molar absorptivities of capecitabine ε_L/dm^3 · mol^{-1} · cm^{-1} and ε_{HL}/dm^3 · mol^{-1} · cm^{-1} calculated for 30 wavelengths of 23 spectra (figure 3d) constitute $(2 \times 30)+1 = 61$ unknown regression parameters, which are estimated and refined with SQUAD(84) or SPECFIT32 programs in the first run. The reliability of the parameter estimates may be tested with the use of the following diagnostics:

The *first diagnostic* value indicates whether all of the parametric estimates pK_a and ε_L and ε_{LH} have physical meaning and reach realistic values: for capecitabine $pK_a^T = 8.76$ ($s = 0.01$) at $T = 298.15$ K. As the standard deviations s(pK_a) of parameters pK_a and $s(\varepsilon)$ of parameters ε_L/dm^3 · mol^{-1} · cm^{-1} and ε_{LH}/dm^3 · mol^{-1} · cm^{-1} are significantly smaller than their corresponding parameter estimates, all the variously protonated species are statistically significant at a significance level $\alpha = 0.05$. The physical meaning of the dissociation constant pK_a, and molar absorptivities ε_L/dm^3 · mol^{-1} · cm^{-1} and ε_{LH}/dm^3 · mol^{-1} · cm^{-1} are so examined.

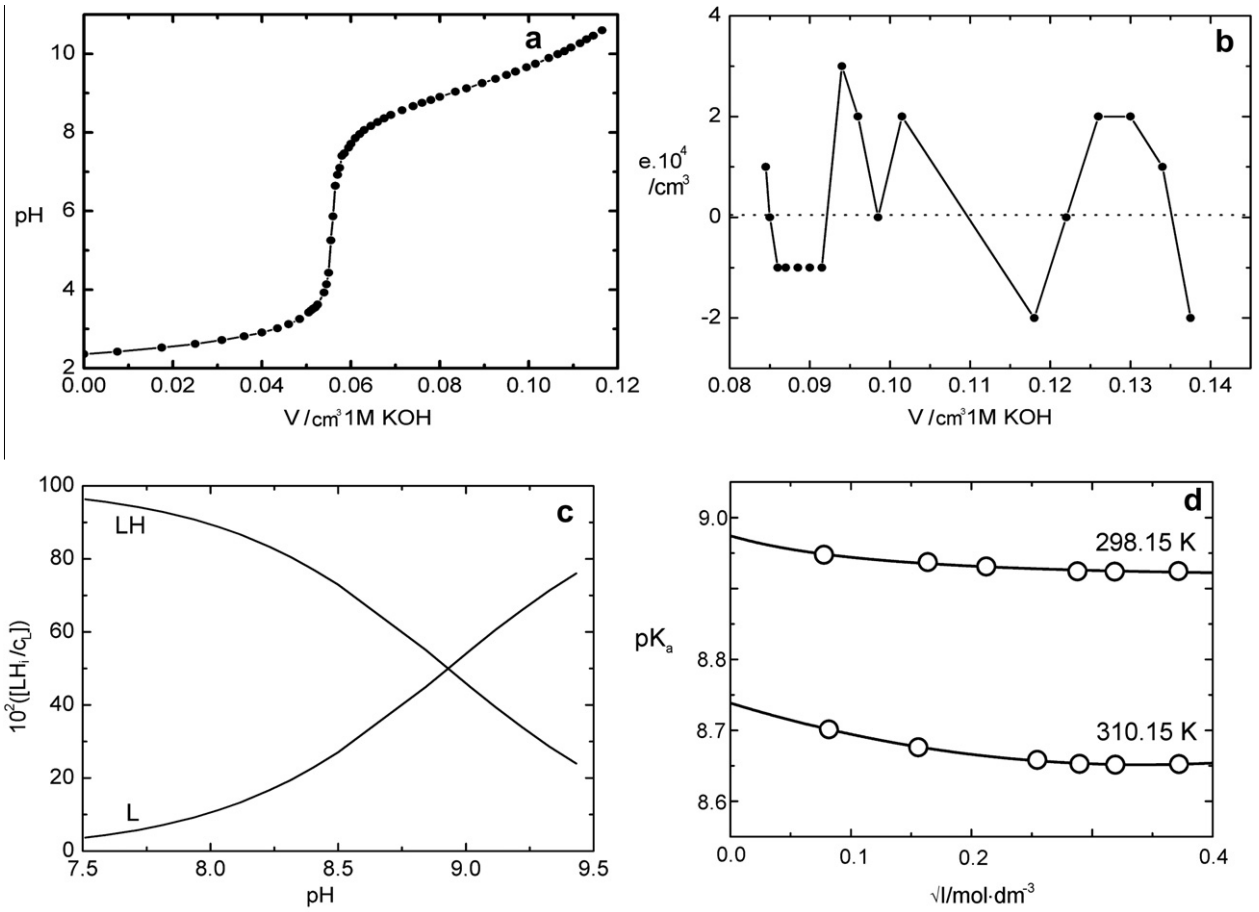

FIGURE 6. Protonation equilibria of capecitabine analyzed with ESAB (a) Plot of the potentiometric titration curve of capecitabine with KOH; $L_0 = 3.772 \cdot 10^{-3}$ mol · dm^{-3}, $H_T = 0.8961$ mol · dm^{-3}, $V_0 = 15.075$ cm^3, $I = 0.005$, $T = 298.15$ K; (b) scatter plot of residuals; (c) distribution diagram of relative presentation of variously pronated species of protonation equilibrium, (d) plot of the dependence of the mixed dissociation constant pK_a of capecitabine on the square root of an ionic strength, which leads to parameter estimates $pK_a^T = 8.97(1)$ at $T = 298.15$ K and $pK_a^T = 8.74(0)$ at $T = 310.15$ K.

The *second diagnostic* tests whether all of the calculated free concentrations of variously protonated species on the distribution diagram of the relative concentration expressed as a percentage have physical meaning, which proved to be the case (figure 3i).

The *next diagnostic* concerns the goodness-of-fit (figures 2b and 3g). The goodness-of-fit achieved is easily seen by examination of the differences between the experimental and calculated values of absorbance, $e_i = A_{exp,i,j} - A_{calc,i,j}$. Examination of the spectra and of the graph of the predicted absorbance response-surface through all the experimental points should reveal whether the results calculated are consistent and whether any gross experimental errors have been made in the measurement of the spectra. One of the most important statistics calculated is the standard deviation of absorbance, $s(A)$, calculated from a set of refined parameters at the termination of the minimization process. It is usually compared with the standard deviation of absorbance calculated by the INDICES program [24], $s_k(A)$, and if $s(A) \leqslant s_k(A)$, or $s(A) \leqslant s_{inst}(A)$, the instrumental error of the spectrophotometer used, the fit is considered to be statistically acceptable. This proves that the $s_2(A)$ value is equal to 0.00058 and is quite close to the standard deviation of absorbance when the minimization process terminates, $s(A) = 0.00061$.

The spectra deconvolution in figure 4 shows the deconvolution of the experimental spectrum of the individual variously protonated species to examine whether the experimental design is efficient. If, for a particular pH range, the spectrum consists of just a single component, further spectra for that range would be redundant, although they could improve the precision. In pH ranges where more components contribute significantly to the spectrum, several spectra should be measured. Such a spectrum provides sufficient information for a regression analysis which monitors at least two species in equilibrium where none of them is a minor species. The minor species has a relative concentration in a distribution diagram of less than 0.05 of the total concentration of the basic component c_L. When, on the other hand, only one species prevails in solution, the spectrum yields quite poor information for a regression analysis and the parameter estimate is rather unsure and definitely not reliable enough. Spectrum deconvolution seems to be quite useful tool in the proposal of an efficient experimentation strategy.

Applying a Debye–Hückel equation to the data in tables 1 and 2 according to the regression criterion, the unknown parameter pK_a^T has been estimated (figure 5). Table 6 brings point estimates of the thermodynamic dissociation constants of capecitabine studied at two temperatures. Because of the small range of ionic strength, the ion-size parameter $\mathring{a}$ and the salting-out coefficient C could not be estimated here.

4.2. Potentiometry

Capecitabine is soluble in water enough to use potentiometry as a second method to determine the pK_a value. For the adjusted value of an ionic strength, the potentiometric titration of a mixture of HCl and capecitabine with potassium hydroxide was carried out. The initial tentative value of the dissociation constant of the drug studied, corresponding to the midpoint value in each plateau of the potentiometric titration curve (figure 6a), was refined by the ESAB and/or the HYPERQUAD programs.

Table 3 shows the results of the ESAB regression analysis of a part of a particular titration curve when the minimization process terminates. Besides the original data $\{V, pa_{H+}\}$, residuals and the Bjerrum protonation function at each point are given.

TABLE 3
ESAB refinement of common and group parameters for a titration of capecitabine with KOH.

n	Volume/cm^3	Residual/cm^3	pa_H+	Protonation function
1	0.0845	0.0001	7.518	0.96
2	0.0850	0.0000	7.586	0.96
3	0.0860	−0.0001	7.702	0.94
4	0.0870	−0.0001	7.803	0.93
5	0.0885	−0.0001	7.923	0.91
6	0.0900	−0.0001	8.023	0.89
7	0.0915	−0.0001	8.111	0.87
8	0.0940	0.0003	8.246	0.83
9	0.0960	0.0002	8.321	0.80
10	0.0985	0.0000	8.401	0.77
11	0.1015	0.0003	8.506	0.73
12	0.1180	−0.0002	8.919	0.51
13	0.1220	0.0000	9.020	0.45
14	0.1260	0.0002	9.122	0.39
15	0.1300	0.0002	9.224	0.34
16	0.1340	0.0001	9.330	0.28
17	0.1375	-0.0002	9.424	0.24

Common parameters refined: $pK_{a1} = 8.97(7)$. *Group parameters refined:* $L_0 = 3.772 \cdot 10^{-3}$ mol · dm^{-3}, $H_T = -0.8961$ mol · dm^{-3}, *Constants:* $H_0 = 3.641 \cdot 10^{-3}$mol · dm^{-3}, $T = 298.15$ K, $pK_w = 13.9799$, $V_0 = 15.075$ cm^3, $s(V) = 0.0001$ cm^3, $j_a = 0.0$ mV, $j_b = 0.0$ mV, $I_0 = 0.0$ mol · dm^{-3} (in vessel), $I_T = 0.8961$ mol · dm^{-3} (in burette).

Both the common and the group parameters are refined and the best curve-fitting is proven by the results of a statistical analysis of the residuals. The strategy of an efficient computation in refinement of the group parameters was described in an earlier publication [30]. The reliability of the protonation constant may be determined according to the goodness-of-fit with increasing number of group parameters are refined a better fit is achieved and therefore a more reliable estimate of protonation constants results.

Figure 6 shows a graphical presentation of regression analysis results showing (a) the potentiometric titration curve of a mixture of HCl and capecitabine at $T = 298.15$ K; (b) the overall scatterplot of classical residuals gives an initial impression of residuals. The true model and the reliable parameter estimates are proven because the residuals exhibit a normal distribution with zero mean and also form a random pattern. No systematic departures from randomness indicate that the proposed model is true and the estimates of the parameter are reliable; (c) the distribution diagram of the relative abundance of variously protonated species seems to be more interesting than a numerical value of only protonation constant. The intersection of both curves gives a value of the protonation constant on the *pH*-axis, (d) dependence of the mixed dissociation constant pK_a of capecitabine on the square root of an ionic strength, which leads to parameter estimates $pK_{a1}^T = 8.97(1)$ at $T = 298.15$ K where in the brackets are estimated standard deviations in last valid digits.

Table 4 (Table 5) shows results for the protonation constant of capecitabine determined at various values of ionic strength and $T = (298.15, 310.15)$ K as a result of regression analysis with two various mathematical approaches. While the program ESAB minimizing residuals $e_i = (V_{exp,i} - V_{calc,i})$ reaches 0.0001 or 0.0002 cm^3, HYPERQUAD2008 minimizing $e_i = (pa_{H+,exp,i} - pa_{H+,calc,i})$ reaches SIGMA value about 1 or less, thus proving an excellent fit.

TABLE 4
Mixed dissociation pK_a constant of capecitabine acid at 298.15 K and various values of an ionic strength I/mol · dm^{-3} estimated by non-linear regression programs ESAB and HYPERQUAD.

I/(mol · dm^{-3})	ESAB		HYPERQUAD	
	pK_a	$\lvert \dot{e} \rvert \cdot 10^{-3}$/cm^3	pK_a	*SIGMA*
0.006	8.947(8)	0.1	8.947(1)	0.362
0.027	8.937(3)	0.1	8.937(1)	0.356
0.045	8.931(8)	0.1	8.931(2)	0.708
0.083	8.924(5)	0.1	8.924(1)	0.287
0.102	8.923(8)	0.1	8.923(2)	0.531
0.138	8.924(5)	0.1	8.924(5)	0.355

Standard deviation of parameter estimates in last valid digits are in brackets.

TABLE 5
Mixed dissociation pK_a constant of capecitabine acid at 310.15 K and various values of an ionic strength I/mol · dm^{-3} estimated by non-linear regression programs ESAB and HYPERQUAD.

I/(mol · dm^{-3})	ESAB		HYPERQUAD	
	pK_a	$\lvert \dot{e} \rvert \cdot 10^{-3}$/cm^3	pK_a	*SIGMA*
0.007	8.701(5)	0.1	8.701(1)	0.366
0.024	8.676(4)	0.1	8.676(1)	0.296
0.065	8.658(4)	0.0	8.658(1)	0.241
0.084	8.653(3)	0.1	8.653(1)	0.295
0.102	8.651(5)	0.1	8.651(1)	0.330
0.138	8.652(6)	0.1	8.652(2)	0.602

Standard deviation of parameter estimates in last valid digits are in brackets.

TABLE 6
Thermodynamic dissociation constants pK_a^T for capecitabine at $T = (298.15, 310.15)$ K.

Method	Thermodynamic dissociation constant pK_a^T		
	Estimated with	Value at 298.15 K	Value at 310.15 K
Spectrophotometry	SPECFIT	8.76(1)	8.62(1)
	SQUAD (84)	8.76(3)	8.62(4)
Potentiometry	ESAB	8.97(1)	8.74(0)
	HYPERQUAD	8.97(1)	8.74(0)

Standard deviation in the last valid digits are in brackets.

Applying the extended Debye–Hückel equation to data from Tables 4 and 5 according to the regression criterion U, the unknown parameter pK_a^T has been estimated (figure 6d). Table 6 shows the point estimates and calculated standard deviations when the minimization process terminates. Thermodynamic dissociation constant $pK_a^T = 8.97$ is estimated with very small standard deviation $s(pK_a^T) = 0.01$ indicating a quite reliable value estimation.

4.3. Enthalpy and entropy

Thermodynamic parameters $\Delta H^\circ/\mathrm{kJ}\cdot\mathrm{mol}^{-1}$ and $\Delta S^\circ/\mathrm{J}\cdot\mathrm{mol}^{-1}$ have been determined from the temperature variation of dissociation constants using Vaṅt Hoffs equation. The value of enthalpy $\Delta H^\circ = 33.9\ \mathrm{kJ}\cdot\mathrm{mol}^{-1}$ shows the dissociation process is endothermic. Positive values of $\Delta G^\circ = 51.2\ \mathrm{kJ}\cdot\mathrm{mol}^{-1}$ at $T = 298.15$ K and $\Delta G^\circ = 51.9\ \mathrm{kJ}\cdot\mathrm{mol}^{-1}$ at $T = 310.15$ K indicate that the dissociation process is not spontaneous, which was also confirmed by a negative value of entropy $\Delta S^\circ = -51.9\ \mathrm{J}\cdot\mathrm{K}^{-1}\cdot\mathrm{mol}^{-1}$.

5. Conclusions

Due to capecitabine's high solubility in water, both spectrophotometry and potentiometry titration to determine thermodynamic dissociation constant were used and the estimated values are in close agreement.

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