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Spectrophotometric study of time stability and acid-base characteristics of chelerythrine and dihydrochelerythrine

Research Article

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Abstract: Time stability, acid-base and UV-VIS spectral properties of dihydrochelerythrine (DHCHE) were studied spectrophotometrically in water:methanol and water:ethanol media. DHCHE is stable in strongly acid milieu (pH < 3) and at the higher amounts (60% v/v) alcohol. Acid-base characteristics and UV-VIS spectral properties of chelerythrine (CHE) were studied in aqueous solutions in the presence of different concentrations of HCl, HNO_3, H_2SO_4, H_3PO_4 and their mixtures. Remarkable shifts of formation parts of absorbance-pH (*A-pH*) curves to the alkaline medium were observed depending on the type and concentration of inert electrolyte (most remarkable for HNO_3 and HCl). The corresponding equilibrium constants pK_{R+} of the transition reaction between charged iminium Q^+ and uncharged QOH (pseudo-base, 6-hydroxy-dihydro derivative) forms of chelerythine were calculated using a numerical interpretation of *A-pH* curves by a SQUAD-G computer program which ranged from 8.51-9.31. The highest changes of ΔpK_{R+} (0.75 and 0.53) were observed for H_3PO_4 and H_2SO_4, respectively. The priority effect of ionic species and ionic strength was confirmed in the presence of additions of NaCl and KCl. The strength of interaction of CHE with biomacromolecular compounds (*i.e.*, peptides, proteins, nucleic acids *etc.*) may be affected because of the observed influence of both cation and anion of the inert electrolyte on acid-base behavior.

Keywords: *Chelerythrine • Dihydrochelerythrine • Time stability • UV-VIS spectral characteristics • Equilibrium constants*

1. Introduction

Quaternary benzo[c]phenanthridine alkaloids (QBAs) are present mainly in plants of *Fumariaceae*, *Papaveraceae*, *Ramunculaceae* and *Runaceae* families. Sanguinarine (SA) and chelerythrine (CHE) are the only commercially available members of QBAs, in addition to minor members (*i.e.*, sanguirubine, chelirubine, sanguilutine, chelilutine and macarpine). QBAs, a group of low molecular compounds with *N*-heteroatom, belong to the highly bioactive substances [1]. Some side effects were reported (toxic effect on some cellular level [2-5]) in addition to the beneficial pharmacological actions (antimicrobial, antiapoptotic, antiinflamatory *etc.* effects [6,7]). Standardized plant extracts have been exploited as anti-plaque components of dental hygiene preparations (mouth-wash, tooth paste *etc.*), as active components for myopathy preparations and in veterinary preparations.

Many very extraordinary properties were observed in the frame of detail studies of acidobasic and electrophoretic behavior of sanguinarine (SA) and chelerythrine (CHE) [8-11] and mainly their dihydroderivatives (dihydrochelerythrine, dihydrosanguinarine, etc., the former being recognized as the first metabolite of detoxification of sanguinarine in rats [2]) in the presence of inert electrolytes [3,4,10,11]. They exhibit very characteristic spectral features that can be used for their direct determination, for studies of their interactions with biomacromolecules using UV-VIS spectrophotometry, fluorimetry, mass spectrometry *etc.* [12-15]

The exact knowledge of physico-chemical characteristics is very important for correct interpretation of experiments concerning studies of interactions and behavior of the alkaloids at nearly physiological conditions. It is evident that the study of interactions [3-6] with biologically important macromolecules (*i.e.*, receptors, transport proteins, nucleic acids *etc.*), as

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well as the often mentioned formation of intercalation complexes with cyclodextrins and biopolymers, belongs to this class of experiments.

The main goals of our effort were (i) study of the acid-base behavior and time stability of CHE and DHCHE as the function of experimental conditions (pH, ionic strength, concentration of electrolyte and its composition, *etc.*), (ii) determination of UV-VIS spectral characteristics and their "true" pK_{R+} constants, (iii) identification of experimental conditions and requirements that qualify the possibility and correctness of interaction studies with these compounds in almost neutral and weakly basic solutions (close to physiological conditions).

2. Experimental Procedure

2.1. Materials and methods

Stock solution of chelerythrine (c = 130 μmol L^{-1}) was prepared by dissolution of chelerythrine chloride (Sigma Aldrich) in distilled water. Working solutions of CHE (c = 6.5 or 9.4 μmol L^{-1}) were prepared by dilution of the stock solution with cold freshly boiled distilled water. Dihydrochelerythrine (DHCHE, MP 189-191°C, 99% purity – checked by HPLC and controlled by calculation using molar absorptivity) was prepared from CHE by reduction with $NaBH_4$ in methanol [16]. Stock solutions of dihydrochelerythrine (c_m = 60 μg mL^{-1}) were prepared by dissolution of DHCHE in methanol or ethanol. Working solutions (c_m = 2.4 μg mL^{-1}) were prepared freshly by dilution of the stock solution with methanol, ethanol and/ or freshly boiled distilled water. All the solutions were stored in the refrigerator and were protected from light.

Stock solutions of electrolytes were prepared from HCl, HNO_3, H_2SO_4, NaCl, KCl, (all Penta, Chrudim, Czech Republic), H_3PO_4, ethanol and methanol (Lach-Ner, Neratovice, Czech Republic), all of p.a. purity. Tris-(hydroxymethyl)aminomethane (TRIS – ultra pure grade, Amresco®, Solon, Ohio, USA) and sodium hydroxide (reagent grade purity, Penta, Chrudim, Czech Republic) solutions were used for the pH adjustment in a pH interval pH = 2 – 11 with steps ΔpH = 0.2 – 0.5. All other experimental conditions were in accordance to previous paper [17].

All spectrophotometric measurements were performed using a UV-VIS Lambda 25 double beam spectrophotometer controlled by UV WinLab software (range 190 -1100 nm, slit 1 nm, fixed, quartz cuvette 10 mm, Perkin Elmer, Shelton, USA) or a Helios Beta UV-VIS single beam spectrophotometer (range 190 – 1100 nm, slit 1 nm, fixed, quartz cuvette 10 mm, Unicam, Cambridge, UK) controlled by a Vision software. The final pH of the solutions was controlled using a pH-meter model WTW pH 527 with a WTW SenTix 21 combined electrode. The electrode was regularly calibrated (several times per day, at least at the beginning and at the end of *A-pH* curve measurement) using a set of standard buffer solutions of pH = 4.01, 7.00 and 9.01 (all WTW GmbH, Wilheim, Germany).

The pK_{R+} constants were calculated from the absorbance values at six selected wavelengths between 268 and 405 nm (268, 272, 280, 316, 339 and 405 nm) using the SQUAD-G computer program [18]. The absorbance values of both alkaloids were measured three times at each pH and the mean values from these measurements were used to calculate of pK_{R+}. For more details see our previous paper [17].

3. Results and Discussion

3.1. UV-VIS spectral and acid-base behavior of dihydrochelerythrine (DHCHE)

Very complex absorption spectra of DHCHE were registered in the wavelength interval 220 – 380 nm in presence of 60% (volume per volume *v/v* percentage is

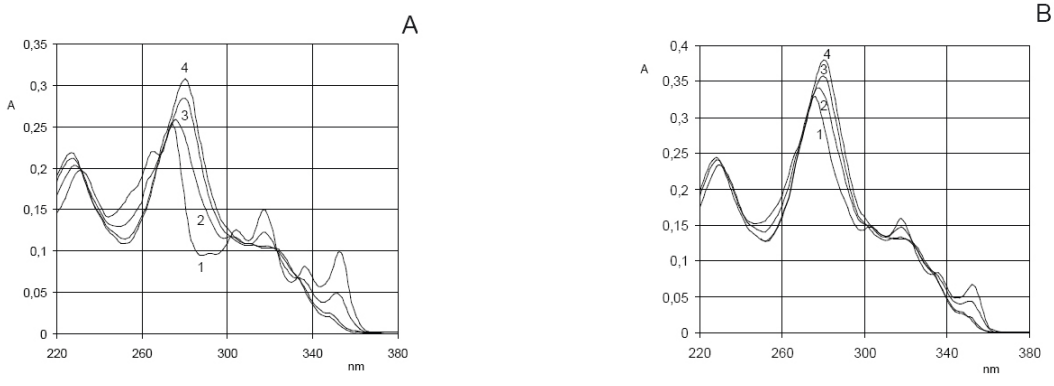

Figure 1. Absorption spectra of dihydrochelerythrine (c_{DHCHE} = 2.4 μg mL^{-1}, c_{HCl} = 0.01 mol L^{-1}, the pH was adjusted by NaOH) in 60% methanol (A) and in 60% ethanol (B): A: 1 – pH = 2,10; 2 – pH = 3,11; 3 – pH = 4,05; 4 – pH = 8,97; B: 1 – pH = 2,08; 2 – pH = 2,95; 3 – pH = 3,64; 4 – pH = 9,58.

used through the text if not stated differently) methanol or ethanol (Fig. 1) and 0.01 mol L^{-1} HCl in the pH range 2 – 10 with ΔpH = 0.3 – 0.5 steps (pH was adjusted by a stepwise addition of a NaOH solution). Distinct absorption maxima at 230, 274, 317 and 353 nm are present in acidic media pH < 3. Only two distinct absorption maxima appear at 227 and 280 nm in pH region 4 – 10. The absorption maximum at 230 nm is shifted to shorter wavelengths (λ_{max} = 227 nm) and the absorption maximum at 274 nm is shifted to longer wavelengths (λ_{max} = 280 nm) when pH was increased (pH > 3). Presence of isosbestic points at 232, 268, and 333 nm confirmed reversible acid-basic equilibrium between the charged iminium Q^+ and uncharged QOH forms of DHCHE at pH 4 - 10.

3.1.1. Influence of alcohol content on time stability

Time stability of DHCHE solutions was registered in 4, 10, 30 and 60% methanol or ethanol at pH = 2 (0.01 mol L^{-1} HCl), in the presence of formiate (pH = 2.8), acetate (pH = 4.2) and phosphate buffers (pH = 6.7) at four selected wavelengths 274, 278, 317 and 353 nm. The mean values (three replicates) of absorbance rapidly decreased in 4% aqueous methanol to about 38, 14 and 12% after 30, 60 and 120 min, respectively, in all cases. The same trend (but much slower) was observed for higher concentrations of methanol and ethanol (10 – 30%). The solutions in 60% methanol and 60% ethanol were mostly stable in the time interval (≤ 120 min) and insignificant increase of absorbance at 317 and 353 nm was observed (approx. up to 104 % of their initial values) in methanolic solutions. The graphs for time stability of DHCHE in 4, 10, 30 and 60% methanol (A) and methanol (B) at λ = 274 nm are depicted in Fig. 2. Higher time stability of DHCHE in ethanol is evident from the Fig. 2B.

3.1.2. Influence of acidity on time stability.

The solutions of DHCHE are relatively stable only in strongly acidic milieu at all contents of methanol or ethanol. At pH 2 in the presence of 0.01 mol L^{-1} HCl absorbance decreased between 95-92% of their initial values after 30 min in 4% methanol and ethanol, respectively. In the presence of formiate, acetate and phosphate buffers (pH 2.8, 4.2 and 6.7, respectively) the decreases were more evident (see Fig. 3 for 4% water/methanol – A - and water/ethanol mixtures - B) due to lower stability at higher pH values and probably due to presence of components of buffers.

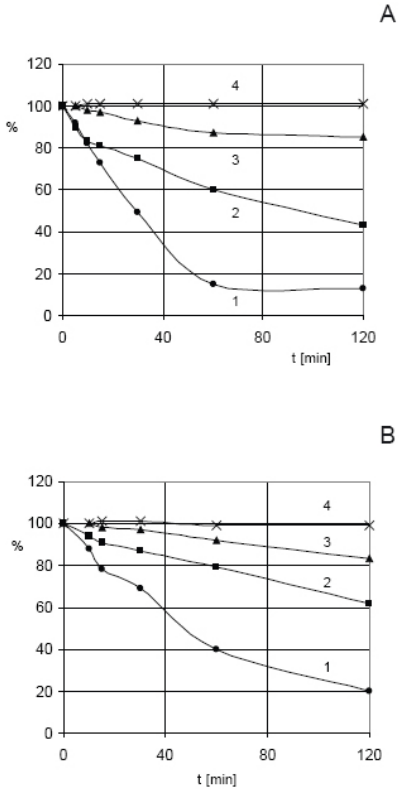

Figure 2. Time stability of solutions containing 2.4 μg mL^{-1} DHCHE in aqueous methanol (A) and ethanol (B) media and measured at 274 nm. Alcohol content (in % v/v): 1 – 4%; 2 – 10%; 3 – 30%; 4 – 60%

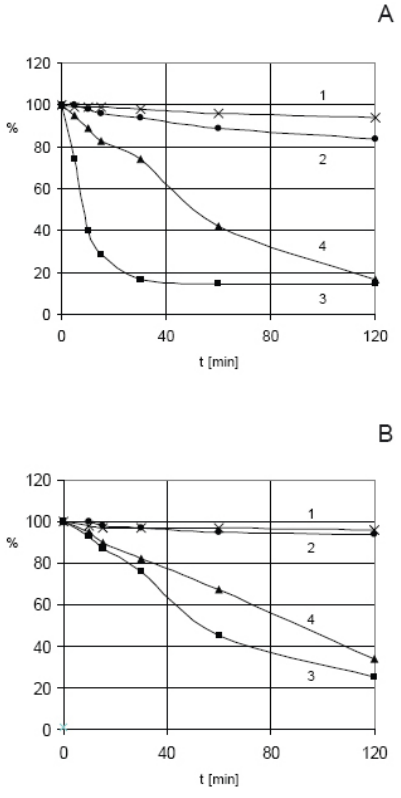

Figure 3. Time stability of DHCHE solutions containing 2.4 μg mL^{-1} DHCHE in 4% aqueous methanol (A) and in 4% aqueous ethanol (B) measured at 274 nm. Curve 1 – 0.01 mol L^{-1} HCl (pH = 2); 2 – formiate buffer (pH = 2.8), 3 – acetate buffer (pH = 4.2); 4 – phosphate buffer (pH = 6.7)

From the results it is clearly evident that the solutions of DHCHE are stable only in more then 60% methanol/ethanol and with a decreasing content of alcohols the stability decreases. Time stability is also seriously increased by acidity. The highest time stability was observed in strongly acidic media (0.01 mol L^{-1} HCl for methanol and ethanol and in formiate buffer of pH 2.8 for ethanol). In slightly acidic, neutral and alkaline media the solutions are unstable and stability decreases with decreasing content of alcohols and increasing pH.

3.2. UV-VIS-spectral and acid-base properties of chelerythrine

3.2.1. Absorption spectra

Absorption spectra of CHE (c = 6.5 µmol L^{-1}) were registered between 220 – 430 nm and pH 2.5 - 11 in the presence of 10 mmol L^{-1} concentrations of HCl, HNO_3, H_2SO_4 and H_3PO_4. Two distinct absorption maxima at 268 and 316 nm and less promoted maxima at 339 and 405 nm are present in the pH interval pH = 2.5 – 8. Absorption maxima shifted to longer wavelengths in alkaline media. The two distinguished absorption bands at 280 nm and a less distinguished one at 316 nm characterize the spectrum of the alkaline solutions (pH 8 – 11). Absorption maxima at 339 and 405 nm disappear with increasing pH (see Fig. 4). The reversible transition reaction between a charged iminium Q^+ and an uncharged QOH (pseudo-base, 6-hydroxy-dihydroderivative) forms of chelerythrine is confirmed by the presence of three sharp isosbestic points at 239, 275 nm and 302 nm. The molar absorption coefficients ε_1 a ε_2 for both acidobasic forms of CHE in 10 and 100 mmol L^{-1} HCl (calculated using SQUAD-G) are given in Table 1.

3.2.2. Absorbance-pH curves

Absorbance-pH curves (*A-pH* curves) were measured at constant concentration of chelerythrine c = 6.5 µmol L^{-1} and 10-100 mmol L^{-1} HCl (see Fig. 5), HNO_3, H_2SO_4 and H_3PO_4 (not graphically presented). The data were collected in pH interval pH = 3 – 11 with steps ΔpH = 0.3 – 0.5 for six wavelengths (268, 272, 280, 316, 339 and

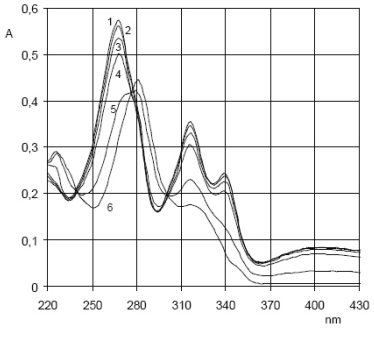

Figure 4. Absorption spectra of chelerythrine (c_{CHE} = 6.5 µmol L^{-1}, c_{HCl} = 0.01 mol L^{-1}, pH adjusted by NaOH). Curve - pH: 1 – 3.16; 2 – 5.16; 3 – 7.07; 4 – 8.03; 5 – 9.05; 6 – 10.03

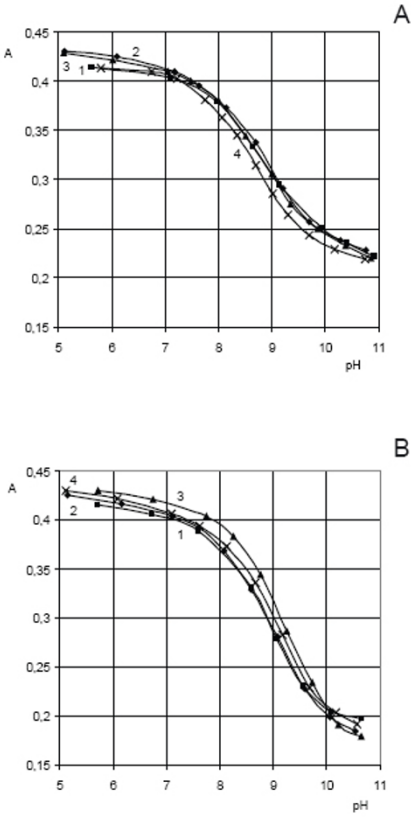

Figure 5. Absorbance-pH curves of chelerythrine (c_{CHE} = 6.5 µmol L^{-1}) in the presence of mineral acids at c = 0.01 mol L^{-1} measured at λ = 268 nm, neutralization with NaOH (a) or TRIS (b). Curve – acid: 1 – HCl; 2 – HNO_3; 3 – H_2SO_4; 4 – H_3PO_4

Table 1. Values of molar absorptivity coefficients ε_1 and ε_2 and their average standard deviation over the whole data set for the charged iminium Q^+ (ε_1) and the uncharged QOH (pseudo-base, 6-hydroxy-dihydroderivative, ε_2) forms of chelerythrine calculated using a numerical interpretation of the A-pH curves by a SQUAD-G program. (c_{CHE} = 6.5 µmol L^{-1}, c_{HCl} = 0.01 or 0.1 mol L^{-1}, pH adjusted by NaOH).

Medium	ε_1(268 nm) [L mol^{-1} cm^{-1}]	ε_2(268 nm) [L mol^{-1} cm^{-1}]	ε_1(316 nm) [L mol^{-1} cm^{-1}]	ε_2(316 nm) [L mol^{-1} cm^{-1}]
0.01 mol L^{-1} HCl	59 660 ± 220	33 520 ± 370	37 060 ± 120	16 790 ± 260
0.1 mol L^{-1} HCl	59 970 ± 200	34 350 ± 270	38 230 ± 150	17 990 ± 200

Table 2. Values of pK_{R+} (and their average standard deviation over the whole data set) of chelerythrine in dependence on the type and concentration of inorganic acids calculated using a numerical interpretation of the A-pH curves by a SQUAD-G program. (c_{CHE} = 6.5 μmol L^{-1}, c_{HA} = 0.01 or 0.1 mol L^{-1} mineral acid, pH adjusted by NaOH).

Medium	pK_{R+} [a]	s(A)[b]	U[c]	Medium	pK_{R+} [a]	s(A)[b]	U[c]
	0.01 mol L^{-1}				0.1 mol L^{-1}		
HCl	8.80 ± 0.072	0.0100	0.0030	HCl	9.30 ± 0.037	0.0039	0.0005
HNO_3	8.81 ± 0.046	0.0180	0.0183	HNO_3	9.26 ± 0.017	0.0023	0.0002
H_2SO_4	8.77 ± 0.038	0.0158	0.0177	H_2SO_4	9.31 ± 0.021	0.0033	0.0004
H_3PO_4	8.51 ± 0.053	0.0240	0.0450	H_3PO_4	9.29 ± 0.031	0.0046	0.0011

[a] (pK_{R+} = 8.9 – 9.0 [6], pK_{R+} = 9.0 [9]; pK_{R+} = 8.77 ± 0.028 and 9.14 ± 0.040 were obtained for 0.01 and 0.1 mol L^{-1} acetic acid (this work); [b] the average standard deviation of absorbance (s(A)) over the whole data set; [c] the sum of squares of absorbance residuals $U = \Sigma (A_{exp,i} - A_{calc,i})^2$ for i = 1 – n, where n is the total number of absorbance data for all solutions and wavelengths used

405 nm) corresponding to the absorption maxima of both acidobasic forms. The formation parts of the *A-pH* curves (and of course the corresponding pK_{R+} values) were shifted to the more alkaline medium with increasing initial concentration of acids (ionic strength) and depended on the character of the corresponding anion of the acid. The corresponding pK_{R+} values calculated using a numerical interpretation of the *A-pH* curves by the SQUAD-G program (see Table 2) varied from 8.51 – 9.31. The most serious shift of the pK_{R+} was found in the presence of H_3PO_4 (ΔpK_{R+} = 0.75) and H_2SO_4 (ΔpK_{R+} = 0.53), while the less distinct shift was observed for HCl (ΔpK_{R+} = 0.50), HNO_3 (ΔpK_{R+} = 0.46) and the smallest one for CH_3COOH (ΔpK_{R+} = 0.37). From the results it can be concluded that the corresponding pK_{R+} values are influenced by the character (type) of anions and also by total mass concentration of anions in the order $PO_4^{3-} > SO_4^{2-} > Cl^- > NO_3^- > CH_3COO^-$.

To verify the influence of cationic species on pK_{R+} values, the pH of the CHE solutions in HCl, HNO_3, H_2SO_4 and H_3PO_4 at the concentrations *c* = 10 mmol L^{-1} was adjusted by stepwise addition of NaOH or TRIS solutions in the interval 2 - 10. The corresponding pK_{R+} values (see Table 3 and Fig. 5) were again shifted to the more alkaline medium with increasing concentration of acids and they were significantly higher for TRIS (mainly in the presence of H_2SO_4 and H_3PO_4) compared to the values obtained for the *A-pH* curves in presence of NaOH. The lowest differences ΔpK_{R+} between values for *A-pH* curves neutralized with TRIS or NaOH were observed in the presence of HCl and, on the other hand, the differences were comparable in the presence of other acids.

Table 3. Values of pK_{R+} of chelerythrine in dependence on the type and concentration of inorganic acids (c_{CHE} = 6.5 μmol L^{-1}, c_{HA} = 0.01 mol L^{-1} mineral acid, pH adjusted by NaOH or TRIS).

medium	pK_{R+} TRIS	pK_{R+} NaOH
HCl	8.85 ± 0.064	8.80 ± 0.072
HNO_3	8.89 ± 0.063	8.81 ± 0.046
H_2SO_4	9.33 ± 0.067	8.77 ± 0.038
H_3PO_4	9.27 ± 0.068	8.51 ± 0.053

To confirm the influence of the type of anions on corresponding pK_{R+} values, the *A-pH* curves were measured in the mixtures of acids at constant total concentration 100 mmol L^{-1} and the corresponding pK_{R+} values were again calculated using a numerical interpretation of the *A-pH* curves by the SQUAD-G program (see Table 4). Mixtures of HCl + HNO_3, HCl + H_2SO_4, HCl + H_3PO_4, HNO_3 + H_2SO_4, HNO_3 + H_3PO_4 and H_2SO_4 + H_3PO_4 for mass concentration ratios 0.07 mol L^{-1} + 0.03 mol L^{-1}, 0.05 mol L^{-1} + 0.05 mol L^{-1} and 0.03 mol L^{-1} + 0.07 mol L^{-1}, respectively, were tested. The corresponding pK_{R+} values confirm that the priority effect of dominant anion of mineral acid is highly questionable since the pK_{R+} chelerythrine are practically the same.

3.3. Influence of ionic strength and character of the electrolyte

The changes in acid-base behavior of CHE depending on ionic strength and character of inert electrolyte were evaluated in the presence of NaCl and KCl at *I* = 0.01, 0.10 and 1.0 and at the constant concentration of HCl, HNO_3, H_2SO_4, H_3PO_4 (*c* = 0.01 mol L^{-1}). The corresponding pK_{R+} values are presented in Table 5 and in the graphical form in Fig. 6. The formation parts of *A-pH* curves and also the corresponding pK_{R+} values were shifted to the alkaline region with increasing ionic strength in the range ΔpK_{R+} = 0.17 – 0.61. The highest effect was observed in the presence of H_2SO_4 while the lowest in the presence of HCl. A notable influence was observed in the presence of KCl (ΔpK_{R+} = 0.69 – 1.01) with the extreme effects in the presence of H_3PO_4 (highest) and HNO_3 (lowest), respectively.

Table 4. Values of pK_{R+} of chelerythrine in dependence on the type and concentration of inorganic acids mixtures calculated using a numerical interpretation of the A-pH curves by a SQUAD-G program (c_{CHE} = 6.5 μmol L^{-1}, c_{HA} = 0.1 mol L^{-1} mineral acid, pH adjusted by NaOH).

Medium	pK_{R+}	s(A)[a]	U[b]
0.03 mol L^{-1} HCl + 0.07 mol L^{-1} HNO_3	9.02 ± 0.028	0.0100	0.0076
0.05 mol L^{-1} HCl + 0.05 mol L^{-1} HNO_3	9.03 ± 0.019	0.0081	0.0051
0.07 mol L^{-1} HCl + 0.03 mol L^{-1} HNO_3	8.99 ± 0.021	0.0081	0.0051
0.03 mol L^{-1} HCl + 0.07 mol L^{-1} H_2SO_4	8.99 ± 0.031	0.0122	0.0115
0.05 mol L^{-1} HCl + 0.05 mol L^{-1} H_2SO_4	8.99 ± 0.030	0.0130	0.0134
0.07 mol L^{-1} HCl + 0.03 mol L^{-1} H_2SO_4	8.90 ± 0.033	0.0148	0.0180
0.03 mol L^{-1} HCl + 0.07 mol L^{-1} H_3PO_4	8.85 ± 0.041	0.0190	0.0290
0.05 mol L^{-1} HCl + 0.05 mol L^{-1} H_3PO_4	8.91 ± 0.034	0.0120	0.0190
0.07 mol L^{-1} HCl + 0.03 mol L^{-1} H_3PO_4	8.97 ± 0.032	0.0149	0.0172
0.03 mol L^{-1} HNO_3 + 0.07 mol L^{-1} H_2SO_4	8.84 ± 0.027	0.0063	0.0031
0.05 mol L^{-1} HNO_3 + 0.05 mol L^{-1} H_2SO_4	8.96 ± 0.032	0.0070	0.0034
0.07 mol L^{-1} HNO_3 + 0.03 mol L^{-1} H_2SO_4	9.11 ± 0.037	0.0078	0.0047
0.03 mol L^{-1} HNO_3 + 0.07 mol L^{-1} H_3PO_4	8.91 ± 0.031	0.0158	0.0193
0.05 mol L^{-1} HNO_3 + 0.05 mol L^{-1} H_3PO_4	8.99 ± 0.021	0.0102	0.0080
0.07 mol L^{-1} HNO_3 + 0.03 mol L^{-1} H_3PO_4	9.06 ± 0.014	0.0072	0.0040
0.03 mol L^{-1} H_2SO_4 + 0.07 mol L^{-1} H_3PO_4	8.87 ± 0.028	0.0060	0.0025
0.05 mol L^{-1} H_2SO_4 + 0.05 mol L^{-1} H_3PO_4	8.89 ± 0.023	0.0053	0.0022
0.07 mol L^{-1} H_2SO_4 + 0.03 mol L^{-1} H_3PO_4	8.91 ± 0.024	0.0058	0.0026

[a] the average standard deviation of absorbance (s(A)) over the whole data set; [b] the sum of squares of absorbance residuals $U = \Sigma (A_{exp,i} - A_{calc,i})^2$ for i = 1 – n, where n is the total number of absorbance data for all solutions and wavelengths used

Table 5. Values of pK_{R+} of chelerythrine in dependence on the type and concentration of inert electrolyte (ionic strength - as NaCl or KCl additions) calculated using a numerical interpretation of the A-pH curves by a SQUAD-G program (c_{CHE} = 6.5 μmol L^{-1}, c_{HA} = 0.01 mol L^{-1} mineral acid, pH adjusted by NaOH).

Medium	pK_{R+}			
	HCl	HNO_3	H_2SO_4	H_3PO_4
no addition of Cl^-	8.80 ± 0.072	8.81 ± 0.046	8.77 ± 0.038	8.51 ± 0.053
0.01 mol L^{-1} NaCl	8.84 ± 0.021	8.82 ± 0.039	8.83 ± 0.035	8.80 ± 0.035
0.10 mol L^{-1} NaCl	8.97 ± 0.026	9.11 ± 0.028	9.04 ± 0.013	8.97 ± 0.021
1.00 mol L^{-1} NaCl	9.13 ± 0.031	9.33 ± 0.018	9.38 ± 0.014	9.00 ± 0.055
0.01 mol L^{-1} KCl	8.81 ± 0.088	8.88 ± 0.034	8.89 ± 0.038	8.65 ± 0.043
0.10 mol L^{-1} KCl	9.08 ± 0.017	9.15 ± 0.024	9.21 ± 0.019	9.09 ± 0.020
1.00 mol L^{-1} KCl	9.53 ± 0.055	9.48 ± 0.024	9.49 ± 0.022	9.52 ± 0.012

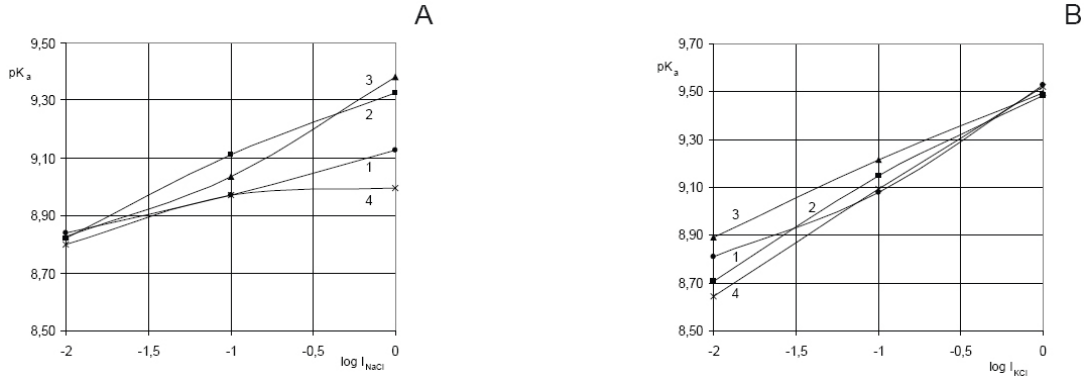

Figure 6. Dependence of pK_{R+} values of chelerythrine on the character of inert electrolyte and ionic strength (NaCl – A, KCl – B), in the presence of 10 mM HCl (curve 1), HNO_3 (curve 2), H_2SO_4 (curve 3) and H_3PO_4 (curve 4)

4. Conclusions

Changes in acid-base characteristics of chelerythrine were studied by measuring the absorbance-pH curves at constant concentration of chelerythrine with varying character (type) and concentration of strong inorganic acids (HCl, HNO_3, H_2SO_4, H_3PO_4). The formation parts of the absorbance-pH curves shifted to a more alkaline region depending on the character and initial concentration of acids and this resulted in a corresponding increase in equilibrium constants pK_{R+}.The most remarkable shifts were observed in the presence of phosphoric acid (H_3PO_4). The abovementioned results indicate that it is practically impossible to identify the priority effect of anions since the pK_{R+} values of chelerythrine (for concentration of individual acids 0.1 mol L^{-1}) differed very little from each other.

The character and total concentration (ionic strength) of inert electrolytes (NaCl and KCl) also significantly influence the pK_{R+} values of chelerythrine. The pK_{R+} values increased with increasing ionic strength of solutions (NaCl and KCl additions I = 0.01 – 1.0) in HCl, HNO_3, H_2SO_4 and H_3PO_4.

DHCHE solutions in 4% methanol and ethanol are time stable only at pH = 2 (0.01 mol L^{-1} HCl). However in the presence of formiate (pH = 2.8), acetate (pH = 4.2) and phosphate buffers (pH = 6.7) their absorbance rapidly decreases from 92% – 17% in 4% methanol and 95-25% in 4% ethanol after 120 minutes (see Fig. 3). DHCHE solutions are stable at relatively high contents of methanol or ethanol (60%, not presented graphically).

The results confirmed that the experimental conditions (pH, ionic strength, inert electrolyte concentration, type and concentration of buffers and organic solvents, *etc.*) seriously influence the acid-base and UV-VIS spectral properties of chelerythrine and mainly its dihydro-derivative. None of the investigated components in solutions (anions, cations *etc.*) is indifferent to the alkaloids, thus, exact evaluation of the side effects as well as the determination of pH-dependencies of spectral and/or acidobasic properties and time stability of the alkaloids in different media is highly important before starting any interaction studies with biomacromolecules. Application of different types and concentrations of buffers, electrolytes and components of solution, acid-base properties and interactions could be very carefully studied.

This study points out that the behavior of chelerythrine and especially its dihydro-derivative in aqueous solutions is more complex than be expected [7,8,10,11].

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