Thermodynamic parameters for the complexation of water-soluble betulin derivatives with (2-hydroxypropyl)-β-cyclodextrin determined by affinity capillary electrophoresis

Viktoria V. Sursyakova[a,*], Vladimir A. Levdansky[a], Anatoly I. Rubaylo [a,b,c]

[a]Institute of Chemistry and Chemical Technology SB RAS, Federal Research Center "Krasnoyarsk Science Center SB RAS", Akademgorodok 50/24, Krasnoyarsk 660036, Russia

[b]Siberian Federal University, Svobodny pr. 79, Krasnoyarsk 660041, Russia

[c]Federal Research Center "Krasnoyarsk Science Center SB RAS", Akademgorodok 50, Krasnoyarsk 660036, Russia

*Corresponding author.

E-mail address: viktoria_vs@list.ru (V.V. Sursyakova)

Abbreviations: ACE, affinity capillary electrophoresis; ASB, betulin 3-acetate-28-sulfate; BGE, background electrolyte; CD, cyclodextrin; CE, capillary electrophoresis; DMSO, dimethyl sulfoxide; DSB, betulin 3,28-disulfate; EOF, electroosmotic flow; HP-β-CD, (2-hydroxypropyl)-β-cyclodextrin; HP-γ-CD, (2-hydroxypropyl)-γ-cyclodextrin;

Abstract

The interaction between (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) and water-soluble betulin derivatives, betulin 3,28-disulfate (DSB) and betulin 3-acetate-28-sulfate (ASB), belonging to the class of pentacyclic lupane triterpenoids, was studied using affinity capillary electrophoresis. It was found that 1:1 and 1:2 complexes were formed. The stability constants of the complexes in the temperature range of 293.15-318.15 K were determined. The values obtained are sufficiently large; log K (1:1) and log K (1:2) are 4.25-5.02 and 6.08-7.59, respectively. This phenomenon can be explained by the presence of broad hydrophobic regions in the molecules of the compound studied. The stability constants decrease with increasing temperature. The stability constants for ASB complexes are slightly higher as compared to the constants for DSB complexes. The thermodynamic parameters for the complexation were calculated from the van't Hoff plots. The complexation was found to be controlled by the enthalpy change. The obtained values of stability constants at 298 K were compared with values for the β-CD complexes of the compounds under study and for the HP-β-CD an d β-CD complexes of water-insoluble betulin derivatives. It was found that water-soluble betulin derivatives form more stable complexes with CDs as compared to water-insoluble derivatives (betulonic and betulinic acids), and the HP-β-CD complexes are more stable than the β-CD complexes.

Keywords:

Binding constants; Cyclodextrins; Drug delivery; Enthalpy; Gibbs energy; Inclusion complexes

1. Introduction

In recent years, betulin (Fig. 1a) and its derivatives, belonging to the class of pentacyclic lupane triterpenoids, have attracted attention due to a number of useful properties such as antitumor, antibacterial, anti-HIV activities [1], and wide abundance in nature. One of the main sources of betulin is birch bark, the betulin content in the external part of which is 10-35 %. The bioavailability and pharmacological activity of betulin and its derivatives can be increased by obtaining inclusion complexes of the compounds with cyclodextrins. Cyclodextrins (CDs) are natural cyclic molecules formed from residues of α-1,4-bonded D-glucopyranose. The CD molecule is a truncated cone with a hydrophobic cavity, due to which CDs can form inclusion complexes or host-guest complexes with various compounds [2, 3]. The thermodynamic parameters for the complexation of CDs with betulin derivatives have not been studied enough. Recently, the interaction of water-soluble betulin derivatives with β-CD [4] and the interaction of betulinic and betulonic acids (water-insoluble) with β-CD [4] and (2-hydroxypropyl)-β and γ-

cyclodextrins [5] have been studied. The stability constants for the complexes studied have been determined at 298 K.

To determine stability constants and thermodynamic parameters, a number of techniques are used [6, 7], including UV-Vis [8-10] and fluorescence [8, 9, 11] spectroscopy, conductometry [12, 13], isothermal calorimetry [14-17], phase solubility study with UV-Vis spectroscopy [14, 18, 19] or high-performance liquid chromatography [20], potentiometry [21], capillary electrophoresis [22-39], and other techniques. Capillary electrophoresis (CE) has advantages such as rapidity, high selectivity, a small value of samples, and low-cost of analysis. Kinetically labile complexes give one peak in electropherograms, the effective electrophoretic mobility of which, μ_{eff}, is the average weighted over the mole fractions of species [27]:

$$\mu_{eff} = \sum_i^N \mu_i \alpha_i \quad (1)$$

where μ_i is the ionic mobility of i^{th} species (for anions $\mu_i < 0$); α_i is the mole fraction of i^{th} species, which depends on the ligand concentration in background electrolyte (BGE). In affinity capillary electrophoresis (ACE, sometimes named as mobility shift assay) [22, 28-34] to study complexation, several electropherograms of the compound studied are obtained with varying concentration of ligand in BGE. Based on the values of electrophoretic mobilities and ligand concentration in BGE, the stability constants, also called binding, formation, or association constants, are calculated. Sometimes the compound is added to BGEs and the ligand is injected as a sample. ACE and related electromigration techniques are used to determine the thermodynamic parameters of the complexation on the basis of temperature dependencies of stability constants [36-39].

The aim of this study was to determine thermodynamic parameters of the complexation of water-soluble betulin derivatives with (2-hydroxypropyl)-β-cyclodextrins using affinity capillary electrophoresis.

2. Experimental

2.1. Instrumentation

The study was carried out using capillary electrophoresis systems with a diode-array detector Agilent ^{3D}CE G1600A and Agilent 7100 (Agilent Technologies, Waldbronn, Germany) of the Krasnoyarsk Regional Center of Research Equipment, Federal Research Center "Krasnoyarsk Science Center SB RAS". Untreated fused silica capillaries with 50 μm id and the total/effective lengths of 80.5/72 cm were used (Agilent Technologies). The capillary temperature was kept constant at T ± 0.04 K; T was 293.15, 298.15, 303.15, 310.15, and 318.15 K. The data acquisition and processing were performed with the computer programs ChemStation Rev.A.10.02 and OpenLab CDS ChemStation Edition C.01.08. The separation was achieved by applying a voltage of + 30 kV. The positive voltage was applied to the capillary

inlet. The direct detection was made at 200 nm with the bandwidth of 6-10 nm. The samples were injected hydrodynamically for 10 sec at a pressure of 50 mbar. All experiments were repeated 3 times.

A new capillary was first flushed with 1 M NaOH for 10 min, then with ultra pure water for 10 min. At the beginning of each day, the capillary was first flushed with 0.1 M NaOH for 5 min, twice with ultra pure water for 10 min and with running BGE for 15 min. Between the runs the capillary was flushed with BGE for 5 min.

All pH measurements were made using a calibrated precise pH instrument «Expert-001-1» (Econix-Expert, Moscow, Russia) with a precision of 0.005 pH units.

2.2. Chemicals

The used reagents were analytical grade purity. (2-Hydroxypropyl)-β-cyclodextrin with an extent of labeling equal to 1 molar substitution (average molecular weight 1540) was purchased in Sigma-Aldrich (Moscow, Russia). HP-β-CD was dissolved in BGEs. The solution of 0.002 % dimethyl sulfoxide (DMSO) dissolved in samples or BGEs was used as an electroosmotic flow (EOF) marker. Deionized water with electrical conductivity less than 0.1 $\mu S \cdot cm^{-1}$ from a water purification system Direct-Q3 (Millipore, France) was used for the solution preparation. BGEs were filtered through 0.45 mkm filters.

The water-soluble betulin derivatives (sodium salts of betulin 3,28-disulfate and betulin 3-acetate-28-sulfate) were synthesized in Institute of Chemistry and Chemical Technology SB RAS, Federal Research Center “Krasnoyarsk Science Center SB RAS” as described in article [4]. Stock solutions of the compounds with a concentration of 1 g/L were prepared by dissolution of accurate weights in deionized water. Samples (12 mg/L) were prepared by dilution of the stock solutions with BGEs before electrophoretic separation.

2.3. Separation conditions and calculations

The sequence of steps for electrophoretic separation in a thermostated capillary segment was as follows [40]. A sample containing a neutral marker (N_1) and the anionic compound studied (A) was injected into a capillary filled with BGE. The injected sample was transferred to the thermostated segment of the capillary by applying a pressure of 50 mbar for time t_{tr}. The voltage was applied to electrophoretically separate N_1 and A for time t_{migr}, but such that they did not reach the detector window. A neutral marker band (N_2) was injected for time t_{inj}. Finally, a pressure of 50 mbar was applied to transfer the separated bands and marker N_2 past the detector window.

For the capillary electrophoresis systems used, about 11.5 cm at the detector side and 8.5 cm at the capillary inlet were poorly thermostated due to the design of cassette and alignment interface for detection. For the calculation the time t_{tr} needed to transfer the sample from the inlet

to the thermostated capillary segment by applying a pressure of 50 mbar, the following equation was used:

$$t_{tr} = l_{tr}/v_p \quad (2)$$

where l_{tr} is the distance on which the sample is shifted from the capillary inlet. In this study, it was assumed that l_{tr} = 9 cm. υ_p is the velocity of electrolyte flow generated by applying a pressure of 50 mbar. The approximate value of υ_p for the calculation of t_{tr} was experimentally estimated separately for each temperature using Eq. (3):

$$v_p = l_{eff}/t_{EOF,\ P} \quad (3)$$

where l_{eff} is the effective capillary length (a distance from capillary inlet to detector), $t_{EOF,\ P}$ is the migration time of EOF marker at 0 kV and 50 mbar.

The time of applying a voltage, t_{migr}, was estimated using Eq. (4):

$$t_{migr} = \frac{ll_{term}}{U\mu_{EOF}} \quad (4)$$

where l is the total length of capillary, l_{term} is the migration distance of a neutral EOF marker (because it is registered first) when electrophoretic separation is carried out in the thermostated capillary segment, U is the voltage, μ_{EOF} is the electroosmotic mobility. In this study, for the capillary with a total length of 80.5 cm, it was assumed that l_{term} = 58 cm, U = +30 kV. The approximate value of μ_{EOF} for the calculation of t_{migr} was experimentally estimated separately for each temperature using Eq. (5):

$$\mu_{EOF} = \frac{ll_{eff}}{Ut_{EOF}} \quad (5)$$

where t_{EOF} is the migration time of EOF marker at 30 kV and 0 mbar.

The effective electrophoretic mobility from experimental data obtained with electrophoretic separation in the thermostated segment of the capillary was calculated as follows [40]:

$$\mu_{eff} = \frac{l \cdot l_A}{U(t_{migr} - t_{ramp\ -up}/2 - t_{ramp\ -down}/2)} \quad (6)$$

$$l_A = (t_A - t_{N_1})v_m \quad (7)$$

$$v_m = \frac{l_{eff}}{t_{N_2} + t_{inj}/2 - t_d} \quad (8)$$

where l_A is the distance between the peaks of anion A and neutral marker N_1, t_{migr} is the time during which the voltage is applied, $t_{ramp\text{-}up}$ and $t_{ramp\text{-}down}$ are the times during which the voltage changes linearly from zero to the desired value or from the desired value to zero, respectively, t_A is the recorded mobilization time for the anion, t_{N_1} is the recorded mobilization time for the neutral marker from the first injection, v_m is the final pressure mobilization velocity, t_{N_2} is the recorded mobilization time for the neutral marker from the second injection, t_{inj} is the time of

injection of EOF marker N_2, t_d is the time delay between the beginning of the final mobilization step and the start of the data acquisition process.

The effective electrophoretic mobility from experimental data obtained by the conventional way (when voltage is applied just after sample injection) was calculated using the following equation:

$$\mu_{eff} = \frac{l \cdot l_{eff}}{U}\left(\frac{1}{t} - \frac{1}{t_{EOF}}\right) \quad (9)$$

where t is the migration time measured at the top of peak.

The factor allowing the corrections for viscosity change, υ, for each BGE was calculated by eq. (10) [4]:

$$\upsilon = t'_{EOF,\ P}/t^0_{EOF,\ P} \quad (10)$$

where $t'_{EOF,\ P}$ and $t^0_{EOF,\ P}$ are the times for DMSO peaks obtained at voltage of 0 kV and hydrodynamic pressure of 50 mbar in the BGEs with the HP-β-CD addition and without it, respectively.

For a case when the studied compound D forms 1:1 and 1:2 complexes with CD, the system is described using following equations [27, 35]:

$$D + CD \rightleftarrows D/CD, K_{11} = \frac{[D/CD]}{[D][CD]} \quad (11)$$

$$D + 2CD \rightleftarrows D/CD_2, K_{12} = \frac{[D/CD_2]}{[D][CD]^2} \quad (12)$$

where K_{11} and K_{12} are the stability constants of D/CD and D/CD_2, respectively. Thus, Eq. (1), taking into account the viscosity correcting factor and mole fractions derived from Eqs. (11) and (12), is transformed into Eq. (13):

$$v_i \cdot \mu_{eff,\ i} = \frac{\mu_D + \mu_{11}K_{11}[CD] + \mu_{12}K_{12}[CD]^2}{1 + K_{11}[CD] + K_{12}[CD]^2} \quad (13)$$

where μ_D, μ_{11} and μ_{12} are the ionic mobilities of D, D/CD, and D/CD_2, respectively. The values of μ_D were determined experimentally using BGE without the HP-β-CD addition. The stability constants and ionic mobilities μ_{11} and μ_{12} were determined from the nonlinear regression fitting of the program MS Excel by minimizing the differences in viscosity corrected experimental and theoretical electrophoretic mobilities (Eqs. (6), (10), (13)) because it has been shown that the nonlinear fitting is more accurate and precise than linearized equations [41].

The enthalpy and entropy changes were calculated from the van't Hoff plot equation, whereas $K \approx K^0$ assuming the equality of activity coefficients of the studied compounds and their complexes (because HP-β-CD is neutral) [13, 21]:

$$\ln K^0 = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (14)$$

$$\Delta H^0 = -b * R, \Delta S^0 = a * R \quad (15)$$

where ΔH^0 and ΔS^0 is the enthalpy and entropy changes, respectively, related to the formation of an inclusion complex, T is the temperature, R is the gas constant, b and a are the slope and intercept of the dependence of ln K on T^{-1}, respectively. The Gibbs free energy at 298.15 K, ΔG^0_{298}, was calculated as follows:

$$\Delta G^0_{298} = -RT \ln K_{298} \quad (16)$$

3. Results and discussion

3.1 Choice of background electrolyte and separation conditions

At first, BGE was chosen such that the compounds studied recorded with the largest sensitivity. The studied sodium salts of betulin derivatives dissociate in solutions, giving sodium ions and anions of DSB and ASB (Fig. 1). In previous study, we used phosphate BGE (20 mM phosphoric acid with the addition of 1 M NaOH up to pH 2.5) to separate these anions [4]. However, for the water-insoluble betulin derivatives it was found that borate BGE showed the largest sensitivity [5]. The electropherograms of water-soluble betulin derivatives (DSB and ASB) were recorded using phosphate and borate BGEs (10 mM borax, pH 9.18). It was found that the signal-to-noise ratio was higher for borate BGE. Concentration of BGE in the range of 2-20 mM did not affect the signal-to-noise ratio. Thereby, 10 mM borax solution was used as BGE in subsequent experiments.

In CE, capillary is known to heat up when voltage is applied. Therefore, capillary is usually thermostated. However, some segments of the capillary are poorly thermostated. To accurately determine the effective electrophoretic mobilities, Williams B.A. et al. suggested carrying out electrophoretic separation in a thermostated capillary segment [40]. The sample shift in this segment after sample injection and the shift of separated zones after switching-off voltage towards to detector are carried out by applying pressure. In our study, a comparison for 293.15 and 318.15 K was done between the values of effective electrophoretic mobilities of DSB obtained by the conventional way (Eq. 9) and those obtained when electrophoretic separation was carried out in the thermostated capillary segment (Eq. 6). For 293.15 K there was no difference in the values obtained: 25.5 (± 0.2) and 25.6 (± 0.1)·10^{-9} m^2 V^{-1} s^{-1}, respectively. However, for 318.15 K, the value obtained by the conventional way was lower than that obtained with the thermostated capillary segment: 39.4 (± 0.2) and 40.0 (± 0.1)·10^{-9} m^2 V^{-1} s^{-1}. Thereby, the subsequent measurements of mobility were carried out using the thermostated capillary segment.

It may seem that the separation in the thermostated capillary segment takes a long time (about 30 min) as compared with the usual separation (10 min). But this is not the case, because the evaluation of the factors allowing the corrections for viscosity change for each BGE (Eq. 10) is needed. This evaluation takes about 30 min per one parallel only and requires several parallels.

For separation in the thermostated capillary segment, the evaluation is made from the same runs as for the calculation of mobility. As a result, the time consumption for both cases is comparable.

The separation was carried out in a long capillary (80.5 cm of total length) because even for this capillary with an effective length of 72 cm, the thermostated capillary segment is about 57-58 cm. But when the compounds studied and DMSO are separated, this distance is the migration of the DMSO zone, while DSB passes about 24 cm. For a shorter capillary, this distance will be smaller, and the error of electrophoretic mobility measurements will be greater [42].

3.2 Complexation of betulin derivatives with HP-β-CD

Electropherograms of water-soluble betulin derivatives were recorded at different temperatures and using BGEs with different HP-β-CD content. Fig. 2 and 3 show examples of the obtained electropherograms and dependencies of the viscosity corrected electrophoretic mobility of DSB and ASB on the HP-β-CD concentration in BGEs, respectively. As can be seen from Fig. 3, these dependencies for all the values of temperature under study do not have a plateau after sharp decay, mobility decreases with increasing HP-β-CD concentration. For a fixed temperature, the decrease in mobility in the range of 2-10 mmol·kg^{-1} HP-β-CD equals to 3.4 - 10 % with respect to the adjacent value. This is significantly higher than the error of the effective mobilities measurements (0.2-0.7 %) and indicates that besides the 1:1 interaction between betulin derivatives and HP-β-CD, 1:2 complexes are formed [36].

The stability constants and ionic mobilities of the complexes calculated form Eq. (13), as well as the values of μ_D determined experimentally using BGE without the HP-β-CD addition, are shown in Table 1 and 2. As can be seen from Fig. 3, the theoretical dependencies, based on Eq. (13), the calculated values of stability constants and ionic mobilities, are in good agreement with the experimental points. The stability constants decrease with increasing temperature (Table 1). This is typical behavior for the CD complexes [7]. It should be noted that the values obtained concern with the complexation of ionic forms of betulin derivatives with HP-β-CD. The stability constants for ASB complexes are slightly higher as compared with the constants for DSB complexes. This is logical because DSB is a doubly charged ion in solution, while ASB is a singly charged ion (Fig. 1), and an increase in the analyte charge usually leads to a decrease of the CD complex stability [43]. Figure 4 shows fraction diagrams for the HP-β-CD complexes of DSB and ASB as a function of HP-β-CD concentration.

Since HP-β-CD is a neutral molecule, and the charge of the complexes is equal to the charge of the anions studied, assuming the activity coefficients of the studied anions and their complexes are equal, the obtained values of stability constants can be equated to the thermodynamic stability constants, and the thermodynamic parameters of the complexation can

be calculated. Fig. 5 shows van't Hoff plots of ln K versus T^{-1} (Eq. 14). The thermodynamic parameters of the complexation are given in Table 3. As seen from Table 3, the calculated values of enthalpy changes and Gibbs free energies for the 1:1 complexes have close values; the complexation is controlled by the enthalpy change. For the 1:2 complexes, the complexation is also controlled by the change in enthalpy because the entropy change is negative.

Table 4 shows the values of stability constants for the β-CD complexes of the compounds under study at 298 K, along with the obtained values for the HP-β-CD complexes, as well as stability constants for such complexes of water-insoluble betulin derivatives (betulonic and betulinic acids). As can be seen from Table 4, the HP-β-CD complexes are more stable than the β-CD complexes. The obtained values of stability constants for the 1:1 complexes are sufficiently large. Generally, the logarithms of stability constants for the CD complexes are in the range from 1 to 4 [7, 44]. This phenomenon is possibly caused by the presence of broad hydrophobic regions in the molecules of the compounds studied and the additional stabilization of the HP-β-CD inclusion complexes due to the hydrophobic interactions of the compounds with the alkyl chains of 2-hydroxypropyl groups. The water-soluble betulin derivatives form more stable complexes with CDs compared to the water-insoluble derivatives (betulonic and betulinic acids). For the β-CD complexes, this can be explained by the additional stabilization of the complexes due to the formation of hydrogen bonds between sulfonate and acetate groups of betulin derivatives and hydroxyl groups of CD. In HP-β-CD, the hydroxypropyl groups possibly create the steric hindrances for the complex formation with water-insoluble betulin derivatives because the complexes of the compounds with β-CD are formed. The fact that the equilibrium between betulin derivatives and HP-γ-CD was reached after 3 days of agitation, irrespective of HP-γ-CD concentration, while for β-CD, the equilibrium was reached after 2 hour, is an argument in favor of the steric hindrances [4, 5].

4. Conclusions

In this paper, using affinity capillary electrophoresis, the complexation between (2-hydroxypropyl)-β-cyclodextrin and water-soluble betulin derivatives (betulin 3,28-disulfate betulin 3-acetate-28-sulfate) was studied. It was found that borate background electrolyte (10 mM borax, pH 9.18) allows recording the compounds studied with the largest sensitivity. It was shown that electrophoretic separation should be carried out using the thermostated capillary segment in order to obtain accurate values of electrophoretic mobility. Besides 1:1 interaction between betulin derivatives and HP-β-CD, the 1:2 complexes were found to form. Based on the calculated values of electrophoretic mobilities and ligand concentration in BGE, the stability constants in the temperature range of 293-318 K and thermodynamic parameters of complexation were determined. The complexation was found to be controlled by the enthalpy

change. The obtained values of stability constants at 298 K were compared with values for the β-CD complexes of the compounds under study and for the HP-β-CD and β-CD complexes of water-insoluble betulin derivatives. It was found that water-soluble betulin derivatives form more stable complexes with CDs as compared to water-insoluble derivatives (betulonic and betulinic acids). The HP-β-CD complexes are more stable than the β-CD complexes.

Conflicts of interest

Authors declare there are no competing financial conflicts.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] T.G. Tolstikova, I.V. Sorokina, G.A. Tolstikov, A.G. Tolstikov, O.B. Flekhter, Biological activity and pharmacological prospects of lupane terpenoids: I. Natural lupane derivatives, Rus. J. Bioorg. Chem. 32 (2006) 37-49. https://doi.org/10.1134/S1068162006010031.

[2] G. Crini, Review: A history of cyclodextrins, Chem. Rev. 114 (2014) 10940-10975. https://doi.org/10.1021/cr500081p.

[3] S. Jacob, A.B. Nair, Cyclodextrin complexes: Perspective from drug delivery and formulation, Drug Dev. Res. 79 (2018) 201-217. https://doi.org/10.1002/ddr.21452.

[4] O.V. Popova, V.V. Sursyakova, G.V. Burmakina, V.A. Levdansky, A.I. Rubaylo, Determination of stability constants of inclusion complexes of betulin derivatives with β-cyclodextrin by capillary electrophoresis, Dokl. Chem. 461 (2015) 67-69. https://doi.org/10.1134/S0012500815030039.

[5] V.V. Sursyakova, N.G. Maksimov, V.A. Levdansky, A.I. Rubaylo, Combination of phase-solubility method and capillary zone electrophoresis to determine binding constants of cyclodextrins with practically water-insoluble compounds, J. Pharm. Biomed. Anal. 160 (2018) 12-18. https://doi.org/10.1016/j.jpba.2018.07.038.

[6] P. Mura, Analytical techniques for characterization of cyclodextrin complexes in aqueous solution: A review, J. Pharm. Biomed. Anal. 101 (2014) 238-250. https://doi.org/10.1016/j.jpba.2014.02.022.

[7] M.V. Rekharsky, Y. Inoue, Complexation thermodynamics of cyclodextrins, Chem. Rev. 98 (1998) 1875-1917. https://doi.org/10.1021/cr970015o.

[8] M. Murugan, A. Anitha, K. Sivakumar, R. Rajamohan, Supramolecular interaction of primaquine with native β-cyclodextrin, J. Solution Chem. 47 (2018) 906-929. https://doi.org/10.1007/s10953-018-0768-2.

[9] R. Rajamohan, S.K. Nayaki, M. Swaminathan, Investigation on association behavior between 1-aminoisoquinoline and β-cyclodextrin in solution and solid state, J. Mol. Liq. 220 (2016) 918–925. https://doi.org/10.1016/j.molliq.2016.04.118.

[10] W. Misiuk, Investigation of inclusion complex of HP-γ-cyclodextrin with ceftazidime, J. Mol. Liq. 224 (2016) 387-392. https://doi.org/10.1016/j.molliq.2016.10.009.

[11] M.S. Mokhtar, F.E.O. Suliman, A.A. Elbashir, Experimental and molecular modeling investigations of inclusion complexes of imazapyr with 2-hydroxypropyl(β/γ) cyclodextrin, J. Mol. Liq. 262 (2018) 504-513. https://doi.org/10.1016/j.molliq.2018.04.088.

[12] B.K. Barman, S. Barman, M.N. Roy, Inclusion complexation between tetrabutylphosphonium methanesulfonate as guest and α- and β-cyclodextrin as hosts investigated by physicochemical methodology, J. Mol. Liq. 264 (2018) 80-87. https://doi.org/10.1016/j.molliq.2018.04.148.

[13] E.A. Gomaa, R.M. Abu-Qarn, Ionic association and thermodynamic parameters for solvation of vanadyl sulfate in ethanol-water mixtures at different temperatures, J. Mol. Liq. 232 (2017) 319-324. https://doi.org/10.1016/j.molliq.2017.02.085.

[14] F. D'Aria, C. Serri, M. Niccoli, L. Mayol, V. Quagliariello, R.V. Iaffaioli, M. Biondi, C. Giancola, Host–guest inclusion complex of quercetin and hydroxypropyl-β-cyclodextrin. A calorimetric study, J. Therm. Anal. Calorim. 130 (2017) 451-456. https://doi.org/10.1007/s10973-017-6135-5.

[15] C.-L. Zhang, J.-C. Liu, W.-B. Yang, D.-L. Chen, Z.-G. Jiao, Experimental and molecular docking investigations on the inclusion mechanism of the complex of phloridzin and hydroxypropyl-β-cyclodextrin, Food Chem. 215 (2017) 124-128. https://doi.org/10.1016/j.foodchem.2016.07.155.

[16] F.H. Xavier-Júnior, C.T. Tavares, M.M. Rabello, M.Z. Hernandes, B.P. Bezerra, A.P. Ayala, O.D.L. Pessoa, R.M. Ximenes, N.S. Santos-Magalhães, Elucidation of the mechanism of complexation between oncocalyxone A and cyclodextrins by isothermal titration calorimetry and molecular modeling, J. Mol. Liq. 274 (2019) 165-172. https://doi.org/10.1016/j.molliq.2018.10.129.

[17] A. Stepniak, A. Buczkowski, L. Zavodnik, S. Belica-Pacha, B. Palecz, Study of the interaction of β-cyclodextrin with albendazole in aqueous solutions, J. Mol. Liq. 248 (2017) 19-23. https://doi.org/10.1016/j.molliq.2017.09.100.

[18] I.L. Nikolic, I.M. Savic, M.M. Popsavin, S.J. Rakic, T.M. Mihajilov-Krstev, I.S. Ristic, S.P. Eric, I.M. Savić-Gajic, Preparation, characterization and antimicrobial activity of inclusion complex of biochanin A with (2-hydroxypropyl)-β-cyclodextrin, J. Pharm. Pharmacol. 70 (2018) 1485-1493. https://doi.org/10.1111/jphp.13003.

[19] F. Fateminasab, A.K. Bordbar, S. Shityakov, S. Gholami, Diadzein complexation with unmodified cyclodextrins: A detailed experimental and theoretical study, J. Mol. Liq. 271 (2018) 80-95. https://doi.org/10.1016/j.molliq.2018.08.124.

[20] X. Ding, M. Zheng, J. Lu, X. Zhu, Preparation and evaluation of binary and ternary inclusion complexes of fenofibrate/hydroxypropyl-β-cyclodextrin, J. Inclusion Phenom. Macrocyclic Chem. 91 (2018) 17-24. https://doi.org/10.1007/s10847-018-0793-1.

[21] K.D. Bhesaniya, S. Baluja, Potentiometric determination of dissociation constant and thermodynamic parameters of dissociation process of some newly synthesized pyrimidine derivatives in MeOH/DMF–water medium at different temperatures, J. Mol. Liq. 190 (2014) 190-195. https://doi.org/0.1016/j.molliq.2013.11.015.

[22] C. Jiang, D.W. Armstrong, Use of CE for the determination of binding constants, Electrophoresis 31 (2010) 17-27. https://doi.org/10.1002/elps.200900528.

[23] M. Olabi, M. Stein, H. Wätzig, Affinity capillary electrophoresis for studying interactions in life sciences, Methods 146 (2018) 76-92. https://doi.org/10.1016/j.ymeth.2018.05.006.

[24] C. Qian, S. Wang, H. Fu, R.F.B. Turner, H. Li, D.D.Y. Chen, Pressure-assisted capillary electrophoresis frontal analysis for faster binding constant determination, Electrophoresis 39 (2018) 1786-1793. https://doi.org/10.1002/elps.201800049.

[25] S. Krait, A. Salgado, B. Chankvetadze, F. Gago, G.K.E. Scriba, Investigation of the complexation between cyclodextrins and medetomidine enantiomers by capillary electrophoresis, NMR spectroscopy and molecular modeling, J. Chromatogr. A 1567 (2018) 198-210. https://doi.org/10.1016/j.chroma.2018.06.010.

[26] S. Štěpánová, E. Procházková, L. Čechová, J. Žurek, Z. Janeba, M. Dračínský, V. Kašička, Separation of rotamers of 5-nitrosopyrimidines and estimation of binding constants of their complexes with β-cyclodextrin by capillary electrophoresis, J. Chromatogr. A 1570 (2018) 164-171. https://doi.org/10.1016/j.chroma.2018.07.083.

[27] V.V. Sursyakova, A.I. Rubaylo, Stability constants of adducts of succinate copper(II) complexes with β-cyclodextrin determined by capillary electrophoresis, Electrophoresis 39 (2018) 1079-1085. https://doi.org/10.1002/elps.201700378.

[28] P. Dubský, M. Dvořák, M. Ansorge, Affinity capillary electrophoresis: the theory of electromigration, Anal. Bioanal. Chem. 408 (2016) 8623-8641. https://doi.org/10.1007/s00216-016-9799-y.

[29] S.E. Deeb, H. Wätzig, D.A. El-Hady, Capillary electrophoresis to investigate biopharmaceuticals and pharmaceutically-relevant binding properties, Trends Anal. Chem. 48 (2013) 112-131. https://doi.org//10.1016/j.trac.2013.04.005.

[30] Z. Chen, S.G. Weber, Determination of binding constants by affinity capillary electrophoresis, electrospray ionization mass spectrometry and phase-distribution methods, Trends Anal. Chem. 27 (2008) 738-748. https://doi.org/10.1016/j.trac.2008.06.008.

[31] K.L. Rundlett, D.W. Armstrong, Methods for the determination of binding constants by capillary electrophoresis, Electrophoresis 22 (2001) 1419-1427. https://doi.org/10.1002/1522-2683(200105)22:7<1419::AID-ELPS1419>3.0.CO;2-V.

[32] X. He, Y. Ding, D. Li, B. Lin, Recent advances in the study of biomolecular interactions by capillary electrophoresis, Electrophoresis 25 (2004) 697-711. https://doi.org/10.1002/elps.200305727.

[33] Y. Tanaka, S. Terabe, Estimation of binding constants by capillary electrophoresis, J. Chromatogr. B 768 (2002) 81-92. https://doi.org/10.1016/S0378-4347(01)00488-1.

[34] N.H.H. Heegaard, R.T. Kennedy, Identification, quantitation, and characterization of biomolecules by capillary electrophoretic analysis of binding interactions, Electrophoresis 20 (1999) 3122-3133. https://doi.org/10.1002/(SICI)1522-2683(19991001)20:15/16<3122::AID-ELPS3122>3.0.CO;2-M.

[35] V.V. Sursyakova, G.V. Burmakina, A.I. Rubaylo, Composition and stability constants of copper(II) complexes with succinic acid determined by capillary electrophoresis, J. Coord. Chem. 70 (2017) 431-440. https://doi.org/10.1080/00958972.2016.1270450.

[36] S.-H. Wu, W.-H. Ding, Application of cyclodextrin-mediated capillary electrophoresis to determine the apparent binding constants and thermodynamic parameters of the alkylnaphthalene derivatives, Electrophoresis 26 (2005) 3528-3537. https://doi.org/10.1002/elps.200500354.

[37] N. Pang, Z. Zhang, Y. Bai, H. Liu, A study of the interaction between enantiomers of zolmitriptan and hydroxypropyl-beta-cyclodextrin by capillary electrophoresis, Anal. Bioanal. Chem. 393 (2009) 313-320. https://doi.org/10.1007/s00216-008-2446-5.

[38] J.-Y. Hsiao, S.-H. Wu, W.-H. Ding, Application of cyclodextrin-mediated capillary electrophoresis to simultaneously determine the apparent binding constants and thermodynamic parameters of naphthalenesulfonate derivatives, Talanta 68 (2006) 1252-1258. https://doi.org/10.1016/j.talanta.2005.07.029.

[39] Q.-F. Zhang, H.-Y. Cheung, X. Shangguan, G. Zheng, Structure selective complexation of cyclodextrins with five polyphenols investigated by capillary electrokinetic chromatography, J. Sep. Sci. 35 (2012) 3347-3353. https://doi.org/10.1002/jssc.201200528.

[40] B.A. Williams, G. Vigh, Fast, accurate mobility determination method for capillary electrophoresis, Anal. Chem. 68 (1996) 1174-1180. https://doi.org/0003-2700/96/0368.

427 [41] M.T. Bowser, D.D.Y. Chen, Monte Carlo simulation of error propagation in the
428 determination of binding constants from rectangular hyperbolae. 2. Effect of the maximum-
429 res.ponse range, J. Phys. Chem. A 103 (1999) 197-202. https://doi.org/10.1021/jp982917e.
430 [42] V.V. Sursyakova, A.I. Rubaylo, New peak broadening parameter for the characterization of
431 separation capability in capillary electrophoresis, J. Sep. Sci. 38 (2015) 690-696.
432 https://doi.org/10.1002/jssc.201401109.
433 [43] E. Siimer, M. Kurvits, A. Köstner, Thermochemical investigation of β-cyclodextrin
434 complexes with benzoic acid and sodium benzoate, Thermochim. Acta 116 (1987) 249-256.
435 https://doi.org/10.1016/0040-6031(87)88185-6.
436 [44] K.A. Connors, The stability of cyclodextrin complexes in solution, Chem. Rev. 97 (1997)
437 1325-1357. https://doi.org/10.1021/cr960371r.

Figure

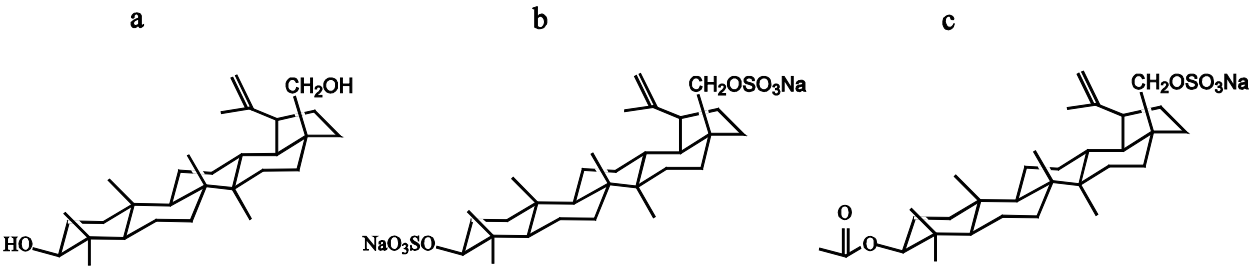

Fig. 1. Structural formulas of betulin (a), sodium salts of betulin 3,28-disulfate (b) and betulin 3-acetate-28-sulfate (c).

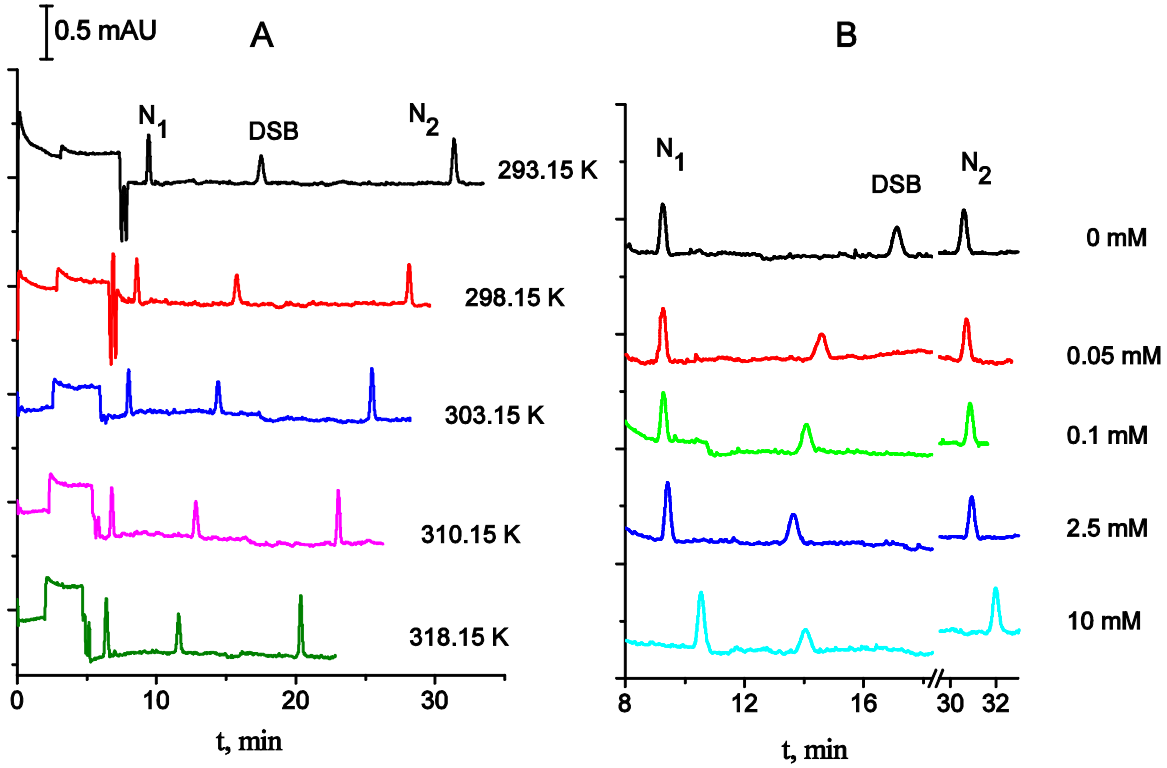

Fig. 2. Examples of electropherograms of DSB recorded at 293.15-318.15 K using BGE without the HP-β-CD addition (A) and at 293.15 K using BGEs with different HP-β-CD concentration (B). N_1 and N_2 are DMSO from 1st (together with DSB) and 2nd sample injections, respectively (see Section 2.3).

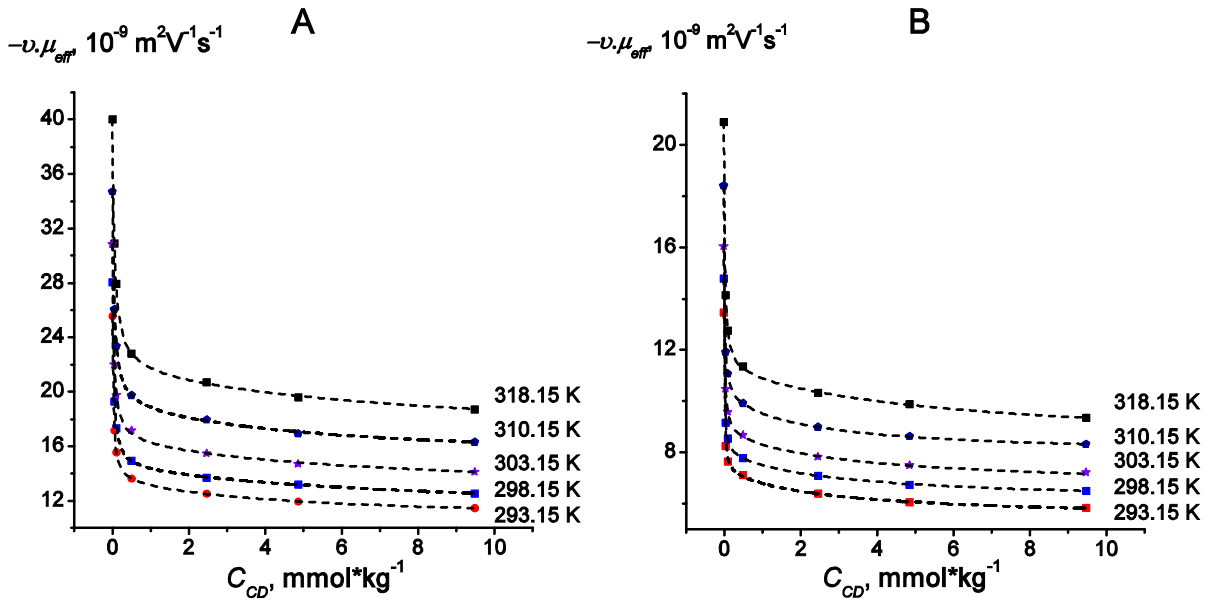

Fig. 3. Experimental points and theoretical curves of the viscosity corrected electrophoretic mobility of DSB (A) and ASB (B) on HP-β-CD concentration for the capillary temperatures of 293.15-318.15 K.

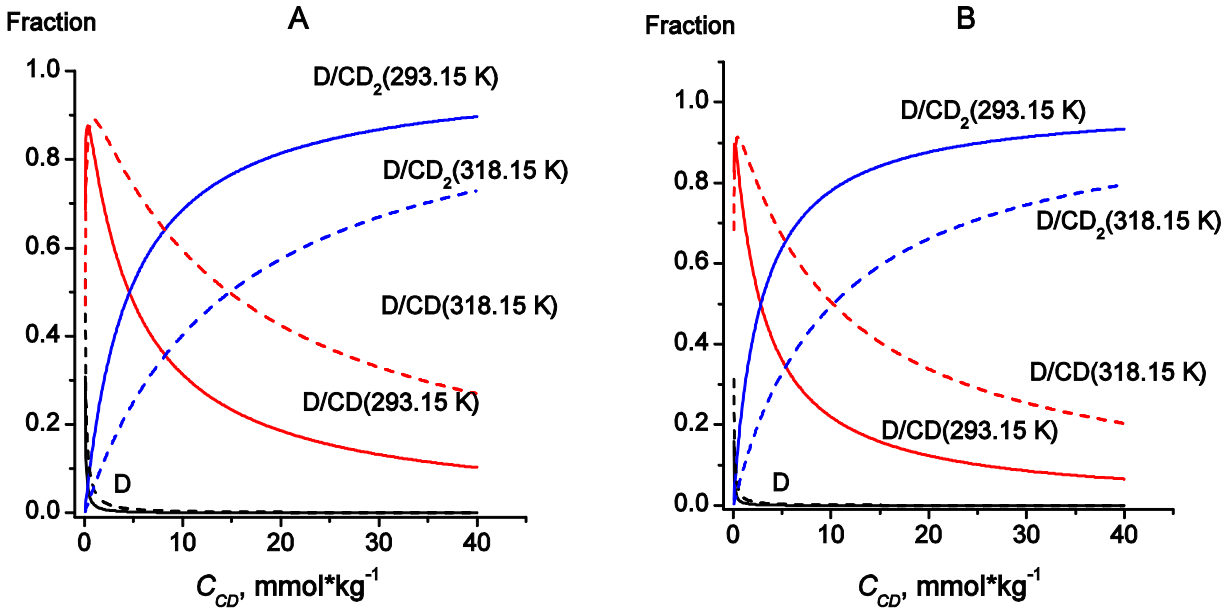

Fig. 4. Fraction diagrams as a function of HP-β-CD concentration for the HP-β-CD complexes of DSB (A) and ASB (B) at 293.15 and 318.15 K (solid and dash lines, respectively).

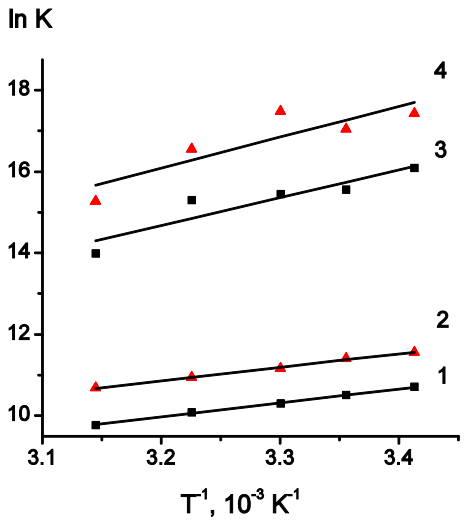

Fig. 5. van't Hoff plots of ln *K* versus T^{-1} for the formation of the HP-β-CD complexes of betulin derivatives. Lines 1 and 3 are ln *K* for the 1:1 and 1:2 HP-β-CD complexes of DSB, respectively; lines 2 and 4 are ln *K* for the 1:1 and 1:2 HP-β-CD complexes of ASB, respectively.

Tables

Table 1

Logarithm of stability constants for the complexes between betulin derivatives and HP-β-CD (1 molar substitution) at different temperatures (with 95 % confidence interval).

Compound	*T*, K				
	293.15	298.15	303.15	310.15	318.15
	log K_{11}				
DSB	4.65 (±0.02)	4.56 (±0.01)	4.47 (±0.02)	4.38 (±0.02)	4.25 (±0.01)
ASB	5.02 (±0.07)	4.96 (±0.04)	4.85 (±0.06)	4.75 (±0.04)	4.64 (±0.02)
	log K_{12}				
DSB	6.99 (±0.09)	6.75 (±0.23)	6.71 (±0.36)	6.64 (±0.31)	6.08 (±0.31)
ASB	7.57 (±0.15)	7.40 (±0.15)	7.59 (±0.18)	7.19 (±0.22)	6.63 (±0.10)

Table 2

Ionic mobilities (10^{-9} m^2 V^{-1} s^{-1}) of betulin derivatives and their 1:1 and 1:2 HP-β-CD complexes at different temperatures (with 95 % confidence interval).

Mobility	*T*, K				
	293.15	298.15	303.15	310.15	318.15
			DSB		
$-\mu_D$	25.62	28.0	30.72	34.7	40.0
	(±0.07)	(±0.2)	(±0.05)	(±0.1)	(±0.1)
$-\mu_{11}$	13.47	14.51	16.4	19.0	21.1
	(±0.08)	(±0.07)	(±0.4)	(±0.3)	(±0.1)
$-\mu_{12}$	10.4	11.2	12.6	14.6	14.4
	(±0.2)	(±1.1)	(±0.9)	(±1.4)	(±3.1)
			ASB		
$-\mu_D$	13.49	14.8	16.09	18.4	20.9
	(±0.03)	(±0.1)	(±0.05)	(±0.1)	(±0.1)
$-\mu_{11}$	7.17	7.91	8.9	9.8	11.1
	(±0.07)	(±0.08)	(±0.1)	(±0.2)	(±0.1)
$-\mu_{12}$	5.4	5.9	7.0	7.6	7.1
	(±0.2)	(±0.1)	(±0.1)	(±0.2)	(±0.4)

Table 3

Thermodynamic parameters for the complexation between betulin derivatives and HP-β-CD (1 molar substitution) (with 95 % confidence interval).

Compound	ΔH^0 (kJ·mol^{-1})	ΔS^0 (J·mol^{-1}·K^{-1})	r^2	ΔG^0_{298} (kJ·mol^{-1})
		1:1 complexes		
DSB	-28.5 ± 0.6	-8.1 ± 1.9	0.997	-26.04 ± 0.08
ASB	-27.5 ± 0.7	2.5 ± 2.4	0.996	-28.2 ± 0.2
		1:2 complexes		
DSB	-56 ± 10	-59 ± 32	0.86	-38 ± 1
ASB	-63± 15	-67 ± 49	0.77	-42.3 ± 0.9

Table 4

Comparison of the obtained stability constants with the data available in the literature for the CD complexes of betulin derivatives at 298 K.

Compound	Solubility	log K_{11}	
		β-CD	HP-β-CD
DSB	soluble	3.87 ± 0.01 [4]	4.56 ± 0.01
ASB		4.00 ± 0.02 [4]	4.96 ± 0.04
Betulinic acid	insoluble	2.40 ± 0.04 [4]	* [5]
Betulonic acid		2.48 ± 0.03 [4]	* [5]

* The complexes are not enough stable or they are formed very slowly