Basicity, Ionic Associations and their Effect on Solubility of the Antineoplastic Benflurone

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Associations of benflurone, *i.e.* 5-(2-dimethylammonioethoxy)-7-oxo-7*H*-benzo[c]fluorene chloride, were studied by UV VIS spectrophotometry in aqueous solutions and its $pK_a = 7.51$ was determined by potentiometry. The UV VIS spectra of the acidified benflurone solutions simulated the second protonation, but it was disproved by the IR spectra of the prepared crystalline salt of the benflurone base (B) B₂(HClO₄)₃. Multiple association equilibria were evidenced, in diluted aqueous solutions they started with dimerization of the benflurone cations. On the addition of increasing excess of KCl (or another strong electrolyte), gradual formation of ion pairs BH⁺Cl⁻, their dimers (BH⁺Cl⁻)₂, and higher colloidal associates was manifested, after some time followed by the precipitation. The determined association constants showed that the preferred associate in the diluted solution was the dimer (BH⁺)₂ of the amphiphilic benflurone cations. The unusually sharp solubility decrease observed on the addition of KCl indicated that while the cationic dimerization supported the benflurone solubility, the uncharged ion pairs and their multimers were very poorly soluble.

Benflurone (Bf) was originally prepared as an antineoplastic drug [1, 2] and exhibited interesting pharmacodynamic properties [3] and biotransformation pathways [4]. According to the reported partition coefficient [5], the nonprotonated base of benflurone is a very hydrophobic compound. In the form of the studied chloride salt (Formula 1), benflurone is, however, well soluble in water, but it is surface-active and its more concentrated dark-orange solutions reveal even visually their colloidal nature, like solutions of many amphiphilic salts. The previous potentiometric measurements of aqueous solutions of benflurone with the chloride ion-selective electrode at concentrations 10^{-2} — 10^{-1} mol dm⁻³ indeed showed extensive associations [6] in the form of the cationic heteroassociate $(BH^+)_2 Cl^-$ However, this finding was based only on the measurements of the activity of chloride ions in the mentioned concentration range of benflurone.



Formula 1. Benflurone (BHCl).

Association phenomena affect greatly solubility [7, 8] and biological availability of drugs [9, 10], thus this work was intended as a study of the association equilibria of benflurone by the UV VIS spectrophotometry in more diluted aqueous solutions and also in the presence of another electrolyte. The effect of the formation of the ion-associates on the solubility was followed as well. Since benflurone is a chloride of the protonable base, the associations may be connected with the proton transfer [9]. Therefore measurements of benflurone basicity by potentiometry and verification of the protonation site by IR spectra were also included in the presented work.

EXPERIMENTAL

The orange substance of benflurone, *i.e.* 5-(2dimethylammonioethoxy)-7-oxo-7*H*-benzo[c]fluorene chloride, was of the same provenience (compound *V* in [2]) and purity, as stated in [5, 6]. Other chemicals were of reagent grade and water was redistilled.

Absorption spectra of the solutions in the UV VIS region were measured on the spectrophotometer Specord M 40 (Zeiss, Jena), using quartz cells with optical path lengths 1—50 mm. IR spectra were recorded on the instrument Specord 75 (Zeiss, Jena) in the KBr pellets. Elemental analyses were performed on the instrument C. Erba 1102. Melting points were measured with a proper correction on the microstage Boetius 83.

Measurements of pH were done with pH-meter PHM-26 (Radiometer, Copenhagen) equipped with a combined glass-calomel electrode GK 2301. The electrode was standardized [11] by the potassium tetraoxalate buffer with pH 1.68 for measurements in acidic solutions, and by usual standard buffers with pH 4.00, 7.00, and 9.22, respectively, for other measurements. Potentiometric, spectrophotometric, and solubility measurements were carried out at ambient temperature ($25 \,^{\circ}$ C).

Benflurone Perchlorate

We tried to prepare crystalline salts where benflurone base would bind more than one molecule of acid. Precipitation of benflurone ($c_{Bf} = 0.1 \text{ mol dm}^{-3}$) with HCl or HNO₃ did not yield defined products. However, precipitation with the solution of HClO₄ ($c = 1 \text{ mol dm}^{-3}$) on intense cooling yielded red crystalline precipitate, after standing it was filtered off and dried over silica gel at room temperature.

The composition of the prepared red salt from benflurone was established as $B_2(HClO_4)_3$, m.p. = 227 °C. It follows from the potentiometric titration (glass electrode) of *ca.* 0.052 g of the precipitate in 50 cm³ of acetone with aqueous NaOH ($c = 0.1 \text{ mol } \text{dm}^{-3}$). The average consumption was 102.6 %, with respect to the formula $B_2(HClO_4)_3$. For $C_{42}H_{41}N_2O_{16}Cl_3$ (M_r = 936.15) $w_i(\text{calc.})$: 53.89 % C, 4.41 % H, 2.99 % N; $w_i(\text{found})$: 52.59 % C, 4.26 % H, 2.98 % N. The analyses confirmed relatively well the formula $B_2(HClO_4)_3$ of the prepared salt, allowing a slight excess of the perchloric acid in the sample. A salt with higher stoichiometric content of $HClO_4$ did not precipitate from the benflurone solutions.

The elemental analysis of the original sample of benflurone (C₂₁H₂₀NO₂Cl, $M_r = 353.83$, m.p. = 268 °C) was carried out for the sake of comparison and the result was as follows: w_i (calc): 71.28 % C, 5.69 % H, 3.96 % N; w_i (found): 71.20 % C, 5.79 % H, 4.14 % N.

Determination of the Benflurone Solubility in Water and in Solutions of KCl

Solubility in water. Series of graduated test tubes with constant samples of benflurone and various volumes of water were gently shaken for 8 h. As the saturated solution was regarded that one, where the sample was completely dissolved and the solution was at most slightly iridiscent but did not reprecipitate after two weeks standing and occasional stirring at constant temperature. Two weeks of equilibrating was a necessary condition, since benflurone formed supersaturated solutions quite easily and later they again precipitated.

Solubility in the presence of KCl. The aqueous so-

lutions of benflurone and KCl were mixed together in such a way that the initial concentrations of benflurone were $c_{\rm Bf} = 0.001$ or 0.005 mol dm⁻³, whereas the concentrations of KCl gradually increased in the range $c_{\rm KCl} = 0.005$ —0.2 mol dm⁻³ On mixing, only a change of the colour shade occurred, indeed some of the solutions with $c_{\rm Bf} = 0.001$ mol dm⁻³ were used for the spectrophotometric measurements of associations.

However, in the course of several hours or days, massive precipitation started in the solutions with higher concentrations of KCl, so that the supernatant over the orange precipitate of benflurone remained only light yellowish. The mixtures were stirred occasionally, left to equilibrate for two weeks and then the precipitate was removed. The final benflurone concentration in the remaining solutions was determined spectrophotometrically at $\lambda = 465$ nm, where the molar absorption coefficient is not too sensitive to the concentration and to the presence of KCl.

RESULTS

Protonation and Basicity of Benflurone

IR Spectra of Benflurone and Perchlorate Salt

In order to elucidate the protonation of the benflurone base, some assigned vibration modes [12] seen in the recorded absorption IR spectra of the original benflurone BHCl and its prepared perchlorate salt $B_2(HClO_4)_3$ are summarized in Table 1. The spectra are mutually very similar and the protonated tertiary amino group is recognized in both cases. Although the perchlorate salt has higher content of $HClO_4$, and it was precipitated at pH ≈ 0 , no signs of eventual second protonation of the benflurone base are seen in the IR spectra. The wavenumbers of the suspected carbonyl (or etheric) group are practically the same in both the salts.

Under the studied conditions the benflurone base

Table 1. IR Spectra of Benflurone Salts

Vibration mode	$\tilde{\nu}/\mathrm{cm}^{-1}$		
	BHCl	$B_2(HClO_4)_3$	
ν(C—H) _{arom}	3080 w, 3015 w	3057 w, 3012 w	
ν (C—H) _{aliph}	2955 w, 2900 w	2943 w, 2900 w	
ν (N ⁺ —H)	2580 w, 2440 w	2670 w, 2450 w	
ν (C=O)	1710 s	1713 s	
$\nu(C=C)_{arom}$	1580 s	1580 s	
$\delta_{\rm s}({\rm CH_3N^+})$	1431 m	1427 m	
$\delta_{\rm as}(\rm N^+-H)$	1403 m	1400 m	
$\nu_{as}(C - O - C)$	1270 m	1270 m	
$\nu_{s}(C - O - C)$	1048 m	1049 m	
$\nu_{\rm as}({\rm C}_3{\rm N}^+)$	996 m, 977 m 984 m, 936 m		

- strong, m - medium, w - weak.

B thus accepts only one proton and forms a cation BH^+ The extra molecule of $HClO_4$ in the solid salt $B_2(HClO_4)_3$ is probably a part of the crystalline lattice.

Dissociation Constant (pK_a)

The acid-base dissociation of benflurone cation, BH⁺ \rightarrow B + H⁺ was followed by the potentiometry in methanol—water medium ($\varphi_{\rm r} = 1$ 1) and the corresponding dissociation constant $K_{\rm a}$, or

$$pK_{a} = pH + \log ([BH^{+}]/[B])$$
 (1)

was determined.

Potentiometry was chosen because the investigated proton dissociation is not reflected in the UV VIS spectrum of benflurone and, on the other hand, the spectrum is sensitive to the addition of another electrolyte. The 50 % methanol—water medium was necessary due to the very poor aqueous solubility of the benflurone base B. The methanol—water solution with total concentration of benflurone $c_{\rm Bf} = 0.002$ mol dm⁻³ was titrated with aqueous NaOH (c = 0.1 mol dm⁻³) and pH was measured with the glass electrode. The obtained titration curve fitted well to the one-proton dissociation of the benflurone cation BH⁺

The pK_a values were calculated from five selected titration curves using eqn (1), as described in [13], with some modifications. Because of the used methanol—water medium, the involved activity coefficient γ_{H^+} had to be determined by analogous five potentiometric titrations. In this case, HCl (c = 0.1mol dm⁻³) in the same methanol—water medium was titrated with the above-mentioned aqueous NaOH and log $\gamma_{H^+} = pH_c - pH_m$, where pH_m was measured and pH_c was calculated from the concentration of the nonneutralized HCl.

The found average result was $pK_a = 7.51 \pm 0.04$. This value is somewhat lower than expected for pK_a of the aliphatic tertiary amino group in the benflurone side chain probably due to the used methanol—water medium [13]. In aqueous solution, the benflurone basicity may be somewhat higher.

Spectrophotometric Investigation of Associations

UV VIS Absorption Spectra of Benflurone

The UV VIS spectrum of the orange aqueous solution of benflurone, measured at the concentration $c_{Bf} = 2 \times 10^{-4} \text{ mol dm}^{-3}$, exhibits absorption bands at wavelengths (λ /nm) 221, 232, 271, 292, and 484 and the molar absorption coefficients at the respective maxima are ($\varepsilon/(\text{cm}^2 \text{ mmol}^{-1})$) 2.74 × 10⁴, 2.31 × 10⁴, 2.96 × 10⁴, 2.90 × 10⁴, and 806. Especially in the visible region, the spectrum markedly depends on



Fig. 1. Absorption curves of aqueous solutions of benflurone at various concentrations $c_{Bf}/(mol dm^{-3})$: 1. 0.0002 (5 cm cell), 2. 0.00025 (4 cm cell), 3. 0.0005 (2 cm cell), 4. 0.001 (1 cm cell), 5. 0.002 (0.5 cm cell), 6. 0.01 (0.1 cm cell).

concentration and on the eventual presence of another electrolyte in the solution.

Some absorption curves in the visible region, at various concentrations of benflurone ($c_{Bf} = 10^{-4}$ — 10^{-2} mol dm⁻³), are shown in Fig. 1. When the benflurone concentration increases, the band at $\lambda = 484$ nm diminishes, while at first a shoulder and then a new absorption maximum develops at $\lambda = 417$ nm. The effect of the admixture of KCl or HCl into the diluted solution of benflurone ($c_{Bf} = 0.001 \text{ mol dm}^{-3}$) is shown in Figs. 2 and 3, respectively. The effect of the growing excess of KCl or HCl on the absorption curves is strikingly similar to the concentration increase of benflurone, the band at $\lambda = 484$ nm again diminishes and the new maximum develops at $\lambda = 417$ nm, although the concentration of benflurone remains low.

The absorption curves in Figs. 1 and 2 thus indicate that some associations occur at the increasing concentration of benflurone and the addition of the excess of chloride anions. The measured spectra were sufficiently time-independent and therefore they were used for the investigation of the association equilibria, although some of the measured solutions precipitated later on. With respect to the determined pK_a , the acid-base dissociation could not substantially interfere in the association studies, since the measured solutions of benflurone, or benflurone with KCl, were in the range of pH = 6.0-6.5.

On the other hand, the absorption curves measured in the solutions of benflurone with HCl (Fig. 3) might



Fig. 2. Absorption curves of the solutions of benflurone ($c_{Bf} = 0.001 \text{ mol } dm^{-3}$) with the excess of KCl. $c_{KCl}/(mol dm^{-3})$: 1. 0.0, 2. 0.005, 3. 0.015, 4. 0.04, 5. 0.1, 6. 0.2, 7. 0.6, 8. 1.0.



Fig. 3. Absorption curves of the solutions of benflurone (c_{Bf} = 0.001 mol dm⁻³) with the excess of HCl. Concentrations of HCl are the same as c_{KCl} in Fig. 2.

simulate another acid-base equilibrium of benflurone, besides of the above investigated dissociation of the BH⁺ cations. However, the eventual second protonation of benflurone, *e.g.* on its ketone carbonyl (Formula 1), has been disproved by the IR spectra of the prepared crystalline perchlorate salt (Table 1). Aromatic ketones are usually too weak bases to be protonated at pH above or about zero [14], structurally similar fluorenone [15] has $pK_a \approx -6$. Therefore the spectral changes observed on the addition of both KCl and HCl in the benflurone solution are regarded as the manifestation of associations of benflurone with chloride anions, although the corresponding absorption curves in Figs. 2 and 3 differ somewhat at the higher respective concentrations of KCl and HCl.

Absorption curves quite similar to those in Figs. 2 and 3 were also observed in the benflurone solutions in the presence of the excess of other strong electrolytes, namely KNO_3 , HNO_3 , KClO_4 , and HClO_4 , before the precipitation occurred. Benflurone apparently associates readily with a number of inorganic anions. In this work we investigated in particular associations in the diluted aqueous solutions of benflurone, without and with the presence of KCl.

Solutions of Benflurone with the Excess of KCl

Solutions with constant concentrations of benflurone $(c_{Bf}/(mol dm^{-3}) = 0.0001, 0.0002, and 0.001)$ in the presence of increasing excess of KCl ($c_{\rm KCl}$ = $5c_{Bf}$ -1.0 mol dm⁻³) were examined at first, using convenient modifications of the spectrophotometric methods for the study of chemical equilibria [16]. Careful inspection of the measured visible spectral region reveals that the absorption curves can be grouped into two different families, one at lower ($c_{\rm KCl} < 0.06$ mol dm⁻³) and the other at higher ($c_{\rm KCl} > 0.06$ mol dm^{-3}) concentrations of KCl; some examples are seen in Fig. 2. Curves of each family intersect in their own isosbestic point, although the two isosbestic points are close together. In Fig. 2, the curves 1-4 intersect at $\lambda = 460$ nm and the curves 5–8 at $\lambda = 475$ nm. It points out that two different, though partially overlapping association equilibria of benflurone with chloride anions take place under the studied conditions.

The molar absorption coefficients ε at three selected wavelengths, namely $\lambda = 385$ nm, 417 nm, and 484 nm, were used to investigate the manifested association equilibria. In Fig. 4, two examples of the plots of log { ε } against log { $c_{\text{Cl}-}$ } are shown, where { ε } $= \varepsilon/(\text{cm}^2 \text{ mmol}^{-1})$ and { $c_{\text{Cl}-}$ } $= c_{\text{Cl}-}/(\text{mol dm}^{-3})$. Symbol $c_{\text{Cl}-}$ stands for the increasing total concentration of chlorides ($c_{\text{Cl}-} = c_{\text{KCl}} + c_{\text{Bf}}$) in the series of the measured solutions with constant concentration of benflurone. The two different equilibria are well documented especially by the shape of the curve 1 ($\lambda =$ 385 nm) in Fig. 4.

The association equilibrium occurring at lower concentrations of KCl was established as ion pairing of the benflurone cations with chloride anions, $BH^+ + Cl^- \rightarrow BH^+Cl^-$ and the corresponding association constant was denoted as K_2

$$K_2 = [BH^+Cl^-]/([BH^+][Cl^-])$$
(2)

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Table 2. Association Constants K_i of Benflurone

 ${K_i} = K_i / (\text{mol}^{-1} \text{ dm}^3)^q$ for i = 1, 2, and 4, q = 1; for i = 3, q = 3; for i = 5, q = 2

Equilibrium	Constant	Slope	λ/nm	
$2BH^+ \rightarrow (BH^+)_2$	$\log{\{K_1\}}$			
	2.87 ± 0.09		385	
	2.77 ± 0.04		417	
	2.89 ± 0.08		484	
$BH^+ + Cl^- \rightarrow BH^+Cl^-$	$\log{\{K_2\}}$			
	2.07 ± 0.19	0.93 ± 0.08	385	
	2.03 ± 0.19	0.97 ± 0.16	417	
	1.89 ± 0.16	1.07 ± 0.07	484	
$2BH^+ + 2Cl^- \rightarrow (BH^+Cl^-)_2$	$\log\{K_3\}$			
	6.15 ± 0.18	1.96 ± 0.18	385	
	5.94 ± 0.19	1.98 ± 0.19	417	
	6.64 ± 0.22	1.56 ± 0.30	484	
$2(\mathrm{BH^+Cl^-}) \rightarrow (\mathrm{BH^+Cl^-})_2$	$\log\{K_4\}$			1.1 1.1 1.1.
	2.01		385	
	1.88		417	
$(\mathrm{BH}^+)_2 + 2\mathrm{Cl}^- \rightarrow (\mathrm{BH}^+\mathrm{Cl}^-)_2$	$\log\{K_5\}$			
	3.28		385	
	3.05		417	



Fig. 4. Molar absorption coefficient $(\varepsilon/(\text{cm}^2 \text{ mmol}^{-1}))$ of benflurone $(c_{\text{Bf}} = 0.001 \text{ mol } \text{dm}^{-3})$ in the solutions with the excess of KCl – a logarithmic plot. Total chloride concentration $c_{\text{Cl}^-} = c_{\text{KCl}} + c_{\text{Bf}}, c_{\text{Cl}^-}/(\text{mol } \text{dm}^{-3})$. 1. $\lambda = 385 \text{ nm}, 2$. $\lambda = 484 \text{ nm}.$

It was evaluated by means of the logarithmic function

 $\log \{Y\} = \log \{K_2\} + \log \{c_{\rm KCl}\}$ (3)

where Y is an auxiliary variable defined as

$Y = (\varepsilon - \varepsilon_{\rm BH^+})/(\varepsilon_{\rm ip} - \varepsilon)$

The molar absorption coefficient ε_{BH^+} of the benflurone cations BH⁺ was found from the measurements of the solutions without KCl, by a slight extrapolation to the zero benflurone concentration. Values of ε were measured in the solutions with constant concentration of benflurone and the excess of KCl, $c_{\rm KCl} = 5c_{\rm Bf}$ —0.05 mol dm⁻³ The molar absorption coefficient ε_{ip} of the ion pairs BH⁺Cl⁻ had to be found by an iteration least-squares procedure, because of overlapping with the following association equilibrium. The resulting values of $\log \{K_2\}$, determined at the three wavelengths and $c_{\rm Bf} = 0.0001 \text{ mol dm}^{-3}$, are in Table 2. The slopes of the dependences of $\log \{Y\}$ on log $\{c_{\text{KCl}}\}$ are also given in Table 2, and their values are close to 1. It confirms the 1 1 stoichiometry in accordance with eqn (3), which means the formation of the uncharged ion pairs BH⁺Cl⁻

The association equilibrium prevailing at $c_{\rm KCl} > 0.06 \text{ mol dm}^{-3}$ was established as the formation of the dimerized ion pair from the monomers, $2\text{BH}^+ + 2\text{Cl}^- \rightarrow (\text{BH}^+\text{Cl}^-)_2$. The corresponding association constant was denoted as K_3

$$K_3 = [(BH^+Cl^-)_2]/([BH^+]^2[Cl^-]^2)$$
(4)

It was evaluated using the logarithmic function

$$\log\{Y\} = \log\{K_3\} + 2\log\{c_{\rm KCl}\}$$
(5)

and here the variable Y was defined as

$$Y = (\varepsilon - \varepsilon_{\rm BH^+})(\varepsilon_{\rm ipd}/2 - \varepsilon_{\rm BH^+})/(2c_{\rm Bf}(\varepsilon_{\rm ipd}/2 - \varepsilon)^2)$$

The molar absorption coefficient $\varepsilon_{\rm BH^+}$ was the same as in eqn (3), coefficients ε were measured at constant concentration of benflurone $c_{\rm Bf} = 0.001 \text{ mol dm}^{-3}$ and the increasing concentrations of KCl, $c_{\rm KCl} = 0.08-0.6$ mol dm⁻³ The molar absorption coefficient $\varepsilon_{\rm ipd}$ of the ion pair dimer (BH⁺Cl⁻)₂ had to be found again by iterations, because at concentrations of $c_{\rm KCl} \approx 1$ mol dm⁻³, the pattern of the benflurone association again changed. It is indicated by the upward course of the right-hand end of curve 1 in Fig. 4. Visually the solutions became slightly iridiscent, which manifested the starting colloidal aggregation.

The resulting values of log $\{K_3\}$, determined at the three wavelengths, are summarized in Table 2, together with the slopes of the respective dependences of log $\{Y\}$ on log $\{c_{\text{KCl}}\}$. For the respective measurements at $\lambda = 385$ nm and 417 nm the evaluated slopes are close to 2, confirming in accordance with eqn (5) the formation of the uncharged dimerized ion pairs $(\text{BH}^+\text{Cl}^-)_2$. More devious value of the slope was found at $\lambda = 484$ nm, at this wavelength the conditions are obviously less favourable for the determination, so the corresponding value of log $\{K_3\}$ is regarded as less reliable.

Besides of the formation from monomeric ions, the considered associate $(BH^+Cl^-)_2$ may also arise by the dimerization of the ion pairs, $2(BH^+Cl^-) \rightarrow$ $(BH^+Cl^-)_2$. This equilibrium is described by the association constant K_4

$$K_4 = [(BH^+Cl^-)_2]/[BH^+Cl^-]^2$$
(6)

The constant K_4 can be readily calculated from the determined K_2 and K_3

$$\log\{K_4\} = \log\{K_3\} - 2\log\{K_2\}$$
(7)

Values of log $\{K_4\}$, corresponding to the convenient wavelengths $\lambda = 385$ nm and 417 nm, are also presented in Table 2.

Solutions of Benflurone as a Single Solute

Some results of the spectrophotometric measurements of aqueous solutions of benflurone as a single solute are seen in Figs. 1 and 5. The curves in Fig. 5 indicate that associations of benflurone begin gradually at $c_{Bf} \approx 10^{-4}$ mol dm⁻³ and proceed continuously at higher concentrations. It is therefore reasonable to suppose that the associations start with the formation of either ion pairs or cationic dimers. With respect to the determined value of the constant K_4 , any substantial ion pairing without the presence of another electrolyte can be disregarded at such dilution. For that reason, the dimerization of the amphiphilic benflurone cations, $2(BH^+) \rightarrow (BH^+)_2$, was examined and indeed established at concentrations up to $c_{Bf} = 0.001$ mol dm⁻³ The symbol K_1 was reserved for the dimerization constant

$$K_1 = [(BH^+)_2] / [BH^+]^2 \tag{8}$$

and it was evaluated as a slope of an origin-passing line

$$Y = K_1 c_{\rm Bf} \tag{9}$$

The variable Y was given here by the formula

$$Y = (\varepsilon_{\rm BH^+} - \varepsilon)(\varepsilon_{\rm BH^+} - \varepsilon_{\rm d}/2)/(2(\varepsilon - \varepsilon_{\rm d}/2)^2)$$

where the molar absorption coefficient $\varepsilon_{\rm BH^+}$ of the monomer cations was found by the same way as in eqn (3), the coefficients ε were measured at $c_{\rm Bf} = 0.0001 - 0.001$ mol dm⁻³ and the molar absorption coefficient $\varepsilon_{\rm d}$ of the dimerized cations (BH⁺)₂ was found by iterative fitting the experimental data into eqn (9).

Values of the dimerization constant, determined at the three used wavelengths, are again given in Table 2 in the form of log $\{K_1\}$. Values of ε measured at $c_{\rm Bf} > 0.001$ mol dm⁻³ did not fit in the linear relationship (9). At higher concentrations (the righthand side of Fig. 5), the benflurone associations apparently proceed beyond dimerization, towards bigger aggregates. However, due to the limitations of the used spectrophotometry, we could not reliably establish the association equilibria at higher benflurone concentrations. The results of this work thus can-



Fig. 5. Molar absorption coefficient $(\varepsilon/(\text{cm}^2 \text{ mmol}^{-1}))$ of benflurone $(c_{\text{Bf}}/(\text{mol} \text{ dm}^{-3}))$ in aqueous solutions – a logarithmic plot. 1. $\lambda = 385$ nm, 2. $\lambda = 484$ nm.

not be directly compared with the previous potentiometric study [6], but they are not in contradiction.

From the above determined constants it is possible to calculate also the equilibrium constant of the association of the dimerized benflurone cation with two chloride ions, $(BH^+)_2 + 2Cl^- \rightarrow (BH^+Cl^-)_2$. The value of the corresponding constant

$$K_5 = [(BH^+Cl^-)_2]/([(BH^+)_2][Cl^-]^2)$$
(10)

follows from the relationship

$$\log \{K_5\} = \log \{K_3\} - \log \{K_1\}$$
(11)

Values of log $\{K_5\}$ calculated after eqn (11) from the data determined at the two favourable wavelengths are listed in Table 2.

The Effect of Associations on the Benflurone Solubility

The found saturated concentration of benflurone in water was in average $c_{\rm Bf} = 0.19 \text{ mol } \mathrm{dm^{-3}}$ The addition of KCl caused extremely sharp decrease of the benflurone solubility, which did not obey the solubility product law. The dependence of the determined solubility of benflurone on the KCl concentration in the range of $c_{\rm KCl} = 0$ —0.2 mol dm⁻³ was therefore expressed in the form of the following semilogarithmic empirical function

$$\log \{c_{\rm Bf}\} = -0.724 - 6.053 \{c_{\rm KCl}\}^{1/3}$$
(12)

The mean deviation of the experimental solubilities from the used empirical function (log $\{c_{Bf}\}$) was ± 0.041 . Eqn (12) enables practical calculations of the benflurone solubility in the presence of KCl. In the approximately physiologically isosmotic solution, corresponding to $c_{\rm KCl} = 0.15$ mol dm⁻³, the benflurone solubility is only $c_{\rm Bf} = 1.1 \times 10^{-4}$ mol dm⁻³, after eqn (12). Mixing the benflurone solutions with other electrolytes should be therefore avoided.

Although there is a certain time lag (see Experimental) before the precipitation of benflurone starts, the presence of KCl brings about the solubility decrease which is by several orders of magnitude more profound than required by the solubility product law. The observed good solubility of benflurone in water without the presence of KCl is apparently a consequence of the formation of cationic dimers or bigger positively charged associates of benflurone. On the other hand, the uncharged ion pairs and their multimers, formed on the addition of KCl or another electrolyte in the solution, are apparently poorly soluble, their poor solubility in water is similar to that of the nonprotonated base of benflurone.

DISCUSSION

Values of the association constants K_1 , K_2 , and K_4 , or their logarithms, can be mutually compared because of the same stoichiometry of the corresponding association reactions (Table 2, q = 1). Among them, the biggest value of log $\{K_1\}$ points out that the dimerization of the amphiphilic monoprotonated cations BH⁺ is the prevailing form of the benflurone associations in diluted solutions. The comparison of $\log \{K_1\}$ and $\log \{K_2\}$ shows that the dimerization of the benflurone cations, in spite of the charge repulsion, exceeds their ion pairing with chloride counterions, when no other electrolyte is present in the solution. Formation of the dimerized cations and larger charged associates may be also responsible for the relatively high solubility of benflurone in water. But since $\log \{K_2\}$ is only somewhat smaller than $\log \{K_1\}$, the admixture of chlorides shifts the equilibrium largely towards the formation of the poorly soluble uncharged ion pairs. The consequence is the observed extremely sharp decrease of the benflurone solubility in the presence of KCl, which cannot be explained by the solubility product law alone.

The solubility promotion effect of the dimerization of an amphiphilic cation was recently reported also with dexverapamil, which is structurally quite different from benflurone, but it is also the chloride of a protonable amphiphilic base [8]. The sharp solubility decrease on addition of a strong electrolyte observed with an anionic anticancer agent brequinar [7] may be another example of the solubility decreasing effect of the transformation of the charged ionic associates into the ion pairs or larger uncharged associates. On the other hand, small admixture of an inorganic electrolyte into the solutions of micellizing ionic surfactants, like amphiphilic quaternary ammonium salts [10, 17], promotes micellization (decreases CMC) and does not decrease dramatically the solubility, since the micelles are the soluble form.

In the case of benflurone, log $\{K_1\}$ is also higher than log $\{K_4\}$, so the dimerization of the benflurone cations BH⁺ is obviously more favoured than the dimerization of the uncharged ion pairs BH⁺Cl⁻, where the repulsion should not play a role. Rather surprisingly, ion pairing with chlorides does not promote but hinders the dimerization of the benflurone cations.

Certain insight into the mechanism of the benflurone associations is provided by the measured electronic spectra. The dimerization of both the free cations and the ion pairs of benflurone is characterized by the development of a new absorption maximum at $\lambda = 417$ nm. Spectral characteristics of the dimerization of the benflurone cations or ion pairs thus resemble the protonation of the carbonyl oxygen of the structurally related ketones, 9-fluorenone or anthraquinone in concentrated sulfuric acid, where a strong absorption maximum develops at $\lambda \approx 410$ nm [15]. The analogous protonation of the benflurone carbonyl is undoubtedly excluded in the measured aqueous solutions with pH about 6. But the similarities of the spectral changes suggest possible intermolecular hydrogen bonding of the type N⁺H··O=C, between the positively charged protonated amino group and the carbonyl oxygen of benflurone cations on their association. In this way, the investigated associations of the amphiphilic benflurone cations are also facilitated by the proton transfer.

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REFERENCES

- Křepelka, J., Roubík, J., Holubek, J., and Vančurová, I., Collect. Czech. Chem. Commun. 47, 1258 (1982).
- Křepelka, J., Vančurová, I., Holubek, J., Mělka, M., and Řežábek, K., Collect. Czech. Chem. Commun. 47, 1856 (1982).
- Miko, M., Křepelka, J., and Nobilis M. Anti-Cancer Drugs 3, 63 (1992).
- Nobilis, M., Anzenbacher, P., Pastera, J., Svoboda, Z., Hrubý, K., Květina, J., Ubik, K., and Trejtnar, F., J. Chromatogr., B 681, 143 (1996).
- Pešák, M., Havlenová, V., Heřmanský, M., Vančurová, I., and Křepelka, J., Cesk. Farm. 34, 123 (1985).

- Kopecký, F. Vojteková, M., and Pešák, M., Collect. Czech. Chem. Commun. 51, 2437 (1986).
- King, S.-Y. P., Basista, A. M., and Torosian, G., J. Pharm. Sci. 78, 95 (1989).
- Surakitbanharn, Y., McCandless, R., Krzyzaniak, J. F., Dannenfelser, R.-M., and Yalkowsky, S. H., J. Pharm. Sci. 84, 720 (1995).
- Attwood, D., in Aggregation Processes in Solution. (Wyn-Jones, E. and Gormally, J., Editors.) P 211. Elsevier, Amsterdam, 1983.
- 10. Kopecký, F., Pharmazie 51, 135 (1996).
- 11. Bates, R. G., Determination of pH. Theory and Practice. P. 62. Wiley, New York, 1964.
- Horák, M. and Papoušek, D., Infračervená spektra a struktura molekul. (Infrared Spectra and Molecular Structure.) P 648. Academia, Prague, 1976.
- Albert, A. and Serjeant, E. P., The Determination of Ionization Constants. P 9 and 23. Chapman and Hall, London, 1971.
- 14. Freiberg, V J. Prakt. Chem. Chem. Ztg. 336, 565 (1994).
- Edward, J. T. and Wong, S. C., J. Am. Chem. Soc. 99, 4229 (1977).
- Sommer, L., Analytical Absorption Spectrophotometry in the Visible and Ultraviolet Region. The Principles. P. 121. Akadémiai Kiadó, Budapest, 1989.
- Greksáková, O., Oremusová, J., Vojteková, M., and Kopecký, F., Chem. Papers 48, 300 (1994).

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