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Cyclodextrin assisted nanophase determination of alkaloid salts

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Abstract

The poor water solubility of the free base and the high dissociation constant (K_a) hinder mainly the assay of alkaloid salts. We have elaborated an enviroment friendly method that can be carried out in aqueous media. The stability difference of the cyclodextrin (CD) complexes of free and protonated bases were used for this purpose. The base is included into the hydrophobic cavity of the CD (which serves as an apolar solvent phase on molecular level) and its solubility in water is increased. Since the base forms more stable inclusion complex than its protonated species, the pK_a is decreased and the potentiometric titration is promoted by this way, too. Six different hydrohalide alkaloid salts have been investigated and the most appropriate CDs were chosen (depending on the size of the molecules and/or substituents). The results of the assays agree well with those obtained by the direct nonaqueous titrations. The stability constants of the inclusion complexes have been also computed.
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Keywords: Assay of halide salts of organic bases; Inclusion complexes of cyclodextrins; Apolar solvent phase on molecular level

1. Introduction

The determination of alkaloid bases as well as their salts formed with different acids has been a problem of pharmaceutical assays for a long time. The earliest methods separated the bases from alkaline solution by solvent extraction, then re-extracted the free alkaloid with a known amount of acid into aqueous phase and the excess of the acid was determined [1–3]. A modified version of this method is the "biphasic titration" where the alkaloid salt is titrated directly by standard alkali solution in presence of an organic solvent preventing the precipitation of the free base [1–3]. Both methods are accurate but the chloroform needed as a second phase has environmental polluting effects. (On the other hand, the first method is rather time-consuming, and only the acid component of the salt is determined in the second one.) The nonaqueous titrations of alkaloid bases [2–4] are also very accurate but the determination of their hydrohalide salts needs mercury(II) acetate [2–4] and the general policy is to avoid the application of toxic reagents. One of the possible solutions is the use of glacial acetic acid/acetic anhydride or formic acid/acetic anhydride mixtures instead of the mercuric acetate method [5]. (The background of this possibility is discussed separately [6,7].) Very interesting that the *Japanese Pharmacopoeia* 14 uses acetic anhydride e.g. in the assay of papaverine hydrochloride [8] while the *United States Pharmacopoea* 26 applies the mercuric acetate method [9].

To eliminate the use of toxic reagents, the method of alkalimetry in alcoholic medium can be favoured in the presence of a small, known amount of added HCl with potentiometric end-point detection (which is a modified version of the biphasic titration). This method is widely recommended by the *European Pharmacopoeia* (*Ph. Eur*. 4) and applied to several salts of organic bases [10]. However, the error caused by the high solubility of carbon dioxide in the alcoholic solution [11] has to be eliminated, and another problem is that the first inflexion (i.e. the end of the titration of excess HCl) is hardly observable in the case of very weak bases. The explanation is that the acidic dissociation constant of the protonated form (K_a) is increasing with increasing alcohol content and the protonated form is co-titrated with HCl [12]. It is pointed out that the titrations in alcoholic solution give good results without addition of HCl, too [13].

Surveying the statements: a method applicable in aqueous media would be the most preferable, but the assay of alkaloid salts is hindered (i) by the poor water solubility of the free base (since titrating in the presence of a precipitate is nearly impossible), (ii) sometimes by the relatively high K_a value (in this case the protonated base is co-titrated with the excess of HCl) or, (iii) oppositely, by the too low K_a value (in this case the second

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inflexion is overshadowed by the presence of the strong base). Based on our earlier experiences, these problems can be solved mostly by the use of cyclodextrins.

Cyclodextins (CDs) as cyclic oligosaccharides are able to form inclusion complexes with a series of organic molecules [14]. β-CD and γ-CD are built up from seven and eight glucopyranose units, respectively. The diameters of the ring are 780 pm (β-CD) or 950 pm (γ-CD), and the volumes can be estimated as 0.60 nm^3 (β-CD) or 0.74 nm^3 (γ-CD). Their solubilities (in water, at room temperature) are very different: 1.85 and 23.2%, respectively. The glucose units of β-CD are methylated in average at two (mainly at 2 and 6) positions in the so-called dimethyl β-CD. Its solubility (in cold water) surpasses that of β-CD several times, the saturated solution is estimated of 20–25%.

The water solubility of the guests is usually increased when reacting with CDs and this fact can be accepted as the proof of inclusion complex formation [14]. Further proofs are the changes in spectral properties and NMR spectra, etc. All of these methods were used e.g., in the study on the interaction between papaverine and different CDs [15]; several ^{1}H-NMR spectra are published about the quinine–β-CD [16] as well as the pilocarpine–CDs [17] interactions.

Since the cavity of CDs is rather hydrophobic, the inclusion of uncharged species is highly preferred against the (corresponding) charged ones. This effect was established by potentiometric investigation of some organic acids rather long ago [18] (while some very interesting exceptions were shown with normal aliphatic dicarboxylic acids [19]). Less investigations are known with the base, protonated base pairs, but similar effects could be proved with aniline and its derivatives [20].

Our working hypothesis was that the two effects of CDs (the increase of water solubility and K_a of the alkaloid base) make an alkalimetric titration possible even in aqueous media using potentiometric end-point detection. Like the method of *Ph. Eur.* 4, we have also used a small excess of HCl.

2. Experimental

2.1. Materials

The selected substances were the hydrohalide salts of codeine (**1**) [(−)-(5*R*,6*S*)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-morphinan-6-ol], ephedrine (**2**) [(−)-(1*R*,2*S*)-2-metylamino-1-phenylpropan-1-ol], homatropine (**3**) {DL-endo-(-hydroxybenzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester}, papaverine (**4**) [1-(3,4-dimethoxy-benzyl)-6,7-dimethoxy-isoquinoline], pilocarpine (**5**) {(+)-(3*S*,4*R*)-3-ethyl-4-[(1-methyl.5-imidazolyl)-metyl]-tetrahydrofuran-2-on}, quinine (**6**) [(−)-(8*S*,9*R*)-6′-methoxy-cinchonanol], and were of pharmacopoeial grade [3]. The base content of the alkaloid salts were checked by nonaqueous titration: the accurately weighted samples were titrated with 0.1 M perchloric acid in anhydrous acetic acid solution in the presence of mercury(II) acetate [3].

In aqueous medium, the effect of different CDs (β-, γ- and dimethyl β-CDs) with different cavity size and solubility were studied. The β-CD was recrystallized from hot water.

All the other materials were of analytical grade and used without further purification. Carbonate free NaOH was prepared by the Sörensen method. The solutions were made in doubly distilled water. The titrant was standardized against sublimed benzoic acid.

2.2. Methods

Potentiometric measurements were carried out with a Radelkis OP 208/1 pH-meter fitted with a Radelkis OP 0808P combined glass electrode and a Schott-Geräte T80/20 automatic burette. The equipment and the titration was computer controlled. The system was calibrated with two different buffers (pH 4.008 and 9.180) and checked with a third buffer (pH 7.000).

Accurately weighed amounts of alkaloid salts (corresponding to a concentration of $3–5 \times 10^{-3}$ M) were dissolved in distilled water and mixed with 1.00–5.00 cm^3 0.01 M HCl and NaCl [to a final concentration of 0.2 M (Na)Cl] as well as with the appropriate amount of the given CD and finally diluted up to 25.00 cm^3. Twenty cubic centimetre of these solutions were titrated with 0.02 M carbonate free NaOH solution under stirring with nitrogen. The temperature was kept at 25 ± 0.1 °C.

3. Results and discussion

The complex forming equilibria of a free base and its protonated form are shown in Scheme 1 and the interactions can be characterized by the general relationship

$$pH^+ + qB + rD \Leftrightarrow H_pB_qD_r^{p+} \quad (1)$$

where H^+ stands for the (hydrated) proton, B is the alkaloid base and D represents the CD. The formation constant is defined as

$$\beta_{pqr} = \frac{\left[H_pB_qD_r^{p+}\right]}{[H^+]^p[B]^q[D]^r} \quad (2)$$

and the expressions of the (known) total concentrations are as follows:

$$c_B = [B] + \Sigma q\beta_{pqr}[H^+]^p[B]^q[D]^r \quad (3)$$

$$c_{CD} = [D] + \Sigma r\beta_{pqr}[H^+]^p[B]^q[D]^r \quad (4)$$

and

$$c_H = [H^+] + \Sigma p\beta_{pqr}[H^+]^p[B]^q[D]^r \quad (5)$$

In every point of the titration, the $[H^+]$ is measured and the concentration of the added sodium hydroxide titrant (c_{NaOH})

$$\begin{array}{ccc} & K_A & \\ B & \leftrightarrow & BH^+ \\ K_{011} \updownarrow & & \updownarrow K_{111} \\ B{\cdot}D & \leftrightarrow & BH^+{\cdot}D \\ & K_A' & \end{array}$$

Scheme 1.

Table 1
Stability contstants of 1:1 complexes

Alkaloid	$\log \beta_{110}$ (pK_a)	β-CD		Dimethyl β-CD		γ-CD	
		$\log K_{111}$	$\log \beta_{011}$	$\log K_{111}$	$\log \beta_{011}$	$\log K_{111}$	$\log \beta_{011}$
Codeine	8.21 ± 0.03	1.60 ± 0.06	2.74 ± 0.08	0.9 ± 0.1	2.44 ± 0.05	1.4 ± 0.1	3.19 ± 0.03
Homatropine	9.88 ± 0.05			2.1 ± 0.1	3.3 ± 0.1	–	–
Papaverine	6.40 [18]			0.4 ± 0.1	1.99 ± 0.05	–	–
Pilocarpine	7.17 ± 0.01	1.0 ± 0.1	1.98 ± 0.07	1.45 ± 0.08	2.02 ± 0.03	1.65 ± 0.04	1.95 ± 0.04
Quinine	8.52 [18]	1.0 ± 0.1	3.09 ± 0.08	0.9 ± 0.1	3.10 ± 0.05	see text	see text

is known, so the concentration of hydrogen ions bound in the undissociated species c^*_H is also known and can be expressed as

$$c^*_H = c_H - c_{NaOH} - [H^+] = \Sigma p \beta_{pqr} [H^+]^p [B]^q [D]^r \quad (6)$$

Assuming a set of p, q and r, as well as β_{pqr} values, the best fit between the measured and calculated values [$c^*_{(meas)}$ and $c^*_{(calc)}$, based on Eq. (6)] can be searched using a computer program.

We found in the present work that $q = r = 1$, i.e. 1:1 base:CD supramolecules are formed. Table 1 summarizes the computed equilibrium constants together with their standard deviations ($\pm 3\sigma$). The first column gives the pK_a ($\log \beta_{110}$) values, which characterize the acid–base property of the given alkaloid in aqueous solution and they agree well with the values in the literature [21]. The next columns of Table 1 include the formation constant of the CD, protonated base complex ($\log K_{111}$)

$$K_{111} = \frac{\beta_{111}}{\beta_{110}} = \frac{[BH^+D][B][H^+]}{[B][H^+][D][BH^+]} = \frac{[BH^+D]}{[BH^+][D]} \quad (7)$$

and the formation constant of the complexed base ($\log \beta_{011}$)

$$\beta_{011} = \frac{[BD]}{[B][D]} \quad (8)$$

When β_{011} is of an appropriate size, the base is kept in the (aqueous) solution, i.e. hindrance (i) is overcome. The further possibilities (ii and iii) are connected to the change of K_a values.

The acid–base property of the base is characterized unambiguously by β_{110} (=1/K_a)

$$\beta_{110} = \frac{[BH^+]}{[B][H^+]} \left(= \frac{1}{K_a} \right) \quad (9)$$

while that of the complexed species

$$\frac{[BH^+D]}{[BD][H^+]} = \frac{\beta_{111}}{\beta_{011}} = \frac{[BH^+D][B][D]}{[BD][B][H^+][D]} \left(= \frac{1}{K'_a} \right) \quad (10)$$

The data of Table 1 prove our working hypothesis, i.e. really the chargeless bases form more stable supramolecules

$$\beta_{011} > K_{111} \quad (11)$$

Multiplying both sides by (β_{110}/β_{011}) we get

$$\frac{\beta_{011} \times \beta_{110}}{\beta_{011}} = \beta_{110} \left(= \frac{1}{K_a} \right) > \frac{K_{111} \times \beta_{110}}{\beta_{011}} = \frac{\beta_{111} \times \beta_{110}}{\beta_{110} \times \beta_{011}} = \frac{\beta_{111}}{\beta_{011}} \left(= \frac{1}{K'_a} \right) \quad (12)$$

It is clear, that the complexed acid is a stronger acid than its parent.

Investigating the selected substances, two inflexions can be detected on every potentiometric titration curves, the first one due to the excess HCl and the second one corresponding to the protonated alkaloid.

Codeine hydrochloride (**1**, having appropriate K_a) can be titrated in presence of different CDs. It could be expected that the larger cavity of γ-CD is more suitable for a larger molecule (like codeine), more stable complexes would be formed allowing the measurements with smaller excess of the CD. The results prove the expectation: three-fold excess of γ-CD is enough for codeine to get well-separated inflexions on the titration curve. The smaller cavity of β-CD results in a less stable complex (see Table 1) and the lower solubility in water does not allow more than three-fold excess. The consequence is that the second inflection is less characteristic. The third possibility for the titration of codeine is the use of dimethyl β-CD. Since the cavity sizes of β- and dimethyl β-CDs are the same, the stabilities of their inclusion complexes are very similar, but the solubility of dimethyl β-CD exceeds that of β-CD several times. Using 10-fold excess of dimethyl β-CD, the shape of the titration curve is excellent (Fig. 1).

As mentioned, the detection of the first inflexion (the end of the titration of excess HCl) is disturbed by the (relatively) high K_a (especially in alcoholic medium). Among the alkaloids studied, papaverine (**4**) has the highest K_a and this value (as evidenced by the data of Table 1) is further increased by complex formation, but the increased solubility could help the separation of the first titration step. Unfortunately, β-CD does not fit for the assay of papaverine, since the stability of the inclusion complex is not sufficiently high to keep the base near the end of the

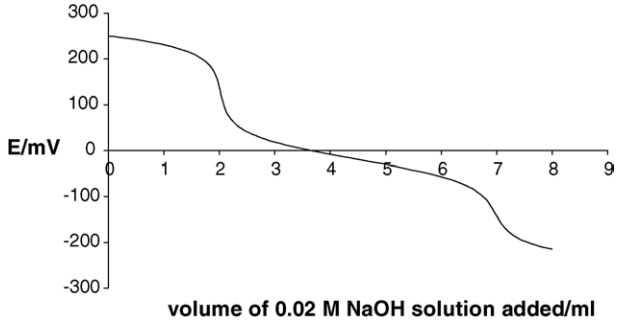

Fig. 1. Titration curve of 0.005 M codeine·HCl in presence of 0.05 M dimethyl β-CD.

Table 2
Percentage content of salts of alkaloids measured by potentiometric titration in presence of different CDs and nonaqueous direct acidimetry

Alkaloid salt	Method			
	β-CD	Dimethyl β-CD	γ-CD	Nonaqueous titration
Codeine hydrochloride	105 ± 1	103.1 ± 0.9	101.0 ± 0.4	100.3 ± 0.3
Homatropine hydrobromide	–	105 ± 2	–	100.0 ± 0.2
Papaverine hydrochloride	–	101.4 ± 0.5	–	100.7 ± 0.5
Pilocarpine hydrochloride	101 ± 1	102.9 ± 0.7	102.9 ± 0.9	99.5 ± 0.3
Quinine hydrochloride	103.5 ± 0.3	103.3 ± 0.5	–	97 ± 3

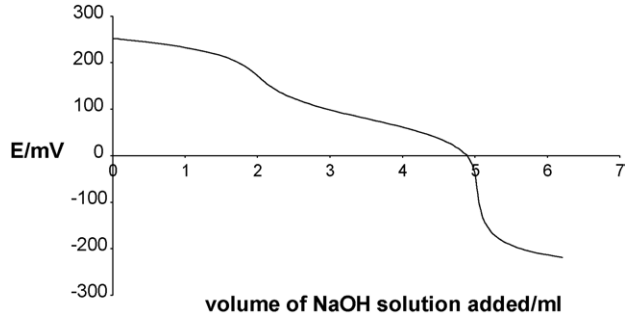

Fig. 2. Titration curve of 0.003 M papaverine·HCl in the presence of 0.03 M dimethyl β-CD.

titration in the solution. Due to the poor water solubility and low stability, papaverine needs a 25-fold excess of γ-CD but the increased viscosity of this solution makes the measurement unreliable. Dimethyl β-CD is better, even if its 20-fold excess is necessary (Fig. 2). (Knowing that papaverine is one of the substances where the method of *Ph. Eur*. 4 does not work, the use of dimethyl β-CD can provide an alternative way.)

Among the alkaloids studied, homatropine (**3**) has the lowest K_a and its assay is hindered by the flat (practically nonexistent) second inflexion. Similarly to papaverine, the potentiometric determination of homatropine is not aided neither by β- nor by γ-CDs, but the second inflexion is observable (although not well expressed) in the presence of dimethyl β-CD (in 20-fold excess, see Fig. 3).

Another example of substances, which cannot measured by the method of *Ph. Eur*. 4, is pilocarpine (**5**). We have found that pilocarpine can be directly titrated in aqueous media (in 5 mM concentration) without any auxiliary material, because of its good water solubility and convenient pK_a value. The effect of CDs was investigated as well but no significant change was caused in the shape of the titration curves since the difference is too small between the stabilities of the neutral and the protonated species.

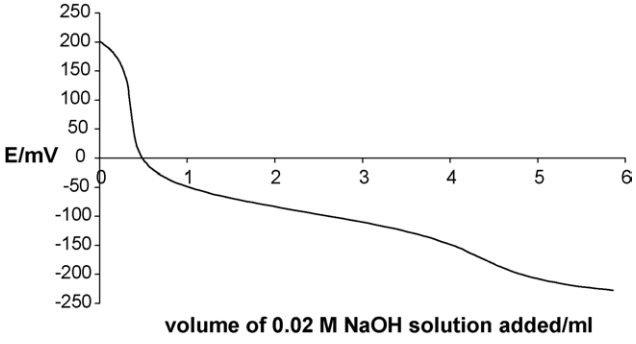

Fig. 3. Titration curve of 0.004 M homatropine·HBr in presence of 0.1 M dimethyl β-CD.

Quinine (**6**), γ-CD supramolecule precipitates during titration providing a nice example of GES (when the guest enforces its poor solubility on the host) [22]. In contrast to γ-CD, both β- and dimethyl β-CDs are suitable for the determination of quinine as represented in Table 2.

The determination of ephedrine (**2**) failed with all of the investigated CDs. (The effect of α-CD was also studied, considering the smaller size of the base.) The lack of succes is probably caused by an unusual side reaction which will be discussed elsewhere.

4. Conclusions

The average content and the standard deviation for five substances examined by the two methods (direct nonaqueous acidimetry and alkalimetric titration in presence of different CDs by potentiometric end-point detection) are given in Table 2. Nonaqueous acidimetry gives slightly lower results than the new method described above, and the reproducibility is better, but it requires a highly toxic reagent. The use of CDs can provide an alternative, environment friendly assay for many salts of weak bases in aqueous media.

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