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Ion-pair formation of pholedrine and phenylephrine with bis(2-ethylhexyl)phosphoric acid in benzene

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Dedicated to Prof. Dr. G. Zessin, Halle (Saale), on the occasion of his 65th birthday

Sympathomimetics of the catecholamine, hydroxyphenylalkylamine and hydroxyphenylaminoalcohol type are ampholytes. The differences between the ionization constants of the phenolic and the amino group are less than 3 units of pK_a and, additionally, the neutral molecules exist to a high degree in the zwitter-ion form. Therefore, a sufficient extraction by ion-suppression is not possible and sample pretreatment is the critical step of an analytical procedure and a general problem especially in the bioanalysis of this type of compounds. The diversity of methods used in this context is representing the problem [1]. The formation and extraction of ion-pairs with a lipophilic counter ion is a suitable tool to overcome the problem. Bis(2-ethylhexyl)phosphoric acid [HDEHP], introduced by Temple and Gillespie [2], proved to be useful as an ion-pairing reagent for the extraction of amphiprotic substances with chloroform [2–7], ethyl acetate [8, 9] and benzene [10, 11]. Compared with chloroform and ethyl acetate, benzene is more selective for the extraction from complex materials, e.g. biological samples. Owing to the lower polarity the co-extraction of interfering compounds can be reduced [1]. In contrast to chloroform [3] and ethyl acetate [8] the composition of the ion-pairs of ampholytes with HDEHP formed in benzene has not yet been investigated.

HDEHP is a weak acid (pK = 3.2). Dissolved in organic solvents extraordinarily strong intermolecular hydrogen bonding can occur for the compound. With diluents of low polarity such as benzene, hexane and carbon tetrachloride HDEHP forms dimers, but it is monomeric in alcohols and of intermediate aggregation in acetone and chloroform.

Assuming that at pH = 5.5 the amphiprotic species exist only in the aqueous phase and HDEHP exists only in the organic phase the extraction of the ampholyte A^+ in the cationic form with the dimeric ion-pairing and adduct-forming agent H_2X_2 (= $[HDEHP]_2$) can be illustrated by the following formula

$$A^+_{aq} + nH_2X_{2\,org} \rightarrow (AHX_2 \times n - 1\,H_2X_2)_{org} + H^+ \quad (1)$$

The extraction constant is

$$K_{ex} = \frac{[AHX_2 \cdot n - 1\,H_2X_2]_{org} \cdot [H^+]}{[A^+]_{aq} \cdot [H_2X_2]^n_{org}} \quad (2)$$

and the distribution constant of the ampholyte is

$$D = \frac{[AHX_2 \cdot n - 1\,H_2X_2]_{org}}{[A^+]_{aq}} \quad (3)$$

The combination of both constants gives

$$D = \frac{K_{ex} \cdot [H_2X_2]^n_{org}}{[H^+]} \quad (4)$$

At constant pH value the logarithmic form gives the simplification

$$\lg D = n[H_2X_2]_{org} + \text{const.} \quad (5)$$

where the slope n represents the number of H_2X_2 in the ion-pair.

The Fig. shows the results for pholedrine and phenylephrine. For pholedrine the slope is calculated to be 0.97 corresponding to an extracted species from the AHX_2 type. The slope of the line for the more polar phenylephrine is calculated to be 1.24 indicating the simultaneous formation of the AHX_2 type and the $AHX_2 \times H_2X_2$ type. This phenomenon has been generally reported by Modin et al. [3]. The results should be emphasized to be only valid for the precondition mentioned above. As expected the extraction efficiency of the more polar phenylephrine is lower than that for pholedrine. To achieve the same efficiency in extraction of phenylephrine the concentration of HDEPH has to be more than the 3-fold compared with pholedrine.

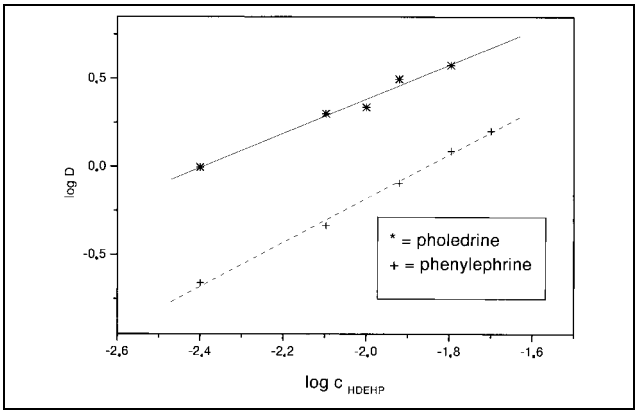

Fig.: Extraction of pholedrine and phenylephrine with HDEHP in benzene

Experimental

Pholedrine sulfate (Isis-Chemie, Zwickau/Germany) and phenylephrine hydrochloride (Synopharm, Barsbüttel/Germany) were of pharmacopoeial quality. Bis(2-ethylhexyl)phosphoric acid [HDEHP] (pract.; Fluka, Buchs/Switzerland) was used as received.

The extraction experiments were carried out in centrifuge tubes shaking equal volumes of an aqueous solution of the ampholyte ($c = 2 \times 10^{-5}$ M) in phosphate buffer (pH 5.5; 0.05 M) and solutions of HDEHP in benzene ($c = 4 \times 10^{-3} - 2 \times 10^{-2}$ M) at 25 °C for 30 min. After centrifugation the aqueous phase was separated and analyzed by HPLC (Mobile phase: 2.5 mM heptanesulfonic acid and 0.1 M acetic acid, adjusted to pH 5.5 with ammonia, containing 0.2% (v/v) diethylamine and 15% (v/v) acetonitrile; Column: LiChrosorb RP-18, 10 µm, 250 × 4.6 mm i.d.; Detection: $UV_{270\,nm}$).

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