

STUDY OF THE CONDITIONS FOR EXTRACTION OF TOXIC ORGANIC BASIS AND METHODS OF DETERMINATION I. EXTRACTION OF STRYCHNINE WITH CHLORINATED HYDROCARBONS

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Received November 12, 1991

Accepted March 21, 1992

The conditions for the extraction of strychnine with 1,2,2-tetrachloroethane, chloroform, 1,2-dichloroethane and carbon tetrachloride were studied and described. The distribution and extraction constants were calculated for strychnine in selected solvents from the dependence of the extraction yield on the pH at various ionic strengths. In addition, a study was carried out of the extraction properties of the ion associates of strychnine with selected groups of azodyes with identical basic skeleton but different numbers of sulfo groups.

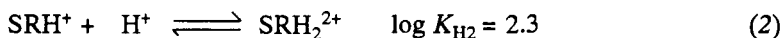
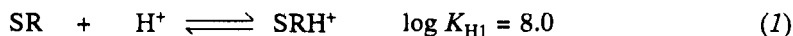
The large group of compounds containing ternary amine or heterocyclic nitrogen include both natural and synthetic substances. They have very different structures and specific effects on living organisms. A great many of these substances are employed in human and veterinary medicine, while the high toxicity of others leads to their classification as general poisons. Some of these substances have been abused as psychotropic drugs or psychoactive poisons. This work considers the methods for the analysis of those substances that have negative consequences for the vital functions of human beings. Cocaine is a well-known alkaloid that was originally used as a local anaesthetic and later as a narcotic drug. The first effective synthetic hallucinogenic substance was diethyl lysergamide, known as LSD 25 (ref.¹). The only poisonous substances with marked psychotropic effects is 3-quinuclidinylbenzilate, denoted by the code BZ (ref.²). The alkaloid strychnine is generally toxic, without specific applications.

All these substances have the common characteristic that they can be protonated in aqueous solutions and form ion associates with an appropriate counterion that can be extracted by suitable organic lipophilic solvents. Thus, these compounds can be analysed by extraction spectrophotometry, which is characterized by simplicity, suitable sensitivity and simple installation in mobile analysers.

A great many works deal with the formation of ion associates between the cation of a base and the anion of an acidic dye³⁻⁶. The existence of every associate is deter-

mined by dissociation, protonation and extraction equilibria, which can differ individually. In addition, the test substances include strychnine, a great many of whose properties have already been studied. However, for example, no comparison has yet been carried out of the properties of its extraction with 1,1,2,2-tetrachloroethane or other chlorinated hydrocarbons in dependence on the ionic strength and the ability to form and extract ion associates with azodyes, which have the same basic skeletal structure, but a different number of sulfo groups per molecule.

Strychnine (SR) is protonated in aqueous solutions in two stages, depending on the pH (ref. ⁷):



Hernandez⁸ studied the distribution of strychnine and a number of other alkaloids and found that it is dependent on their basicity. However, the extraction of the base in dependence on the ionic strength of the aqueous phase has not yet been considered in connection with the possibility of formation of ion associates of the strychnine cations with chloride.

The formation of coloured associates with the anions of several azodyes for the determination of codeine in pharmaceuticals was described by Serrat and Perez⁹. Vinues and Serrat¹⁰ compared the use of orange II and methyl orange for the determination of a selected range of alkaloids including strychnine. They found that these dyes can be employed for quantitative determination of the test substances. No study has been carried out on the use of dyes with as greater number of sulfonate groups or of a wider range of solvents. For the determination of strychnine, the study of these works led primarily to a method for carrying out the experiments. The values of the extraction and distribution constants are usually found by linearization of the dependence of the logarithm of the distribution ratio $\log D_B$ on pH (ref.⁸) or the conditional extraction constant is determined at a given pH value assuming that the products are not further modified¹¹. The EXLET program¹² is commonly employed to study extraction equilibria of chelates for the calculation of constants corresponding to the given protonation, dissociation, distribution and extraction equilibria.

The determination must be preceded by a determination of the extraction conditions, including the determination of suitable pH and ionic strength values of the medium. This can be achieved in extraction by decreasing the relative permittivity of the solution and especially by decreasing the water activity, increasing the stability of the extracted species and facilitating the exchange of a water molecule in its hydration shell. This favourable action of the indifferent electrolyte on the extraction is termed salting-out¹³. A change in the ionic strength leads to a change in the extraction charac-

teristics of the individual bases. The work of Shimer et al.¹⁴ describes the effect of the ionic strength and temperature on the formation of ion associates.

The extraction spectrophotometric determination of very toxic compounds requires a complete study of the conditions for the extraction of the individual substances. Strychnine is the first substance in this group and is studied in this paper.

EXPERIMENTAL

Instruments and Chemicals

A mixture of the aqueous solutions and the solvents was shaken at 25 °C in an TE III laboratory agitator (Czechoslovakia) and the pH of the aqueous phase after the extraction was measured using an MW 870 instrument (Präcitronic, Dresden, F.R.G.).

The absorbance of the aqueous and organic phases were read from the recording from a double-beam Specord M 40 UV VIS spectrophotometer (Zeiss, Jena, F.R.G.) in the ultraviolet spectral region. The absorbance of the ion associates was read on a single-beam Spekol 11 spectrophotometer (Zeiss, Jena, F.R.G.). The extraction characteristics were evaluated from the dependence of $\log D_B$ on the pH using the EXLET program¹² on an AT/386 personal computer.

Strychnine nitrate (Zdravotnické zásobování, Prague, Czechoslovakia) was controlled by measuring the absorbance of aqueous solutions, $c = 0.1 \text{ mmol l}^{-1}$ and comparison of the molar absorption coefficients at a wavelength of 254 nm with the values in tables¹⁵. In addition, a volume of 5 ml of an aqueous solution of strychnine nitrate was titrated with a standard 0.01 mol l^{-1} solution of sodium tetraphenyl borate. The electromotor force was measured on the OP-208/1 instrument (Radelkis, Budapest, Hungary), with an OP-0830 reference electrode and coated wire measuring electrode¹⁶. It was confirmed that it has a purity of 99.6% active component.

Selected azodyes, sodium 4-[(2-hydroxy-1-naphthyl)azo]-1-naphthalene sulfonate (*I*), disodium 1-[(1-naphthyl)azo]-2-hydroxy-6,8-naphthalene disulfonate (*II*), trisodium 1-[(4-sulfo-1-naphthyl)azo]-2-hydroxy-6,8-naphthalene disulfonate (*III*) and tetrasodium 1-[(4-sulfo-1-naphthyl)azo]-2-hydroxy-3,6,8-naphthalene trisulfonate (*IV*) were recrystallized three times from methanol using the commercial products of the BASF Co., F.R.G. (*I* and *III*), Francolor, France (*II*) and Cassella, F.R.G. (*IV*). Their purity was controlled by ascending thin-layer chromatography on Silufol plates (Kavalier Sázava, Czechoslovakia). The mobile phase consisted of ethyl acetylacetate, methanol and aqueous 28% ammonia, mixed in a volume ratio of 5 : 3 : 2. It was found that the dyes do not contain impurities. The remaining chemicals from Lachema Brno, Czechoslovakia were at least of p.a. purity.

Procedure

An amount of 50 ml of the base with a concentration of 50 mmol l^{-1} , an NaCl solution and HCl were pipetted into a round-bottomed flask so that the final volume of 100 ml had a pH value of 2 or 4 and the ionic strength was 0.3 mol l^{-1} . An amount of 100 ml of solvent was added and the solution was extracted for 1 h. After the extraction, the absorbance of the organic layer was measured at a wavelength of 254 nm and the strychnine concentration was measured. Then 20 ml of the organic phase and 20 ml of standardized AgNO_3 , $c = 0.01 \text{ mol l}^{-1}$, were added. The mixture was shaken for 5 min and the concentration of excess AgNO_3 was determined using a standard solution of NH_4SCN , $c = 0.01 \text{ mol l}^{-1}$.

An amount of 1 ml of an aqueous solution of strychnine with a concentration of 2.5 mmol l^{-1} was transferred to a 100 ml round-bottomed flask, 10 to 100 μl NaOH or HCl were added, the ionic strength was adjusted to a value of 0.01 to 0.3 by addition of 0.1 to 3 ml of an aqueous 2 mol l^{-1} NaCl solution.

The aqueous phase was then diluted to a final volume of 20 ml by addition of distilled water. After shaking with an equivalent amount of freshly redistilled solvent, the pH of the aqueous layer was determined and the absorbance of the aqueous and organic phases were measured at a wavelength of 254 nm.

To determine the extraction curves of the ion associates, 1 ml each of the base and dye *I* with the same concentration of base c_B and dye $c_L = 5 \text{ mmol l}^{-1}$ were pipetted into a 100 ml round-bottomed flask. Then 0 to 100 μl NaOH or HCl was added and the ionic strength *I* was adjusted to $I = 0.01, 0.1$ or 0.3 mol l^{-1} in the 20 ml of the aqueous phase. After shaking with an equal volume of 1,1,2,2-tetrachloroethane, the absorbance of the aqueous and organic phases were measured at a wavelength of 515 nm. The extraction curves of the remaining associates were measured with the following dye and base concentrations: c_L (II) = 3 mmol l^{-1} , $c_B = 6 \text{ mmol l}^{-1}$ at a wavelength of 515 nm; c_L (III) = 5 mmol l^{-1} , $c_B = 15 \text{ mmol l}^{-1}$ at a wavelength of 508 nm; c_L (IV) = 10 mmol l^{-1} , $c_B = 40 \text{ mmol l}^{-1}$ at a wavelength of 524 nm.

RESULTS AND DISCUSSION

Calculation of the contents of the individual components in the extraction of strychnine was based on concepts of the protonation, distribution and extraction equilibria of strychnine and its associates with chloride ions. Argentometry at pH 2.0 and 4.0 confirmed only the presence of the associate $\{\text{SRH}^+, \text{Cl}^-\}_{\text{org}}$ which is extracted by the organic solvent,



where the extraction constant K_{EX} can be expressed by the equation

$$K_{\text{EX}} = [\text{SRH}^+, \text{Cl}^-]_{\text{org}} [\text{H}^+]_{\text{aq}}^{-1} [\text{SR}]_{\text{aq}}^{-1} [\text{Cl}]_{\text{aq}}^{-1} \quad (4)$$

and calculated from the dependence of the distribution ratio of the base D_B on the pH using the EXLET program¹². The ability to form an ion associate between strychnine and the chloride ions depends on their concentrations in the aqueous phase. Fig. 1 depicts the extraction curves for strychnine for the individual solutions with an ionic strength of $I = 0.01, 0.1$ and 0.3 mol l^{-1} after extraction with chloroform.

An increase in the ionic strength *I* greatly increases the yield of the extraction *R* as a consequence of the transfer of the associate $\{\text{SRH}\cdot\text{Cl}\}_{\text{org}}$ to the organic phase. The solvents employed do not all behave identically; as the polarity changes, the yield also changes. At an ionic strength of 0.3, 1,1,2,2-tetrachloroethane was the best extraction agent, followed by chloroform, 1,2-dichloroethane and finally carbon tetrachloride, as depicted in Fig. 2.

The contents of the individual forms, i.e. the protonated bases, free strychnine and its associate for chloroform, 1,1,2,2-tetrachloroethane, 1,2-dichloroethane and carbon tetrachloride are apparent from the distribution diagrams in Fig. 3.

The extraction constants K_{EX} were calculated for the individual systems and are listed in Table I.

TABLE I
Logarithms of the extraction (K_{EX}) and distribution (K_D) constants of strychnine after extraction with various solvents in media with various ionic strengths

I mol l^{-1}	$\log K_D / \log K_{EX}$			
	1,1,2,2-tetra chloroethane	1,2-dichloroethane	carbon tetrachloride	chloroform
0.01	2.00 ± 0.19	2.08 ± 0.03	1.25 ± 0.01	2.73 ± 0.07
	7.62 ± 0.15	6.36 ± 0.04	5.70 ± 0.04	7.08 ± 0.07
0.10	1.81 ± 0.09	1.79 ± 0.05	1.21 ± 0.01	2.91 ± 0.10
	8.21 ± 0.07	6.73 ± 0.05	5.75 ± 0.01	7.53 ± 0.08
0.30	1.70 ± 0.11	1.76 ± 0.08	1.12 ± 0.01	2.82 ± 0.10
	8.54 ± 0.06	6.97 ± 0.08	5.76 ± 0.01	7.86 ± 0.06

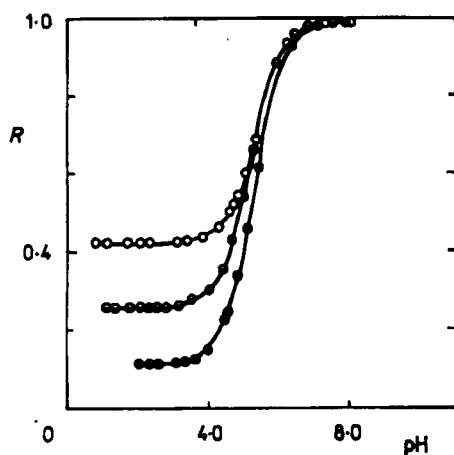


FIG. 1
The effect of the pH of the aqueous phase on the yield of the extraction of strychnine in the water-chloroform system, at $I = 0.01$ (●), 0.1 (◐), and 0.3 (○) mol l⁻¹

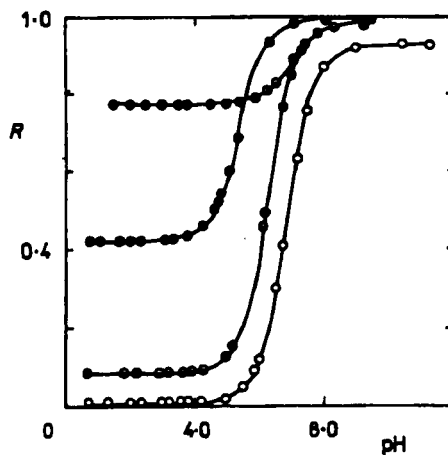


FIG. 2
The effect of the pH of the aqueous phase on the yield of the extraction of strychnine in the water-carbon tetrachloride (○), 1,2-dichloroethane (◐), chloroform (●) and 1,1,2,2-tetrachloroethane (⊙) systems at $I = 0.3$ mol l⁻¹

An increase in the ionic strength also appears as a change in the value of the logarithm of the extraction constant $\log K_{EX}$ for 1,1,2,2-tetrachloroethane and chloroform. A smaller change was found for 1,2-dichloroethane. The associate of strychnine chloride is extracted by carbon tetrachloride without any great change at ionic strength values of 0.01 to 0.3 mol l⁻¹.

A study was also carried out of the ability to form the associate between strychnine and the coloured anions. The selected azodyes were denoted as acid red (*I* – *IV*). These are the sulfo derivatives of 1-[(naphthyl)azo]-2-naphthol, differing in the number and positions of the sulfo groups. Fig. 4 indicates that only the associate of strychnine with the monosulfo derivative (*I*) is extracted quantitatively. As the number of sulfo functional groups increases, the yield *R* decreases, as can be seen in Fig. 4.

The measurements were carried out at a constant ionic strength of 0.01 mol l⁻¹. It can be seen from Fig. 5 that, when the ionic strength is adjusted to a value of 0.3 mol l⁻¹ in this system, only the yield of the associate of strychnine and the monosulfonated dye (*I*) remains unchanged. In contrast, a marked decrease was observed for the associate of strychnine with the polysulfonated dyes *II* to *IV*. It can be assumed that an increase in the ionic strength will lead to a shift in the equilibrium in favour of the formation of the

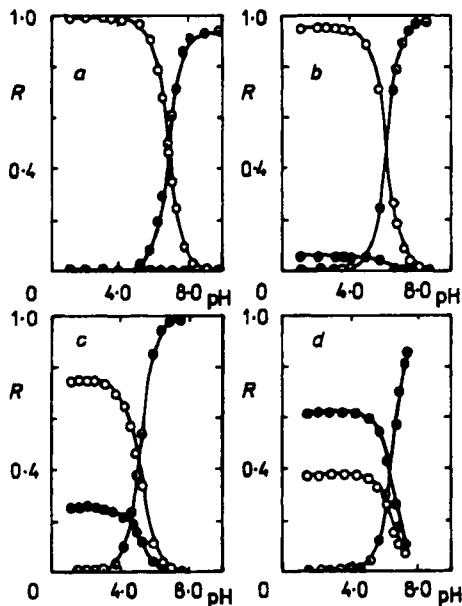


FIG. 3
The effect of the pH of the aqueous phase on the yield of the extraction of strychnine as the protonated form (○), the free base (⊙) and the associate of strychnine with chloride ions (●) in the water-carbon tetrachloride (a), 1,2-dichloroethane (b), chloroform (c) and 1,1,2,2-tetrachloroethane (d) systems at $I = 0.3 \text{ mol l}^{-1}$

ion associate of strychnine with chlorides and thus a decrease in the amount of dye associates.

It follows from the figures that only the monosulfonated dye (*I*) is favourable for the determination of strychnine. For the associates of the base with dyes with a higher number of sulfo groups, the yield is decreased with an increase in the ionic strength. They cannot be considered useful for the determination of these organic bases as the method requires maintenance of constant conditions including the ionic strength.

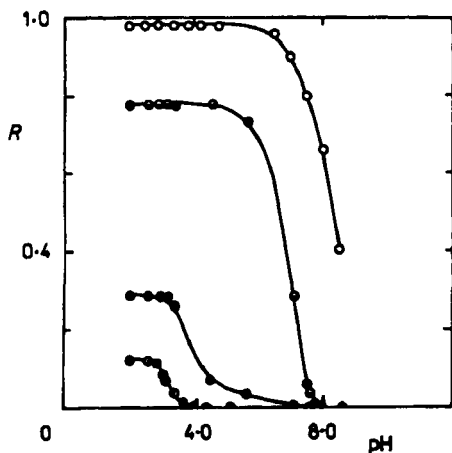


FIG. 4

The effect of the pH of the aqueous phase on the yield of the ion associate of strychnine with dye *I* (○), *II* (◐), *III* (●) and *IV* (⊙) in the water-1,1,2,2-tetrachloroethane system at $I = 0.01 \text{ mol l}^{-1}$

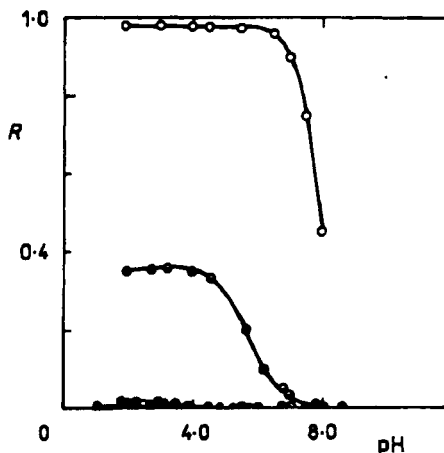


FIG. 5

The effect of the pH of the aqueous phase on the yield of the ion associate of strychnine with dye *I* (○), *II* (◐), *III* (●), and *IV* (⊙) in the water-1,1,2,2-tetrachloroethane system at $I = 0.3 \text{ mol l}^{-1}$

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Translated by M. Štulíková.