Fluorometric Determination of Non-Fluorescent Amines by Ion-Pair Extraction

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Summary. An ion pair extraction method is described for quantitative fluorometric determination in the ng/ml range of non-fluorescent amines used as drugs. As counter ion, reagent, anthracene-2-sulphonate was used. Its extracting ability of ion pairs with amines was studied by determination of extraction constants and its fluorescence properties by determination of quantum yield of fluorescence. The properties of a suitable organic solvent is discussed and methylene chloride was found to be advantageous. Conditions for quantitative extraction and determination are given and the method was applied to determination of amitriptyline in the range 10–100 ng/ml giving an extraction degree of 100% and a precision of 5%.

Introduction

Fluorometric measurements are widely used in the analysis of physiologically active compounds. This is mainly due to the high sensitivity that can be obtained by this technique. To make fluorometry generally applicable to a group of substances it is however necessary to evaluate methods for transformation of non-fluorescent or weakly fluorescent compounds into compounds that can be determined in low concentrations. In a previous paper we have used ion pair extraction for this transformation in determination of anions [1]. In the present investigation the same principles are applied to extraction and determination of amines and quaternary ammonium ions by using anthracene-2-sulphonate as counter ion. Glazko et al. [4] have used a similar technique in determination of diphenhydramine but they used a divalent sulphonic acid, Tinopal GS, as anion component which is unfavourable particularly in the low concentration range. The ion pair extraction technique has also been used in determination of tubocurarine [3].

The ion pair extraction of ionizable organic molecules has been extensively studied by Schill et al. [7, 9, 12]. In these papers the partition of the ion pairs are studied quantitatively by determination of extraction constants and other constants of importance for the partition. By this theoretical approach it is possible to calculate the degree of selectivity of the analytical method which is of particular importance in the analysis of complicated samples e.g. biological materials. Ion pair extraction is generally applicable to ionizable substances and besides by choosing suitable conditions a high degree of selectivity can be obtained.

Experimental

Apparatus

The fluorometric measurements were performed with an Aminco-Bowman Spectrophotofluorometer 4-8202 B equipped with a Hanovia 150 W Xenon lamp and a 1P 21 photomultiplier. Unless otherwise stated a bandwidth of 24 nm was used for exciting light and fluorescence and the measurements were made at 20°C. Variations in the instrumental sensitivity were compensated for by calibration with known solutions.

Photometric measurements were performed with a Zeiss Spectrophotometer PMQ II and the pH determinations with a Radiometer pH Meter 4.
Chemicals and Reagents

Sodium anthracene-2-sulphonate was synthesized according to Liebermann [6]. The product was recrystallized three times from water. The identity was checked by IR-spectrum and the purity was controlled by ion pair extraction according to the method in [2].

The amines used were kindly supplied by the manufacturers.

All other chemicals were of analytical grade. The methylene chloride was of Fisher Certified quality and distilled and equilibrated with water before use.

Determination of Partition Ratio and Determination of Extraction Constants

The partition experiments were performed in centrifuge tubes in a thermostated bath at 25°C when chloroform was used as organic phase and at 20°C with methylene chloride. The tubes were shaken to partition equilibrium for at least 30 min and after centrifugation the phases were separated by a capillary siphon. The concentration of anthracene-sulphonate in the aqueous phase was determined fluorometrically or photometrically. In most cases a determination of the ion pair in the organic phase was also made.

The determinations of extraction constants of the ion pairs were performed in a pH-range where the partition of the components in the ion pair in other forms were negligible.

Discussion and Results

Principles of Ion Pair Extraction

The most simple case of ion pair extraction is extraction of an aprotic cation e.g. a quaternary ammonium ion with an aprotic anion e.g. the anion of a strong acid. This case can be described by the following expressions.

\[ \text{HA}^+ + \text{X}^- \rightarrow \text{HAX} \]  

The equilibrium is illustrated by the extraction constant, \( E_{\text{HAX}} \),

\[ E_{\text{HAX}} = \frac{[\text{HAX}]_{\text{org}}}{[\text{HA}]_{\text{aq}} \cdot [\text{X}^-]_{\text{aq}}} \]  

The partition ratio, \( D_{\text{HAX(X)}} \), of HA + as ion pair with X - is then

\[ D_{\text{HAX(X)}} = \frac{[\text{HAX}]_{\text{org}}}{[\text{HA}]_{\text{aq}} \cdot [\text{X}^-]_{\text{aq}}} = E_{\text{HAX}} \cdot [\text{X}^-]_{\text{aq}} \]  

The degree of extraction, with a given organic phase, can be varied by the kind and concentration of the anion, X -, and if the extraction constant, \( E_{\text{HAX}} \), is known the partition ratio is easily calculated. In most cases the conditions are more complicated than in the example given above. When side-reactions occur that influence the distribution of the components in the ion pair it is convenient to make use of conditional extraction constants [7] which include compensation for side-reactions such as protolysis, dimerization of the ion pair in the organic phase, dissociation of the ion pair in the organic phase etc. The extraction constants are in such cases calculated by graphical methods [7, 11]. It must be emphasized that these side-reactions are not to be interpreted as disturbances but instead they can improve both selectivity and degree of extraction.

Methods of Measurement

Photometric measurements have been without comparison the most common technique in studying ion pair extraction. The anions used in amine extraction have therefore often been chosen among substances with high molar absorptivities and to make the measurement more selective among substances that absorb visible light. The sensitivity obtained by photometry depends on the anion used and with suitable anions the \( 10^{-3} \) M concentration range can be studied with good precision [7]. This concentration corresponds to the microgram/ml range for substances of moderate molecular weights (> 200).

The main reasons of using fluorometric measurements are to increase the sensitivity in order to make quantitative determinations in the nanogram/ml range possible and to be able to study the extraction process in the low concentration range.

The Anion Component

The choice of the anion component, the reagent, in extraction of ammonium compounds followed by fluorometry has to be based both on the ability of the anion to give extractable ion pairs and on the optical properties of the anion i.e. the anion must give such a size of the extraction constants [Eq. (2)] and must be possible to use in such a concentration that a quantitative extraction is possible. An extraction degree of \( 99\% \) is obtained when the partition ratio, \( D_{\text{HAX(X)}} \), defined in Eq. (3) is equal to 100 and equal phase volumes are used. Besides the anion must give the ion pair such properties that it can be measured fluorometrically in low concentrations.

In this study anthracene-2-sulphonate was chosen as a suitable anion both with respect to ion pair extraction and to fluorometric measurement.

The ability of anthracene-sulphonate to give extractable ion pairs with ammonium compounds of moderate size was studied by determination of the extraction constant with tetrabutylammonium ion with chloroform as organic solvent. The constant is given in Table 1 that also reports extraction constants with other anions previously used in ion pair extraction. Anthracene-sulphonate as sodium salt can be used in a concentration of \( 10^{-3} \) M which means that quantitative extraction of an ammonium compound is possible when the extraction constant is more than \( 10^2 \) according to Eq. (3).