J. Pharm. Pharmac 1970, **22**, 500–506 *Received December* 22, 196

Selective determinations of amines and quaternary ammonium compounds as ion pairs with methyl orange by an automated method applicable to single dose analysis

LARS NYBERG

Department of Analytical Chemistry, Faculty of Pharmacy, Uppsala University, Box 6804, *S*-113 86 *Stockholm, Sweden*

Aliphatic amines and quaternary ammonium compounds can be determined photometrically after extraction as ion pairs with methyl orange. Extraction conditions are calculated from constants for the extraction procedure and side-reactions. Methods for the selective determination of ammonium ions of different degrees of substitution are given. Quaternary ammonium ions are isolated by extraction at pH 12. The amines are extracted as ion pairs at pH 4·6 and selective determinations can be made after primary and secondary amines are masked by acetylation and primary amines by coupling with salicylic aldehyde. An automated application of the method on a Technicon AutoAnalyzer is presented. A complete analysis of a sample containing the four kinds of ammonium ions requires four separate runnings. The same basic manifold is used for all the runnings with some rearrangements for the masking procedures. The method has been tested on benzhexol, diethylpropion, dodecylamine, emepronium, *N*-hydroxyethylpromethazine, promethazine and protriptyline. Recoveries of about 100% with a relative variation of 1–2% were obtained.

Several automated methods for determination of amines and quaternary ammonium compounds based on ion pair extraction have been published (Häussler & Hajdù, 1964; Kabasakalian, Karl & Townley, 1967; Kuzel, 1968; Stevenson & Comer, 1968). The applicability of these methods is limited because of the empirical approach to extraction conditions. A theoretical approach in the method construction (Schill, 1965) gives more rapid and reliable results. The conditions are then calculated from measured values of extraction constants of the ion pairs, and from partition coefficients and dissociation constants of the amines and the anion used.

This principle was applied by Eriksson & Nyberg (1967) in an automated method on the AutoAnalyzer intended for analysis of single tablets. The organic ammonium ions were extracted by chloroform as ion pairs with methyl orange. The method gave the total ammonium content. In the present paper this method has been developed to give possibilities for selective determinations of ammonium ions of different degrees of substitution at the nitrogen.

The ion pair extraction

The ion pair extraction can be illustrated by the following reaction

$$HA^{+}_{aq} + M^{-}_{aq} = HAM_{org}$$

where HA^+ is an ammonium ion, M^- the anion of methyl orange and HAM the extracted ion pair. The equilibrium is expressed by the extraction constant, E_{HAM},

defined by the following equation

$$\frac{(HAM)_{org}}{(HA^+)_{aq} \times (M^-)_{aq}} = E_{HAM} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

The extraction can be influenced by different side reactions such as protolysis, partition of the amine as base or dimerization of the ion pair in the organic phase. It is convenient to combine the effects of all processes influencing the extraction into a conditional extraction constant, E^x_{HAM}, valid under stated conditions (e.g. pH or concentration range). The conditional extraction constant is defined by

$$\frac{C'_{HAM\ org}}{C'_A \times C'_M} = E^x_{HAM} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$C'_{HAM\ org}$ is the total concentration of the amine present as ion pair with methyl orange in the organic phase. C'_A and C'_M represent the concentrations of all other forms of amine and methyl orange. The degree of extraction of the amine as ion pair can be expressed by the partition ratio, D_{HAM}.

$$D_{HAM} = \frac{C'_{HAM\ org}}{C'_A} = E^x_{HAM} \times C'_M \quad .. \quad .. \quad .. \quad (3)$$

The percentage degree of extraction, P, is given by

$$P = 100\left(1 + \frac{q}{D_{HAM}}\right)^{-1} \quad .. \quad .. \quad .. \quad (4)$$

where q = volume of aqueous phase/volume of organic phase. The compounds studied (1–7 in Fig. 1) represent the degrees of substitution at the nitrogen from quaternary to primary. The constants necessary for the calculation of the conditional extraction constants of their methyl orange ion pairs are given in Table 1. (The determinations of the constants will be discussed in a separate paper.)

Table 1. *Constants of extracted ammonium compounds*

Substance	Kind	Org. phase	log E_{HAM}	log $k_{2(HAM)org}$	log $K'_{HA} \times k_{d(A)}$
Emepronium ..	quatern.	$CHCl_3$	4·94	—	—
N-Hydroxyethyl-promethazine ..	quatern.	$CHCl_3$	4·74	—	—
Benzhexol ..	tertiary	$CHCl_3$	6·90	—	—3·06
Diethylpropion ..	tertiary	$CHCl_3$	4·24	—	—4·58
Promethazine ..	tertiary	$CHCl_3$	6·76	—	—3·00
		$C_2H_4Cl_2$	6·13	—	—3·73
Protriptyline ..	secondary	$CHCl_3$	5·27	4·48	—4·92
		$C_2H_4Cl_2$	4·68	4·71	—5·68
n-Dodecylamine ..	primary	$CHCl_3$	4·78	4·74	—4·74

$$K'_{HA} = \frac{a_{H^+} \times (A^-)}{(HA)} \qquad k_{d(A)} = \frac{(A)_{org}}{(A)_{aq}} \qquad k_{2(HAM)org} = \frac{(H_2A_2M_2)_{org}}{(HAM)_{org}}$$

The properties of methyl orange were discussed by Eriksson & Nyberg (1967). It is a sulphonic acid and an aromatic tertiary amine with $pK_a = 3{\cdot}43$ and has a solubility in water of $1{\cdot}3 \times 10^{-4}$ mol/litre. The limiting solubility of its sodium salt in 0·1M NaCl is about $10^{-2{\cdot}8}$ mol/litre. Methyl orange is not extracted from an aqueous phase by chloroform or ethylene dichloride. The degree of extraction of

the amine as ion pair will depend on E^x_{HAM} and C'_M according to equation (3). The value of the conditional constant, E^x_{HAM}, will change with pH due to protolysis and partition of the amine and protolysis of methyl orange. Furthermore, the concentration of methyl orange in the aqueous phase, C'_M, cannot exceed $10^{-2.8}$ because of its limited solubility. Calculations based on these assumptions are summarized in Fig. 1 which gives the relations between partition ratio, D_{HAM}, and pH for the compounds studied. The dimerization of primary and secondary amine ion pairs has not been considered. It is, however, a favourable effect giving a slight increase of the partition ratio in the actual concentration range.

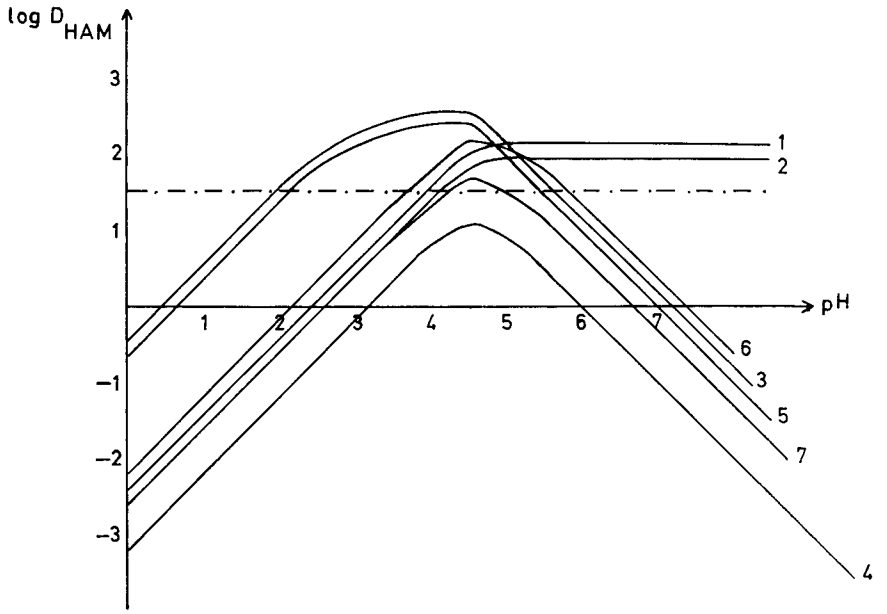

FIG. 1. Relations between maximal partition ratio and pH at the extraction of methyl orange ion pairs. 1. Emepronium. 2. *N*-Hydroxyethylpromethazine. 3. Benzhexol. 4. Diethylpropion. 5. Promethazine. 6. Protriptyline. 7. Dodecylamine. Side-reactions: protolysis and partition of the amines, protolysis and precipitation of methyl orange. Maximum concentration of methyl orange $10^{-2.8}$ M.

An extraction of more than 99% is obtained according to equation (4) if $q/D_{HAM} \leq 0{\cdot}01$. Since a volume ratio, q, of 1/3·5 is used in the automated extraction method, a partition ratio of 30 or more ($\log D_{HAM} \geq 1{\cdot}5$) is then necessary for an almost complete extraction. This limit is represented by the broken line in Fig. 1.

From Fig. 1 it follows that an appropriate extraction of all the compounds is obtained at pH 4·5. With increasing pH the partition ratio of the ion pairs of the amines decreases rapidly while the quaternary ammonium ion pairs are unaffected. Thus, an extraction at pH 12 will only give the quaternary ammonium ions.

Masking reactions

Primary and secondary amines can be excluded from ion pair extraction by masking procedures giving aprotic derivatives of the amines.

Acetylation. The primary and secondary amines were acetylated with acetic anhydride to aprotic amides in a two-phase system of chloroform and an aqueous

citrate buffer with pH 4·6. The anhydride was dissolved in the chloroform in a molar concentration of 5×10^{-3} while the amine $(0{\cdot}4\text{–}1{\cdot}0) \times 10^{-4}$ was added to the aqueous phase. The phase volume ratio, chloroform : aqueous phase, was 2. The amines used in this investigation were quantitatively acetylated within 10 min at room temperature with continuous shaking of the reaction mixture.

Azomethine formation. The primary amine was coupled with salicylic aldehyde to an azomethine with only weakly basic properties. The reaction was in ethylene dichloride containing 10^{-1} mol salicylic aldehyde/litre and an aqueous borate buffer of pH 9·0 and a salicylic aldehyde concentration of 2×10^{-2}M. The phase volume ratio was 1. The amine $(0{\cdot}7\text{–}2{\cdot}0) \times 10^{-5}$M, was added to the aqueous phase and the reactants shaken at room temperature for 15 min to complete the reaction. Ethylene dichloride (b.p. 84°) was used as the organic phase, since it will admit a higher reaction temperature which might be necessary with less reactive primary amines.

Determination procedure

The reactions discussed can be combined to automated procedures for the selective determination of different kinds of amines as ion pairs with methyl orange. The principles are summarized in Table 2. A complete analysis of a mixture of

Table 2. *Principles for selective determinations of ammonium compounds*

Running	Circuit 1		Circuit 2	
	Reaction	Extraction of amines as bases	pH of aqueous phase	Determination as ion pairs
A	—	—	12·0	Quaternary ammonium ions
B	—	All	4·6	Total amine content
C	Acetylation of secondary and primary amines	Tertiary	4·6	Tertiary amines
D	Coupling of primary amines with salicylic aldehyde	Tertiary and secondary amines	4·6	Tertiary and secondary amines

four ammonium ions of different degrees of substitution requires four separate runnings. The scheme contains two circuits, one for separation and one for determination. In the determination step, the ammonium ions are transformed to methyl orange ion pairs in the organic phase which are measured photometrically at 420 nm.

Quaternary ammonium ions (running A) only require one circuit which gives both separation and determination since all amines are excluded by the use of an aqueous phase with pH 12. At this pH the amines are extracted as bases and only quaternary ammonium ions give methyl orange ion pairs. The total amine determinatio (running B) begins with an extraction of the amines as bases. The organic phase containing the amines is then treated with an aqueous solution of methyl orange with pH 4·6, which transforms the bases to methyl orange ion pairs.

In running C primary and secondary amines are acetylated and the alkalinized aqueous phase is then extracted with chloroform. In the organic phase only tertiary amines have protolytic properties giving ion pairs by treatment with methyl orange as in running B.

In running D primary amines are coupled with salicylic aldehyde. The alkalinized aqueous phase is then treated with ethylene dichloride which extracts the azomethine as well as secondary and tertiary amines as bases. Quaternary ammonium ions can also to some extent be extracted as ion pairs with salicylic aldehyde. They can be eliminated by a second treatment with an alkaline aqueous phase. The organic phase is then treated with methyl orange at which secondary and tertiary amines are transformed to ion pairs.

Reagents

NaM, stock solution (3×10^{-3}M): 0·1000 g of purified methyl orange (acid form) is dissolved in equivalent amount of NaOH, 0·1N and water to 100·00 ml. *Purified methyl orange* is prepared from a solution of the sodium salt in water by addition of HCl to pH 1, at which the acid precipitates (Eriksson & Nyberg, 1967).

Sodium borate: 0·2M borate buffer, pH 9·0, prepared from boric acid and NaOH.

Sodium citrate: 0·1M citrate buffer, pH 4·6, prepared from disodiumhydrogen citrate and NaOH. *Sodium phosphate*: 0·1M phosphate buffer, pH 12·0, prepared from trisodium phosphate and HCl. *NaM, pH* 12: a mixture of two volumes of NaM, stock solution, one volume of sodium phosphate and one volume of water. *NaM, pH* 4·6: a mixture of equal volumes of NaM, stock solution, and sodium citrate.

Acetic anhydride chloroform (Ac_2O–$CHCl_3$): 5×10^{-3}M solution of acetic anhydride in water-saturated chloroform. Freshly prepared. *SA–$C_2H_4Cl_2$*: 0·1M solution of salicylic aldehyde in water-saturated ethylene dichloride. Freshly prepared. *SA–H_2O*: saturated solution of salicylic aldehyde in water. Freshly prepared. *Brij:* 20 drops of Brij 35 (10% in water) in 1000 ml of water. All substances should be of analytical or equivalent grade.

Samples. Aqueous solutions of salts of the ammonium compounds listed in Fig. 1. In the present investigation the samples were diluted so that in each running the sum concentration of the ammonium compounds to be determined was 7×10^{-5}M.

Standards. Water solutions of a salt of an ammonium compound not being masked in the actual running. Concentrations: $3{\cdot}5 \times 10^{-5}$, $7{\cdot}0 \times 10^{-5}$ and $10{\cdot}5 \times 10^{-5}$.

Methodology

The flow schemes for a complete analysis comprising four separate runnings are given in Fig. 2. The parts of the common manifold used in each running are indicated by letters and arrows. The lettering system is the same as in Table 2. The samples are segmented with organic phase. The extractions take place in double mixing coils. Between the circuits and before measurement, desegmentation comes about in phase separators (BO in the flow scheme) at which the lighter aqueous phase goes to waste. The acetylation of primary and secondary amines takes place in a

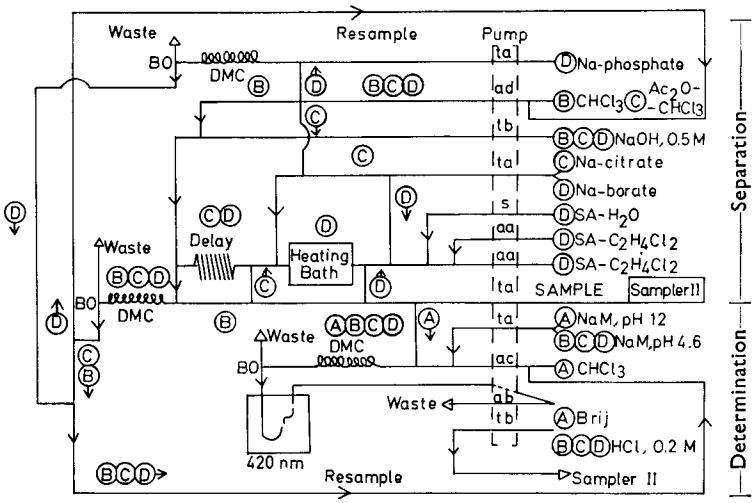

FIG. 2. Total manifold for selective determinations of ammonium ions by four separate runnings. Notes for reading the scheme: 1. Horizontally only readings from right to left should be made, except in the 2 cases of resampling. 2. Vertically the directions are shown by arrows. 3. The parts of the manifold used in each running are indicated by letters on or at the lines. Abbreviations: aa = Acidflex 0·056″; ab = 0·065; ac = 0·073; ad = 0·081; ta = Tygon 0·035; tb = 0·045; s = Solvaflex 0·056; DMC = Double Mixing Coil. Transport tubes and time delay coil are made of polyethene (bore 1·5 mm, wall 0·6 mm).

time delay coil and for the coupling between salicylic aldehyde and the primary amine both a heating bath and the time delay coil are used. In the present investigation no heating was necessary.

The rates of analysis are 40/h for runnings A and B, 30/h for running C and 20/h for running D. The Acidflex tubes are rinsed by pumping 0·1N HCl in ethanol followed by ethanol. Tygon and Solvaflex tubes are rinsed by water.

RESULTS

The determination step of the procedure (running A) was tested on the quaternary ammonium ions (pH 12 in the aqueous phase) and the amines (pH 4·6 in the aqueous phase) using samples containing only one substance. In all cases recoveries of 100·0% were obtained with a relative variation of about 1%. Results from tests of the masking procedures are given in Table 3. The acetylation procedure (running

Table 3. *Tests of the masking procedures in the automated method.* Standard: promethazine. The indications of the runnings refer to Table 2

Running	Sample	Concentrations × 10^4 added	found
C	Promethazine	1·60	1·60
	Dodecylamine	3·20	
D	Promethazine	0·310	0·317
	Dodecylamine	0·540	
D	Promethazine	0·350	0·707
	Protriptyline	0·350	
	Dodecylamine	0·350	

Table 4. *Complete analysis of ammonium compounds by the automated method*

Running	Determined components	Concentration × 10^5 added	Concentration × 10^5 found	Standard deviation	Number of determinations
A	*N*-hydroxyethyl-promethazine	6·21	6·34	0·10	30
C	Promethazine	7·00	7·14	0·05	30
B	Dodecylamine Protriptyline Promethazine	6·26	6·16	0·07	30
D	Protriptyline Promethazine	6·96	6·88	0·11	10

Sample: dodecylamine, 7·03 × 10^{-5}M; protriptyline, 6·91 × 10^{-5}M; promethazine, 7·00 × 10^{-5}M; *N*-Hydroxyethylpromethazine, 6·21 × 10^{-5}M. Standards: running A—*N*-hydroxyethyl-promethazine; running B and D—protriptyline; running C—promethazine. The sample is diluted in running B (3 + 7) and in running D (1+1).

Recoveries: dodecylamine 96%; protriptyline 96%; promethazine 102%; *N*-hydroxyethyl-promethazine 102%.

C) seems to give complete masking while the results obtained in running D which comprises the azomethine formation are too high. A complete analysis of a sample containing ammonium ions of four degrees of substitution is reported in Table 4.

The relative variation in each of the runnings is about 1% but the results of dodecylamine and protriptyline have a relative variation of about 2% since they are calculated as differences, dodecylamine from runnings B and D, protriptyline from runnings D and C. The deviations from 100% recovery are partly due to the slightly incomplete masking of the primary amine. They might, however, also be referred to the evaluation of several components by one single standard, since the compounds have been found to give slightly different peak forms.

Acknowledgements

The work has been supported by a grant from Apotekarsocieteten which is gratefully acknowledged. I also want to thank Drs Göran Schill and Olle Eriksson for interest and valuable discussions and AB KABI and Technicon, Sweden, for supplying me with laboratory equipment and materials.

REFERENCES

ERIKSSON, O. & NYBERG, L. (1967). *Automation in analytical chemistry, Technicon Symposia* 1967, Vol. II, pp. 269–273, White Plains, New York: Media Inc.

HÄUSSLER, A. & HAJDÙ, P. (1964). *Z. analyt. Chem.*, **205**, 455–460.

KABASAKALIAN, P., KARL, M. & TOWNLEY, E. (1966). *Automation in analytical chemistry, Technicon Symposia* 1966, Vol. I, pp. 232–234, White Plains, New York: Media Inc.

KUZEL, N. (1968). *J. pharm. Sci.*, **57**, 852–855.

SCHILL, G. (1965). *Acta pharm. suecica*, **2**, 13–46.

STEVENSON, C. E. & COMER, J. (1968). *J. pharm. Sci.*, **57**, 1227–1230.