EXTRACTION-SPECTROPHOTOMETRIC DETERMINATION OF AMMONIUM ION BY DIBENZO-18-CROWN-6 AND CALMAGITE

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Abstract

An extraction-spectrophotometric method for the determination of trace amounts of ammonium ion is described. Ammonium ion is effectively extracted as a neutral ternary complex NH_4. DB18C6. calmagite at pH 3.0 into chloroform, followed by spectrophotometric determination at λ_{max} = 485 nm. Molar absorptivity of the ternary complex at this wavelength is 1.08×10^3 l mol^{-1} cm^{-1}. Beer's law is obeyed over the range 0.5-9.0 μg ml^{-1} and the Sandell's sensitivity for 0.001 absorbance unit is 16.7 ng cm^{-2}. The method is simple, rapid and free from interferences of several cations and anions which are usually present with NH^+_4 ion in real samples.

Introduction

Although growing plants need to assimilate nitrogen, ultimately in the fully reduced form, excessive concentrations of ammonia or ammonium ions are toxic to plants. An increase in the concentration of free ammonia may disrupt various aspects of plant metabolism [1-3].

The toxicity of ammonia to animals has also been demonstrated [4]. It derives from the deamination of amino acids and glutamine, diffuses into the tubule lumen and there reacts with a hydrogen ion to form an ammonium ion. The ammonium ion then may react with a variety of anions and thus carry out not only an excess hydrogen ion, but an anion as well. By this operation, the pH of the body remains constant and so prevents wasting of the essential cations such as sodium ion. Ammonia production normally occurs at a relatively low level unless the body is presented with large amounts of the hydrogen ion.

Because of the high solubility of ammonia in water, ammonium ion can be found anywhere, in sea water, soil, the body, plants, synthetic materials and so on. The determination of ammonium ion then can be a measure of the ammonia in the subject of interest.

Among the different analytical methods reported for the determination of ammonium ion [5-10], extraction-spectrophotometric methods have found a relatively widespread use, especially for the determination of NH^+_4 in sea water [11], soil [12-14], plants [15, 16] and biological materials [17]. However, despite the widespread use of crown ethers and their functionalized molecules in the separation and determination of various metal ions [18-22], their application to the extraction-spectrophotometric determination of ammonium ion is quite sparse [23].

In this paper, a simple, rapid and sensitive method for the determination of NH^+_4 ion is described. The method is based on the extraction of dibenzo-18-crown-6-ammonium-calmagite ternary complex in chloroform, followed by spectrophotometric measurement at 485 nm.

Keywords: Ammonium; Dibenzo-18-crown-6; Calmagite; Extraction spectrophotometry

Experimental Section

Reagents

The chemicals used in this study were of the highest

purity available and used without further purification except for vacuum drying over P_2O_5. Triply distilled deionized water was used throughout.

Ammonium stock solution (1000 μg ml^{-1}) was prepared by dissolving 2.972 g ammonium chloride (Merck) in a 1000 ml volumetric flask and diluting to the mark with water.

Calmagite solution ($5.6x10^{-3}$ M) was prepared by dissolving 2.0 g of the dyestuff (Merck) in a 1000 ml flask and diluting to the mark with water.

Crown ether solution ($5.9x10^{-1}$ M) was prepared by dissolving 2.125 g dibenzo-18-crown-6 (DB18C6, Merck) in a 1000 ml flask and diluting to the mark with pure chloroform (Merck).

Apparatus

The spectra were recorded on a Beckman DK-2A ratio recording spectrophotometer and the absorbance measurements at fixed wavelength were made with a Perkin-Elmer model 35 spectrophotometer. The pH measurements were made with a Corning 125 pH meter using a combined electrode.

Procedure

An aliquot of the sample solution containing 5-90 μg of ammonium ion was placed in a calibrated 10 ml flask. To this was added 3ml of $5.6x10^{-3}$ M calmagite of pH 3.2 and the solution was diluted to the mark with water. The solution was transferred into a 50 ml separatory funnel, 10 ml of chloroform containing 0.015 M DB18C6 was added and the mixture was shaken vigorously for 7 min. The phases were allowed to separate, the organic layer was collected and its absorbance was measured at 485 nm against a reagent blank.

Results and Discussion

Absorption Spectra

The absorption spectra of calmagite in the absence and presence of ammonium ion in the aqueous phase were recorded (Fig. 1). Both solutions showed the same spectra having a maximum at 540 nm. The same solutions were then extracted with chloroform containing DB18C6 and the corresponding spectra in organic phase were recorded (Fig. 1). As is seen, the extracted complex of ammonium shows a maximum at 485 nm with a molar absorptivity of $1.08x10^3$ l mol^{-1} cm^{-1}, whereas the reagent blank shows a negligible absorbance at this wavelength, In this case, calmagite as a large polarizable molecule can act as a counter ion [24] for the efficient extraction of NH^+_4-DB18C6 complex, as a ternary neutral adduct NH_4-DB18C6-calmagite, into the organic phase.

Effect of pH

The optimum pH for the extraction of ammonium with DB18C6 and calmagite was investigated over the range 1.0-10.0, using either HCl or NaOH solutions, and the results are shown in Figure 2. It is seen that the absorbance-pH plot shows two maxima at about pH 3 and 8. The first maximum, due to the extraction of NH_4-DB18C6-calmagite adduct, is more intense than the second one. Since calmagite with a pK_a value of 7.92 [25] bears two negative charges at pH 8, it can be assumed that the second maximum corresponds to the extraction of NH^+_4 ion in the form of $(NH_4$-DB18C6$)_2$-calmagite into the organic phase. However, at higher pH values, a fraction of ammonium ion is converted to ammonia and, therefore, a lower level of free NH^+_4 ion is available for the complex formation and extraction into the organic phase. Consequently, the existence of a less intense absorbance at pH 8 is not unexpected.

Effect of Calmagite Concentration

The influence of calmagite concentration on the extraction of ammonium ion is shown in Figure 3. It was found that the maximum absorbance occurs at a calmagite to ammonium mole ratio of about 6. A further excess of

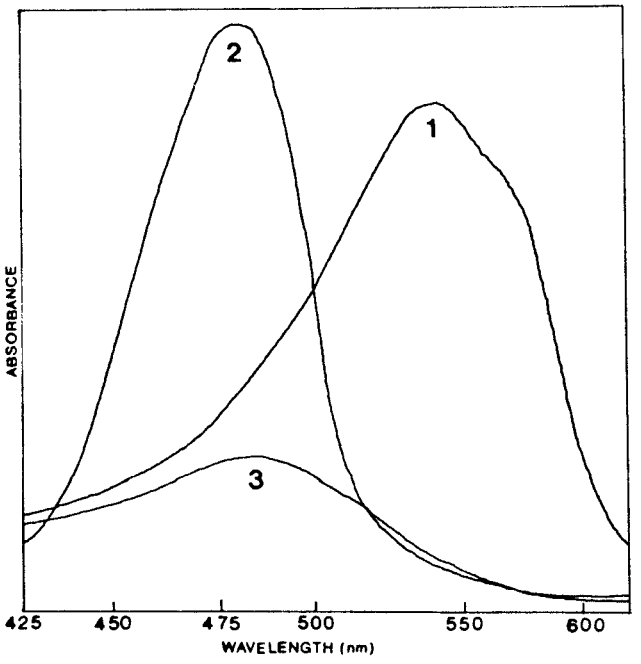

Figure 1. Absorption spectra of: 1, calmagite in water; 2, NH_4-DB18C6- calmagite complex in chloroform; 3, reagent blank in chloroform

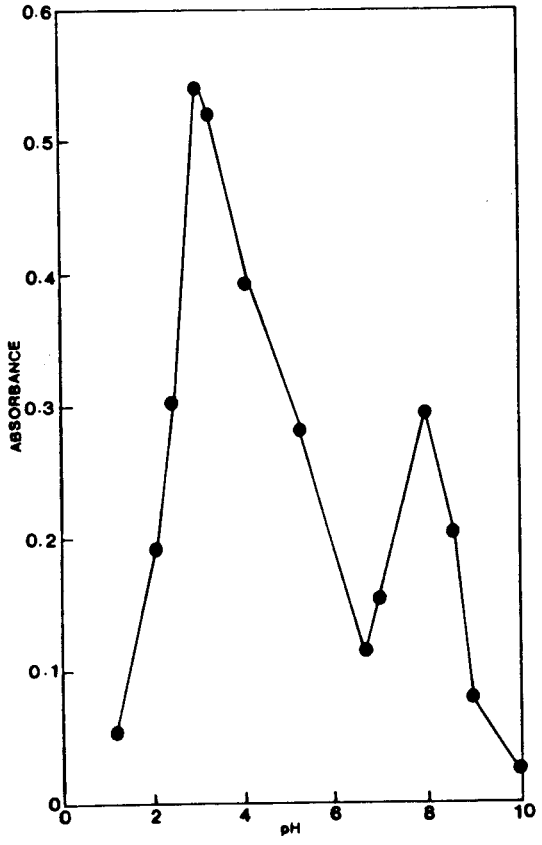

Figure 2. Effect of pH on the extraction of ammonium. Conditions: NH^+_4, 10 μg; ml^{-1}; calmagite, $1.40x10^{-3}$ M; DB18C6, $5.9x10^{-3}$ M; λ_{max}, 485 nm

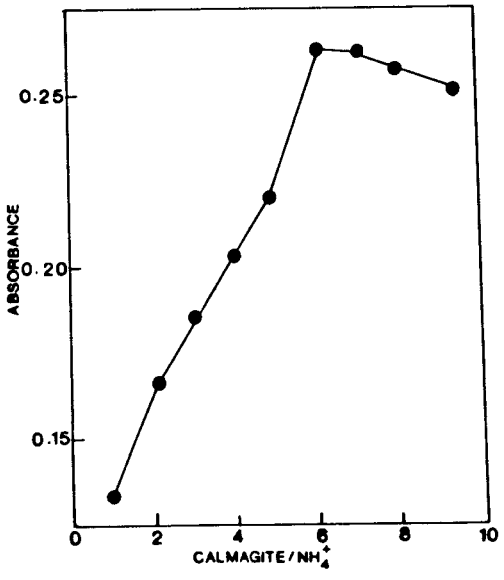

Figure 3. Effect of calmagite concentration on the extraction of ammonium. Conditions: NH^+_4, 5 μg ml^{-1}; pH, 3.0; DB18C6, $5.9x10^{-3}$ M; λ_{max}, 485 nm

calmagite causes a gradual decrease in absorbance as a result of some emulsion formation during the extraction process.

Effect of DB18C6 Concentration

The influence of DB18C6 concentration on the extraction of NH^+_4 ion was investigated and the results are shown in Figure 4. As is seen, the absorbance of ternary complex in the organic phase increases with an increase in DB18C6 concentration in chloroform. Maximum extraction occurs when the reagent to ammonium mole ratio is about 55. A further excess of DB18C6 has no considerable effect on the fraction of the complex extracted. Such a high concentration of the crown ether required for efficient extraction of ammonium seems to be due to the formation of a relatively weak complex between DB18C6 and NH^+_4 ion [26]. Consequently, a higher concentration of the crown ether is needed to push the complex formation equilibrium to completion.

Effect of Ionic Strength

The ionic strength of the aqueous solution, adjusted by

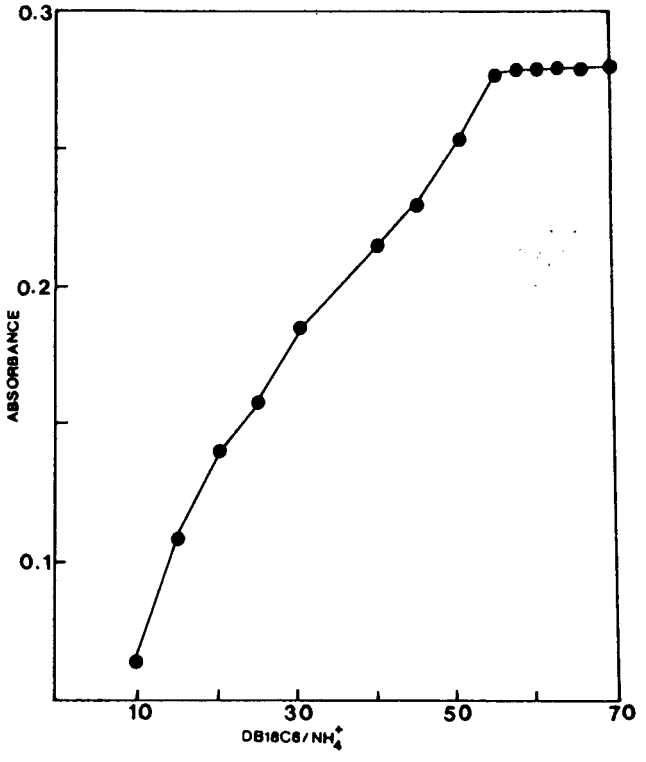

Figure 4. Effect of DB18C6 concentration on the extraction of ammonium. Conditions: NH^+_4, 5 μg ml^{-1}; pH, 3.0; calmagite, $1.67x10^{-3}$ M; λ_{max}, 485 nm

LiCl, was found to affect the extraction of ammonium ion with DB18C6 and calmagite into chloroform. There is an inverse relationship between the extent of extraction and the ionic strength of the aqueous phase, similar to the results reported by Kolthoff [27] on the effect of ionic strength on the extraction of K^+-18C6 complex into the organic solvents.

Effect of Shaking Time

The extraction of ammonium under the conditions recommended in the procedure is rapid. A shaking time of 7 min. was found to be sufficient for the complete extraction of NH^+_4 ion. Longer shaking times did not have any considerable effect on the absorbance measured.

Effect of Organic Solvents

The extraction process was performed at optimal conditions with some common organic solvents such as chloroform, carbon tetrachloride, benzene, isobutanol and 1,2-dichloroethane. Among the organic solvents tested, chloroform was found to be the best one because of some advantages such as the effective and fast extraction, relatively high solubility of DB18C6 [28] and low cost and availability of the solvent.

Effect of Different Crown Ethers

The extraction of ammonium ion was examined according to the recommended procedure using several crown ethers. The results are shown in Table 1. Among the 18 crowns used (i.e. 18-crown-6, dicyclohexyl-18-crown-6, 1,10-diaza-18-crown-6 and dibenzo-18-crown-6), DB18C6 with the lowest tendency for complex formation with NH^+_4 [26] showed the highest extractability. However, it is well known that the extent of extraction depends not only on the complexation equilibria but also on the solubilities and partition coefficients of various uncomplexed and complexed species. Thus, the higher extractability of DB18C6 can be related to its higher lipophilicity and better solubility of the resulting ammonium complex in the organic phase.

Table 1. Effect of crown ether structure on the extraction of ammonium ion. Conditions: NH^+_4, 5 μg ml^{-1}, pH, 3.0; calmagite, 1.67×10^{-3} M; crown ether, 1.5×10^{-2} M; λ_{max}, 485. nm

Crown ether	Absorbance
Dibenzo-18-crown-6	0.281
Dibenzo-30-crown-10	0.111
Dicyclohexyl-18-crown-6	0.027
18-crown-6	0.017
Benzo-15-crown-5	0.006
1,10-Diaza-18-crown-6	0.005

In contrast to 18-crowns, which have proper cavity sizes for NH^+_4 ion [29], benzo-15-crown-5 and dibenzo-30-crown-10 have too small and too large cavity sizes for this cation. Thus, the negligible extraction of ammonium with benzo-15-crown-5 is not unexpected.

It is interesting to note that, in the case of dibenzo-30-crown-10, despite the large cavity of the ligand, its extractability is considerably high (i.e. second in the series after DB18C6). It is well known that, large crown ethers such as dibenzo-30-crown-10 are very flexible molecules with enough oxygen atoms in the ring to enable them to twist around a cation of suitable size, to envelope it completely and form a stable three-dimensional "wrap around" complex [31-33]. Thus, considering the possible formation of such a stable "wrap around" complex between NH^+_4 and DB30C10 and the relatively high lipophilicity of the large crown ether, the observed extractability order is not surprising.

Beer's Law Study

A calibration graph for ammonium was obtained under the optimum conditions. Beer's law was obeyed over the concentration range of 0.5-9.0 μg ml^{-1} of NH^+_4 at 485 nm. The Sandell's sensitivity [34] for 0.001 absorbance unit was found to be 16.7 ng cm^{-2}. The relative error (95% confidence level) for 5.0 μg ml^{-1} of ammonium was ±2.4% (7 replicates).

Effect of Diverse Ions

Ammonium ion was extracted according to the recommended procedure in the presence of several cations and anions which are usually present with NH^+_4 ion in real samples [11-17]. The results are shown in Table 2. An error of ±4% in absorbance reading was considered tolerable. As is seen, moderate amounts of the cations and anions commonly associated with ammonium ion are tolerated under the optimum conditions.

Application

In order to evaluate the applicability of the method to the real samples, it was applied to the determination of ammonium in river waters. 100 ml of the sample river water (from Red Cedar River, East Lansing, Michigan, USA) was preconcentrated to about 20 ml by gentle evaporation. The sample was the transferred to a 25 ml calibrated flask and diluted to the mark with triply distilled deionized water. The ammonium content of the resulting solution was determined by the recommended procedure.

Table 2. Effect of diverse ions on the extraction of ammonium ion with DB18C6 and calmagite. Conditions: NH^+_4, 5 μg ml^{-1}; calmagite, 1.67x10^{-3}M; DB18C6, 5.9x10^{-3}M; pH, 3.0; λ_{max}, 485. nm

Foreign ion	Salt added	Tolerable concentration (g ml^{-1})
Li^+	LiCl	200
Na^+	NaCl	100
K^+	KCl	11
Cs^+	$CsNO_3$	37
Mg^{2+}	$MgCl_2$	50
Ca^{2+}	$CaCl_2$	50
Ba^{2+}	$BaCl_2.2H_2O$	100
Sr^{2+}	$Sr(No_3)_2$	100
Cu^{2+}	$Cu(NO_3)_2.3H_2O$	20
Fe^{3+}	$Fe(NO_3)_3.9H_2O$	50
Cd^{2+}	$Cd(NO_3)_2.4H_2O$	107
Hg^{2+}	$HgCl_2$	102
Zn^{2+}	$ZnCl_2.H_2O$	500
SCN^-	NaSCN	100
CH_3COO^-	$NaCH_3COO$	100
EDTA	Sodium Salt	100
NO^-_3	$LiNO_3$	100
Cl^-	LiCl,	200

The resulting values (0.21, 0.21 and 0.22 μg ml^{-1}) were in satisfactory agreement with those obtained by a standard method [35] (i.e. 0.22, 0.22, 0.20 μg ml^{-1}).

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