

Conclusion

Hypochlorite, the unstable reagent required for the CL reaction, can easily be electrogenerated on-line, and this novel method has many potential applications. Isoniazid acts as an enhancer of the luminol-hypochlorite CL system. Moreover, the proposed method is suitable for the automatic and can be applied in pharmaceutical analysis for the quick, simple and sensitive determination of isoniazid. Further work on this subject, aiming at liquid chromatographic applications is in progress.

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Interference with ammonium determination by the indophenol-type reaction of salicylate and dichloroisocyanurate

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Abstract Two widely accepted indophenol-type reactions with ammonium, which use phenol/hypochlorite and salicylate/dichloroisocyanurate as reagents, were compared for possible interferences with other naturally occurring nitrogen-containing compounds. Unlike the first method, the second showed strong interference from all amino acids and peptides tested. As much as 57 mole % of threonine and 49 mole % of serine were degraded to ammonium. Pyrimidines, purines, nucleosides and urea were negative in both reactions. The wide distribution and importance of free and combined amino acids in naturally occur-

ring waters favours the application of the classical phenol/hypochlorite reaction with ammonium rather than the modified salicylate/dichloroisocyanurate reaction.

Introduction

Ammonium is present in natural and artificial aqueous ecosystems, exhibiting particularly high concentrations in the water of sewage plants, caused by ammonification processes, and in anoxic hypolimnia and sediments of lakes as the result of ammonification and dissimilatory nitrate reduction. Oxidic lake water, ground water and sea water also contain ammonium, but the concentrations typically found are rather low in the nanomolar to micromolar range. Despite these low concentrations, ammonium exhibits the highest uptake rates of all inorganic nitrogenous species in lake and sea water. The outstanding importance of ammonium makes necessary a reliable determination method that works satisfactorily at the low concentrations occurring naturally.

One suitable method is the Berthelot reaction [1] which has been subjected to numerous modifications [2]. The original procedure was conducted with phenol and hypochlorite as oxidizing reagent [3], but later this method was changed to overcome the toxicity of the reagents and minor reaction products (chlorophenols) and the instability of hypochlorite. Phenol was replaced by salicylate and hypochlorite by dichloroisocyanurate [4, 5]. This version of the reaction is recommended in several analytical handbooks [6, 7], has been worked out as a standard method [8] and has gained wide application as a convenient method.

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Table 1 Organic nitrogenous compounds were applied in 20 μM concentrations and analysed for ammonium reactivity by the salicylate/dichloroisocyanurate (DCI) and the phenol/hypochlorite reaction. The reaction yield of ammonium is given in percent of the nitrogen in the compound. The mean of three determinations and the standard deviations are presented

Compound	Ammonium yield (%)	
	Salicylate/DCI	Phenol/HClO
Glycine	42.85 \pm 1.95	0.35 \pm 0.25
L-Alanine	28.20 \pm 0.95	0.20 \pm 0.10
L-Valine	9.80 \pm 0.55	0.05 \pm 0.05
L-Leucine	12.15 \pm 1.00	0.10 \pm 0.20
L-Threonine	56.75 \pm 0.40	-0.10 \pm 0.20
L-Serine	48.75 \pm 0.90	0.05 \pm 0.08
L-Glutamic acid	24.00 \pm 1.75	-0.35 \pm 0.05
L-Aspartic acid	18.00 \pm 0.35	-0.10 \pm 0.25
L-Glutamine	9.10 \pm 0.90	0.13 \pm 0.05
L-Asparagine	3.40 \pm 0.15	-0.15 \pm 0.17
L-Phenylalanine	17.10 \pm 0.85	-0.40 \pm 0.05
L-Cystine	23.18 \pm 0.50	0.10 \pm 0.15
L-Methionine	34.95 \pm 1.80	0.10 \pm 0.30
L-Tryptophane	7.53 \pm 0.15	0.03 \pm 0.04
L-Histidine	10.30 \pm 0.43	0.18 \pm 0.07
L-Proline	4.45 \pm 0.50	0.08 \pm 0.30
L-Arginine	0.78 \pm 0.08	0.02 \pm 0.03
L-Lysine	8.48 \pm 0.03	0.05 \pm 0.15
Guanine	-0.05 \pm 0.01	0.03 \pm 0.04
Thymine	-0.20 \pm 0.05	0.03 \pm 0.05
Uric acid	-0.11 \pm 0.05	0.05 \pm 0.07
Cytidine	-0.17 \pm 0.03	0.18 \pm 0.05
Guanosine	-0.09 \pm 0.01	0.08 \pm 0.03
Uridine	-0.23 \pm 0.02	-0.03 \pm 0.05
Urea	0.03 \pm 0.12	0.15 \pm 0.17
Spermidine	9.50 \pm 0.38	-0.02 \pm 0.03
Spermine	5.65 \pm 0.36	0.01 \pm 0.02
Peptone, tryp. dig.	5.00 \pm 0.10	-0.15 \pm 0.30
Peptone, acid dig.	23.35 \pm 0.45	11.05 \pm 1.70
Bovine serum albumin V	0.05 \pm 0.05	-0.30 \pm 0.15

During amino acid uptake experiments in lake water, it was noticed that a large number of naturally occurring nitrogenous compounds interfere with the modified rather than with the original procedure and this observation has given rise to serious doubts as to the reliability of the salicylate/dichloroisocyanurate method.

Experimental

Nitrogenous organic compounds were dissolved at 20 μM concentration in deionized water free of detectable ammonium. If necessary, the compounds were first dissolved by adding 0.1 M HCl or NaOH and subsequently neutralized. The different solutions were analysed in parallel with the analytical procedure using phenol and hypochlorite in the version as published by Solórzano [3] and with salicylate and dichloroisocyanurate as outlined in German standard methods [8]. The volumes of the samples were 25 mL and 40 mL, respectively. The compounds used were purchased from Merck, Darmstadt, Germany and Fluka, Buchs, Switzerland. Acid-digested (8.3% total N) and tryptic-digested peptone (13% total N) from casein were from

Fluka and bovine serum albumine V from Sigma. The total N of the latter compounds was used to calculate 20 μM concentrations.

Results and discussion

The modified indophenol-type reaction of ammonium with salicylate/dichloroisocyanurate has been optimized for the determination of ammonium in freshwater [7, 8] and sea water [9]. Only primary amines have been reported also to give ammonium-positive reactions [8], but because this class of compounds has not yet been found in appreciable concentrations in natural waters this interference was judged to be unimportant. However, during field experiments it was noticed that amino acids and peptides also severely interfere with this method.

To evaluate the specificity of both methods, 20 μM concentrations of various nitrogenous compounds reported to occur in natural waters were analysed with each method for ammonium (Table 1). All amino acids, but particularly glycine, the hydroxy- and sulphur-containing amino acids threonine, serine, and methionine, cystine gave high signals for ammonium in the salicylate/dichloroisocyanurate reaction whereas in the phenol/hypochlorite reaction negligible amounts of ammonium were produced from these compounds. In the salicylate/dichloroisocyanurate reaction as much as 57% and 49% of threonine and serine were oxidised to give ammonium. Acid-hydrolysed and tryptic-digested casein but not bovine serum albumine were also reactive. The interference by these compounds is particularly important because the major part of naturally occurring amino acids occurs in the combined rather than free form. Purines, pyrimidines, nucleosides and urea did not react with either method. The low interference of amino acids in the phenol/hypochlorite reaction has been observed before [10] and is corroborated in this study. A reduction in colour formation has been reported for several amino acids and various laboratory chemicals [11], but this reaction took place at concentrations 500 times higher than in the present study and these values are far beyond naturally occurring concentrations.

The strong interference of amino acids and peptides, which are ubiquitous and present in natural waters in varying concentrations, makes the application of the rather unspecific salicylate/dichloroisocyanurate method questionable.

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