

A Flow Injection Spectrophotometric Determination of Ammonium in Natural Water

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A determinação espectrofotométrica de amônio em um sistema de injeção em fluxo pode ser realizada utilizando-se salicilato como reagente, que gera o produto azul de indofenol. Assim, o emprego de fenol é evitado e as soluções usadas podem ser descartadas sem nenhum tratamento. As melhores condições para a determinação de amônio foram estabelecidas utilizando-se nitroprusiato como catalisador e um sistema de aprisionamento da zona de amostra, que possibilitou aumentar o tempo de residência sem modificar a dispersão da amostra. Dependendo do intervalo de aprisionamento programado, até cem medidas podem ser realizadas por hora, com um desvio padrão relativo inferior a 2,0% para uma amostra típica ($50 \text{ ng NH}_4^+ / \text{mL}$, $n = 5$). O limite de detecção foi de $7,1 \text{ ng NH}_4^+ / \text{mL}$ ($3 s_{\text{branco}} / \text{coeficiente angular}$). O método empregando salicilato apresentou resultados concordantes com o método convencional utilizando fenol ($r = 0,9810$).

Ammonium can be determined in natural water by a flow injection spectrophotometric procedure based on indophenol blue formation employing salicylate. The use of phenol as a colorimetric reagent is avoided and the resulting solutions can be discarded without treatment. The best conditions for measurements were achieved using nitroprusside as the catalyst and a zone trapping procedure in order to increase the residence time without modifying the sample zone dispersion. Depending on the programmed trapping period, up to 100 measurements can be made in one hour with a relative standard deviation lower than 2.0% for a typical sample ($50 \text{ ng NH}_4^+ / \text{mL}$, $n = 5$). The detection limit was $7.1 \text{ ng NH}_4^+ / \text{mL}$ ($3 s_{\text{blank}} / \text{slope}$). The salicylate method correlates well with the traditional colorimetric reagent containing phenol ($r = 0.9810$).

Keywords: flow injection spectrophotometry, ammonium determination, salicylate

Introduction

The flow injection spectrophotometric determination of low concentrations of ammonium, *i.e.* less than 100 ng/mL , is not an easy task. Bergamin F^o. *et al.*¹ and Santos F^a. *et al.*² used preconcentration in resins packed in microcolumns and measurements by Nessler's reaction to solve this analytical challenge. The ammonium ions could also be determined through a zone trapping procedure and measurements by Berthelot's reaction³. The latter procedure is highly suitable for the implementation of slow colorimetric reactions in flow systems. Thus, the central portion of the sample zone is trapped and maintained in a heated coil. However, the procedure proposed by Krug *et al.*³ involves

the use of phenol, which is toxic and volatile. The use of phenol can also result in the formation of poisonous o-chlorophenol under the reaction conditions.

Searle⁴ emphasizes that the utilization of salicylate is more suitable than phenol because the former is easily dissolved in water, is more stable in solution, and is less toxic.

More recently, Rocha *et al.*⁵ employed the salicylate method for the determination of total nitrogen in meat and meat products. All measurements were made using conventional manual procedures.

Kempers and Kok⁶ presented a complete investigation of the determination of ammonium through the indophenol blue complex using salicylate. It was observed that this

reagent is less reactive than phenol, and that the rate of the formation of the colored product is markedly influenced by the sequence and moment of the addition of the reagents. However, Searle⁴ previously suggested that if the reagents are added rapidly and sequentially, the order of addition has no effect on the formation of the products.

Thus, considering these kinetic aspects, the application of flow injection analysis could be strongly recommended, since in this kinetic method of analysis the perfectly reproducible temporization makes it possible and easy to control of all physicochemical processes that occurred during the sample transport and reagent mixing through the detector^{7,8}.

The aim of this work was to establish conditions for flow injection spectrophotometric determination of low concentrations of ammonium in waters using salicylic acid, nitroprusside and hypochlorite as reagents. The study was conducted using a flow diagram involving zone trapping of the sample zone in a heated coil.

Materials and Methods

A peristaltic pump with at least five channels is required; in this work a pump with six channels was used (Micronal model B332). A UV visible spectrophotometer (Micronal model B382) equipped with a flow cell with a dead volume of 80 μL was employed to signal measurements. The signals were collected in a potentiometer recorder (Metrohm E586 Labograph). To control the trapping period of the sample zone, an electronic commutator (Micronal B352) was used. The trapping period is defined as the time during which the central portion of the

sample zone is trapped at B1 for the development of the colorimetric reaction.

For the FIA system, 0.8 mm i.d. polyethylene tubing was used in the manifold. Propulsion tubes (Technicon) with different internal diameters were also used.

All solutions employed were prepared with distilled-deionized water.

The salicylic acid solution was prepared in a sodium hydroxide medium containing 5% w/v sodium citrate to prevent the precipitation of ions as hydroxides. The concentrations of salicylic acid tested were 0.625%, 1.25%, 2.50%, and 3.75% w/v. These solutions were prepared in three different sodium hydroxide concentrations: 0.175, 0.350, and 0.525 M.

The effect of the nitroprusside concentration was evaluated by using aqueous solutions containing 0.50%, 0.75%, and 1.00% w/v.

The sodium hypochlorite solutions were prepared using domestic bleach. Four solutions were prepared for testing: 0.05%, 0.10%, 0.20%, and 0.40% v/v in active chlorine. All solutions were prepared in 0.15 M sodium hydroxide.

The flow diagram of the ammonium system is shown in Fig. 1. This flow diagram is identical to that proposed by Krug *et al.*³ With this configuration, the analytical solution is aspirated in the sample loop. After commutation, the sample zone is continuously transported towards the detector by a water carrier stream. The reagents are added at confluence points x, y, and z.

The central portion of the sample zone is trapped for the formation of the colored reaction product under heating (36 °C and 45 °C). The trapping periods used were 32, 48,

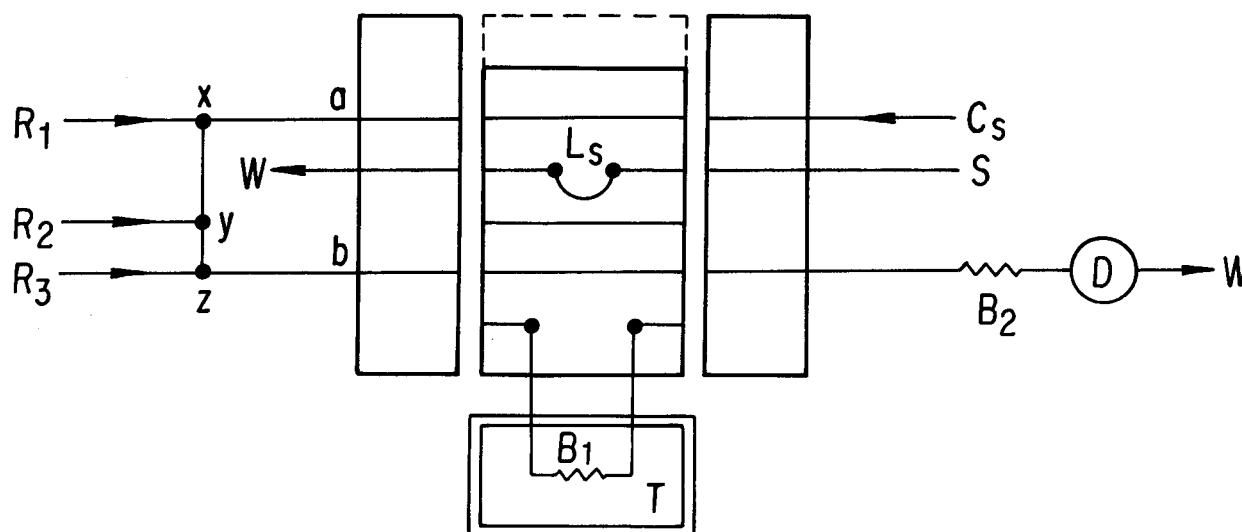


Figure 1. The flow diagram of the system proposed for the determination of ammonium. Dashed lines represent the position of the injector after commutation. S is the standard solution which is aspirated at 3.8 mL / min (the peristaltic pump is not shown). S fills loop L_s, which has a volume of 1.0 mL, then goes to waste W. C_s is the carrier stream (water), which flows at 6.0 mL / min. R₁, R₂, and R₃ are the reagents used, which are pumped at 1.0 mL / min. Transmission lines ax, xy, yz, and zb have lengths of 10, 20, 10, and 10 cm, respectively. B₁ and B₂ are reaction coils, both with a length of 150 cm. B₁ is placed in T, a 36 °C water bath. D is the spectrophotometer detector at 697 nm.

and 64 s. The injection period was fixed at 14 s. After the trapping period, the reaction product is reintroduced into the carrier stream and transported to the detector. The absorbance signal is measured at 697 nm.

The experimental work basically consisted of the establishment of the concentrations of the reagents and the application of this procedure to determine ammonium in water.

Water samples collected from different oxbow lakes of the Mogi Guaçu river were filtered through a 0.45 μm Millipore membrane and refrigerated at 4 °C. The ammonium determinations were made within 24 h after sampling. The data generated in this work is part of a project developed at the Jatai Ecological Station (SP, Brazil), in which the main focus is the understanding of nutrient cycling in a typical river floodplain ecosystem⁹.

Results and Discussion

As mentioned, the salicylic acid solution was prepared in a sodium hydroxide medium, which also contained sodium citrate. The optimum concentration of salicylic acid was 1.25% w/v (Fig. 2). When the concentration was doubled the signal was not affected, but the absorbance signals decreased with higher concentrations of salicylic acid. Searle⁴ also mentioned that a large excess of phenol may decrease the sensitivity.

The effect of the nitroprusside concentration was also evaluated. It is well known that nitroprusside is a good catalyst for Berthelot's reaction. For a solution containing 0.5 mg NH_4^+ /L, the absorbance signal increased only 15% when the nitroprusside concentration was doubled. The

blank signal also increased when the nitroprusside concentration was changed from 0.50% to 1.00% w/v. All other experiments were made fixing the nitroprusside concentration at 0.50% w/v. Considering only the dilution at the confluence points and using the equations proposed by Zagatto *et al.*¹⁰, it can be calculated that the nitroprusside concentration after the confluence point z is about 2.1 mM. This is very different from the optimum amount (*i.e.*, 0.07 M) of nitroprusside recommended by Kempers and Kok⁶. Such difference could be related with kinetic aspects of FIA and batch procedures. As previously mentioned by Kempers and Kok, the indophenol blue complex formation is greatly influenced by the preparation and timing of the addition of reagents.

The concentration of active chlorine also exerted a strong effect on indophenol blue complex formation (Fig. 3). The optimum concentration was 0.10% v/v in active chlorine. Solutions containing higher or lower concentrations of chlorine caused a decrease in sensitivity.

Finally, the effect of sodium hydroxide concentration on sensitivity was also investigated. The effect of sodium hydroxide was evaluated by varying the alkaline concentration of the R1 reagent. Better sensitivity was obtained using a 0.350 M NaOH solution.

The effects of the temperature and trapping period were studied. As expected, the sensitivity improved when either one or both parameters were increased. Hence, by increasing the water bath temperature of the trapping coil from 36 °C to 45 °C the absorbance signals increased 40%. However, gas evolution was more intense at higher temperatures, and a baseline drift was observed, probably due

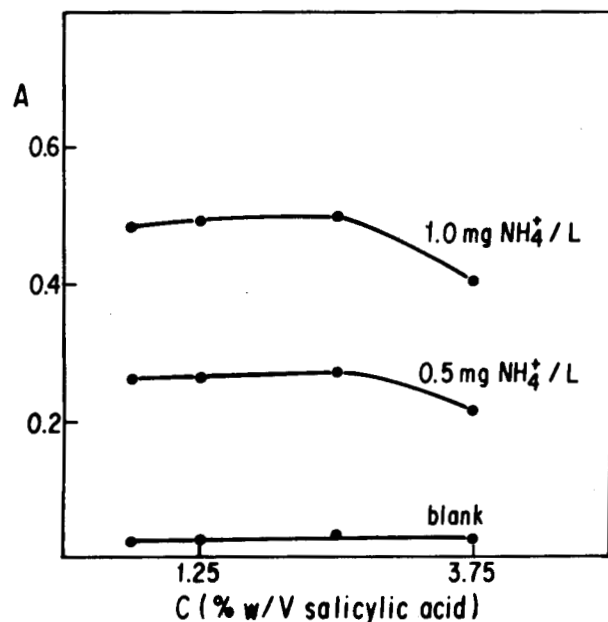


Figure 2. The effect of salicylic acid concentration on ammonium signals.

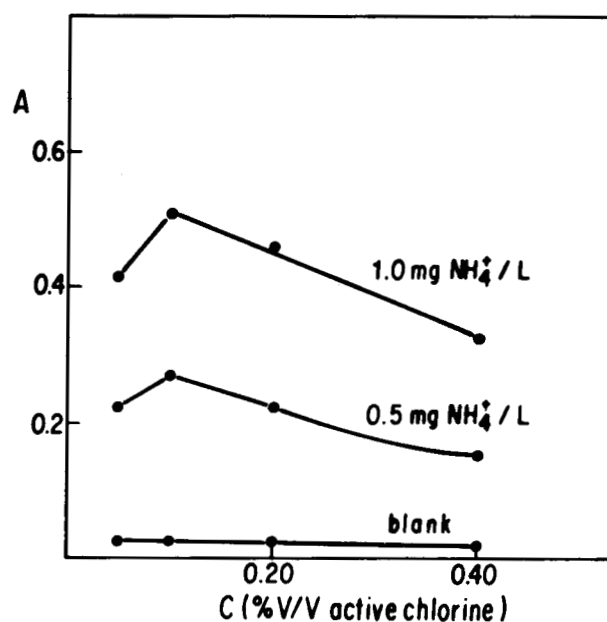


Figure 3. The effect of active chlorine concentration on ammonium signals.

to the accumulation of micro air bubbles in the flow cell. Thus, all measurements were made at 36 °C.

The effect of the trapping period was assessed by fixing this parameter at 32, 48, and 64 s. A twofold increment in the trapping period caused a 50% increase in the absorbance signals. However, this gain in sensitivity was accompanied by a decrease in the sample throughput.

Therefore, the ammonium determination in water samples was carried out using the optimum reagent concentrations described above, a trapping period of 48 s, and a trapping coil temperature of 36 °C.

An analytical curve was obtained using solutions containing from 10 to 160 ng NH₄⁺ / mL. The correlation coefficient was 0.9989 and the absorbance variation with ammonium concentration can be described by the equation:

$$A = 0.00042 C_{\text{ammonium}} + 0.0024$$

A detection limit of 7.1 ng NH₄⁺ / mL was experimentally determined using $3 S_{\text{blank}} / \text{slope}$.

The environmental significance of the ammonium levels determined in the aquatic ecosystem studied will not be discussed here. The water samples contained from 20.6 to 63.4 ng NH₄⁺ / mL. The relative standard deviation was better than 2.0% for all samples (n = 5). The results compare well (r = 0.9810) with those obtained using a colorimetric reagent containing phenol, as proposed by Krug et al.³, which is evidence that interferences did not occur when the salicylate reagent was used. The procedure can also be applied to ammonium determination in rain water.

Conclusion

Flow injection spectrophotometric determination of ammonium ions in natural water through indophenol blue

complex formation using salicylate is as accurate and precise as the method using phenol. However, the latter should be avoided because the analyst is exposed to toxic chemicals and the resulting solutions cannot be discarded without treatment.

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