Individual sample conditioning in flow analysis. Determination of N-total in plant materials

Abstract  A flow-batch system allowing in-line individual sample matrix matching is proposed for analysis of sample lots with high variability in acidity. The feasibility of the approach is demonstrated in the spectrophotometric determination of total nitrogen in Kjeldahl digests, using a column with a slightly soluble reagent (AgCl). The solutions are sequentially injected by means of an 8-port selecting valve and processed in a mixing chamber that is also used as a monitoring unit. The system yields reproducible results (r.s.d. usually < 2.5%) and the sampling rate is 14 samples/h. The analytical curve is linear within 1.00 and 6.00% N (dry basis), and the regression coefficient is > 0.999 (n = 6). Results are in agreement with certified values of standard reference materials and with results obtained by conductometry.

1 Introduction

Research in the field of flow analysis and the overall acceptance of the related procedures have undergone a continuous and exponential increase [1] leading to the proposal of novel concepts and innovations and to the commercial availability of advanced analyzers. Flow injection [2] and sequential injection [3] analyzers as well as modern systems exploiting several active devices in the manifold [4], miniaturized components [5], flow-batch chambers [6] and/or different flow patterns [7] are nowadays recognized world-wide as important tools for answering the ever increasing demand for chemical analyses.

A common feature inherent to flow analysis is that sample processing is generally rigidly fixed, which means that all samples from the lot are submitted to the same conditioning. There are situations, however, where every sample should be processed in a specific manner in view of its individual characteristics. This can be a limiting factor in the analyses of, e.g., sample batches with high variability in the bulk matrix composition.

In-line individual sample conditioning would reduce the required time for the analysis as an additional step of the entire analytical procedure is mechanized. Potentially more accurate results would be obtained, as limitations due to pronounced variability in sample matrix within the sample lot, losses of analyte by volatilization, and contamination effects would be reduced. The proposal is better implemented in a flow-batch analyzer [6], because the identity is maintained inside the flow-batch chamber, thus permitting the individual sample conditioning defined by a feedback procedure. Moreover, the intrinsic advantages of flow analysis such as high sampling rate, low consumption of sample and reagent, low cost, ease of automation, etc. are combined with the wide application range inherent to batch analysis.

In this work, a flow-batch system with in-line individual sample conditioning is proposed for the determination of N-total in plant materials. Since the Kjeldahl digests [8, 9] present high variability in acidity, their analyses usually require a prior sample neutralization step or the addition of highly concentrated buffer solutions [10–12]. The proposed system permits in-line acidity matching and ammonium determination. The latter stage involves passage of the processed sample through a AgCl(s) column, release of Ag⁺ ions as a consequence of diamino argentate(I) formation, addition of color forming reagents and spectrophotometric monitoring [12, 13]. In view of the potentialities of a sequential injection analyzer with a batch chamber [14], the proposed flow-batch system exploited the sequential injection strategy.

2 Experimental

2.1 Reagents and solutions. All solutions were prepared with chemicals of analytical grade quality and deionized water. The AgCl(s) was a commercial product from Synth (São Paulo, Brazil) and the particle size was selected by using 0.35 and 0.5 mm sieves [12].
R₁ reagent (Fig. 1) was a 1.6 x 10⁻⁴ mol/L bromophenol red (BPR) + 1.6 x 10⁻³ mol/L α-phenanthroline (phen) + 0.35 mol/L citric acid solution that could be used for at least four days if stored in a dark glass bottle. R₁ reagent was a 2.0 mol/L NaOH + 0.5 mol/L Na₂B₄O₇ solution. R₂ reagent was a 20 mg/mL thymolphthalein solution 50% (v/v) in ethanol. The sample carrier stream was a 0.25 mol/L Na₂B₄O₇ + 5.0 x 10⁻⁴ mol/L NaCl buffer solution with the pH adjusted to 10.8 with 10 mol/L NaOH.

The stock standard solution (1.333 g/L N) was prepared by dissolving 1.572 g (NH₄)₂SO₄ in 250 mL water. Working standards within the 26.6 and 159.6 mg/L N range (corresponding to 1.00–6.00% (w/w) N, dry basis) were prepared in 0.9 mol/L H₂SO₄.

The sample preparation involved a wet digestion with sulfuric acid and was similar to that already described [9].

### 2.2 The flow-batch system

The main component of the hybrid flow-batch system (Fig. 1) designed for individual conditioning of samples was a reaction chamber positioned inside the spectrophotometer. It was a 0.75-mL Perspex cylinder with a magnetic stirring bar (Fig. 2) positioned perpendicularly in relation to the light beam of a model 432 Femto spectrophotometer. Since monitoring was accomplished during sample processing, the chamber is referred to as a monitoring reaction chamber (MRC).

An Ismatec IPC-04 peristaltic pump was used with a 0.76-mm i.d. Teflon® pumping tube, and the manifold (holding coil and transmission lines) was constructed with 0.8 mm i.d. Tygon® tubing. The flow-batch system also included a Valco E8 8-channel selection valve with indication of port number, a Kynar® y-shaped connector and a magnetic stirrer.

An IBM/PC compatible microcomputer furnished with a PCL-711 interface card was used for controlling the pump, the 8-port selection valve and the magnetic stirrer, as well as for data acquisition and treatment. The program was developed in QuickBasic 4.5 and is available upon request.

The column was prepared by drilling a 15 × 4 mm hole inside a Perspex block, filling it with ca. 0.5 g AgCl, adding two screens at the extremities to avoid reagent losses, and covering it with two Perspex blocks with built-in connections. The components were tightly assembled together by means of screws and springs.

### 2.3 Procedure

The operation of the flow-batch system comprised three stages (Table 1): column/MRC cleaning (steps 1–6), pH adjustment (steps 7–10) and analysis itself (steps 15–21).

Steps 1–3 are related to column washing and steps 4–6 to MRC washing. After addition of sample/R₁ (thymolphthalein) aliquots to MRC (steps 7–9), R₂ (NaOH/Na₂B₄O₇) solution is aspirated (step 10) and initially propelled towards MRC during 2.5 s at a pump rotation speed of 60 (step 11). Under the acidic condition inherent to steps 10 and 11, the volume added to MRC does not cause any alteration in the thymolphthalein (pH-indicator) color and, therefore, a blank signal can be obtained. Next, the pump rotation speed is changed to 10, the signal monitoring begins, and the NaOH solution (R₂) is propelled until the signal reaches the pre-selected value (step 12). After the NaOH addition, the pH of the processed sample approaches 10.8, a suitable value for quantitative conversion of NH₄⁺ to NH₃ and to avoid column leaching [12]. The remaining R₂ solution is thereafter aspirated from the MRC channel link (step 13) and wasted (step 14) without flowing through the column. It should be stressed that the alkaline solution should not flow through the column because for pH >12 a dark powder (probably Ag₂O) can be formed in the column, reducing the lifetime of the column. Once the pH-adjustment stage is completed, the sample solution is aspirated towards the holding coil (step 15). Air is also aspirated from MRC after sample solution drainage, and therefore it is necessary to remove it (step 16) before sample pumping towards MRC through the column (step 17). Diamino argentate(l) is formed while the processed sample flows through the column. Thereafter, an R₁ (BPR/phen/citric acid) aliquot is sampled (step 18) and propelled towards MRC (step 19). Almost neutral conditions (pH ~7) are attained in view of the citric-acid addition, allowing the formation of the colored ternary complex, the chemistry involved being discussed elsewhere [15]. Next, the magnetic stirrer is turned off in order to permit a more stable analytical signal to be achieved. The processed sample is then discarded from MRC (steps 20 and 21).

It should be noted that solutions can be propelled towards MRC through two different ways, directly or passing through the column. A noteworthy feature of this system is that the sample flows through the column only after proper pH adjustment.

Sample digests of different acidity require different volumes of the R₂ reagent for proper neutralization, therefore, the sample digests inside MRC undergo different dilutions. Systematic errors due to the dilutions are avoided by propelling a constant volume of the processed sample through the column and further correcting the analytical signal as a function of dilution.

The strategy for obtaining a constant volume of the processed sample passing through the column is outlined in Fig. 3 that shows the flow patterns inside the holding coil (steps 14–17) related to two sample digests of different acidity. Examination of this figure reveals that a volume larger than the normal volume variation should be removed together with air (step 16) in order to obtain a constant volume of the processed sample to be propelled through the column (step 17). Regarding timing, the time interval for aspirating the processed sample from MRC (step 15) should be enough for complete emptying of the chamber plus transmission lines. With the high rotation speed inherent to step 16, a 4-s propulsion time is suitable to guarantee a constant sample volume through the AgCl(l) reactor. In this situation, a constant volume of the pro-