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Flow injection conductometric system with gas diffusion separation for the determination of Kjeldahl nitrogen in milk and chicken meat

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ABSTRACT

A simple flow injection (FI) conductometric system with gas diffusion separation was developed for the determination of Kjeldahl nitrogen (or proteins) in milk and chicken meat. The sample was digested according to the Kjeldahl standard method and the digest was diluted and directly injected into the donor stream consisting of 4 M NaOH. In alkaline medium, ammonium was converted to ammonia, which diffused through the PTFE membrane to dissolve in an acceptor stream (water). Dissociation of ammonia caused a change in conductance of the acceptor solution, which was linearly proportional to the concentration of ammonium originally present in the injected solution. A conductometric flow through cell and an amplifier circuit was fabricated, which helped improve sensitivity of the conductometric detection system. With using a plumbing Teflon tape as a gas diffusion membrane and without thermostating control of the system, a linear calibration graph in range of 10–100 mg L^{-1} N-NH_4 was obtained, with detection limit of 1 mg L^{-1} and good precision (relative standard deviation of 0.3% for 11 replicate injections of 50 mg L^{-1} N-NH_4). The developed method was validated by the standard Kjeldahl distillation/titration method for the analysis of milk and chicken meat samples. The proposed system had sample throughput of 35 h^{-1} and consumed much smaller amounts of chemical than the standard method (275 mg vs 17.5 g of NaOH per analysis, respectively).

1. Introduction

Proteins are essential for growth and survival of human and animals. Proteins in food are commonly found in peanuts, meat, poultry and seafood. Standard methods for determination of total proteins are based on Kjeldahl method [1] and Dumas method [2]. Both the methods involved the quantitative determination of total nitrogen contents in sample and then the protein contents were calculated using different multiplying factors suitable for different kind of samples [3]. The factors are needed in order to account for different amino acid sequences of different proteins.

The Kjeldahl method consists of the digestion by heating a substance with sulfuric acid to decompose the organic nitrogen to ammonium sulfate, and the distillation of the digest after being alkalized for titrimetric determination of the released ammonia nitrogen. Potassium sulfate is added in the digestion step in order to increase the boiling point of the medium. Some catalysts (mercuric oxide and copper sulfate) were added to speed up the decomposition. The digested

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solution is distilled with sodium hydroxide, which converts the ammonium to ammonia. The ammonia is trapped in an acidic solution, e.g., 4% (w/v) boric acid, and was determined by titration. Although the method is accurate, reproducible, and has been used for standardization of other methods, its drawbacks such as the need to use concentrated sulfuric acid at high temperature and the relatively long testing time (an hour or more), compare unfavorably with the Dumas method.

The Dumas method involved combustion of a sample of known mass in a high temperature (900 °C) chamber in the presence of oxygen. This leads to the release of carbon dioxide, water and nitrogen. The gases are passed over special columns that absorb the carbon dioxide and water, and then nitrogen was separated from residual carbon dioxide and water by another column before being measured by a thermal conductivity detector using helium as a carrier gas. The method is fast (taking a few minutes per measurement) and does not use toxic chemicals or catalysts. However, its instrument and operation costs are high. Both the Kjeldahl and Dumas methods do not give a measure of true protein, as they register non-protein nitrogen in addition. The Dumas method usually gives higher protein contents than the Kjeldahl method [4,5].

Flow injection (FI) technique has been applied to automate the distillation/determination step of the Kjeldahl method [6–9]. The FI methods help shorten analysis time, reduce chemical consumption, and provide more reproducible results with the easy to use, relatively low-cost automated instrumentation. The FI methods are based on separation of ammonium from the Kjeldahl digest by using gas diffusion membrane and then determination of ammonium by different detection techniques, e.g., UV–vis spectrophotometry [8], potentiometry [9], conductometry [6] and bulk acoustic wave-impedance sensor [7]. Although the conductometric detection is not selective, its high sensitivity, wide linear response to the analyte concentration and relatively simple instrumentation are attractive. By employing a gas diffusion membrane, selectivity can be extremely improved. However, gas diffusion efficiency is usually low (<40%), so high temperature with thermostat control is required to improve sensitivity and reproducibility [6,7,10]. Non-linear gas diffusion characteristic is observed [6], which results in a non-linear calibration graph to be used for quantification of ammonium. Potentiometric and bulk acoustic wave-impedance sensor also provides a non-linear response.

In this work, a simple FI conductometric (FIC) system with gas diffusion separation was developed for the determination of Kjeldahl nitrogen (or proteins). A conductometric flow through cell and an amplifier circuit were fabricated, which helped improve sensitivity of the conductometric detection system. Although a simple and low-cost plumbing Teflon tape was used as a gas diffusion membrane in a planar gas diffusion unit and without either using of thermostat bath or optimization for good gas diffusion efficiency, the system could provide a linear calibration graph in range of 10–100 mg L^{-1} $N\text{-}NH_4$, with detection limit of 1 mg L^{-1}, which was appropriate for determination of Kjeldahl nitrogen in food samples. A good precision (0.3% R.S.D. for 11 replicate injections of 50 mg L^{-1} $N\text{-}NH_4$) was achieved. Moreover, the system provided sample throughput of 35 h^{-1} and consumed very small amounts of NaOH (275 mg per analysis), which is much smaller than the standard Kjeldahl method. The proposed method was applied to the determination of Kjeldahl nitrogen in milk and chicken meat samples.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade. Deionized water (obtained by a system of Milli-Q, Millipore, Sweden) was used throughout. Stock standard solution of ammonium (1000 mg L^{-1} $N\text{-}NH_4$) was prepared by dissolving 0.3821 g of ammonium chloride (Merck, Germany) and adjusting the volume to 100 mL with water. Working standard solutions were daily prepared by diluting the stock solution with water. Sodium hydroxide solution (4 M) was prepared by dissolving 16.00 g NaOH (Merck, Germany) in 100 mL of water. The solutions used for the Kjeldahl technique were prepared according to the reference method [1].

2.2. Instrument

FI conductometric system (Fig. 1a) consisted of a peristaltic pump (Model ISM 935, Ismatec, Switzerland) with pump tubing, an injection valve (Upchurch, USA), a home-made gas diffusion unit (GDU) [11], a home-made flow through conductometric cell as depicted in Fig. 1(b), a conductometer (Model 712, Metrohm, Switzerland), a lab-built amplifying and data collecting unit, and a personal computer. The planar GDU was fabricated by drilling a groove of 1 mm wide × 30 cm long × 0.5 mm deep on each of 2 Perspex plastic blocks, inserting a PTFE membrane (a commercial PTFE (Teflon) tape for household and plumbing purposes) between the Perspex blocks to form two channels opposite to each other. The conductometric cell was built by drilling a Perspex block to form a channel of 1.5 mm i.d. for solution to flow in and out, and for inserting of two Cu wires (1 mm o.d.) to be used as the electrodes, as shown in Fig. 1(b). The amplifying and data collecting unit was built employing a Basic Stamp 2SX microcontroller (Parallex, USA), as similar to the previously reported one [12], excepting the new software based on Visual Basic 6.0 (Microsoft, USA) was used instead of Microsoft excel. The unit accepted the analog input signal in the range of 0–5 V. PTFE tubing of 0.5 mm i.d. was used to assemble the FI system, including a mixing coil (C1) and back pressure coils (C2 and C3).

A block digester instrument (Model DK6, VELP Scientifica, Italy) was used for Kjeldahl digestion and a steam distillation unit (Model UDK132, VELP Scientifica, Italy) was employed for ammonia distillation in the Kjeldahl standard method. Dumas method was performed by using an automated system (FP-528 Protein/Nitrogen Determinator, LECO, USA).

2.3. FI conductometric procedure

Using the FIC system as shown in Fig. 1(a), a standard/sample solution was injected via an injection valve into the stream of 4 M NaOH donor stream. Ammonia gas was produced during the injected zone flowed through a mixing coil (C1) to the GDU, where the gas diffused though a PTFE membrane to dissolve in