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Stepwise injection spectrophotometric determination of cysteine in biologically active supplements and fodders

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ABSTRACT

A stepwise injection analysis system was developed to monitor the concentration of L-cysteine in biologically active supplements (BAS) and fodders. It is based on the rapid redox reaction of L-cysteine with 18-molybdo-2-phospahte heteropoly anion (18-MPA) and the detection of the formed heteropoly blue with a spectrophotometry. The method has relatively wide optimal ranges of pH and 18-MPA concentration −5.0 to 7.3 and 0.4 to 2.0 mM, respectively. Under optimized operating conditions the performance of the SWIA system was linear up to a concentration of L-cysteine of 0.1 mM ($R^2 = 0.994$) with a detection limit of 0.003 mM and a sample frequency of 20 h^{-1}. The SWIA system was employed to determine the concentration of L-cysteine in BAS and fodder. The obtained data were in good agreement with those measured by a HPLC method.

1. Introduction

The biologically active food supplement market has been one of the fastest growing segments in the last several years. The number of registered biologically active food additives in Russia is now over 2000. The attractiveness of biologically active food supplements is due to the fact that they are relatively easy to develop and launch into production. This is one of the reasons why new analytical methods for their control have to be developed.

Cysteine and its salts are widely used as supplements (E 920) in food and agricultural products. Cysteine is a non-essential α-amino acid containing nonpolar sulphydryl (—SH) group that provides its participation in a variety of biochemical reactions. The sulphydryl group can be easily oxidized to form disulfide bonds that can be converted back to corresponding thiols through enzymatic activities and plays important structural roles in proteins [1]. The lack of cysteine is responsible for many kinds of different diseases. It causes slow hair growth, depigmentation, damage of liver and muscles. Increased levels of cysteine lead to the disturbance of normal brain function [2].

The methods reported up to now for the determination of cysteine include batch spectrophotometric [3–7], fluorimetric [8,9], chemiluminescence [10–12], HPLC [13–16], capillary electrophoresis [17,18], and electrochemical procedures [19–23]. These methods are generally laborious and time consuming. In addition, chromatographic procedures require expensive and complicated instrumentation that make them unattractive to routine analysis. For the FIA/SIA determination of cysteine different detection techniques, including electrochemical [24,25], chemiluminescent [10,26], enzymatic [27,28] and spectrophotometric [29–33] were used.

Lately, the significance of the Wells–Dawson heteropoly anion (HPA) 18-molybdo-2-phosphate $P_2Mo_{18}O_{62}^{6-}$ (18-MPA) for the determination of a number of reducing agents in batch and sequential or stepwise injection systems was shown [34–37]. It should be noted that the history of the application of HPAs in analysis began with intensive use of complexes having Wells–Dawson structure in biochemical analysis. The 18-tungsto-2-phosphate HPA (Folin uric acid reagent, phospho-18-tungstic acid) was for a long time one of the main reagent for the determination of cystine and cysteine [38–42].

Flow analysis techniques are the well-established analytical tool for solving problems of routine analysis and quality assurance control. Especially in quality control of pharmaceuticals or other samples with simple matrix such as biologically active supplements, flow analysis techniques with spectrophotometric detection can easily and effectively replace complicated and expensive chromatographic separation methods since the measured active ingredient usually exists in high concentrations and the common excipients cause no serious interferences.

Stepwise injection analysis (SWIA) is one of the universal solutions for the automation of analytical reactions in which the equilibration in the reaction is reached but dispersion of the reactants is prevented [36,43]. SWIA manifold is a hybrid analyzer exploiting characteristics of both flow and batch systems. It combines the advantages of automated control of flows such as high throughput, complete and precise control of reactant volumes and timings of operations, low cost, low consumption of the reagents and low effluent production with the flexibility and the versatility of mixing chamber (MC).

The scheme of the SWIA manifold is close to that for flow-batch analysis (FBA) system [44]. At the beginning of each analytical cycle,

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the sample solution as well as certain volumes of all of the reagent solutions specified by analytical procedure is sequentially supplied with the use of a peristaltic pump through corresponding channels of multiway valve into MC. As opposite to this, a multicommutation system consisting usually of three-way solenoid valves is built into the FBA manifold for directing the fluids towards the MC. On the other hand, similar to the SIA setup, in SWIA all chemistries are applied through a single-channel manifold. MC is the main component of the SWIA manifold providing intense and effective mixing of a reagent and sample solutions by the gas flow. The reaction mixture is kept in MC the time needed for the completion of all of the chemical and physical processes, including mixing, chemical reaction, extraction, etc. Magnetic stirrer is generally used in FBA systems for mixing but it complicates the configuration. In addition, using the MC greatly simplifies the execution of some analytical operations. For instance, gaseous samples can be analyzed without inclusion into the scheme of flow system any additional absorption devices [45–48]. The contents of the mixing (reaction) chamber are pumped through the flow detector using the reversible pump and directed to waste. All the instrumental parameters of SWIA manifold are computer controlled.

We have found that intensively colored heteropoly blue is formed rapidly and selectively in the reaction between cysteine and 18-MPA. The aim of this study was to develop simple, fast, and robust automated SWIA spectrophotometric method for the determination of cysteine in biologically active supplements and fodders which allows selective and sensitive determination of the analyte in a wide concentration range with minimum sample pretreatment and low operational cost.

2. Experimental

2.1. Reagents

Ammonium salt of 18-molybdo-2-phosphate HPA $(NH_4)_6P_2Mo_{18}O_{62}\times 14H_2O$ was synthesized according to the procedure described in Ref. [36]. About 0.01 M solution of 18-MPA was prepared by dissolving 0.7855 g of the synthesized salt and diluting to 25 mL with distilled water. The stock solutions of 0.01 M cysteine chloride hydrate (Senn Chemical, Switzerland) was daily prepared by dissolving accurately weighed amounts in 0.01 M HCl solution and stored in a refrigerator. The acetate buffer solution of pH 5.0 was used for adjusting the pH of the samples. All chemicals were of analytical-reagent grade.

2.2. Apparatus

The absorbance was measured by means of SHIMADZU UVmini-1240 (Shimadzu Scientific Instruments, Kyoto, Japan) spectrophotometer equipped with a standard quartz cells with an optical path length of 10 mm. The pH of solutions was measured by an I-500 potentiostat (Akvilon, Russia) using glass indicator electrode and Ag/AgCl as a reference electrode.

The SWIA manifold (Fig. 1) was based on a flow analyzer PIAKON-30-1 (Rosanalit, Saint-Petersburg, Russia). It included a bidirectional peristaltic pump ensuring a reverse flow, a six-port titanium valve, a mixing chamber which had cylindrical shape and was funnel-shaped at the bottom (glass tube 350 mm in height and 10 mm in inner diameter), a spectrophotometric detector with flow cell (optical path length of 5 cm), and communication tubes (PTFE, 0.5 mm in inner diameter). The analyzer was operated automatically by means of a computer.

2.3. Procedure for the SWIA determination of cysteine

According to the scheme of stepwise injection analysis (Fig. 1), at the first step, 0.2 mL of sample solution, 0.2 mL of acetate buffer solution (pH 5.0) and 0.2 mL of 4×10^{-4} M solution of 18-MPA were sequentially passed through multiway valve by means of peristaltic pump into MC. After that, the flow of argon gas was introduced into the system to mix reaction mixture in MC. The flow rate was maintained at 3.5 mL min^{-1}. After 60 s, the solution from MC was passed through port **e** by the reverse flow to the flow cell of spectrophotometric detector. Absorbance of analytical form was measured in stopped-flow mode. After measurement of analytical signal, the solution was passed away. At the next step, the washing with distilled water of hydraulic communications of the manifold and MC was performed. The measurement of analytical signal for blank solution was carried out by the above mentioned algorithm. But in this case instead of the sample solution, distilled water was passed through the port **f**. To provide the automated control of sequence and duration of all stages of analysis by personal computer, the operating program was written (Table 1).

2.4. Sample preparation

For the analysis of biologically active supplements, the content of one capsule was ground, then 0.35–0.40 g of obtained powder was dissolved in distilled water, transferred to a 25 mL volumetric flask, and the volume was adjusted to the mark with distilled water. The solution was then centrifuged at 5000 rpm for 15 min and filtered through a 0.45 μm membrane filter for separation of insoluble components of biologically active supplements. Before analysis, sample was tenfold diluted two or three times and appropriate aliquot of this solution was used for the analysis.

Sample preparation for the analysis of fodders included an acidic hydrolysis of samples [49,50]. The amount of sample (0.35–0.40 g)

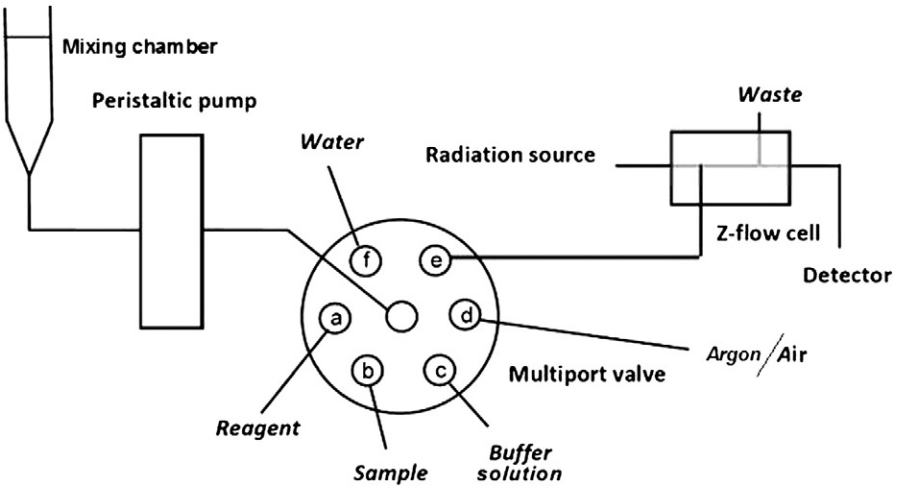

Fig. 1. The scheme of the manifold for SWIA determination of cysteine.