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Expanding the separation capability of sequential injection chromatography: Determination of melamine in milk exploiting micellar medium and on-line sample preparation ☆

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ABSTRACT

Silica, C8 and C18 monolithic column were the only option for separation on a sequential injection chromatography (SIC) system due its low-pressure operation. Therewith, the separation of molecules poorly retained by this stationary phase was a difficult task. This work presents the first use of micellar chromatography on a SIC system, with the applicability demonstrated by the determination of melamine in milk. The mobile phase was composed by aqueous sodium dodecyl sulfate (SDS) solution and propanol (92.5:7.5). The adsorption of SDS monomers on the C18 stationary phase modified its chromatographic properties, enabling satisfactory melamine separation from the milk components. The sample pretreatment procedure was on-line implemented by dilution of the sample with SDS using a multiposition valve. Calibration equations obtained from melamine solutions prepared in different types of milk and water presented similar sensitivity, indicating absence of matrix effects. A linear response was observed within 2.0 and 6.0 mg L^{-1} of melamine with a detection limit estimated at 0.6 mg L^{-1} and coefficients of variation at 2.9% ($n = 6$). The procedure was suitable for fast determination of melamine below the limits established by FDA and WHO (2.5 mg L^{-1}), with minimized reagent consumption. Results for different milk samples agreed with those obtained by high performance liquid chromatography at the 95% confidence level.

1. Introduction

Sequential injection chromatography (SIC) is based on sequential injection analysis systems coupled to short chromatographic monolithic columns to perform separations of simple mixtures, operating at low pressures (up to 250 psi) [1]. The main limitation of these systems was the lack of chemical variety of stationary phases, being silica, C8 and C18 the only commercially available options, which makes the separation of polar and closely related compounds a difficult task. Recently, the introduction of fused-core particle columns increased the applicability of SIC systems. They present similar symmetry and peak resolution to the monolithic columns but a lower height equivalent to a theoretical plate [2]. These columns can be operated at pressures higher than 250 psi but still lower than conventional columns and some chemically different stationary phases are available (e.g. amine, pentafluorophenylpropyl and phenyl-hexyl). Chocholouš et al. presented the use of fused-core particle columns with three different stationary phases (C18, amide and phenyl-hexyl) for the determination of phenolic acids [3]. The amide column provided the best chromatographic performance (peak symmetry ≤ 1.33, resolution ≥ 1.87, height equivalent to theoretical plate ≤ 10 μm), which demonstrates the potential of application of these new columns in comparison to conventional C18 monolithic phases used in SIC [3].

The chromatographic performance critically depends on chemical and physical characteristics of the stationary phase. The addition of surfactants to the mobile phase can change these properties, thus resulting on different selectivity to the analytes. This is due to the adsorption of the surfactant monomers to the stationary phase and to the formation of micelles in the mobile phase above the surfactant critical micellar concentration (CMC) [4]. The partitioning of the analyte between micelles, mobile and stationary phases depends on the nature and concentration of surfactants, as well as pH and ionic strength [5]. The use of aqueous surfactants solutions as mobile phases is a very attractive alternative due to their low toxicity, costs and environment impacts, although it is necessary the addition of small volume of an organic solvent to the micellar phase to improve resolution [6]. All these features make the micellar chromatography a good option to expand the separation capability of SIC. This artifice increases the possibilities to perform

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separations at low pressures of analytes that would be hardly achieved by the C18 phases of monolithic columns.

Melamine is a toxic triazine, used in the manufacture of plastics, resins, and adhesives because of their thermotolerant properties [7,8]. It is also used as a milk adulterant due to its high nitrogen content (66.7% *w*/*w*) and low cost [7]. The addition of melamine at 1% (*w*/*w*) increases the apparent protein content of feed in 4% [9]. Melamine has a low oral toxicity, but excessive exposure causes formation of kidney stones and bladder; children being more affected due to the immaturity of their organs [10]. In 2008 in China and the United States were found the main cases of melamine adulteration in milk and pet food, respectively, leading to death in children and small animals [11]. The World Health Organization [12] and the US Food and Drugs Administration [13] established daily intake limits for melamine by adults and children as 2.5 and 1.0 mg kg^{-1} of body weight, respectively, as well as the threshold limit of 2.5 mg L^{-1} in milk.

The adulteration of milk by melamine cannot be detected by conventional methods, such as Kjeldahl [14] and Dumas [15], which do not differentiate proteic and non-proteic nitrogen. Several procedures for determination of melamine in different matrices have been developed in which the main group is based on high performance liquid chromatography (HPLC) [16–21]. Other procedures have exploited capillary electrophoresis [16,22], cation-exchange chromatography [23], gas chromatography coupled to mass spectrometry [10], voltammetry [22], MALDI-TOF [24], chemiluminescence [25,26], immunoassays [27], Raman [7] or fluorescence [28] spectrometry. These procedures generally involve time-consuming sample preparation, including extraction and preconcentration steps, in addition to the high consumption of organic solvents.

The objective of this work was to expand the separation capability of SIC by exploiting micellar chromatography. The approach was applied for determination of melamine in milk exploiting on-line sample preparation.

2. Experimental

2.1. Apparatus

A SIChrom™ equipment (FIAlab Instruments®, Bellevue, WA, USA) with an 8-port high-pressure stainless-steel selection C5H valve (Valco Instrument Co., Houston, TX, USA) and an S17 PDP syringe pump (Sapphire™ Engineering, MA, USA) with a 4.0 mL reservoir was used to develop the present work. All tubes were of PEEK with 0.25 mm i.d. The detection system was a multi-channel CCD spectrophotometer (model USB4000, Ocean Optics®, Dunedin, FL, USA), with a deuterium light source (model DH-2000, Ocean Optics®) and optical fibers with a core diameter of 600 μm and SMA connectors ended (CeramOptec®, East Longmeadow, MA, USA). The spectrophotometer was coupled to a Z-flow cell with 9 μL inner volume and 20-mm optical path (FIAlab Instruments®). The SIC system was controlled by a microcomputer equipped with FIAlab® 5.9 software. The chromatographic separations were performed on a monolithic column (Phenomenex® Onyx™, monolithic C18, 50 mm length and 4.6 mm d.i.). A manometer was coupled to the SIC system for pressure monitoring during the chromatographic separations.

2.2. Chemicals and solutions

All solutions were prepared in ultrapure water (18 MΩ cm). Melamine stock solution (Merck, Germany) was prepared by dissolving 100.0 mg of the reagent in ca. 40 mL of water at 40 °C and diluting up to 100 mL. Reference working solutions were daily prepared from dilutions of the stock in milk or water. The evaluation of the chromatographic performance was carried out at isocratic elution conditions. The mobile phase was composed by 0.05 mol L^{-1} sodium dodecyl sulphate (Merck, Germany, 99% purity) prepared in 1.0 mmol L^{-1} phosphate buffer (Merck, Germany) at pH 3.0 plus 7.5% (*v*/*v*) 1-propanol (Merck, Germany). This solution was degassed before use by sonication for 5 min.

Different types of milk (whole, whole powdered, skimmed and semi-skimmed) were obtained at the local market. The powdered milk was prepared according to package instructions. Samples were analyzed without any pretreatment.

2.3. Procedure

The SIC system (Fig. 1) was operated according to the routine described in Table 1. Initially the pump aspirated the mobile phase (S_1) for conditioning the chromatographic column followed by the sequential aspiration of SDS (S_2), sample (S_3) and again SDS (10 μL each) to perform the on-line pretreatment. Then, the mixture was introduced into the monolithic column and detection was performed at 210 nm. Measurements were based on peak heights as in previous works with SIC [29] and carried out in triplicate. All experiments were performed at room temperature (25 °C).

2.4. Reference procedure

The reference procedure for accuracy assessment was based on micellar chromatography as previously proposed [11], except for a short C18 chromatographic column (0.46 × 10 cm, 3.5 μm) and calibration performed in a milk sample medium to compensate matrix effects.

3. Results and discussions

3.1. Chromatographic conditions

Melamine is a hydrophilic compound that is poorly retained by typical reversed-phase columns (e.g. C18 or C8) [30]. An alternative to perform the chromatographic separation is the micelle mediated separation, which changes the properties of the original stationary phase. The chromatographic evaluation was performed by using an SDS aqueous solution containing propanol and phosphate buffer as mobile phase, which had its concentration optimized to achieve acceptable chromatographic resolution between melamine and the matrix components.

Separation by micellar liquid chromatography is based on the same components of a reversed phase liquid chromatography system, a polar mobile phase and a non-polar stationary phase. However, two different media compose the mobile phase: the micellar aggregates and the aqueous–organic solution that contains surfactant at the CMC levels, forming a microscopically heterogeneous media as represented in Fig. 2. Additionally, the stationary phase is modified by the interaction between the carbon chains of the surfactant monomers and the

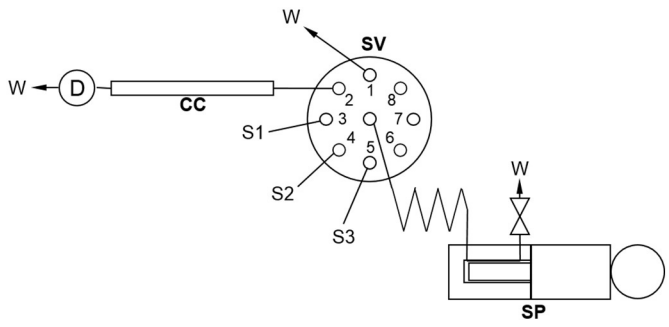

Fig. 1. Diagram of the SIC system for melamine determination. SV: selection valve; SP: syringe pump; CC: monolithic chromatographic column; D: detection system; S_1: mobile phase (0.05 mol L^{-1} SDS in 1.0 mmol L^{-1} phosphate buffer pH 3.0 plus 7.5% (*v*/*v*) 1-propanol); S_2: 0.2 mol L^{-1} SDS; S_3: milk samples or reference solutions; W: waste vessel.