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Determination of linear aliphatic aldehydes in heavy metal containing waters by high-performance liquid chromatography using 2,4-dinitrophenylhydrazine derivatization

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ABSTRACT

A simple and sensitive method is described for the determination of picomolar amounts of C_1–C_9 linear aliphatic aldehydes in waters containing heavy metal ions. In this method, aldehydes were first derivatized with 2,4-dinitrophenylhydrazine (DNPH) at optimized pH 1.8 for 30 min and analyzed by HPLC with UV detector at 365 nm. Factors affecting the derivatization reaction of aldehydes and DNPH were investigated. Cupric ion, an example of heavy metals, is a common oxidative reagent, which may oxidize DNPH and greatly interfere with the determination of aldehydes. EDTA was used to effectively mask the interferences by heavy metal ions. The method detection limits for direct injection of derivatized most aldehydes except formaldehyde were of the order of 7–28 nM. The detection limit can be further lowered by using off-line C_{18} adsorption cartridge enrichment. The recoveries of C_1–C_9 aldehydes were 93–115% with a relative standard deviation of 3.6–8.1% at the 0.1 μM level for aldehydes. The HPLC–DNPH method has been applied for determining aldehyde photoproducts from Cu(II)–amino acid complex systems.

1. Introduction

Quantitative determination of aldehydes is an important measurement because they are involved in several important biotic and abiotic processes in natural waters [1–4]. However, the analysis of aldehydes using HPLC is complicated by three factors. First, they often lack significant chromophores. Second, the lower molecular weight aldehydes are highly polar and therefore difficult to retain by reversed-phase HPLC columns. Third, aldehydes are highly reactive so there are quantification problems.

Derivatization overcomes these problems. Many of derivatization reagents for aldehydes have been introduced [5]. One of the most commonly used derivatization reagents is 2,4-dinitrophenylhydrazine (DNPH). Carbonyl groups of aldehydes react with an amino group of DNPH under acidic conditions forming the corresponding 2,4-dinitrophenylhydrazone (DNP-hydrazone) derivatives. The resulting DNPH derivatives have greatly increased UV sensitivity and improved hydrophobic retention, so they can be easily separated by reversed-phase liquid chromatography and detected using UV/visible spectrophotometry at wavelengths between 340 and 380 nm. In recent years, the DNPH–HPLC method has been established as the most widely used standard procedure for the analysis of aldehydes in ambient air [6], cigarette smoke [7], automotive exhaust [7,8], drinking water [9], alcoholic beverages [10,11], human saliva [12], and aquatic products [13].

A significant disturbance of the conventional method has been reported for measuring aldehydes by the presence of ozone [14,15]. Ozone is known to degrade both unreacted DNPH and the already formed DNP-hydrazones. Since hydrazine is easily oxidized by O_3, H_2O_2, or copper [16–18], the traditional method reported could be subject to analytical errors for the determination of picomolar amounts of aldehydes in real environmental samples containing heavy metal ions such as acid mine drainage, hydrothermal waters, and industrial wastewaters. However, interferences from heavy metal ions on the traditional method have not been reported.

One goal of our research is to investigate the photochemical reactions of copper(II)/amino acid complexes in aquatic systems. Photochemical reactions of Cu(II) complexes of organic ligands have significant impacts on the redox speciation of copper and the degradation of organic ligands in aquatic systems [19]. Photoproduction of Cu(I) from Cu(II)/dicarboxylate complexes has been observed in model solutions [20,21]. In nature waters, the highest concentrations of Cu(I) were found in surface water layers, since most ligands with carboxylate and amino functional groups form Cu(II)

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complexes that are photoreactive. Photochemical processes have been shown to cleave small moieties from chromophoric dissolved organic matter [22]. Small photoproducts that have been observed include carbon monoxide, carbon dioxide, ammonia, and carbonyl compounds. Among the carbonyl compounds, aldehydes are receiving increasing attention as pollutants and as intermediates in photochemical reactions since their photochemical production is closely correlated with their biological uptake in the natural waters [1]. To date, preliminary studies in our laboratory indicate that photoproduction of aldehydes from the Cu(II)/amino acid complex systems (with 2–50 mM amino acids and ionic strength 0.10 M) was observed under monochromatic radiation at 313 nm. To be able to assess the photoreactivity of Cu(II)/amino acid systems and characterize such photoreactions, the photoformation of aldehydes must be precisely determined under extreme conditions with high concentrations of Cu(II).

In this study, a method based on derivatization with DNPH was developed to determine picomolar amounts of C_1–C_9 linear aliphatic aldehydes in waters containing heavy metal ions. Factors affecting the derivatization reaction of aldehydes and DNPH in the method were optimized. Interference of heavy metal ions with the determination of aldehydes was discussed. We then applied this method to measure aldehyde photoproducts from Cu(II)–amino acid complex systems.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical or reagent grade, or the highest purity available from several suppliers. 2,4-Dinitrophenylhydrazine (>70%, Sigma, St. Louis, MO, USA) was recrystallized in hexane-dichloromethane with the ratio of 7 to 3 (v/v) prior to use [23]. Formaldehyde (36% in water/methanol), acetaldehyde (>98%), and L-alanine (>99%) were obtained from Fluka (Buchs, SG, Switzerland). Propanal (>98%), butanal (>98%), pentanal (>98%), hexanal (>98%), heptanal (>97%), octanal (>98%), and nonanal (>98%) were purchased from Merck (Darmstadt, Germany). Standard solutions of heavy metal ions including Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), and Pb(II) (1000 ppm in 0.5 M nitric acid) were from Merck. Na_2EDTA (>99%) was also from Merck. Sodium hydroxide, HCl (36.5–38.0%) and acetonitrile (HPLC degree) were from J.T. Baker (Phillipsburg, NJ, USA). Other reagents used were 2-nitrobenzaldehyde (>98%, Aldrich) and sodium chloride (>99.8%, Riedel-deHaën, Seelze, Germany).

Stock solutions of aldehydes except acetaldehyde were prepared in pure acetonitrile, each in 1.0 mM. The stock solutions were stored at 4 °C and stable for several weeks. The reliable concentration of acetaldehyde was difficult to be prepared due to its high volatility. Alternatively, hydrazone standard of acetaldehyde was prepared according to a procedure based on the work of Behforouz et al. [24]. For the synthesis of acetaldehyde derivative, approximate 0.2 mL acetaldehyde was placed in 5 mL vial with 200 mg DNPH and 1 mL HCl added. The mixture was shaken for at least 30 min to ensure complete reaction. Acetaldehyde hydrazone was purified and dried at 40 °C for further quantitative use. A 0.2 M solution of EDTA was prepared from Na_2EDTA. Extreme care is necessary to make low-level standards, freshly deionized water must be used and exposure to ambient air avoided. Such standards must be used immediately after preparation. Doubly deionized water prepared with a Milli-Q system (Millipore, Bedford, MA, USA) or doubly deionized-distilled water was used exclusively for all solutions ($\geq$18.2 MΩ cm resistivity). Solution pH was measured with a Radiometer analytical Ioncheck 45 m and combination glass electrode (Mettler Toledo Inlab 439/120). The pH of sample solutions was adjusted by adding aliquots of 1 or 0.1 M NaOH to the desired pH. The pH of the buffer was checked periodically and readjusted when necessary.

2.2. Apparatus

The binary gradient HPLC system consisted of the following components connected in series: a microvolume double-plunger pump (LC-9A, Shimatzu, Tokyo, Japan), a Rhenodyne (Cotati, CA, USA) Model 7125 injector with 100 μL loop, a guard column and a Beckman Ultrasphere C_{18} column (150 mm × 4.6 mm; 5 μm particle size), and a Spectra-Physics SP-8450 UV/vis detector set at 365 nm. The data were collected and processed by chromatographic data station software (SISC 32.3.1, Taiwan).

Ultraviolet–visible absorbance measurements were made with a double-beam scanning spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) and a custom-built constant-temperature (25 °C, BL-20, TIT recirculator) variable-path-length aluminum cuvette holder (black-anodized). Steady-state illuminations at 313 nm used a 1000 W O_3-free Hg–Xe lamp, a monochromator (entrance and exit slits = 2.5 mm; full band width at half peak height ≈ 7–8 nm; Spectral Energy Corp., Kratos, Schoeffel), two 2.5-mm Hoya UV-30 optical glass filters to filter light exiting the monochromator, and 20.0 mL of sample solution in a custom-built constant-temperature (25 °C, BL-20, TIT recirculator) variable-path-length aluminum cuvette holder (black-anodized) as described previously [20,21].

2.3. Analysis

The reagent solution was prepared according to Kieber and Mopper [1] by dissolving 4 mg DNPH in 4.0 mL of a solution containing concentrated HCl (~12 M), water, and acetonitrile in the ratio 1:2.5:0.5 (v/v/v) in 10 mL vial. Samples were not filtered prior to derivatization since filtration would result in unexpected increase of aldehyde concentrations, which has been observed in previous studies. 5 μL of 0.2 M solution of EDTA was added in 490 μL sample solution. The 495 μL solution was derivatized by adding 5 μL derivatizing reagent in 2 mL vial at pH 1.8. The vial was capped and shaken slowly, and the reaction was allowed to proceed for 30 min at ambient temperature. The HPLC system was operated with binary gradients consisting of acetonitrile and acidified water with H_2SO_4 at pH 3.0. The gradient was carried out with 45% acetonitrile for 1 min, increased linearly to 65% acetonitrile in 4 min, then increased linearly to 100% acetonitrile in 4 min, and eventually held for 7 min. The flow rate was 1.0 mL min^{-1}. The column was at ambient temperature.

Photochemical redox reactions of copper(II)/amino acid complexes were studied in N_2-purged aqueous solutions. After illuminated at 313 nm for 0, 2, 4, 6, 8, and 10 min, the photolyzed solutions were analyzed without any previous treatments or filtration.

3. Results and discussion

3.1. HPLC separation of hydrazones

Fig. 1 shows a typical liquid chromatogram for aldehyde standards (1.0 μM each). In the experimental conditions described above, excellent separation has been achieved for DNPH and nine linear aliphatic aldehydes in less than 18 min using a Beckman Ultrasphere C_{18} column. As expected, the most polar compound with the shortest alkyl group is eluted first, and the sequence of retention times parallels the increasing of hydrophobic alkyl groups. From the detail observation in Fig. 1, at least 5 min peaks appeared. These small peaks could be explained by the formation of Z-isomers of DNP-hydrazones from unsymmetrical carbonyl