Miniaturized and direct spectrophotometric multi-sample analysis of trace metals in natural waters

Gemma Albendín [a], José A. López-López [b, *], Juan J. Pinto [b]

[a] Laboratory of Toxicology Faculty of Marine and Environmental Sciences, Universidad de Cádiz, Cádiz 11510, Spain
[b] Department of Analytical Chemistry, Faculty of Marine and Environmenal Sciences, Universidad de Cádiz, Cádiz 11510, Spain

ARTICLE INFO

ABSTRACT

Trends in the analysis of trace metals in natural waters are mainly based on the development of sample treatment methods to isolate and pre-concentrate the metal from the matrix in a simpler extract for further instrumental analysis. However, direct analysis is often possible using more accessible techniques such as spectrophotometry. In this case a proper ligand is required to form a complex that absorbs radiation in the ultraviolet−visible (UV−Vis) spectrum. In this sense, the hydrazone derivative, di-2-pyridylketone benzoylhydrazone (dPKBH), forms complexes with copper (Cu) and vanadium (V) that absorb light at 370 and 395 nm, respectively. Although spectrophotometric methods are considered as time- and reagent-consuming, this work focused on its miniaturization by reducing the volume of sample as well as time and cost of analysis. In both methods, a micro-amount of sample is placed into a microplate reader with a capacity for 96 samples, which can be analyzed in times ranging from 5 to 10 min. The proposed methods have been optimized using a Box–Behnken design of experiments. For Cu determination, concentration of phosphate buffer solution at pH 8.33, masking agents (ammonium fluoride and sodium citrate), and dPKBH were optimized. For V analysis, sample (pH 4.5) was obtained using acetic acid/sodium acetate buffer, and masking agents were ammonium fluoride and 1,2-cyclohexanediaminetetraacetic acid. Under optimal conditions, both methods were applied to the analysis of certified reference materials TMDA-62 (lake water), LGC-6016 (estuarine water), and LGC-6019 (river water). In all cases, results proved the accuracy of the method.

© 2015 Elsevier Inc. All rights reserved.

During the past decades, monitoring trace metal concentrations in natural waters has gained increasing attention due to the effects that they present even at low concentration [1]. Some of them (e.g., zinc, nickel, copper, vanadium) can be considered as essential metals because they are needed for some biological processes, but they are toxic at higher concentration [2]. For this reason, important efforts have been made in the development of methods for their analysis in different natural water matrices [3].

Analysis of metal traces presents some drawbacks that are associated with the low concentration level they present in natural waters but also with the effects of sample matrices on the instrumental determination. Currently, most research is focused on development of sample preparation methods that allow isolation of the metal from the sample matrix as well as pre-concentrate the metal for instrumental analysis [3,4]. Nevertheless, alternatives that avoid the sample treatment step would be desirable.

Among the techniques that offer the possibility of direct analysis of trace metals, spectrophotometric methods have particular interest. They have been traditionally used for analysis of metals by complexation with an organic ligand [5]. However, in the case of analysis of natural waters, selection of a proper spectrophotometric reagent is of critical importance. In some cases, newly synthesized and non-commercial ligands are required to guarantee sufficient sensitivity [6,7]. This is the case of the hydrazone derivative di-2-pyridylketone benzoylhydrazone (dPKBH), which has been used in this work, as a reagent for the spectrophotometric analysis of traces of copper (Cu) and vanadium (V) [8,9]. However, these methods are normally time- and reagent-consuming. Recently, alternatives for traditional spectrophotometric analysis based on the use of microliter plate readers have renewed interest in spectrophotometry in environmental analysis [10].

The aim of this work was in miniaturization of spectrophotometric analysis of Cu and V that could be extended to other metals. To do this, a microliter plate reader has been used. In this way, samples can be arranged in the microliter plate and reagents can be added simultaneously using a multi-channel micropipette. Consequently, volume of sample required is reduced to a few microliters, the use of volumetric materials and laboratory facilities is minimized, and time of experiment can be shortened. In addition, the environmental profile of the method can be improved [11].

Multivariate optimization has been used to establish the chemical conditions for application of the methods [12]. In particular a Box−Behnken design of the experiment has been used for enhancement of the response of Cu and V complexation by dPKBH and to identify possible synergies between the factors affecting the response [13]. Finally, both methods have been successfully applied for the determination of Cu and V in natural water samples.

Materials and methods

Reagents and solutions

Unless otherwise stated, all reagents were analytical reagent grade. Sodium citrate (99.0%), ammonium fluoride (99.0%), 1,2-cyclohexanediaminetetraacetic acid (CDTA, 99%), potassium phosphate dihydrate (99.0%), sodium phosphate monohydrate (99.0%), acetic acid (99.0%), and sodium acetate (99.0%) were purchased from Merck (Darmstadt, Germany). Standards of Cu and V used for calibration were prepared daily by dilution from a commercial stock solution of 1000 mg L^{-1} (Merck). Solutions were prepared using high-purity deionized water with resistivity lower than 18.2 MΩ cm (Milli-Q, Millipore, USA).

Certified reference material for analysis of metals in lake water (TMDA-62) was obtained from the National Water Research Institute (Canada). Certified reference materials for estuarine (LGC-6016) and river water (LGC-6019) analysis were obtained from the Measurement Standards Laboratory (New Zealand).

The spectrophotometric reagent dPKBH was synthesized from an equimolar mixture of di-2-piridyl ketone (99%) and benzoyl-hydrazide (99%) dissolved in ethanol. The synthesis procedure of dPKBH can be found elsewhere [14].

Buffer solutions of Na_2HPO_4/KH_2PO_4 (pH 8.33) and $CH_3COOH/NaCH_3COO$ (pH 4.55) were prepared to adjust sample pH for the spectrophotometric determination of Cu and V, respectively.

Apparatus

Spectrophotometric signal was measured using the ultraviolet−visible (UV−Vis) microplate reader Benchmark Plus (Bio-Rad, USA). This spectrophotometer is suitable for measurement of microplates containing up to 96 samples that can be measured simultaneously. The Cu−dPKBH complex was measured at a wavelength 370 nm, and the V−dPKBH complex was measured at a wavelength 395 nm [8,9]. Sample pH was measured using a Jenway 4330 conductivity and pH meter (Jenway, UK).

Procedure

Water samples were dropped into their corresponding vessels in the microplate. For Cu analysis the sample volume was 210 µl, and for V analysis the sample volume was 227 µl. Once water samples were placed in the microplate, the appropriate volumes of buffer solution, masking agents, and dPKBH (in that order) were added onto the samples using a multi-channel micropipette. Next, the microplate was placed inside the microplate reader autosampler,

and absorbances of the samples at 370 nm for Cu determination and at 395 nm for V determination were registered simultaneously.

Optimization

Multivariate optimization using a response surface method (RSM) was applied for enhancement of the absorbance of the Cu−dPKBH and V−dPKBH complexes obtained [13]. In both cases, a Box−Behnken experimental design was developed. This experimental design allows taking into account the possibility of interaction between factors as well as the existence of curvature in the response. All calculations were carried out using the software Statgraphics 5.1 (Statpoint Technologies, USA).

The Box−Behnken design of experiments involves a three-level factorial design in which only the first- and second-order coefficients of the mathematical model are selected. Curvature is represented using a number of center points (N_c). This simplifies the number of experiments to be carried out, showing similar efficiency as the three-level factorial design. The number of experiments required to complete a Box−Behnken design is represented by Eq. (1), where N_c is the number of center points and n is the number of factors [12]:

$$2n(n-1) + N_c \tag{1}$$

As a result, a mathematical model that represents the experimental analytical response as a function of the factors can be obtained. This model can be used to calculate the optimal values of the factors. In the optimization of each method, absorbance was selected as the response and buffer concentration, reagent concentration, and concentration of masking agents to avoid interferences in the analytical signal were selected as the factors to be optimized. Finally, normalized analysis of variance (ANOVA) was used to evaluate the fit of the model to the experimental response. ANOVA was also used to establish which factors presented a significant effect on the signal, and results were summarized in a Pareto chart [15].

A seawater sample from the Mediterranean Sea (in north Morocco) spiked with 20 µg L^{-1} of Cu and V ions was used for optimization. This way, it was ensured that an appropriate instrumental signal would be obtained when real conditions were taken into account during optimization. Prior to optimization, study of interferences was carried out and masking agents were selected to ensure selectivity of the methods.

Results and discussion

Optimization of Cu−dPKBH signal

Complex formation between Cu and dPKBH is favored at slightly basic conditions (pH 8.33). For this reason, Na_2HPO_4/KH_2PO_4 buffer was used to keep the pH constant. In addition, sodium citrate and NH_4F were used to control the effect of chemical interferences on the analytical signal, according to the results reported previously [8]. The spectrophotometric reagent, dPKBH, was dissolved in ethanol and was added to adjust the final ethanol concentration in the sample at 10% (v/v), as described in previous work [8].

For Box−Behnken design, buffer, dPKBH, and concentration of masking agents were optimized using 3 center points. In consequence, a total of 27 experiments were carried out. Lower and higher values of the factors were selected based on previous information regarding the application of dPKBH to the complexation of copper ions [8] (Table 1). Two replicates of every experiment were executed, and a mathematical model that fitted the 87% of the experimental response was obtained (Eq. (2)):