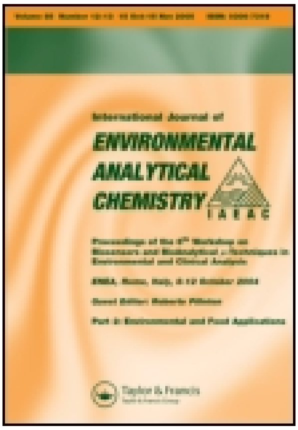

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/geac20

A pre-concentration procedure employing a new imprinted polymer for the determination of copper in water

Mostafa Khajeh [a] & Esmael Sanchooli [a]

[a] Department of Chemistry, University of Zabol, PO Box 98615-538, Zabol, Iran

To cite this article: Mostafa Khajeh & Esmael Sanchooli (2011): A pre-concentration procedure employing a new imprinted polymer for the determination of copper in water, International Journal of Environmental Analytical Chemistry, 91:13, 1310-1319

To link to this article: http://dx.doi.org/10.1080/03067310903020334

Intern. J. Environ. Anal. Chem.
Vol. 91, No. 13, November 2011, 1310–1319

A pre-concentration procedure employing a new imprinted polymer for the determination of copper in water

Mostafa Khajeh* and Esmael Sanchooli

Department of Chemistry, University of Zabol, PO Box 98615-538, Zabol, Iran

(*Received 7 March 2009; final version received 5 May 2009*)

This work describes the development by response surface methodology (RSM) of a procedure for copper determination by inductively coupled plasma optical emission spectrometry (ICP OES) in water samples after extraction by copper imprinted polymer. Results of the two-level full factorial design (2^4) based on an analysis of variance demonstrated that only the solution pH; amount of polymer and adsorption time were statistically significant. Optimal conditions for the extraction of copper samples were obtained by using Box-Behnken design. Solution pH; amount of polymer and adsorption time were regarded as factors in the optimisation study. The working conditions were 4.6, 0.03 g and 3.5 h, for solution pH, amount of polymer; and adsorption time, respectively. Under the optimised experimental conditions, the detection limit of the proposed method followed by ICP OES was found to be 0.8 $\mu g L^{-1}$. The relative standard deviation (RSD) was found to less than 0.81%. The pre-concentration factor was 22.5. The accuracy of the optimised procedure was evaluated by analysis of certified reference material. The method was applied to the determination of copper in water samples.

Keywords: copper; Box-Behnken design; imprinted polymer; water samples

1. Introduction

Copper is an environmental pollutant, which has been tested and assessed over the past few years from both the toxicological and human health viewpoints [1]. While the slight quantity of copper is essential for normal physiological processes, the extra amount causes a significant threat to human health. The maximum tolerable daily intake for copper is 0.5 $mg kg^{-1}$ body weight [2]. The development of new methods for quantifying trace metals is required. In some common procedures for the determination of low concentrations of heavy metals a pre-concentration step is needed before performing determination. The traditional separation and pre-concentration methods for metal ions include liquid–liquid extraction, coprecipitation, ion exchange, etc. These methods often require large amount of high-purity organic solvents, some of which are harmful to health and cause environmental problems [3].

The molecular imprinting technique is a specific chemical procedure for the generation of explicit nano-cavities that can mimic the behaviour (in terms of binding) of naturally occurring receptor sites, and is suitable for this purpose. One of the foremost applications of molecular imprinting has been in solid phase extraction. In theory, molecular

---

*Corresponding author. Email: m_khajeh@uoz.ac.ir

ISSN 0306–7319 print/ISSN 1029–0397 online

http://dx.doi.org/10.1080/03067310903020334
http://www.tandfonline.com

imprinting provides greatly increased selectivity and sensitivity over conventional solid phase extraction (SPE) packing materials. Furthermore due to the specific nature of the interactions between rebinding template and the chemical functionalities within the pores or cavities of the cross linked polymer matrix, it is expected that molecularly imprinted solid phase extraction has the ability to discriminate between closely related compounds [4]. Lemaire *et al.* [5,6] developed ion imprinted resins based on EDTA and DTPA derivatives for the selective separation of Gd from La, Nd, Eu and Lu ions. Uezu *et al.* [7] reported that post irradiation of imprinted polymer particles by γ-irradiation resulted in better separation of Zn compared with copper. Biju *et al.* [8] reported effect of γ-irradiation of ion imprinted polymer particles for the pre-concentrative separation of dysprosium.

The use of multivariate experimental design techniques is becoming increasingly widespread in analytical chemistry. Multivariate designs, which allow the simultaneous optimisation of several control variables, are faster to implement and more cost-effective than traditional univariate approaches [9]. One of the most popular multivariate designs is two-level full (or fractional) factorial, in which every factor is experimentally studied at only two levels. Due to their simplicity and relatively low cost, full factorial designs are very useful for preliminary studies or in the initial steps of an optimisation, while fractional designs are almost mandatory when the problem involves a large number of factors [10]. On the other hand, since only two levels are used, the models that can be fitted to these designs are somewhat restricted. If a more sophisticated model is required, as for the location of an optimum set of experimental conditions, then one must resort to augment response surface designs, which employ more than two factor levels. Among these, Box-Behnken is a second-order multivariate design based on three-level incomplete factorial designs that received widespread application for assessment of critical experimental conditions, that is, maximum or minimum of response functions. The number of experiments (N) required for the development of this design is defined as $N = 2k(k-1) + C_0$, where (k) is the factor number and (C_0) is the replicate number of the central point [11–17].

This work proposes a pre-concentration procedure using copper imprinted polymer for determination of copper in water samples by inductively coupled plasma optical emission spectrometry (ICP OES). The optimisation of the pre-concentration procedure is performed exploiting the Box-Behnken design.

2. Experimental

2.1 *Apparatus*

The measurements were performed with a simultaneous inductively coupled plasma optical emission spectrometry (ICP OES, Varian Vista-Pro, Springvale, Australia) coupled to a V-groove nebuliser and equipped with a charge coupled device (CCD). The ICP conditions are shown in Table 1. The pH was determined with a model 630 Metrohm pH meter with combined glass-calomel electrode.

2.2 *Materials*

VP, EDMA and AIBN were obtained from Aldrich (Milwaukee, WI, USA). All acids, methanol and EDTA used were of the highest purity available from

Table 1. ICP OES instrumental conditions.

Parameter	Value
RF generator power (kW)	1.5
Plasma gas flow rate ($L\,min^{-1}$)	15.0
Auxiliary gas flow rate ($L\,min^{-1}$)	1.5
Frequency of RF generator (MHz)	40.0
Observation high (mm)	8.0
Nebuliser pressure (KPa)	240
Wavelength (nm)	324.754

Merck (Darmstadt, Germany) and used as received. Reagent grade CuSO4 and nitrate or chloride salts of other cations (all from Merck) were of the highest purity available and were used without any further purification. Reagent grade morin (Merck) was used as received. Nitrogen (99.99% purity), contained in a cylinder, was obtained from Sabalan Co. (Tehran, Iran). Stock solution of Cu ($1000\,mg\,L^{-1}$) was prepared by dissolving the proper amount of $CuSO_4$ in doubly distilled water in a 50 mL flask and diluting to the mark with water. Dilute solutions were prepared by appropriate dilution of the stock solution in doubly distilled water.

2.3 *Preparation of polymer*

The following procedure was used for synthesising the polymer: 0.24 g of $CuSO_4$ and 0.2 g of morin were weighed in a glass tube and dissolved in 15 mL of methanol. Four mmol of VP, 20 mmol of EDMA and 50 mg of AIBN were added to the solution. The oxygen of the solution was removed by bubbling of nitrogen through it for 10 min, then frozen with liquid nitrogen and sealed under vacuum. The polymerisation reaction was performed in a water bath at 60°C for 10 h. The synthesised polymer was ground in a mortar, dried and sieved to get particles with diameters in the range of 55–75 µm. Fine particles were removed by suspending polymer beads in methanol and decantation of methanol for three times. To remove templates, the remaining particles were treated with $2\,mol\,L^{-1}$ of HCl for 72 h, and the excess amount of HCl was washed by methanol. For confidence of complete removal of template from polymer, the concentration of copper ions in aqueous phases after desired treatment periods were measured by ICP OES. Finally the particles were dried at 80°C. Figure 1 schematically shows the synthesis of molecular imprinted polymer.

2.4 *Adsorption and desorption studies*

Adsorption of copper from aqueous solutions was investigated in batch experiments. Adsorptions were performed in test tubes containing 12.5 µg copper ions and 50 µg morin in 50 mL deionised water. According to a preliminary experimental design, the pH was adjusted (2.5, 4.0 and 5.5) by drop-wise addition of $1\,mol\,L^{-1}$ sodium hydroxide and $1\,mol\,L^{-1}$ hydrochloric acid; and appropriate imprinted polymer were added to solutions. After that, the mixture was shaken for an appropriate time (2.5 to 5.5 h). Finally, the concentrations of copper ion in aqueous phases after desired treatment periods were measured by ICP OES. The instrument response was periodically checked with known

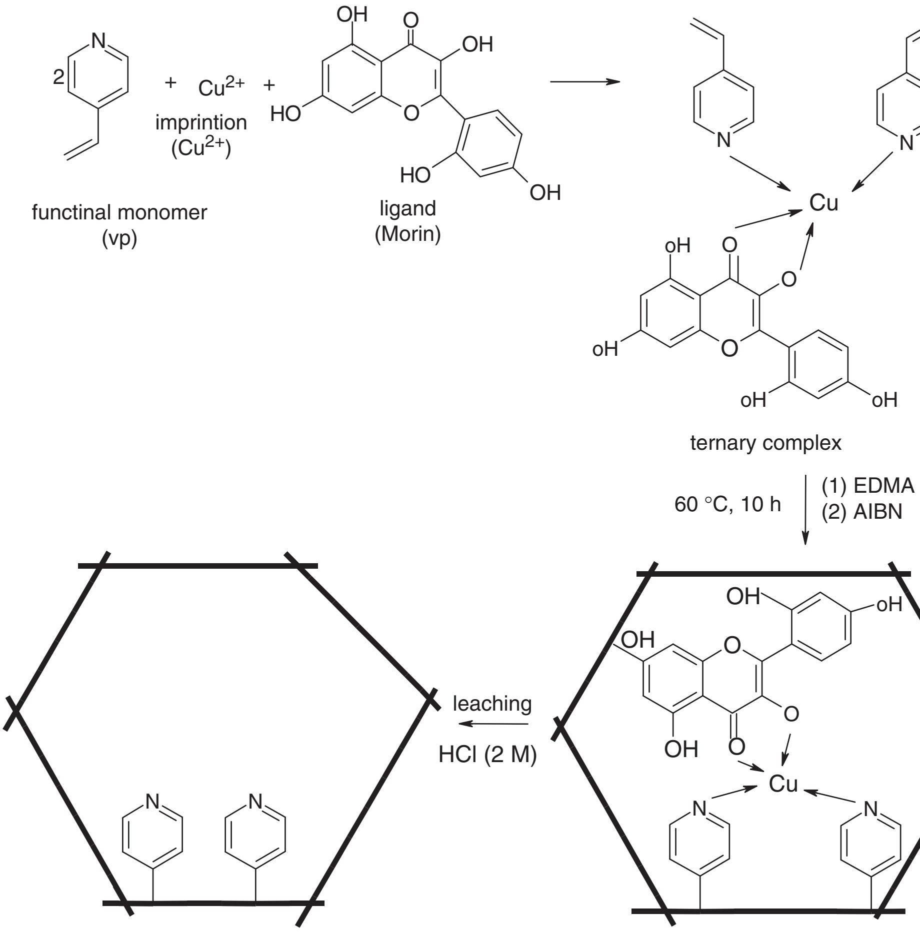

Figure 1. Preparation of ion imprinted polymer material.

copper standard solutions. Three replicate extractions and measurements were performed for each aqueous solution. Percentage extraction of Cu was calculated from this equation:

$$\%\text{Extraction} = (\mathrm{C_0} - \mathrm{C_f}/\mathrm{C_0}) \times 100 \tag{1}$$

where $\mathrm{C_0}$ and $\mathrm{C_f}$ are the concentrations of Cu ion before and after extraction in the solution, respectively.

The phase distribution ratio (D) was calculated using the following equation:

$$\mathrm{D} = \left(\frac{\mathrm{C_0} - \mathrm{C_f}}{\mathrm{C_f}}\right)(V/W) \tag{2}$$

where W is the weight of the polymer in g and V is the volume of the aqueous phase in mL, respectively.

The adsorbed copper complex was desorbed from the copper ions imprinted polymer (IIP) by putting washed polymer particles into a 10 mL of 0.5 mol $\mathrm{L^{-1}}$ EDTA. The imprinted polymer containing Cu were placed in the desorption medium and stirred continuously at 600 rpm at room temperature for 8 h. The final copper concentration in the aqueous phase was determined by ICP OES. The unbound copper amount to the polymer was obtained by subtracting the copper bound amount to the polymer from that of the initial copper loaded to the polymer.

2.5 *Factorial design*

A two level factorial 2^4 design with two replicates of centre point was carried out in order to determine the influence of these factors (pH, amount of polymer, amount of ligand and adsorption time) as their interactions. The factorial design was evaluated using analytical response (A) of copper. The experimental design matrix and the A for each trial are shown in Table 2. An analysis of the variance (ANOVA) demonstrated that, within the experimental range, only pH, amount of polymer (W) and adsorption time (t) were statistically significant as the Pareto chart (Figure 2) interprets it. The results of the Pareto chart demonstrated that the variables pH, amount of polymer and adsorption time are statistically significant. The positive value for the effect of the adsorption time indicated that, the extraction was increased as the adsorption time increases at the levels of interest. The negative values for the effect of the pH and amount of polymer revealed that the extraction was decreased as these variables increase at the levels of interest.

2.6 *Box-Behnken design*

The significant variables like pH; W and t were chosen as the critical variables and designated as X_1, X_2 and X_3 respectively. The low, middle, and high levels of each variable

Table 2. Experimental design: The results obtained in function of the copper analytical signal (A).

Adsorption time (h)	Amount of polymer (g)	pH	Amount of ligand (g)
−1 (2.0)	−1 (0.02)	−1 (3.5)	−1 (1.0)
1 (4.0)	1 (0.04)	1 (5.5)	1 (2.0)

No.	Adsorption time (h)	Amount of polymer (g)	pH	Amount of ligand (g)	A
1	2	0.02	3.5	1	0.03242
2	4	0.02	3.5	1	0.03401
3	2	0.04	3.5	1	0.03426
4	4	0.04	3.5	1	0.03173
5	2	0.02	5.5	1	0.03311
6	4	0.02	5.5	1	0.03034
7	2	0.04	5.5	1	0.03043
8	4	0.04	5.5	1	0.03334
9	2	0.02	3.5	2	0.03174
10	4	0.02	3.5	2	0.04471
11	2	0.04	3.5	2	0.03131
12	4	0.04	3.5	2	0.03359
13	2	0.02	5.5	2	0.02822
14	4	0.02	5.5	2	0.03632
15	2	0.04	5.5	2	0.01744
16	4	0.04	5.5	2	0.02549
17	3	0.03	4.5	1.5	0.03161
18	3	0.03	4.5	1.5	0.03179

were designated as –, 0, and + respectively. The actual design of experiments is given in Table 3.

In a system involving three significant independent variables X_1, X_2, and X_3 the mathematical relationship of the response on these variables can be approximated by the quadratic (second degree) polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (3)$$

where

Y = estimate response,
β_0 = constant,
β_1, β_2 and β_3 = linear coefficients,
β_{12}, β_{13} and β_{23} = interaction coefficients between the three factors,
β_{11}, β_{22} and β_{33} = quadratic coefficients.

A multiple regression analysis is done to obtain the coefficients and the equation can be used to predict the response. The degree of experiments chosen for this study was Box-Behnken, a fractional factorial design for three independent variables. It is applicable once the critical variables have been identified. In the model given in Equation (3), interactions higher than second order have been neglected. A total of 15 experiments were necessary to estimate of the full model.

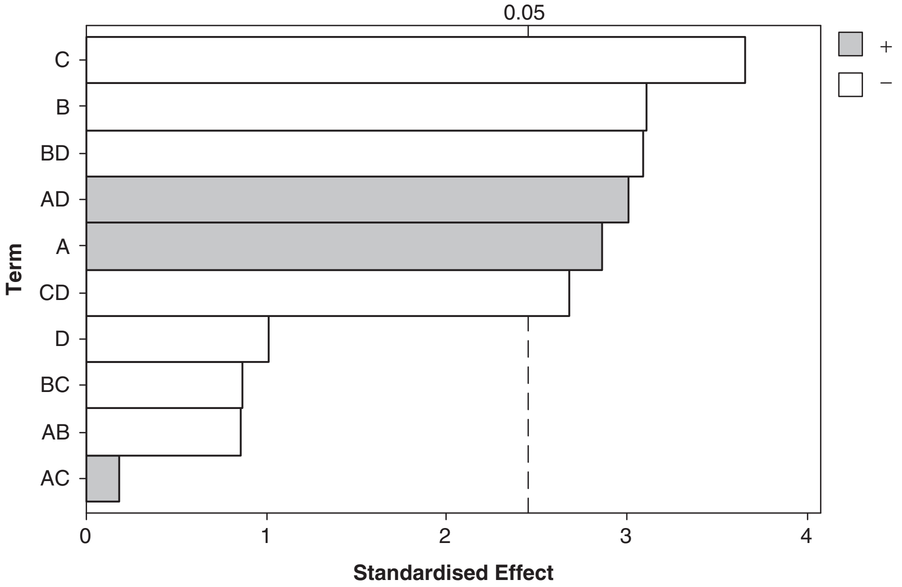

Figure 2. Pareto chart of main effects obtained from 2^4 full factorial designs. The vertical line defines the 95% confidence interval (A = Adsorption time, B = Amount of polymer, C = pH and D = Amount of ligand).

Table 3. Variable levels used for the trials and the Box-Behnken design for the optimization of the IIP.

pH	Amount of polymer (g)	Adsorption time (h)
2.5 (−1)	0.02 (−1)	2.5 (−1)
4.0 (0)	0.03 (0)	4.0 (0)
5.5 (+1)	0.04 (+1)	5.5 (+1)

Trial No.	pH	Amount of polymer (g)	Adsorption time (h)	A (Cu)
1	2.5	0.02	4.0	0.0120
2	5.5	0.02	4.0	0.0332
3	2.5	0.04	4.0	0.0130
4	5.5	0.04	4.0	0.0349
5	2.5	0.03	2.5	0.0122
6	5.5	0.03	2.5	0.0372
7	2.5	0.03	5.5	0.0270
8	5.5	0.03	5.5	0.0488
9	4.0	0.02	2.5	0.0401
10	4.0	0.04	2.5	0.0401
11	4.0	0.02	5.5	0.0486
12	4.0	0.04	5.5	0.0489
13	4.0	0.03	4.0	0.0385
14	4.0	0.03	4.0	0.0379
15	4.0	0.03	4.0	0.0391

3. Results and discussion

3.1 *Optimisation of the pre-concentration procedure*

The optimisation step of the IIP procedure was performed using a Box-Behnken design.

The number of experiments required to investigate the previously noted three parameters at three levels would be 27 (3^3). However, this was reduced to 15 using a Box-Behnken experimental design. The results from this limited number of experiments provided a statistical model, which was used to identify high yield trends for the extraction process. Table 4 shows the matrix and the responses as analytical signals for copper. The equations below illustrate the relationship of the three variables, that is, pH, W, t and analytical response (A).

$$\begin{aligned} \mathrm{A} = {} & -0.0715 + 0.059(\mathrm{pH}) + 0.6008(\mathrm{W}) - 0.0199(\mathrm{t}) \\ & - 0.0063(\mathrm{pH})^2 - 10.5(\mathrm{W})^2 + 0.0031(\mathrm{t})^2 \\ & + 0.0117(\mathrm{pH})(\mathrm{W}) - 0.0004(\mathrm{pH})(\mathrm{t}) + 0.005(\mathrm{W})(\mathrm{t}) \end{aligned} \tag{4}$$

The response surfaces for the variables of pH, W and t in equation (4) are shown in Figure 3. The derivation of this general equation as (pH), (W) and (t) results in three new equations:

$$\frac{\delta(A)}{\delta(pH)} = 0.059 - 0.0126(\mathrm{pH}) + 0.0117(\mathrm{W}) - 0.0004(\mathrm{t}) \tag{5}$$

Table 4. Selectivity factors and distribution ratios of copper.

Foreign ion	Distribution ratio (D)	Selectivity factor (α)
Cu^{2+}	16	–
Cd^{2+}	0.78	20.5
Zn^{2+}	1.3	12.3
Ni^{2+}	0.95	16.8
Pb^{2+}	0.67	23.9
Co^{2+}	0.84	19.0

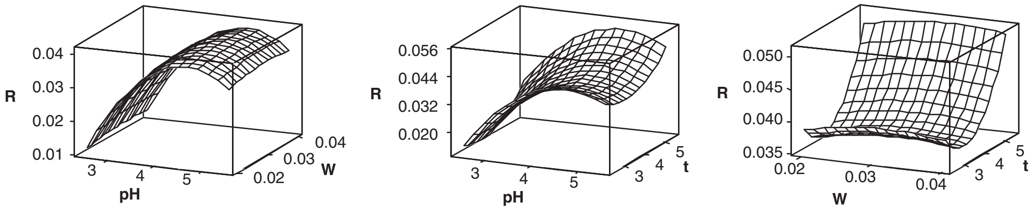

Figure 3. Three-dimensional response surface plot for the effect of pH, W and t vs. analytical response.

$$\frac{\delta(A)}{\delta(W)} = 0.6008 + 0.0117(\mathrm{pH}) - 21.0(\mathrm{W}) + 0.005(\mathrm{t}) \tag{6}$$

$$\frac{\delta(A)}{\delta(t)} = -0.0119 - 0.0004(\mathrm{pH}) + 0.005(\mathrm{W}) + 0.0062(\mathrm{t}) \tag{7}$$

The critical point in the surface response are found by solving these equation systems for the condition of $\delta(A)/\delta(pH) = 0$, $\delta(A)/\delta(W) = 0$ and $\delta(A)/\delta(t) = 0$. The way of calculating these critical points has been published previously [18].

The calculated values for the critical point are as follows: pH = 4.6, W = 0.03 g and t = 3.5 h. The effect of pH on the extraction of copper ions was studied. Results showed that the percentage extraction was nearly constant and quantitative in the pH of 4.6. At the pH values below 4.6, however, the percentage recovery was decreased. The competitive adsorption of hydrogen ions with copper ions for morin groups at lower pH values accounts to the observed low efficiency.

3.2 *Analytical performance*

Under the optimum conditions described, the calibration curve was linear over the concentration range from 10–200 $\mu g\,L^{-1}$. The least square equation at above dynamic linear range was as follows:

$$A = 0.0041\ C\ (\mu g L^{-1}) - 0.0054 \quad (r^2 = 0.996) \tag{8}$$

The limit of detection (LOD) of the proposed method for the determination of copper was studied under the optimal experimental conditions. The LOD obtained from LOD = 3

$(\sigma)_{\text{blank}}/\text{m}$, where σ is the standard deviation of ten consecutive measurements of the blank and m is the slope of the calibration curve. The LOD was 0.8 μg L^{-1}. The limit of quantification (LOQ) defined as $\text{LOQ} = 10\ (\sigma)_{\text{blank}}/\text{m}$ was 2.7 μg L^{-1}. All the statistical calculations are based on the average of triplicate extraction and measurement of each standard solution in the given range. The experimental pre-concentration factor defined as the ratio of the slopes of calibration graphs with and without pre-concentration was 22.5. The accuracy of the procedure was confirmed by analysis of the copper in certified reference material, namely CASS-4 from the National Research Council of Canada. The achieved result was 0.597 ± 1.1 (μg L^{-1}). The certified value is 0.592 ± 0.062 (μg L^{-1}). This results show that the validation of the presented procedure.

Selecting coefficients of copper ions over the other selected inorganic cations that coexisted with copper ions in natural sources were studied by batch procedure. The IIP particles were tested for separation of 25 μg copper ions in 25 ml aqueous solution and 50 μg from Cd^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+} and Ni^{2+} ions either individually or in their mixtures. The concentrations of transition metals were determined by ICP OES. The selectivity of the copper ions versus another cation was determined by the ratio of the two partition coefficients, $\text{D}_{\text{Cu}}^{2+}$and D_{m}^{n+} which is referred to the selectivity factor, α:

$$\alpha = \frac{D_{\text{Cu}}^{2+}}{D_{m}^{n+}} \tag{9}$$

The obtained results are summarised in Table 4. As it is clear from the results, quantitative separation of copper from other cations is possible. Compared to other metal ions, the selectivity factor values demonstrate that the IIP synthesised for the Cu^{2+} has a higher selectivity.

3.3 *Analytical application*

In order to assess the applicability of the method to real samples with different matrices, it was applied to the extraction and separation of copper ions from 100 mL of three different samples. Table 5 shows the extraction of 2.0 μg of added copper ions from 100 mL of different samples. As seen, the results of three analysis of each sample show that the copper recovery was almost quantitative.

Table 5. Recovery of 2 μg copper added to 100 mL of samples.

Sample	Spiked amount (μg L^{-1})	Copper found (μg L^{-1})	Recovery%	RSD%
Tap water	–	–	–	–
Tap water	20.0	19.92	99.6	0.65
Chahnemah water	–	–	–	–
Chahnemah water	20.0	19.84	99.2	0.81
Seawater	–	2.49	–	0.74
Seawater	20.0	22.4	–99.6	0.8
Mineral water	–	1.48	–	0.76
Mineral water	20.0	21.436	99.7	0.75

4. Conclusion

In the present work, a copper imprinted polymer was prepared using the combination of VP as functional monomers, EDMA as cross-linker, AIBN as initiator and Cu-morin complex as template. The prepared IIP revealed high affinity and selectivity towards its template. The Cu-imprinted adsorbent, which was highly selective towards Cu ions, is expected to be a useful material for the interference-free extraction and pre-concentration of Cu ions.

The application of a Box-Behnken matrix became possible, rapid, and an economical and efficient way of an optimisation strategy of the proposed procedure.

References

[1] E. Shams, A. Babaei, and M. Soltaninezhad, Anal. Chim. Acta **501**, 119 (2004).

[2] WHO Technical Report Series, *Evaluation of Certain Food Additives and Contaminants* (WHO, Geneva, 1982, No. 683).

[3] Y. Zni, X. Chang, X. Zhu, H. Luo, Z. Hu, X. Zou, and Q. He, Microchem. J. **87**, 20 (2007).

[4] F. Puoci, C. Garreffa, F. Iemma, R. Muzzalupo, U.G. Spizziri, and N. Picci, Food Chem. **93**, 349 (2005).

[5] O. Vigneau, C. Pinel, and M. Lemaire, Anal. Chim. Acta **435**, 75 (2001).

[6] O. Vigneau, C. Pinel, and M. Lemaire, Chem. Lett. **3**, 202 (2002).

[7] K. Uezu, H. Nakamura, J. Kanno, T. Sugo, M. Goto, and F. Nakashio, Macromolecules **30**, 3888 (1997).

[8] V.M. Biju, J. Mary Gladis, and T. Prasada Rao, Talanta **60**, 747 (2003).

[9] D.C. Montgomery, *Design and Analysis of Experiments*, 4th ed. (Wiley, New York, 1997).

[10] G.E.P. Box, W.G. Hunter, and J.S. Hunter, *Statistics for Experimenters* (Wiley, New York, 1997).

[11] L.A. Portugal, H.S. Ferreira, W.N.L. dos Santos, and S.L.C. Ferreira, Michrochem. J. **87**, 77 (2007).

[12] F. Nazari, S.N. Ebrahimi, M. Talebi, A. Rassouli, and H.R. Bijanzadeh, Phytochem. Anal. **8**, 333 (2007).

[13] S. Buco, M. Morgagues, M. Sergent, P. Doumenq, and G. Mille, Environ. Res. **104**, 209 (2007).

[14] R.E. Santelli, M.A. Bezerra, O.D. SantAna, R.J. Cassella, and S.L.C. Ferreira, Talanta **68**, 1083 (2006).

[15] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandao, E.G.P. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza, and W.N.L. dos Santos, Anal. Chim. Acta **597**, 179 (2007).

[16] H.S. Ferreira, A.C.N. Santos, L.A. Portugal, A.C.S. Costa, M. Miro, and S.L.C. Ferreira, Talanta **77**, 73 (2008).

[17] A.S. Souza, W.N.L. dos Santos, and S.L.C. Ferreira, Spectochim. Acta. Part B – Atom. Spectrosc. **60**, 737 (2005).

[18] W.L. dos Santos, C.M.M.D. Santos, J.L.O. Costa, H.M.C. Andrade, and S.L.C. Ferreira, Microchem. J. **77**, 123 (2004).