



Anal. Bioanal. Chem. Res., Vol. 5, No. 2, 317-330, December 2018.

Chemometrics-enhanced Kinetic Spectrophotometric Method for Simultaneous Determination of Ag^+ , Cu^{2+} and Ni^{2+} Ions in Some Medicinal Plants by Dimethyl 2,2'-(ethan-1,2-diylbis(1))bis(cyclopent-1-ene-1-carbodithioate)

A. Fakhraei^a, M. Abbasi Tarighat^{a,*}, K. Mohammadi^a, G. Abdi^b and A. Rezaei^a

^aDepartment of Chemistry, Faculty of Sciences, Persian Gulf University, Bushehr, 75169, Iran

^bDepartment of Biotechnology, Persian Gulf Institute, Persian Gulf University, Bushehr, 75169, Iran

(Received 2 March 2018, Accepted 18 June 2018)

The metal ions constituents of plants is reliable for determination of their medicinal, nutritional and toxicity properties. A novel, simple and accurate spectrophotometric method was developed for the simultaneous determination of Ag^+ , Cu^{2+} and Ni^{2+} ions in different medicinal plants without prior separation steps. This method was based on the different kinetic characteristics between the reactions of analytes with newly synthesized Schiff base as dimethyl 2,2'-(ethan-1,2-diylbis(1))bis(cyclopent-1-ene-1-carbodithioate (DEBC). All experimental conditions, including DEBC concentration, and the effects of pH and temperature were optimized. Also, orthogonal array design was applied for the construction of concentration. The differential kinetic spectra were monitored and recorded at 397 nm. The reaction orders were estimated with respect to complex mixture of analytes with DEBC. Limit of detection values were 0.013, 0.078 and 0.003 mg l^{-1} and limit of quantification values were 0.043, 0.250 and 0.011 for, Cu^{2+} , Ni^{2+} and Ag^+ , respectively. The recorded data were processed by principal component analysis-back propagation neural networks (PCBPNNs). A set of synthetic mixtures of metal ions was evaluated and the obtained results by PCBPNNs were discussed. The simultaneous analysis was also performed without compressed data and was compared with those obtained by PCBPNNs. The proposed method was successfully applied to the simultaneous determination of metal ions in medicinal plant samples. The results of the proposed method were compared by those obtained on the same samples using Graphite furnace atomic absorption spectrometry.

Keywords: Kinetic, Medicinal plants, Schiff base, Cu^{2+} , Ni^{2+} , Ag^+

INTRODUCTION

During the past decades, herbal medicines attracted attention to a greater extent because of their high pharmacological activities with low toxicity and rare complications. Unlike synthetic drugs, herbal medicines are complicated mixtures that pharmacognostical analyses of medicinal herbs remain challenging issues for analytical chemists. As the plants are directly in contact with different environmental effects (air, water and soil) the constituents of these sources might contaminate the plants.

Heavy metal content in medicinal plants has been a subject of great interest [1]. Heavy metal contents in spices and medicinal plants depend on climatic factors, plant species, air pollution and other environmental factors. Several factors may influence on the accumulation of contaminants, such as plant species, contaminant level and duration of exposure to contaminant, and topography. Three main mechanisms have been proposed for contamination of medical herbal products by heavy metals; contamination during cultivation, accidental cross contamination occurring during processing, and/or the deliberate introduction of heavy metals as a therapeutic ingredient [1]. Heavy metal

*Corresponding author. E-mail: matarighat@pgu.ac.ir

central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Accumulation properties of toxic elements such as mercury, arsenic, lead, nickel and cadmium which might be present in some medicinal plants can threaten the consumer health. Whereas other elements such as calcium, magnesium, zinc, manganese, copper and iron are also usually present in plants that are useful for human health. Hence, accurate determination of heavy metals may help us elucidate their authenticity, safety, efficacy and intrinsic quality of medicinal plants [2].

Also, there is a general agreement that metals may react directly with DNA. The large number of nucleophilic centers in nucleic acids implies a high affinity for metals. The most serious interaction between metals and DNA is, probably, crosslinking between the DNA strands after exposure to Cu^{2+} , Zn^{2+} , Co^{2+} and Mn^{2+} . Ions such as Ni^{2+} can react with the phosphate groups as well as with the bases of DNA. Nickel has been reported to induce a variety of DNA lesions. These include DNA strand damage as well as DNA protein cross-links. Nickel has been shown to inhibit DNA synthesis following either in vitro or in vivo exposure. There are different levels of phytotoxicity in various heavy metals, and the order of toxicity in plants follows the order of $\text{As}^{5+} < \text{As}^{3+} < \text{Cr}^{6+} < \text{Co}^{2+} < \text{Ni}^{2+} < \text{Cu}^{2+} < \text{Ti}^{+} < \text{Hg}^{2+} < \text{Cd}^{2+} < \text{Ag}^{+}$ [3,4]. The higher concentrations of these metals in plant cells results in alterations at the physiological, biochemical and cellular levels leading to the severe damage to plants.

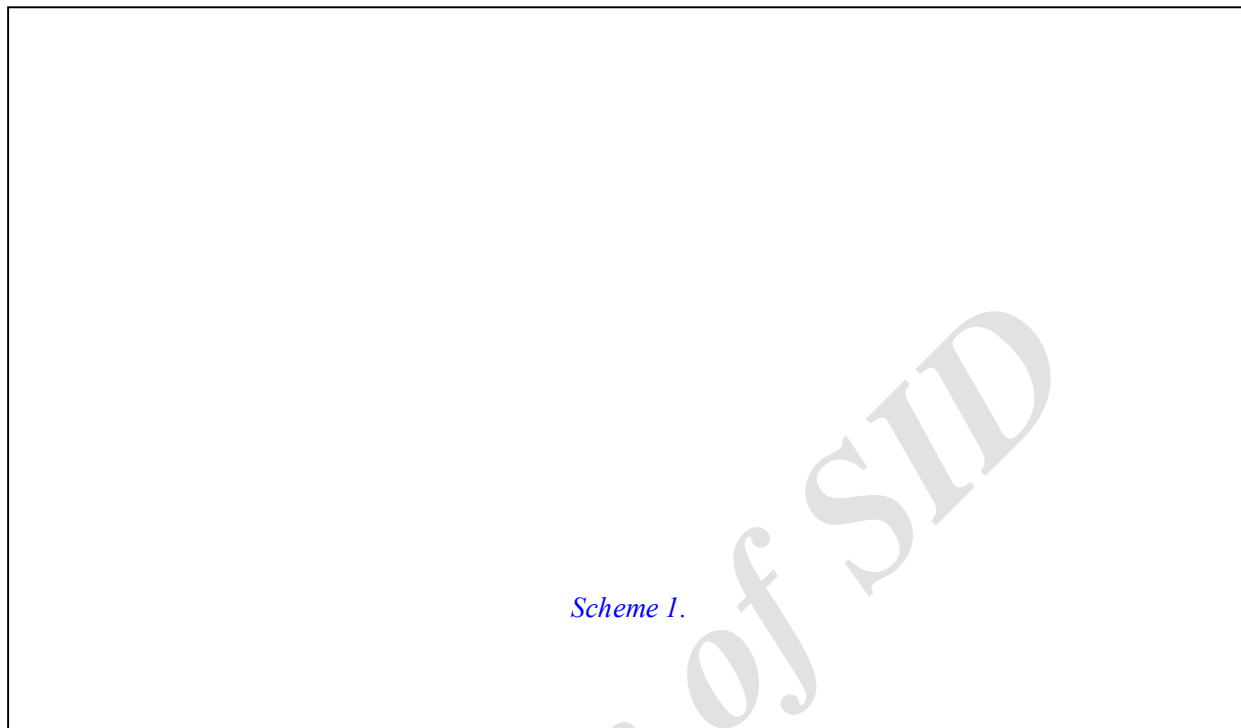
Therefore, the advanced and effective quality evaluation system establishment is necessary. There is an ongoing requirement to develop analytical methodologies for the sensitive and selective determination of heavy metal constitute in many sample types. Many analytical approaches such as high performance liquid chromatography (HPLC) [5], thin layer chromatography (TLC) and 2D TLC [6], hydrophilic interaction chromatography [7,8], infrared spectroscopy and gas chromatography(GC) [9], HPLC–Mass spectrometry(MS) [10], RP-HPLC [11], 2D chromatography combining size-exclusion and reverse phase liquid chromatography [12], electrothermal atomic absorption spectrometry [13] and differential pulse anodic stripping voltammetry [14] have been introduced to evaluate the quality of medicinal plants,

and significant amount of measurement data.

In recent years, there has been an increasing interest in multi-component determinations using chemometric techniques on the grounds of the high computational power of modern computers. These methods include quality evaluation of medicinal plants, such as similar analysis (SA), principle component analysis (PCA)[3] cluster analysis (CA) [15], discriminate analysis (DA) [16], clustering discriminate analysis [17], pattern recognition [18-19], orthogonal projection approach(Sanchez *et al.*, 1996) [20], size moving window evolving factoranalysis (FSMWEFA) [21], PCA and sub-window factor analysis (SFA) [22] and heuristic evolving latent projection [23]. These methods play a vital role in the discrimination and classification of medicinal plants. Therefore, the application of chemometrics in the field of medicinal plants is spontaneous and necessary.

Schiff base containing strong donor sites like phenoxo oxygen atoms as well as imine nitrogen atoms find extensive applications in different fields for their special coordination ability with transition metal ions [24,25]. Schiff bases have been preferred ligand systems in catalysis, biological modeling, the design of molecular ferromagnets, medical imaging and optical materials [26,27].

There are several published works for determination of the mineral content in plants by extracting the plant tissue with a suitable solvent or by removing the organic fraction by oxidation, leaving the inorganic ions for subsequent analysis. The mostly used analytical method for determination of ions is atomic absorption spectrophotometry (AAS). Therefore, due to high demand and importance of medicinal plants determination in laboratories and their effects on human health, expertise have developed a new spectrophotometric method for the simultaneous determination of metal ions at trace level in medicinal plants. Kinetic methods are powerful tools for multi-component analysis as they permit the sensitive and selective determination within no sample pre-treatment in many cases. Hence, kinetic methods have made a great improvement by using the chemometric procedures [24]. Therefore, we proposed suitable, selective and sensitive new synthetic Schiff base coupled with chemometrics assisted kinetic method for simultaneous determination of



Scheme 1.

Ag^+ , Cu^{2+} and Ni^{2+} ions. To the best of our knowledge, this is a first report describing differential reaction rate method associated with artificial neural network for the simultaneous analysis of ternary mixtures of Ag^+ , Cu^{2+} and Ni^{2+} ions using dimethyl 2,2',-(ethan-1,2-diylbis(1))bis (cyclopent-1-ene-1-carbodithioate) (DEBC). For the construction of analytes concentration, orthogonal array design was applied. The chemical reaction orders with respect to Ag^+ , Cu^{2+} and Ni^{2+} mixtures were estimated. The measured kinetic data were processed by the PCBPNN to develop calibration models, and the method was then validated with the use of a set of synthetic samples containing mixtures of the ions (prediction set), thereafter, it was applied to several medicinal samples to demonstrate general applicability.

EXPERIMENTS

Reagents, Standards, Samples

All chemicals were of analytical reagent quality, and freshly deionised water was used throughout. Stock solutions (200.0 mg l^{-1} Ag^+ , Cu^{2+} and Ni^{2+}) were prepared

by dissolving appropriate amounts of $\text{Cu}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and AgNO_3 (Merck, Darmstadt, Germany) and $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Sigma-Aldrich) in water. Pantanone (Sigma-Aldrich) was used for the synthesis of ligand according to literature (See Scheme 1) [28]. The color-forming reagent was a 3.6×10^{-3} M solution at ethanol. The buffer reagents were 0.1 M. The pH studies were carried out using sodium acetate–acetic acid (pH 3.0-5.0), disodium hydrogen phosphate(Na_2HPO_4) -monosodium phosphate (NaH_2PO_4) (pH 6.0-10.0) and ammonium chloride-ammonium buffer solution (pH 11-12).

Apparatus

The UV-Vis spectra were recorded on a AnalyticaJena spectrophotometer, and 1.0 cm quartz cell, a scan rate of 100 nm min^{-1} and a slit width of 2.0 mm were used. Also, atomic absorption absorbance spectrometer (AAS), equipped with graphite furnace (GTA-120), PSD-120 autosampler Varian model AA-240FS (Varian, Victoria, Australia), was used. The recorded spectra were digitized with one data point per nanometer. All calculations were performed by MATLAB (version 7.8, MathWorks, Natick,

Table 1. Composition of Calibration, Prediction, Samples in Tertiary Mixtures for Simultaneous Determination of Ni²⁺, Cu²⁺ and Ag⁺ with BPNNs

Concentration (mg l ⁻¹)								
Calibration set			Prediction set			Validation set		
Ag ⁺	Cu ²⁺	Ni ²⁺	Ag ⁺	Cu ²⁺	Ni ²⁺	Ag ⁺	Cu ²⁺	Ni ²⁺
0.01	0.03	0.5	0.10	0.55	1.20	0.01	0.30	0.50
0.01	0.10	2.0	0.12	0.03	0.50	0.01	0.70	7.50
0.01	0.30	4.0	0.075	0.10	2.00	0.05	0.50	0.50
0.01	0.50	6.0	0.10	0.40	4.00	0.12	0.30	7.50
0.01	0.70	7.5	0.05	0.10	2.30	0.15	0.10	0.25
0.05	0.03	2.0	0.20	0.65	0.50	0.20	0.55	3.10
0.05	0.10	4.0	0.15	0.50	0.75			
0.05	0.30	6.0	0.02	0.07	7.00			
0.05	0.50	7.5	0.04	0.15	4.00			
0.05	0.70	0.5	0.05	0.05	0.50			
0.10	0.03	4.0	0.20	0.70	3.00			
0.10	0.10	6.0	0.03	0.12	0.67			
0.10	0.30	7.5						
0.10	0.50	0.5						
0.10	0.70	2.0						
0.15	0.03	6.0						
0.15	0.10	7.5						
0.15	0.3	0.5						
0.15	0.5	2.0						
0.15	0.7	4.0						
0.20	0.03	7.5						
0.20	0.1	0.5						
0.20	0.3	2.0						
0.20	0.5	4.0						
0.20	0.7	6.0						

MA, USA).

General Procedure

Aliquots of either one of the Ag^+ , Cu^{2+} and Ni^{2+} solution or a mixture of these compounds, 1.0 ml buffer solution (pH 7.0), were transferred into a 5.0 ml volumetric flask. Then, 0.012 ml of DEBC solution 3.6×10^{-3} M was added. This solution was diluted to volume with water and mixed well. After mixing, 3.0 ml of the reaction was immediately transferred to a spectrophotometric cell which was kept at a constant temperature of 25 °C. The absorbance was recorded as a function of time for 300 s at 397 nm against reagent blank. The zero time was taken as the moment the last drop of the sample solution was diluted to the mark with water. The signal ΔA ($A_{\text{total}} - A_{\text{blank}}$) between 1 and 200 s was considered as analytical signal. The same procedure and experimental conditions were used in interference study in the presence of interfering substances.

Concentration Design for Calibration Sets

In order to extract maximum quantitative information about the samples with the use of minimum experimental trials, the orthogonal array design was applied for the construction of the set of calibration samples [29]. Table 1 shows the composition of the 25 calibration samples, which were designed according to a 5-level orthogonal array design. The concentration levels for the analytes were: Ag^+ , 0.01, 0.05, 0.10, 0.15 and 0.20 mg l^{-1} , Cu^{2+} , 0.03, 0.10, 0.30, 0.50 and 0.70 mg l^{-1} and Ni^{2+} , 0.50, 2.0, 4.0, 6.0 and 7.5 mg l^{-1} . These levels were selected to cover a wide distribution range of concentrations, while covering the range of levels found in real samples. The 12 and 6 ternary synthetic mixtures of analytes shown in Table 1 were prepared and used for prediction and validation sets, respectively.

Sample Preparation Procedure

Iranian medicinal plant samples were purchased from local importers of Bushehr in dried form. All samples were treated the same way. To prepare the samples for elemental analysis, 0.50 g of sample was accurately weighed into a pre-cleaned beaker. Concentrated nitric acid (6.0 ml) was added to the sample and the beaker covered with a watch-

glass was heated gently for 3 h on a hot-plate until the digestion process was complete. The digested sample was allowed to cool to room temperature and then transferred quantitatively into the clean 25.0 ml volumetric flasks. The samples were then diluted to the mark with deionized water [3].

RESULTS AND DISCUSSION

Spectral Composition of Complexes

In the present study, the reactions of Ag^+ , Cu^{2+} and Ni^{2+} ions with DEBC reagent were investigated. Spectra of the colored product and the corresponding reagent blank were measured in the range of 250-500 nm at a reaction time of 300 s according to the previously described experimental procedure (Fig. 1a). This figure shows that spectral shapes resulting from the complexes are very similar, which complicates the analysis of the spectral output from their mixtures. Hence, it is impossible to determine the three compounds simultaneously by conventional methodology. The formation of these colored products was utilized in the development of a kinetic spectrophotometric method for the simultaneous determination of metal ions. Preliminary investigations showed that the reaction rates for the three metal complexes with DEBC are different.

As Fig. 1b shows the kinetic profiles of metal complexes in reaction with DEBC are time dependent and the absorption at 397 nm decreases with elapse of time. Thus, the differential kinetic rates should be useful for resolving the spectra from the metal ion mixtures with the use of chemometric methods. In this study, BPNN method was proposed for simultaneous determination of Ag^+ , Cu^{2+} and Ni^{2+} ions. Therefore, a set of calibration samples with known compositions were first prepared and the kinetic measurements were carried out, after which, the mathematical models were established by processing the measured kinetic data. Subsequently, the mathematical models were used for the prediction of unknown samples under the same experimental conditions.

Influence of Experimental Factors

The overall processes are influenced by pH of the solution, concentration of DEBC, and temperature which affects the absorbance of the colored products and reaction

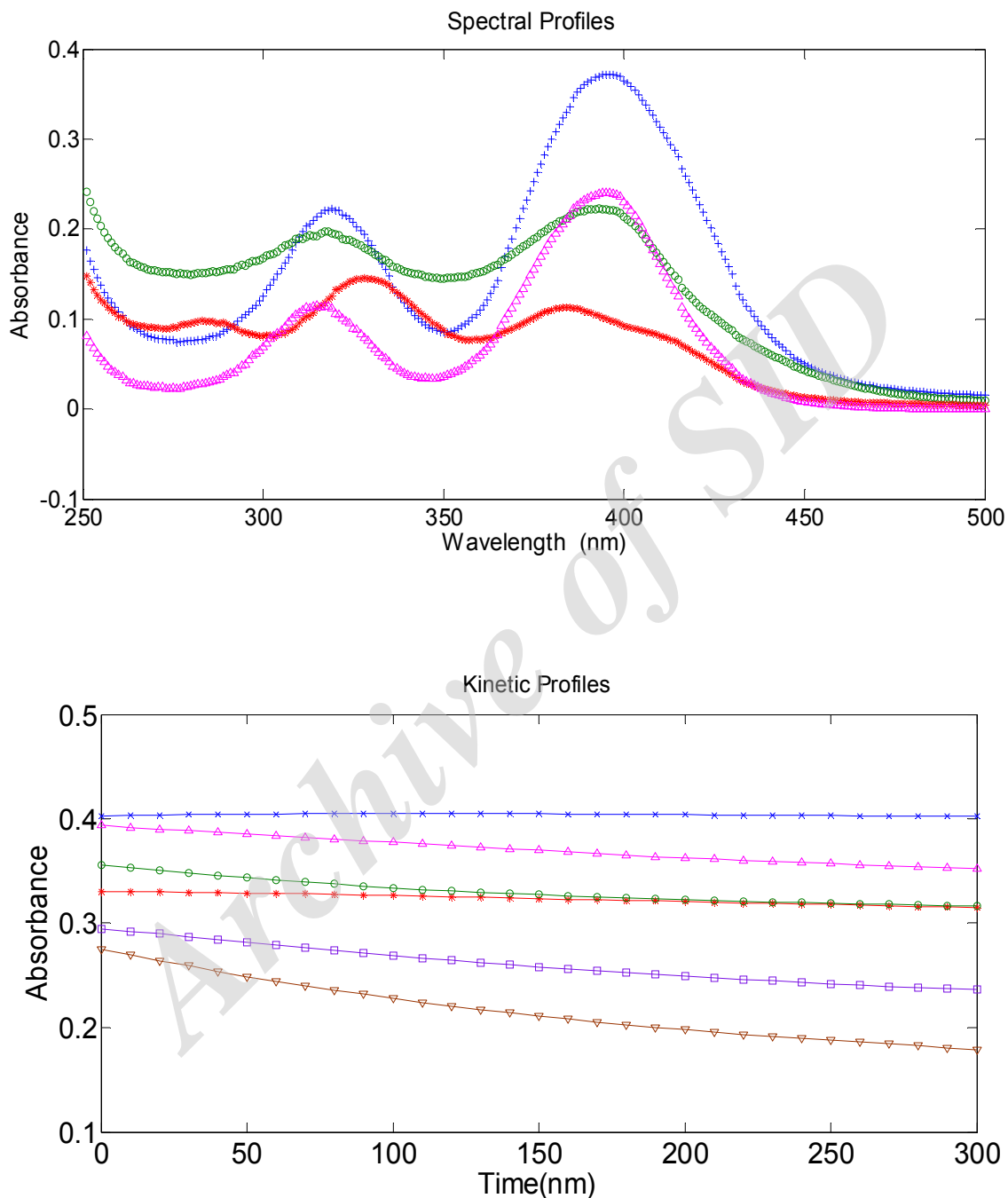


Fig. 1. (a) Spectra of DEBC (\times , 1.4×10^{-4} M) and its complexes with Ni^{2+} (Δ , 1.0 mg l^{-1}), Cu^{2+} ($*$, 1.0 mg l^{-1}), and Ag^+ (\circ , 1.2 mg l^{-1}) at pH 7 at 25 °C (a), (b) Plot of kinetic behavior *versus* of complexation reactions of DEBC (\times , 1.4×10^{-4} M) Ni^{2+} (Δ , 1.0 mg l^{-1}), Cu^{2+} ($*$, 1.0 mg l^{-1}), and Ag^+ (\circ , 1.2 mg l^{-1}), mixture of metal ions (\square , experimental), and mixture of metal ions (\blacktriangledown , expected) with DEBC at 397 nm, pH 7.0 and at 25 °C.

rates. Therefore, the various experimental parameters were carefully studied and optimized.

Effect of pH

The influence of pH on the absorbance of the reaction of individual metal ions with DEBC was studied in order to establish the experimental conditions resulting in the greatest possible discrimination between the kinetic behavior of metal ions and greatest value of signal. In order to find the optimum pH, the effect of pH in the range of 3.0-12.0 on a constant concentration of each metal ions with DEBC was investigated (Fig. 2a).

The pH studies were carried out using sodium acetate-acetic acid (pHs 3.0-5.0), disodium hydrogen phosphate-monosodium phosphate and (pHs 6.0-10.0) and ammonium chloride-ammonia buffers (pH 11.0-12.0). For this purpose, changes in absorbance at different pHs at 397 nm over 200 sec following the initiation of the reaction were monitored and ΔA ($\Delta A_{\text{total}} - \Delta A_{\text{blank}}$) signal is plotted against pH. At pH 7.0 the ΔA signal reached its maximum value. At higher pH for Ag^+ and Ni^{2+} , ΔA of the solution was reduced. In order to keep the high sensibility for determination of metal ions, pH 7.0 was selected for the optimal experimental conditions.

Effect of DEBC Concentration

The concentration of DEBC affects the spectral sensitivity and kinetic behavior of all three compounds. In order to find the optimal concentration of DEBC, the effect of its concentration on the reaction of each metal ions was studied by carrying out the reaction at 25 °C using preselected pH 7.0 and varying concentration of DEBC in the range of 1.6×10^{-6} - 3.26×10^{-5} M. In this case, the reasonable ΔA values were obtained. Therefore, from the ΔA values, it is observed that 1.4×10^{-5} M is sufficient to get maximum sensitivity.

Effect of Temperature

By keeping pH at 7.0 at 1.4×10^{-5} M of DEBC, the influence of temperature on the absorbance of the solution was studied in the range of 10-35 °C. The changes in absorbance at 397 nm in the time interval of 1 to 200 s at different temperatures were monitored. It was found that the ΔA signal for metal ion complexes increases up to 25 °C

and after that levels off or slightly increases. Hence, this temperature was selected to be used in further analysis.

Individual Calibration Graphs of Metal Ions

A set of sample solutions with different metal ion concentrations was prepared and measurements were carried out under optimum conditions according to the experimental procedure described in Sections 3.2. The linear ranges (Fig. 2c), correlation coefficients, LODs and LOQs were determined and presented at Table 2.

Detection of Non-linearity

Synergistic effect. Application of ANNs in multivariate calibrations was proposed when a significant nonlinearities is observed in the data. The nonlinearity due to synergistic effect, which is the main factor to the error of the kinetic analysis, has attracted much attention, and some work has been done for solving this problem in nonlinear systems with the aid of chemometrics [29]. As Fig. 1b shows the kinetic curve of DEBC after reaction with the ternary mixture of the metal complexes (experimental value) has a lower overall intensity than the one computed from the sum of the kinetic curve of DEBC after reaction with the individual metal ions (expected value). Thus, there is a factor(s) which is (are) not accounted for by the simple Beer's and absorbance additively laws. Hence, PCBPNNS models can be used for such nonlinear systems to resolve mixtures.

Partial response plot. Application of ANNs in multivariate calibrations was proposed when a significant non-linearity is observed in the data. Classical method to diagnose nonlinearity in multivariate calibration was used to plot y versus each PC (partial response plot, PRP), or to plot the residuals ($e_{\text{PC1-PCA}}$) of the regression $y = f(\text{PC1}, \dots, \text{PCA})$

$$y = b_0 + b_1\text{PC}_1 + \dots + b_A\text{PC}_A + b_a \text{PC}_a + e_y, \text{PC}_1\text{-PC}_A, a = 1, \dots, A \quad (1)$$

against the fitted (predicted) response \hat{y} (residual plot, RP). A , in Eq. (1), is the total number of PCs used. The relevant PRP plot was obtained by plotting the concentration as a function of PC1. Figure 3a shows the PRP for the calibration of the Ag^+ , using PC1. PRP for the PC1

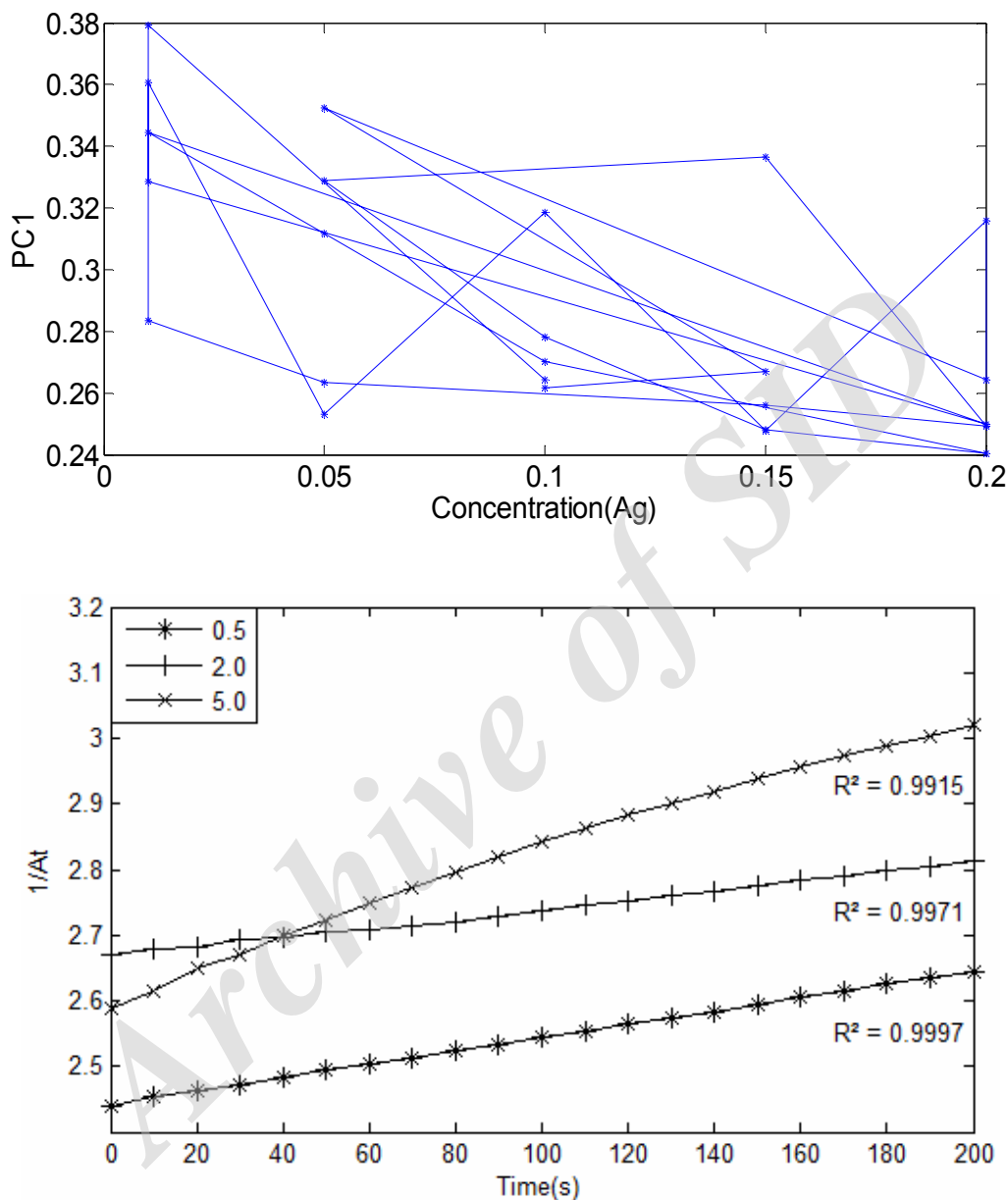


Fig. 2. (a)Effect of pH of the buffer solution, (b) effect of DEBC Concentration on ΔA signal of Ni^{2+} , Cu^{2+} and Ag^+ complexes with DEBC (c) and Individual calibration graphs for Ni^{2+} , Cu^{2+} and Ag^+ at 397 nm, pH 7.0 and at 25 °C.

included in the model shows a nonlinear pattern. It is concluded that all investigated plots are able to detect strong non-linearities presented in the system [30].

Estimation of reaction order. According to Beer-Lambert law, absorbance (A) is directly proportional to the

concentration. The order of the reaction can be obtained for zero order, first order and second order reactions from the absorbance of the solution with time by the following equations:

$$t = \frac{1}{k} [(A_{\infty} - A_t) - (A_{\infty} - A_0)] \quad (2)$$

$$\ln \left[\frac{(A_{\infty} - A_t)}{(A_{\infty} - A_0)} \right] = -kt \quad (3)$$

$$\frac{1}{A_t} = \frac{1}{A_0} + kt \quad (4)$$

in which A_0 , A_t and A_{∞} are absorbance at time zero, t , and at $t \rightarrow \infty$, respectively. For a zero-order reaction the plot of $A_{\infty} - A_t$ as a function of t , fits to a straight line with the slope $-k$. On the other hand, when order is one, the plot

$\ln \left[\frac{(A_{\infty} - A_t)}{(A_{\infty} - A_0)} \right]$, as a function of t , will produce straight

line with slope of $-k$. The plot $[(1/A_t)]$, as a function of t , fits to straight line with slope k for the second order reactions [31]. In other words, these equations should be examined; the equation with the best fit for the experimental data will produce the order of the reaction. At Fig. 3b, $1/A_t$ for different concentrations of analyte (Ni^{2+}) was plotted as a function of t . In this case, the straight line obtained corresponds to the order of the reaction with respect to mixtures that is two.

Optimization of Network Parameters

The back-propagation neural network is a multi-layer feed-forward trained according to error back propagation algorithm and is a kind of network model used extensively. This network is composed of a three-layer feed-forward network: input layer, output layer and hidden layer. The hidden layer can be single or multiple that each layer can have several neurons connected by weight value between the adjacent layers.

Any variations in the spectral due to spectrometer and sampling error should be eliminated prior to data analysis. It is necessary to pre-process data by mean centering, scaling or auto-scaling. In this study, the data matrix is first auto-scaled. Also, decreasing the data volume before using ANNs for non-linear multivariate calibration has been suggested as a preprocessing step in many of the previous studies. Reducing the number of inputs to a network reduces the training time. The most popular method for data

compression in chemometrics is principal component analysis (PCA). A PCA algorithm was used in this study for reducing the kinetic spectroscopic data by constructing a linear combination of the original variable into a few orthogonal principle components containing most of the variability of the data set. Even if there is some non-linearity in data set, all relevant information is usually contained in the first PCs [30].

In this study some factors such as the number of principal components, number of nodes (neurons) in hidden layer, learning function, number of epochs, and momentum were optimized. The aim of calibration is to produce a model that relates the spectral data of calibration mixtures to the concentration data. The number of input nodes was selected as an optimal number of principal components (PC_1 , PC_2 , PC_3 and PC_4) obtained by applying principal component analysis on auto-scaled data. The root-mean-square error prediction (RMSEP) for the prediction of analytes concentration was estimated. Criterion for illustration of the performance of trained network is:

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}} \quad (5)$$

where \hat{y}_i and y_i are the desired output and the actual output sets, respectively, and n is the number of prediction set samples. The validation set was applied to prevent the over fitting of the networks to the noise from the calibration set during training. In order to optimize the PC BPNN architecture, also, the number of hidden nodes was varied from one to twenty five for metal ions. The RMSEP% values were minimum at 8, 4 and 20 nodes for Ag^+ , Cu^{2+} and Ni^{2+} , respectively. The basic transfer function used here consisted of a tansig function which passes the value through a non-linear function for hidden and output layer. The output layer for Ag^+ , Cu^{2+} and Ni^{2+} ions contains one layer. Training the network was performed with several learning rates changing from 0.01 to 1.0. During the learning procedure of the network with the calibration set, the prediction and validation sets were subsequently tested with the learned network. Also, number of iterations (epoch) and momentum was investigated and optimized.

Table 2. Analytical Characteristics of Ag⁺, Cu²⁺ and Ni²⁺ in Ternary Mixtures Using DEBC as a Complexing agent

Analyte	Calibration equation	Correlation coefficient	Linear range (mg l ⁻¹)	LOD (mg l ⁻¹)	LOQ (mg l ⁻¹)
Ag ⁺	A = 0.224C _{Ag} + 0.005	0.996	0.010-2.000	0.003	0.011
Cu ²⁺	A = 0.058C _{Cu} + 0.004	0.999	0.030-0.700	0.013	0.043
Ni ²⁺	A = 0.010C _{Ni} + 0.002	0.996	0.500-7.500	0.079	0.259

Table 3. Optimized Parameters Used for Construction of PCBPNNs and BPNNs for the Simultaneous Determination of Ag⁺, Cu²⁺ and Ni²⁺ Ions

Parameter	Method	Ag ⁺	Cu ²⁺	Ni ²⁺
Input nodes	PC-BPNNs	4	4	4
	BPNNs	Original sets	Original sets	Original sets
Hidden nodes	PC-BPNNs	8	4	20
	BPNNs	44	27	34
Output nodes	PC-BPNNs	1	1	1
	BPNNs	1	1	1
Learning rate	PC-BPNNs	0.40	0.20	0.08
	BPNNs	0.90	0.20	0.25
Momentum	PC-BPNNs	0.85	0.30	0.50
	BPNNs	0.50	0.15	0.45
Number of iteration (epoch)	PC-BPNNs	9000	6000	15000
	BPNNs	21000	20000	26000
Hidden layer transfer function	PC-BPNNs	Tansig	Tansig	Tansig
	BPNNs	Tansig	Tansig	Tansig
Output layer transfer function	PC-BPNNs	Tansig	Tansig	Tansig
	BPNNs	Tansig	Tansig	Tansig

The results for optimized parameters for construction of the network are represented in Table 3.

Using the optimized parameters (Table 3), the PCBPNNs architecture was created for three analytes and concentration of each ion was predicted. RMSEP% values were obtained as 0.51, 1.18 and 4.58 for Ag^+ , Cu^{2+} and Ni^{2+} complexes, respectively. The reasonable relative errors obtained by optimized networks for each analyte indicate the applicability of the proposed method.

In order to compare the PCBPNNs models with BPNNs, original spectrophotometric data, as input nodes, were subjected to FFNNs. The topology parameters were shown in Table 3. Comparison of PCBPNNs with BPNNs models shows that the PCBPNNs models appear the shorter training time because of the fewer input nodes and iterations. Subsequently, RMSEP% values of BPNNs models are shown in Table 4. RMSE% values in BPNNs models were worse than those in PCFFNNs models. The results indicate that the PCFFNNs models have more advantages over BPNNs models. Therefore, reproducibility and lower training time of networks in the PCRFNNs are among the main advantages of these networks compared to PCBPNNs.

Study of Interferences

The effects of foreign species on the simultaneous determination of Cu^{2+} , Ag^+ and Ni^{2+} ions were investigated by measuring the absorbance of solutions containing 2.0 mg l⁻¹ of each metal ion in the presence of various amounts of other ions. The tolerance limit was taken as the amount of added ions causing less than 10% relative error in the determination of Cu^{2+} , Ag^+ and Ni^{2+} ions. The results showed that the ions K^+ , Ca^{2+} , Ba^{2+} , Mg^{2+} , Na^+ , I^- , Cl^- , F^- , SO_4^{2-} , CN^- , PO_4^{3-} , CH_3CO_2^- , NO_3^- and SCN^- did not interfere even when present in 1000-fold-excess over the Ag^+ , Cu^{2+} and Zn^{2+} ions. Ions Al^{3+} , Fe^{3+} , Cr^{3+} , V^{3+} , Be^{2+} , Li^+ , Sn^{2+} , Pb^{2+} did not interfere up to 200 fold-excess. Ions Co_3^{2-} and Cl^- did not interfere up to 150 fold-excess. Ion Co^{2+} did not interfere up to 60 fold-excess. Ions Zn^{2+} , Cd^{2+} , Mn^{2+} did not interfere up to 30 fold-excess. Ion Hg^{2+} did not interfere up to 10 fold-excess.

Application

Results for the analysis of synthetic mixtures by the

proposed methods (RMSE% values) showed satisfactory results for the simultaneous determination of Cu^{2+} , Ag^+ and Ni^{2+} ions. To demonstrate the applicability of the optimized methods to real samples, it was applied to the simultaneous determination of Cu^{2+} , Ag^+ and Ni^{2+} ions in *Zataria multiflora*, *Haplophyllum*, *Canaliculatum*, *Salvia mirzayanii*, *Artemisia sieberi*, *Teucrium polium* and *Sarberenjashk* sample solutions. The concentrations of metal ions were predicted by the optimized PCBPNN models. The results are shown in Table 4.

Note that PCBFNN analysis could not detect the amount of Ni^{2+} ions in the above medicinal plants, because the amount of this ion generally is lower than its LOD. Hence, reliability of the proposed procedure was also checked for determination of Ni^{2+} after addition of a known amount of standard analyte to *Zataria multiflora*, *Haplophyllum*, *Canaliculatum*, *Salvia mirzayanii*, *Artemisia sieberi*, *Teucrium polium* and *Sarberenjashk* solutions. The PCBPNNs analyses were performed and recovery for Ni^{2+} ion in medicinal plants was calculated. The results are presented in Table 5. This procedure was repeated three times for each metal ion. The mean recoveries of the trace metal ions from samples were calculated. The recoveries and R.S.D. values of the metal ions were in the order of 95.1-109% and 1.48-5.41%, respectively. The recovery of spiked samples is satisfactorily reasonable and indicates the capability of the method in determination of Ni^{2+} , in real samples.

The concentrations of Cu^{2+} , Ag^+ and Ni^{2+} were determined using GFAAS. The results of analysis by GFAAS as a standard method are presented in Table 4. Comparison of the results obtained by the proposed method with those obtained by GFAAS method confirmed the accuracy of the results obtained by PCBPNN and GFAAS approaches. Statistical data obtained by using Student's *t*-test are also given in Table 4. The results show that there is no significant difference between the obtained results using PCBPNN and GFAAS. According to the results, the developed method has advantages in selectivity, sensitivity and operation and also can effectively resolve spectra overlapping problem. This method has been applied to the kinetic spectrophotometric determination of the real samples with satisfactory results. The calculated results synthetic ligand and neural networks approach was suitable

Table 4. Simultaneous Determination of Ag⁺, Cu²⁺ and Ni²⁺ in Different Medicinal Plant Samples by the Proposed Method and GFAAS as a Standard Method (mg l⁻¹)

Method	Sample Scientific name	Mean ± SD			t _{cal}			t _{critical} ^b
		Ag ⁺	Cu ²⁺	Ni ²⁺	Ag ⁺	Cu ²⁺	Ni ²⁺	
PCFAAN	<i>Zataria multiflora</i>	0.030±0.001	0.058±0.001	ND ^a	1.960	2.350	-	2.780
	<i>Haplophyllum canaliculatum</i>	0.101±0.009	0.038±0.001	ND	0.630	1.460	-	2.780
	<i>Salvia mirzayanii</i>	0.040±0.003	0.036±0.002	ND	1.290	1.620	-	2.780
	<i>Artemisia sieberi</i>	0.015±0.001	0.066±0.004	ND	1.760	0.650	-	2.780
	<i>Teucrium polium</i>	0.040±0.002	0.067±0.002	ND	0.450	0.990	-	2.780
	<i>Artemisia vulgaris</i>	0.020±0.003	0.073±0.003	ND	2.00	0.900	-	2.780
	GFAAS	<i>Zataria multiflora</i>	0.028±0.002	0.055±0.002	0.053±0.002			
<i>Haplophyllum canaliculatum</i>		0.097±0.010	0.041±0.004	0.021±0.004				
<i>Salvia mirzayanii</i>		0.038±0.001	0.039±0.003	0.024±0.002				
<i>Artemisia sieberi</i>		0.016±0.002	0.065±0.001	0.064±0.007				
<i>Teucrium polium</i>		0.041±0.001	0.068±0.001	0.059±0.000				
<i>Artemisia vulgaris</i>		0.023±0.001	0.070±0.006	0.031±0.001				

^aND: not detected. ^bP = 0.05.

for the simultaneous determination of Cu²⁺, Ag⁺ and Ni²⁺ ions in complex mixtures.

A comparison between the proposed method with the previously reported methods for pre-concentration and simultaneous determination of Cu²⁺ and Zn²⁺ (Table 6) indicates that this method provides a lower detection limit, and R.S.D. values and/or wider linear range for the simultaneous determination of trace quantities of Cu²⁺ and Zn²⁺ in real samples. The method offers good procedures for analyzing the complex mixtures using simple spectrophotometer; this is available in most laboratories.

CONCLUSIONS

Use of plant-based drugs for curing various ailments is

as old as human civilization and is used in all cultures throughout the history. Therefore, determination and monitoring of metals in highly consumed medicinal are of great importance for protecting the public health from the hazards of possible toxic effects and also for informing the population about the nutritional values of the medicinal plants. Metal pollution in medicinal plants is a global problem, and the development of rapid, sensitive and clean up technologies is therefore a significant interest. At the present study, a novel, rapid, sensitive and accurate spectrophotometric method, based on reaction kinetics was researched and developed for the simultaneous determination of three heavy metal ions in medicinal plant samples. The underlying chemistry involved the kinetics of the reaction between Cu²⁺, Ag⁺ and Ni²⁺ ions and newly

Table 5. Determination of Ni²⁺ in Different Spiked Medicinal Plant Samples by the Proposed Method (mg l⁻¹)

Sample	Added	Found	%Recovery	%R.S.D
<i>Zataria multiflora</i>	0.00	0.05	-	-
	2.00	2.11	102.85	3.85
<i>Haplophyllum canaliculatum</i>	0.00	0.02	-	-
	0.50	0.57	109.80	5.41
<i>Salvia mirzayanii</i>	0.00	0.02	-	-
	7.00	7.08	100.80	1.44
<i>Artemisia sieberi</i>	0.00	0.03	-	-
	4.00	4.15	102.15	2.18
<i>Teucrium polium</i>	0.00	0.06	-	-
	1.50	1.50	96.07	3.42
<i>Artemisia vulgaris</i>	0.00	0.03	-	-
	6.00	6.11	1001.33	1.48

Table 6. Comparison of the proposed method with some existing methods

Metal ion	System	Linear dynamic range	LOD	LOQ	RMSE %	Detection method	Ref.
Cu ²⁺ , Zn ²⁺ , Co ⁺ , Ni ²⁺ , Pb ²⁺ , Cd ²⁺	Prrrolydine Dithiocarbamate	-	33.0-55.0 μg ml ⁻¹	-	-	FAAS	[32]
Cu ²⁺ , Co ⁺ , Ni ²⁺	MPKO ^a -Triton X 100	0.01-0.25 ng l ⁻¹	1.60- 2.1 ng l ⁻¹	-	-	1.2-1.3	[33]
Cu ²⁺ , Co ⁺ , Ni ²⁺ , Zn ²⁺	PATS ^b	0.5-10 mg l ⁻¹	0.056-0.123 mg l ⁻¹	0.186-0.410 mg l ⁻¹	1.24- 6.80	Spectroscopy	[25]
Cu ²⁺ , Co ⁺ , Ni ²⁺	DEBC	0.01-7.5 mg l ⁻¹	0.003-0.079 mg l ⁻¹	0.011-0.259 mg l ⁻¹	0.50- 4.58	Spectroscopy	Present method

^aMethyl-2-pyridylketone oxime. ^b3,6-Bis((2-aminoethylsalicyliden)thio)pyridazine.

synthesized Schiff base. The results showed that the key concept of proposed kinetic procedure overcomes the main drawback related to the heavily overlapped spectra of constitute. Proposed kinetic method provides sensitive and selective determination without any separation steps. Also kinetic method showed low LOD and LOQ values which can be considered as an advantage of this study. Investigation of the PCBPNN models and prediction measurements from synthetic mixtures of the analytes

showed that PCBPNNs is the most effective calibration method on the basis of the RMSEP% criteria. These models were computationally fast and yield reproducible results with respect to BPNNs. The other important advantage of PCBPNNs is much shorter training time in comparison to BPNNs. The fast convergence speed of PCBPNN can increase the accuracy and stability of the network. Also, in this work, the non-linear behavior of the analytes' responses and synergistic effects was observed (Fig. 1 c).

Based on the results in such cases, the PBFNN procedure shows the well-known ability of artificial neural network models to accommodate nonlinear data.

ACKNOWLEDGMENTS

The authors declare no conflicts of interest and accepted all principles of ethical and professionals. Also, our research was not involved animals and human participants.

REFERENCES

- [1] M. Rahimi, R. Farhadi, M. Salehi Balashahri, *Intl. J. Agron Plant Prod.* 3 (2012) 154.
- [2] S. Basgel, S.B. Erdemoğlu, *Sci. Total Environ.* 359 (2006) 82.
- [3] G.L. Eichhorn, *Mech. Ageing DeV.* 9 (1979) 291.
- [4] F.V. Assche, H. Clijsters, *Plant Cell and Environ.* 13 (1990) 195.
- [5] A.D. Kaur, V. Ravichandran, P.K. Jain, *J. Pharm. Biomed. Anal.* 46 (2008) 391.
- [6] Ł. Cieśla, A. Bogucka-Kocka, M. Hajnos, *J. Chromatogr. A* 1207 (2008) 160.
- [7] Y. Jiang, B. David, P. Tu, *Anal. Chim. Acta* 657 (2010) 9.
- [8] Y. Jin, T. Liang, Q. Fu, *J. Chromatogr. A* 1216 (2009) 2136.
- [9] I. Bombarda, N.J.P.D. Dupuy, *Anal. Chim. Acta* 613 (2008) 31.
- [10] H. Zhu, Wang, Y.H. Liang, G. Chen, P. Zhao, J. Tao, *Talanta* 81 (2010) 129.
- [11] N. Djabou, M.J. Battesti, H. Allali, L. Phytochemistry 72 (2011) 1390.
- [12] I.A. Suther, D. Fisher, *J. Chromatogr. A* 1216 (2009) 740.
- [13] N.B. Kormušoska, K. Čundeva, T. Stafilov, *J. Environ. Sci. Heal A* 44 (2009) 1274.
- [14] J.T. Kinard, *J. Environ. Sci. Heal A* 12 (1977) 531.
- [15] B.K.P. Huang, L.Q. Huang, L.P. Qin, Z.X. Zhao, H.C. Zheng, L. J. Chin. *Med. Mater.* 31 (2008) 1494.
- [16] H.S. Chen, R.B. Bhavik, K.G. Prem, *Anal. Chim. Acta* 602 (2007) 1.
- [17] F. Gong, Y.Z. Liang, Y.S. Fung, *J. Chromatogr. A* 1029 (2004) 173.
- [18] H.L. Zhai, F.D. Hu, X.Y. Huang, J.H. Chen, *Anal. Chim. Acta* 657 (2010) 131.
- [19] I.A. Sutherl, DerekFisher, *J. Chromatogr. A* 1216 (2009) 740.
- [20] R.T. Tian, P.S. Xie, H.P. Liu, *J. Chromatogr. A* 1216 (2009) 2150.
- [21] F.C. Sanchez, J. Toft, B. Vanden Bogaert, *Anal. Chem.* 68 (1996) 79.
- [22] C.J. Xu, Y.Z. Liang, F.T. Chau, *J. Chromatogr. A* 1134 (2006) 253.
- [23] Y.X. Zeng, C.X. Zhao, Y.Z. Liang, *Anal. Chim. Acta* 595 (2007) 328.
- [24] O.M. Kvalheim, Y.Z. Liang, *Anal. Chem.* 64 (1992) 936.
- [25] A. Afkhami, M. Abbasi Tarighat, *Talanta* 78 (2009) 424.
- [26] A. Afkhami, M. Abbasi Tarighat, H. Khanmohammadi, *Talanta* 77 (2009) 995.
- [27] J. Tisato, F. Refosco, F. Bandoli, *Coord. Chem. Rev.* 135 (1994) 325–397.
- [28] M. Asadi, Kh. Mohammadi, Sh. Esmailzadeh, B. Etemadi, H. Fun, *J. Inorg. Chim. Acta* 362 (2009) 4913.
- [29] Kh. Mohammadi, M. Zahedi, *Global J. Inorg. Chem.* 3 (2012) 1.
- [30] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, *Analyst* 119 (1994) 1669.
- [31] M. Abbasi Tarighat, M.R. Mohammadzadeh, Gh. Abdi, *J. Agri. Food Chem.* 61 (2103) 2632.
- [32] P.S. Hernandez, A.R. Hernandez, M.T. Ramiraz Silva, *Spectrochim. Acta A* 59 (2003) 2667.
- [33] I. Narin, M. Soylak, *Anal. Chim. Acta* 493 (2003) 205.
- [34] M. Ghaedi, A. Shokrollahi, F. Ahmadi, H.R. Rajabi, M. Soylak, *J. Hazard. Mater.* 150 (2008) 533.