Analysis of Complexation Interactions between Metal Ions and Drugs under Pseudo-physiological pH Conditions by a High-throughput Screening Method Using a Solid-phase Extraction Cartridge

Yukiko MORIIWA,† Naoko SUZUKI, Atsushi SHOJI, and Akio YANAGIDA†

Department of Biomedical Analysis, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

A high-throughput screening method for the complexation between metal ions and drugs was established by combining solid-phase extraction (SPE) using a nitrilotriacetic acid (NTA) modified silica spin cartridge with subsequent HPLC analysis. First, a test metal ion solution was passed through the NTA cartridge, then a test drug solution diluted in phosphate buffered saline (pH 7.4) was passed through the metal-chelated NTA cartridge. The complexation behavior between the metal and the drug on the NTA cartridge was evaluated by HPLC quantification of the drug in the SPE eluate. Comprehensive analysis of the complexation behavior between 11 different metal ions and 55 drugs showed that Cu²⁺, Ni²⁺, Co²⁺, Zn²⁺, Cr³⁺ and Fe³⁺ formed complexes with 12, 5, 4, 2, 1 and 1 kinds of drugs, respectively. Bromazepam selectively formed complexes with Cu²⁺, Ni²⁺ and Co²⁺.

Keywords Metal ion, medicinal drug, metal complex formation, solid phase extraction, high-performance liquid chromatography

(Received October 31, 2019; Accepted December 12, 2019; Advance Publication Released Online by J-STAGE December 20, 2019)

Introduction

Metal ions are essential for all living organisms and cell metabolism. Indeed, transition metals, such as iron, copper, manganese and zinc, are essential cofactors for cellular functions. Various biogenic compounds and drugs contain several transition metal binding sites (i.e., Lewis base ligands) and form metal complexes under aqueous physiological conditions. Norfloxacin, ciprofloxacin, ofloxacin, gemifloxacin and enoxacin form complexes with metal ions such as Al³⁺, Mg²⁺, Ca²⁺ and Fe²⁺. These complexes interfere substantially with the intestinal absorption of the drug because of the lower solubility of the metal complex in the intestinal tract. Moreover, metal complexes of some drugs, such as antibacterial and antiviral agents, show stronger activities than their free-forms, perhaps due to the metal ion stabilized drug binding with the target microbial DNA. Metal complexes of the above drugs form under physiological pH and ionic strength conditions. Therefore, understanding the biotransformation, biodistribution and pharmacological actions of drugs requires investigating the complexation behavior and/or potential between a metal ion and the drug under physiological conditions.

Several methods have been developed over the past few decades for confirming complexation between metal ions and drugs, but most have methodological limitations in terms of versatility, operability and throughput efficiency. For example, mass spectrometry and voltammetry have been used to identify the metal complexes of several drugs with high sensitivity but these methods cannot be used under physiological ionic conditions requiring phosphate buffered salt. Conventional ultraviolet (UV)-visible (Vis) absorption analyses can measure metal complexes under physiological pH conditions but only if the shape of the absorption spectrum changes upon metal complex formation. In general, liquid chromatography (LC) is poorly suited for measuring metal complexes because free-metal ions often gradually deteriorate LC stationary phase columns. Immobilized metal ion chromatography (IMAC) has been used for the efficient purification of recombinant proteins under physiological conditions by utilizing the differential affinities of tagged proteins for immobilized metal ions to achieve separation.

In this study, we describe a novel high-throughput method for screening the complexation between metal ions and drugs under pseudo-physiological pH conditions. The method combines solid-phase extraction using a centrifugal spin cartridge, followed by HPLC analysis. This method is superior to the conventional methods mentioned above in terms of simplicity and high-throughput efficiency. In addition, we report the results of comprehensive analyses of the complexation behavior between several different metal ions and drugs.

Experimental

Reagents and chemicals

Acetonitrile (CH₃CN; HPLC grade), sodium chloride (NaCl), potassium chloride (KCl), potassium dihydrogen phosphate
Flecainide 40 Type I 220 1.39 0.74 54 Prednisolone 30 Type I 250 1.60 1.00

Eleven metal chlorides (MgCl₂, AlCl₃, CaCl₂, CrCl₃, MnCl₂, FeCl₃, FeCl₂, CoCl₂, NiCl₂, CuCl₂, and ZnCl₂) were purchased from Wako Pure Chemical Industries. (KH₂PO₄) and disodium hydrogen phosphate (Na₂HPO₄) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Water was purified using a Milli-Q system (Merck-Millipore, Darmstadt, Germany). Phosphate buffered saline (PBS, pH 7.4) was prepared by dissolving 0.8 g of NaCl, 0.02 g of KCl, 270 μg/mL of each drug in 1 mL of aqueous 50% CH₃CN. Stock solutions (1000 μg/mL) of dasatinib (No. 40), folic acid (52) and metoclopramide (55) were prepared by dissolving 1 mg of each drug in 1 mL of a mixture of CH₃CN/1 M hydrochloric acid (1:1 v/v).

A test solution (20 μg/mL) of each drug was prepared by diluting each stock solution with a mixture of PBS and CH₃CN. The percent CH₃CN in each test solution was the same as in the HPLC mobile phase shown in Table 1.

Preparation of each metal ion test solution
Test solutions (500 μg/mL) of the 11 metal chlorides (MgCl₂, AlCl₃, CaCl₂, CrCl₃, MnCl₂, FeCl₃, FeCl₂, CoCl₂, NiCl₂, CuCl₂, and ZnCl₂) were prepared by dissolving 1 mg of each metal chloride in 2 mL of water.

HPLC apparatus and conditions
A conventional reversed-phase HPLC system (Hitachi, Tokyo, Japan) was used for quantitative determination of the drug in the test solution. The system consisted of an L-7100 pump, an L-2200 autosampler, and an L-7455 diode-array detector. A Chromolith HighResolution RP-18 column (100 × 4.6 mm i.d., Merck, Darmstadt, Germany) attached to a guard column.
(5 × 4.6 mm i.d.) was used as the stationary phase.

HPLC analyses were performed in isocratic elution mode using a mixture of the two mobile phase solvents: A (CH₃CN) and B (aqueous solvent: 10 mM acetate buffer (pH 5.0), 10 mM phosphate buffer (pH 7.0), 10 mM acetic acid or 0.1% formic acid). A test solution (20 μL) of a drug was injected and eluted at a flow rate of 2.0 mL/min over 3 min. The absorbance of the eluate was monitored in the range 200 to 400 nm.

Solid-phase extraction cartridge
We used a monolithic silica disk built-in centrifugal spin cartridge for solid-phase extraction (SPE) (“MonoSpin”, GL Sciences, Inc., Tokyo, Japan). Two different cartridges, MonoSpin ME and NTA, were investigated as metal ion adsorbents. The surface of the monolithic silica disk of the ME cartridge was modified with iminodiacetic acid functional groups, and that of the NTA cartridge was modified with nitrilotriacetic acid functional groups.

Centrifugal conditions for SPE using a MonoSpin cartridge
Solutions were passed through the MonoSpin cartridge using centrifugation at 5000 rpm (2300 × g) for 1 min using a Centrifuge 5415R (Eppendorf, Hamburg, Germany).

Pretreatment of the MonoSpin cartridge
The MonoSpin cartridge was pretreated by sequentially passing 200 μL of 1 M nitric acid and 200 μL of 100 mM ammonium acetate buffer (pH 5.5) to condition the ME or NTA moieties on the cartridge.

Measurement of the maximum amount of adsorbed metal ion on the MonoSpin NTA cartridge
We measured the amount of metal ion in the test solution and the concentration of the metal ion in the eluate after passage through a MonoSpin NTA cartridge using a graphite furnace atomic absorption spectrometer (Z-2000, Hitachi, Tokyo, Japan) equipped with a background correction system based on the Zeeman effect. Before each measurement, the graphite tubes and platforms were cleaned by heating for 6 s cycles at 2800°C until a low and stable baseline was achieved. Both the metal ion test solution and the corresponding eluate were diluted to less than 10 ng/mL with water. Next, 10 μL of the diluted solution was pipetted onto the platform and the absorbance was measured. The metal ion concentration in the solution was calculated from a calibration plot generated using dilutions of the standard solution. The absorption wavelength and other operating parameters (drying, atomization, cleanout, flow rate of the carrier Ar gas) for measuring each metal ion were as recommended by the manufacturer. The adsorption amount of a metal ion to NTA moieties on the MonoSpin cartridge was calculated from the decreased concentration of the metal ion after passage through the MonoSpin cartridge.

Measurement of the adsorption capacities of drugs to a metal-chelated MonoSpin NTA cartridge
A schematic of our screening method for complexation between a metal ion and a drug on a MonoSpin NTA cartridge is shown in Fig. 1. A test solution of a metal ion (500 μL, 500 μg/mL) was passed through a MonoSpin NTA cartridge and the metal-chelated cartridge was washed with 500 μL of water. Then, 200 μL of a drug solution (20 μg/mL) was passed through the metal-chelated MonoSpin NTA cartridge and the eluate was collected for HPLC analysis.

The amount (i.e., peak area (PA) on the chromatogram) of the drug in the eluate and in the test solution was measured by reverse-phase HPLC analysis given in the Experimental section. For example, the measured amount (PA) of the drug in the eluate and in the test solution were denoted “PAₑ” and “PAstd”, respectively, as shown in Fig. 1. The drug adsorption capacity (Q, %) to the metal-chelated MonoSpin NTA cartridge was calculated using Eq. (1):
\[ Q = (1 - PAE/PA_{STD}) \times 100 \] (1)

Separately from the above measurements, the same test solution of drug was passed through a bare (non-metal-chelated) MonoSpin NTA cartridge, and the eluate (i.e., metal blank eluate: MBE) was collected for the next HPLC analysis. The measured amount (PA) of the drug in the eluate was denoted “PA_{MBE}”, as shown in Fig. 1.

Results and Discussion

Optimization of HPLC conditions for rapid quantitative measurement of each drug

Prior to optimizing the SPE procedure, we optimized the reversed-phase HPLC conditions for analyzing each target drug in the test solution and SPE eluate. HPLC was performed in isocratic elution mode with a solvent mixture of A (CH3CN) and B (aqueous solvent) at a flow rate of 2.0 mL/min. Each target drug was rapidly eluted within 3 min and detected by the UV detector at its \( \lambda_{\text{max}} \) or shorter (210 - 220 nm) wavelength. The respective HPLC conditions (mobile phase composition, detection wavelength, retention time and \( k \)) for the 55 test drugs are listed in Table 1.

Confirmation of the maximum adsorption amount of each metal ion on a MonoSpin cartridge

Our preliminary study tested two different centrifugal-spin cartridges, MonoSpin ME and NTA, as metal ion adsorbents for our SPE procedure. Compared with an iminodiacetic acid moiety in the MonoSpin ME cartridge, a nitrilotriacetic acid moiety on the MonoSpin NTA cartridge is a bulky sterically functional group, and its density of the functional groups per a NTA cartridge is much less than that per a ME cartridge. Therefore, the maximum amount of metal ion adsorbed on a ME cartridge was larger than on a NTA cartridge, because the difference of the amount is proportional to the difference in density of each functional groups on each cartridge. However, under aqueous PBS (pH 7.4) buffer conditions, the adsorbed metal ion desorbed easily from the ME surface but did not desorb from the NTA surface (Table S1 in Supporting Information) and thus the MonoSpin NTA cartridge was used in all further experiments.

Next, we investigated the maximum amount of metal ion adsorbed on the NTA surface of the MonoSpin NTA cartridge. A 500-μL aliquot of test solution (500-μg/mL, i.e., 250 μg) was loaded onto a MonoSpin NTA cartridge and the concentration of metal ion in the eluate was quantified by atomic absorption spectrometry. The maximum adsorbed amounts (μg) of 10 metal ions on the NTA surface per cartridge were estimated, and ranged from 1.94 μg for Al\(^{3+}\) to 31.77 μg for Zn\(^{2+}\) (Table 2).

The data revealed that the NTA surface of the cartridge was essentially totally chelated by an excess of metal ion (250 μg) following passage of each metal ion test solution. However, the data also shows a large variation of error of a maximum adsorbed amount of each metal ion on the NTA cartridge. The large variations of them shown in Table 2 may correspond closely to the large variation of density of sterically bulky NTA moieties on each cartridge.

Optimization of the procedure for analyzing the interaction between a metal ion and a drug in the MonoSpin NTA cartridge

The procedure and optimized conditions for our screening method for the complexation of a metal ion and a drug on a MonoSpin NTA cartridge are illustrated in Fig. 1. The bare NTA surface in the cartridge is chelated by an excess of metal ion, the metal-chelated cartridge is washed with water, the drug test solution (containing PBS and CH3CN) is passed through the cartridge, then the concentration of drug in the eluate is measured by HPLC analysis. The concentration in the eluate is expressed in terms of the peak area of the drug in the HPLC chromatogram, e.g., “PAx” as shown in Fig. 1. The peak area of the drug in the test solution (e.g., “PA_{STD}”) is measured in advance using the same HPLC protocol. Based on the PAx and PA_{STD} values, the adsorption capacity (%) of the drug to the metal ion (chelated in the cartridge) is calculated using Eq. (1) given in the Experimental section. As shown in Fig. 1, if PAx and PA_{STD} are 10 and 100, respectively, the adsorption capacity (Q, %) of the drug is calculated to be ((1 – 10/100) × 100) = 90%.

Prior to the above experiments, we chose the CH3CN percent concentration for each drug test solution using a bare NTA cartridge as follows. We confirmed virtually no adsorption of a test drug to the bare NTA surface by passing a test solution of a drug through a (non-metal-chelated) NTA cartridge, then measured the drug concentration in the eluate (“PA_{MBE}” as shown in Fig. 1) by HPLC. In this study, it is desirable that the PA_{MBE} of a drug be similar to its PA_{STD}, which means little adsorption of a drug to the bare NTA cartridge (i.e., the Q value to a bare NTA surface is lower than 20%), but this value is very sensitive to the solvent composition of the drug test solution, and especially to the percent CH3CN.

Figure 2 shows the relationship between the adsorption capacity (Q, %) of bromazepam (BMP, No. 46) to a bare NTA cartridge and the CH3CN concentration (%) in the BMP test solution. When the CH3CN concentration was 0%, the Q value was relatively high (about 50%) but the Q values significantly decreased as the CH3CN concentration increased. The results in Fig. 2 suggested that non-specific adsorption of BMP to the bare NTA surface resulting primarily from hydrophobic interactions could be eliminated by adding CH3CN to the BMP test solution. Indeed, the Q values of BMP decreased below 20 or 10% using a BMP test solution containing more than 20% CH3CN or 30% CH3CN, respectively. Based on the results for the 55 drugs tested in this study, the CH3CN percent concentration in each of the 55 drug test solutions was set to the same percent value as the CH3CN concentration in each HPLC mobile phase shown in Table 1. For BMP, the percent CH3CN concentration in the test solution was 30%, the same as in its HPLC mobile phase.

Table 2: Maximum amount of metal ion adsorbed on the NTA surface of a MonoSpin cartridge

| Metal ion | Maximum adsorption amount (per cartridge) μg | SD (n = 3) |
|----------|---------------------------------------------|-----------|
| Mg\(^{2+}\) | 8.60 | 1.35 |
| Al\(^{3+}\) | 1.94 | 0.67 |
| Ca\(^{2+}\) | 13.46 | 3.44 |
| Cr\(^{3+}\) | 3.50 | 2.19 |
| Mn\(^{2+}\) | 3.13 | 0.94 |
| Fe\(^{2+}\) | 4.24 | 0.60 |
| Fe\(^{3+}\) | 8.71 | 0.42 |
| Ni\(^{2+}\) | 5.00 | 3.30 |
| Cu\(^{2+}\) | 19.3 | 1.74 |
| Zn\(^{2+}\) | 31.77 | 12.22 |

a. The loaded amount of each metal chloride was 250 μg (per cartridge).
Characteristics of the interaction between metal ions and bromazepam under pseudo-physiological pH conditions

BMP is a benzodiazepine anxiolytic and is well-known to form metal-complexes with Mn$^{2+}$, Cr$^{3+}$, Fe$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$. The characteristics and behavior of the BMP complex with metal ions have been studied by UV-Vis spectroscopic, crystallographic, electrochemical and thermal analyses, but not under physiological pH and ionic strength conditions. We thus first studied BMP complexation with 11 different metal ions using our present screening method under aqueous PBS conditions. Figure 3A shows HPLC stacked chromatograms of BMP in the eluates after passing a test solution of BMP (containing PBS and CH$_3$CN) through the MonoSpin NTA cartridge. Chromatogram (a) shows BMP in the test solution before passing through the NTA cartridge, (b) shows the BMP remaining in the eluate after passing through the bare NTA cartridge, and chromatograms (c) to (m) shows BMP remaining in the eluate after passing through each metal-chelated NTA cartridge. Based on the amount of free BMP (i.e., the peak areas), the adsorption capacities (%) of BMP to each of the 11 metal ions (chelated on each NTA cartridge) were respectively calculated in accordance with Eq. (1) and the % values are shown as Fig. 3B. The comparative data shown in this figure revealed that the adsorption capacities of BMP for Co$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$ ions were high but very low for Mg$^{2+}$, Al$^{3+}$, Ca$^{2+}$, Cr$^{3+}$ and Fe$^{3+}$ ions (comparable to the bare NTA cartridge). These data strongly indicate that BMP selectively forms metal complexes with Co$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$ ions under pseudo-physiological pH conditions and not with the remaining 8 metal ions. Unfortunately, the value of adsorption capacity of BMP in Fig. 3 represents nothing more than a barometer of the presence or absence of complexation between BMP and a metal ion, and it is difficult to calculate a complex formation constant from an adsorption capacity value. However, a large value of adsorption capacity clearly demonstrates “the presence” of a complexation between BMP and a metal ion, and the comparison of the value shown in Fig. 3 is very useful for studying “the selectivity” of complexation between both compounds.

Previous reports showed that BMP forms metal complexes with Co$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$, as well as with Mn$^{2+}$, Cr$^{3+}$, Fe$^{2+}$, Fe$^{3+}$ and Zn$^{2+}$ ions in simple aqueous conditions. In general, high ionic strength is known to weaken ionic bonds and metal complex formation, and phosphate salts interact as ligands for metal ions, interfering with drug-metal complex formation. Then, we additionally confirmed the UV spectra of BMP with metal ions in a mixture solution of PBS (pH 7.4) and CH$_3$CN (7:3, v/v) (Fig. S1 in Supporting Information). The UV spectroscopic results were in good agreement with our screening results regarding nine kinds of metal ions (complex formation with Co$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$, and no complex with Mg$^{2+}$, Ca$^{2+}$, Al$^{3+}$, Cr$^{3+}$, Mn$^{2+}$ and Zn$^{2+}$) except for Fe$^{2+}$ and Fe$^{3+}$. The comparison between Fig. 3 and Fig. S1 suggests that the complex formation of BMP with Fe$^{2+}$ or Fe$^{3+}$ may be difficult to detect by our screening method using NTA cartridge under pseudo-physiological pH conditions.

![Fig. 2 Relationship between the adsorption capacity (%) of BMP to a bare NTA cartridge and the CH$_3$CN concentration (%) in the BMP test solution.](image1)

![Fig. 3 (A) HPLC stacked chromatograms of BMP in the eluates after passing the BMP test solution through the MonoSpin NTA cartridge. a) Chromatogram of BMP in the test solution before passing through the NTA cartridge; b) after passing through the bare NTA cartridge; c) – m) after passing through each of 11 different metal-chelated NTA cartridges: c) Mg$^{2+}$, d) Al$^{3+}$, e) Ca$^{2+}$, f) Cr$^{3+}$, g) Mn$^{2+}$, h) Fe$^{2+}$, i) Fe$^{3+}$, j) Co$^{2+}$, k) Ni$^{2+}$, l) Cu$^{2+}$, m) Zn$^{2+}$. (B) Comparison of the adsorption capacity (%) of BMP to each of 11 different metal ions chelated onto the NTA cartridge.](image2)
Trial comprehensive interaction analysis between metal ions and 55 drugs

We used our present high-throughput screening method to comprehensively analyze the interactions between 11 different metal ions and 55 drugs under pseudo-physiological pH conditions. Our method has the capacity to facilitate screening of about 20 different combinations of complexation between a metal ion and a drug within 1 h, by simultaneous centrifugal treatment using the MonoSpin NTA cartridge with subsequent rapid HPLC analyses at three minute intervals. Figure 4 lists all combinations between the metals and drugs, and the strength of the adsorption capacity (%) of each drug against each metal ion (chelated to the NTA cartridge) is expressed as color intensity for each % value. Of the 55 drugs, only clonidine (No. 35) could not be measured because of its strong non-specific adsorption to the (non-metal chelated) bare NTA cartridge (47%). Of the remaining drugs, 41 formed very weak complexes and 12 (Nos. 2, 3, 5, 10 – 12, 14, 41, 46, 48, 49) strongly formed complexes with Cu²⁺ (i.e., their adsorption capacities were over 40%). Specifically, bacitracin (5) and tetracycline (14) formed complexes with Co²⁺, Ni²⁺, Cu²⁺ and Zn²⁺, levofloxacin (12) formed complexes with Fe³⁺, Co²⁺, Ni²⁺ and Cu²⁺, ceftriaxone (10) formed complexes with Ni²⁺ and Cu²⁺, and bromazepam (46) formed complexes with Co²⁺ and Cu²⁺. Teicoplanin (2), vancomycin (3), meropenem (11), phentolamine (33), imatinib (41), aminophylline (48) and theophylline (49) selectively formed Cu²⁺-complexes only. Furthermore, only lamotrigine (19) formed a Cr³⁺-complex. The results shown in Fig. 4 are the first reported examples of metal complex formation by drugs under pseudo-physiological pH conditions.

None of the tested drugs formed complexes with light metal ions such as Mg²⁺, Al³⁺ and Ca²⁺, despite previous reports that tetracycline (14) formed complexes with heavy metal ions (such as Co²⁺, Ni²⁺, Cu²⁺ and Zn²⁺) and light metal ions (such as Mg²⁺ and Ca²⁺).⁹⁻¹³ Our present interaction analysis is based on ternary-complex formation between a drug, a metal ion, and an NTA moiety on a MonoSpin cartridge. Interactions between a drug and a light metal ion may be weaker than that between a drug and a heavy metal ion, making it difficult to detect, especially under pseudo-physiological pH conditions.

Conclusions

In this study we established a high-throughput screening method for complexation between a metal ion and a drug by combining SPE treatment using a metal-chelated MonoSpin NTA cartridge with subsequent rapid HPLC analysis of the drug. In our screening method, the MonoSpin NTA was used as the SPE cartridge in all experiments for various kinds of drugs (In addition, the optimum SPE condition using the MonoSpin ME cartridge is currently being tested for other target compounds, such as zwitter amino acids or neutral polyphenols). The method was used to comprehensively analyze the complexation behavior between 11 types of metal ions and 55 drugs under pseudo-physiological pH conditions. Our findings efficiently identified many combinations of complexation between metal ions and drugs and will aid in understanding the pharmacological effects and/or side-effects of medicinal drugs and in evaluating the quality of a drug. Furthermore, our present screening method is broadly applicable not only to drugs but also to other biogenic and related organic compounds. As such, this method will facilitate further research on metal complexation and potentially provide new knowledge that will be useful in the fields of biochemistry, pharmacology and drug discovery.

Acknowledgements

The authors acknowledge Mr. Shigenori Ohta (GL Sciences Inc.) for his technical support for atomic adsorption analyses and advice on SPE treatment using MonoSpin ME and NTA cartridges. No funding was received for this work.

Supporting Information

Table S1 shows the comparison of the adsorption-desorption characteristics of Cu²⁺ or Fe²⁺ chelated with ME or NTA cartridge. Figure S1 shows the UV spectra of BMP with metal ions in a mixture solution of PBS (pH 7.4) and CH₃CN (7:3, v/v). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References

1. M. A. Zoroddu, J. Aaseth, G. Crisponi, S. Medici, M.
1. Peana, and V. M. Nurchi, *J. Inorg. Biochem.*, **2019**, *195*, 120.
2. C. Nouet, P. Motte, and M. Hanikenne, *Trends Plant Sci.*, **2019**, *16*, 395.
3. P. Lehto, K. T. Kivisto, and P. J. Neuvonen, *Br. J. Clin. Pharmac.*, **1994**, *37*, 82.
4. M. W. Pletz, P. Petzold, A. Allen, O. Burkhardt, and H. Lode, *Antimicrob. Agents Chemother.*, **2003**, *47*, 2158.
5. N. Sultana, E. Humza, M. S. Arayne, and U. Haroon, *Quim. Nova*, **2011**, *34*, 186.
6. P. Fernandes, I. Sousa, L. Cunha-Silva, M. Ferreira, B. de Castro, E. F. Pereira, M. J. Feio, and P. Gameiro, *J. Inorg. Biochem.*, **2014**, *131*, 21.
7. I. Turel, *Coord. Chem. Rev.*, **2002**, *232*, 27.
8. J. Nagaj, R. Starosta, and M. Jezowska-Bojczuk, *J. Inorg. Biochem.*, **2015**, *142*, 68.
9. N. Shahabadi, F. Shiri, and S. Hadidi, *Spectrochim. Acta*, Part A, **2019**, *219*, 195.
10. X. Yuan, J. Qin, and L. Lu, *Spectrochim. Acta*, Part A, **2010**, *75*, 520.
11. A. M. Nowicka, M. Mackiewicz, E. Matysiak, B. Krasnodebska-Ostrega, and Z. Stojek, *Talanta*, **2013**, *106*, 85.
12. M. S. Refat, *J. Mol. Struct.*, **2013**, *1037*, 170.
13. A. Kadej, M. Kuczer, E. Czarniewska, A. Urbanski, G. Rosinski, and T. Kowalik-Jankowska, *J. Inorg. Biochem.*, **2016**, *163*, 147.
14. O. P. Chouhan and G. Jacob, *Oriental J. Chem.*, **2014**, *30*, 1501.
15. L. Szyrwiels, J. S. Pap, W. Malinka, Z. Szewczuk, A. Kotynia, and J. Brasun, *Pharm. Biomed. Anal.*, **2013**, *76*, 36.
16. N. Sridevi and K. K. M. Yusuff, *Toxicol. Mech. Meth.*, **2007**, *17*, 559.
17. S. Najma, M. S. Arayne, K. M. Mehboob, and N. Muhammd, *Chin. J. Chem.*, **2011**, *29*, 1933.
18. N. Sultana, M. S. Arayne, M. Nawaz, and Z. U. Rehman, *Med. Chem. Res.*, **2011**, *20*, 531.
19. T. Cecchi, F. Pucciarelli, P. Passamonti, and S. Ferraro, *J. Liq. Chromatogr. Relat. Technol.*, **1999**, *22*, 429.
20. H. Engelhardt and T. Lobert, *Anal. Chem.*, **1999**, *71*, 1885.
21. R. C. F. Cheung, J. H. Wong, and T. B. Ng, *Appl. Microbiol. Biotechnol.*, **2012**, *96*, 1411.
22. G. S. Chaga, *J. Biochem. Biophys. Methods*, **2001**, *49*, 313.
23. M. M. C. Santos, V. Famila, and M. L. S. Goncalves, *Electroanalysis*, **2000**, *12*, 216.
24. J. A. Real, J. Borras, M. C. Munoz, A. Mosset, and J. Galy, *J. Inorg. Biochem.*, **1987**, *31*, 221.
25. J. Hernandez-Mendez, C. Gonzalez-Perez, and M. I. Gonzalez-Martín, *Microchem. J.*, **1985**, *31*, 94.
26. J. D. Sabatino, O. W. Weber, G. R. Padmanabhan, and B. Z. Senkowsksi, *Anal. Chem.*, **1969**, *41*, 905.
27. A. M. Mansour and O. R. Shehab, *Appl. Organometal. Chem.*, **2017**, *31*, 3635.
28. J. A. Real, M. C. Munoz, and J. Borras, *Thermochim. Acta*, **1986**, *101*, 83.
29. J. A. Real and J. Borras, *Synth. React. Inorg. Met. Org. Chem.*, **1984**, *14*, 843.
30. J. A. Real and J. Borras, *Synth. React. Inorg. Met. Org. Chem.*, **1984**, *14*, 857.
31. L. Lambs and G. Berthon, *Inorg. Chim. Acta*, **1988**, *151*, 33.
32. L. Lambs, M. Venturini, B. D. Reverend, H. Kozlowski, and G. Berthon, *J. Inorg. Biochem.*, **1988**, *33*, 193.
33. B. Carlotti, A. Cesaretti, and F. Elisei, *Phys. Chem. Chem. Phys.*, **2012**, *14*, 823.