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Copper sorption efficiency of *p*-tetranitrocalix[4]arene based high performance liquid chromatographic column

Fatih Durmaz[a], Fakhar N. Memon[b], Shahabuddin Memon[b,*], Ayaz A. Memon[b], Naeem A. Memon[b], Hüseyin Kara[a,c]

[a]*Department of Chemistry, Selçuk University, Konya 42031, Turkey, Tel. +90 3322233861; emails: fatihkaan@gmail.com (F. Durmaz), huskara@gmail.com (H. Kara)*
[b]*National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan, Tel. +92 22 9213429 30; email: fakhar_memon2@yahoo.com (F.N. Memon), Fax: +92 22 9213431; emails: shahabuddinmemon@yahoo.com (S. Memon), ayazmemon33@gmail.com (A.A. Memon), naeemahmedmemon12@yahoo.com (N.A. Memon)*
[c]*Faculty of Science, Department of Biotechnology, Necmettin Erbakan University, Konya 42090, Turkey*

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ABSTRACT

Present study deals with the sorption characteristics of *p*-tetranitrocalix[4]arene (**3**) appended silica-based high-performance liquid chromatographic (HPLC) column utilized for preconcentration, determination, and purification of Cu^{2+} from water. The sorption characteristics of the HPLC column were investigated for Cu^{2+} in column agreement. The experiments were performed in five steps that were monitored using UV–visible diode-array detector. However, all the HPLC experimental results were reconfirmed by using atomic absorption spectrophotometer. The effect of concentration on the sorption efficiency of the column was evaluated for Cu^{2+} and data obtained was investigated using Langmuir and Freundlich sorption isotherms. The value of coefficient of determination (R^2), i.e. 0.99, suggested that the Freundlich sorption isotherm was found to be the best fit model for Cu^{2+}. The developed method was validated for system efficiency, accuracy, and precision, while the data obtained revealed that the developed column could be applied for the sorption of Cu^{2+} with adequate efficiency and repeatability.

Keywords: HPLC; AAS; Copper; Sorption; Calixarene

1. Introduction

Heavy metals such as Cd^{2+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, Zn^{2+} Cu^{2+}, and many other toxic metals are responsible for environmental pollution and can be introduced into aqueous (natural or wastewater) and soil environments either naturally or as a result of human activities, i.e. agricultural, industrial, and mining operations [1]. The functions of all metal processing industries, particularly copper-based pesticides (herbicides and fungicides) and Cu fertilizers [2], can give rise to elevated copper concentrations in the water and surface layers of agricultural soil [3]. Copper has broad range applications such as catalysts, pigments, pesticides, fertilizers, and stabilizers for PVC [4]. For plants and animals, Cu^{2+} is considered as third abundant micronutrient trace metal ion after Fe^{2+} and Zn^{2+}, and play significant role in human health [5,6]. Trace

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*Corresponding author.

amount (μg/L) of Cu^{2+} is necessarily required as cofactors for the enzymatic activities in some organisms. However, the access amount of Cu^{2+} (mg/L or more) is considered as prominent toxic ion due to irreversible inhibition of some enzymes [7] present in soils and aqueous systems having harmful effects to the environment and human body [1–8]. Moreover, these metal ions are different from other toxic pollutants and are not biodegradable; they can be accumulated in living tissues, causing various diseases and disorders [9]. Free copper is acutely and chronically effective to aquatic as well as terrestrial life, while excessive ingestion of copper can damage liver or kidney [1] and even its deficiency can influence the microorganisms and force to sluggish growth of plants [6–10]. Therefore, more useful methods for the determination, preconcentration, and removal of Cu^{2+} from the aqueous environment are still an immense challenge for the scientists that need more attention.

A variety of treatment methods have been applied for the removal of heavy metals from aqueous environment including coagulation [11], online dialysis ion chromatography [12], solid-phase extraction (SPE) by different ligands and multi-walled carbon nanotubes [13,14], surface complexation, adsorption, chemical precipitation, electrolytic processes, membrane processes, ion-exchange [15–20] solvent extraction, chemical oxidation/reduction, activated carbon, and reverse osmosis [9]. All the above-listed procedures are known to be conventional methods for the handling of toxic heavy metals from water. Nowadays, the technologies have been moved to advanced, sophisticated, easy to use, and economical ones which give slightest disadvantages as compared to conventional techniques that can meet all the desired requirements along with good efficiency. In this regard, SPE is a versatile and evergreen technique that utilizes the analytical and instrumental methods including high-performance liquid chromatography (HPLC). Many low-cost natural and synthetic sorbents have been used for removal of toxic metals such as lignin, chitosan, Lignocellulosic Materials, peat moss, pectin, clay, saw dust, zeolite, seaweed, bark materials, ironoxide-coated sand [13,14,21–23], and different derivatives of calix[4]arene. The immobilization of calix[4]arene onto functional silica gel material provide a new addition in the field of novel and admirable stationary phases for column chromatography [24–26].

This is the extension of our previously reported work [26]. The purpose of the present work is to utilize the synthetic *p*-tetranitrocalix[4]arene (**3**) based immobilized silica material (**4**) as an efficient stationary phase for HPLC to investigate the removal and recovery of Cu^{2+} under flow conditions and to calculate the breakthrough capacity of the column. In our previous study [6–24], it has been noticed that *p*-tetranitrocalix[4]arene is a versatile derivative to coordinate with metal ions and then it was immobilized with silica for HPLC study.

2. Experimental

2.1. Materials and methods

All the reagents and solvents were of analytical or HPLC grade and used without further purification. Acetonitrile and hydrochloric acid were purchased from Fisher Chemicals (Fair Lawn, NJ, USA). The Cu^{2+} was used in their nitrate salt form and was purchased from Merck (Darmstadt, Germany). Silica resin (250–400 mesh size) was purchased from Sigma–Aldrich Chemie Gmbh. (Steinheim, Germany). Synthesis of compounds **(1–3)** and immobilization of **3** with silica were made according to previously reported methods [24].

2.2. Instrumentation

FT-IR spectra were recorded on a Thermo Nicollet AVATAR 5700 FT-IR spectrometer in the spectral range 4,000–400 cm^{-1}. CHNS analyzer (model Flash EA 1112) instrument (20090; Rodano, Milan, Italy) was used for elemental analyses. Scanning electron microscopic (SEM) studies were performed by using a JSM-6380 instrument. Melting points were determined by using the Gallenkamp apparatus with a sealed capillary tube. Thin layer chromatography was performed on pre-coated silica gel plates (SiO_2, PF254, Merck), and column chromatography was performed using liquid chromatography (Spectra system SCM 1000, Thermo Finnigan) equipped with vacuum degasser, Q Grade A0024752 model quaternary pump, Rheodyne manual injector system with 20 μL loop, and a DAD system. In the last, a Perkin-Elmer atomic absorption spectrophotometer (AAS) Model A Analyst 700 (Norwalk, CT) with deuterium background correction was used for verification of Cu^{2+}. The hollow cathode lamp of particular copper metal was run under the conditions suggested by the manufacturer.

2.3. Immobilization of *p*-tetranitrocalix[4]arene (3) with silica (4)

The synthesis (Fig. 1) of all compounds from **1** to **3** and immobilization of **3** on silica was made according to the previously reported method [26].

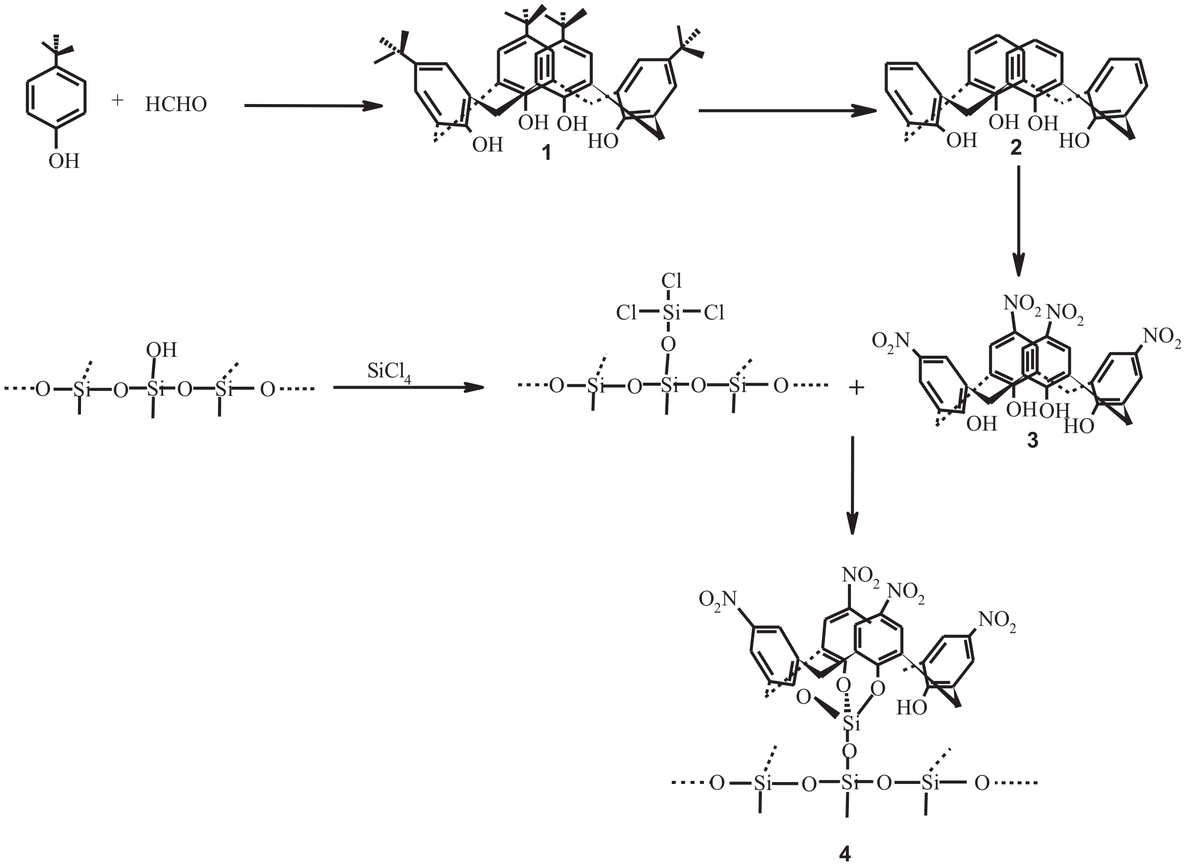

Fig. 1. Synthetic route for *p*-tetranitrocalix[4]arene (3) appended silica [26].

2.4. Column packing

The column was packed with immobilized material by using slurry packing technique followed by a reported procedure [26]. The empty stainless steel column with 150 mm in length and 3 mm in internal diameter was used for packing immobilized material. Before packaging, empty column was washed thoroughly with appropriate solvents i.e. D.I. water, 0.1 M HCl and NaOH, and methanol for removing any impurities. Peristaltic pump was used for filling column at maximum pressure. In order to remove any possible impurity, filled column was washed with 0.01 M HCl and ultrapure water. Consequently, it was conditioned to be used for further experiments.

2.5. HPLC procedure and parameters

A four-step procedure of HPLC for sorption of Cu^{2+} on resin packed column is described in Fig. 2 and illustrated as below; whereas, the chromatograms were recorded at 300 nm at constant flow rate of 0.5 mL/min throughout the analysis and the method was operated by gradient elution system.

Fig. 2 (step **1**) shows that the mixture of acetonitrile and water (30:70) as mobile phase was used for column conditioning for 10 min. After conditioning, Cu^{2+} ions were loaded as the solution of appropriate copper nitrate with different concentrations passed through the column in step **2** and loading process was continued until the column was near to saturation or gets saturated. The column was washed in step **3** with deionized water for five minutes to remove the unloaded copper ions and the process was continued till a stable signal was obtained from the detector. The step **4** shows the stripping process, where loaded copper ions were stripped from the column by flowing 0.1 M HCl solution for 10 min. During the stripping step, all solutions leaving the detector were collected till the end of peak and were subjected to AAS analysis.

2.6. Atomic absorption spectroscopic analysis

The AAS analysis was performed using standard solutions of copper metal with linear concentration range (R^2, 0.99). All calculations were performed by using the data obtained from AAS analysis.

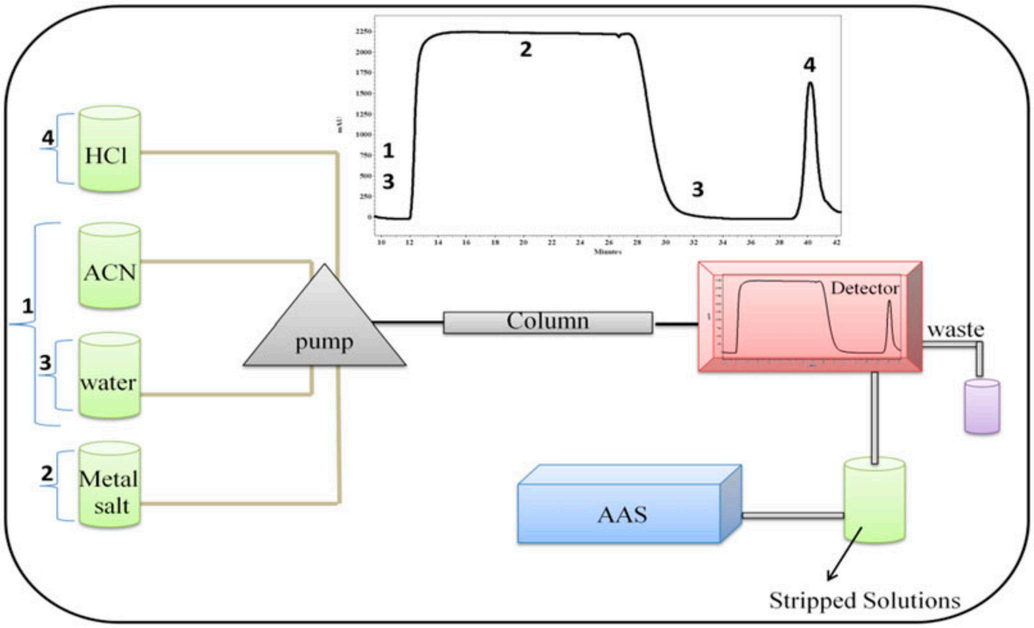

Fig. 2. Chromatographic scheme: (1) Column conditioning: acetonitrile and water (30:70), (2) Loading of metal ion solution to the column: 0.01 M, (3) Washing of unloaded metal ion: H_2O, and (4) Unloading of metal ion solution from the column: 0.1 M HCl solution for 10 min.

3. Results and discussion

3.1. Characterization of immobilized resin

The characterization of the immobilized resin was carried out using FTIR and SEM techniques and the results achieved were in good agreement with that of our recently reported work [24].

3.2. Effect of copper ion concentration

The Cu^{2+} concentration significantly affects the amount of sorbed metal ion. Consequently, the loading solutions of Cu^{2+} were diluted in the range of 0.01–0.0001 M to examine their effect of concentration as shown in Fig. 3. It can be revealed from the Fig. 3 that the curves exhibit sharper profile at higher concentration as compared to lower concentration; the effect may be pronounced due to the fact that at higher concentration, Cu^{2+} ions are in dynamic equilibrium (forward and backward reaction exhibit no net change over time) between the mobile phase and stationary phase. The normalized curves at different concentration of Cu^{2+} are shown in Fig. 3.

3.3. Sorption isotherms

The data obtained from Cu^{2+} concentration profiles was tested using two frequently applied sorption isotherms.

3.3.1. Langmuir sorption isotherm

Langmuir sorption isotherm deals with the process that occurs on homogenous surface without mutual interactions between molecules of the sorbate. This isotherm has the following linear form Eq. (1):

$$\frac{C_e}{C_{\text{ads}}} = \frac{1}{Qb} + \frac{1}{Q}C_e \tag{1}$$

where C_e is concentration of metal ion at equilibrium (mol/L), C_{ads} is concentration adsorbed (mol/g), Q and b becomes Langmuir isotherm constants which are calculated from the plot of (C_e/C_{ads}) vs. C_e in which $1/Q$ is the slope and $1/Qb$ is intercept as shown in Fig. 4.

Fig. 4 shows the Langmuir sorption isotherm of Cu^{2+} and Table 1 represents the parameters calculated from Fig. 4.

Whereas, the separation factor R_L is calculated using Langmuir constant b using the value of C_i that is initial concentration of the metal ion through Eq. (2).

$$R_L = \frac{1}{1 + bC_i} \tag{2}$$

(1) $R_L > 1$, is unfavorable isotherm.
(2) $R_L = 1$, is linear isotherm.

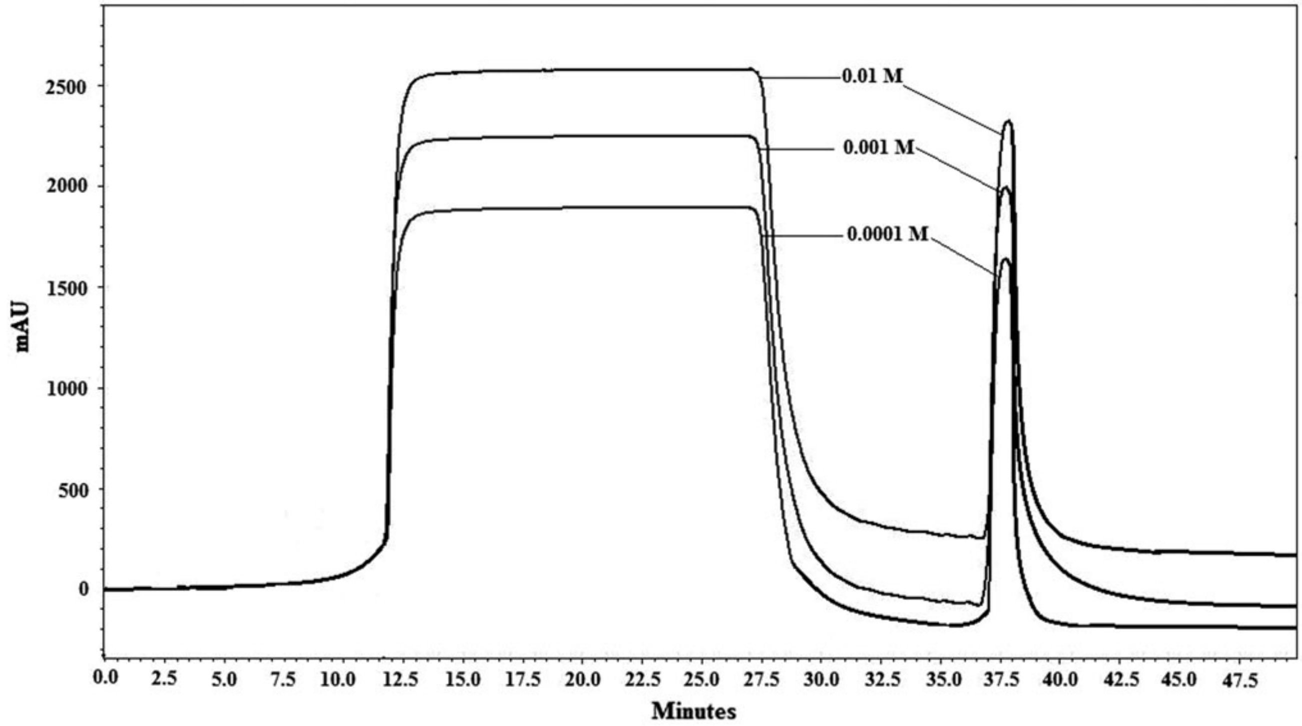

Fig. 3. A real chromatogram showing adsorption and desorption of Cu^{2+} on *p*-tetranitrocalix[4]arene appended silica HPLC column. The loading solutions of Cu^{2+} were diluted in the range of 0.01–0.0001 M.

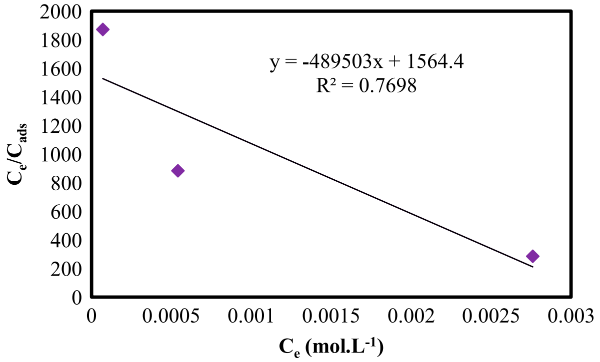

Fig. 4. Langmuir sorption isotherm: 0.01 M Cu^{2+}, wavelength of detection was 300 nm at flow rate of 0.5 mL/min.

(3) $0 < R_L < 1$, is favorable isotherm.
(4) $R_L = 0$, is irreversible isotherm.

However, the linear sorption for Cu^{2+} is evident from calculated value of R_L, that is 1.0 thus $R_L = 1$.

3.3.2. Freundlich sorption isotherm

Freundlich sorption isotherm is used to describe the sorption process on heterogeneous and microporous

Table 1
Isotherm constant and value of regression coefficient (R^2) for Cu^{2+}. 0.01 M Cu^{2+}, wavelength of detection was 300 nm at flow rate of 0.5 mL/min

Isotherms	C_m (mol g^{-1})	$1/n$	R^2
Freundlich	1.201	1.508	0.996
	Q (mol g^{-1})	B (mol L^{-1})	R^2
Langmuir	2.04E-05	1.31E-08	0.769

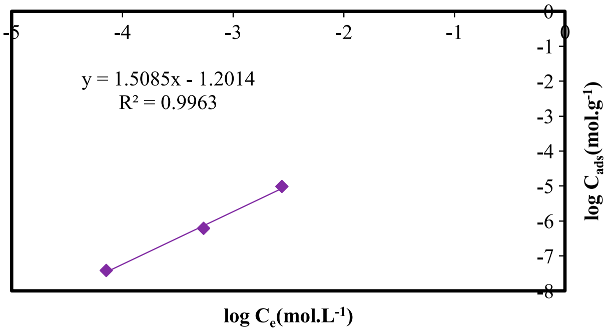

Fig. 5. Freundlich sorption isotherm: 0.01 M Cu^{2+}, Wavelength of detection was 300 nm at flow rate of 0.5 mL/min.

Table 2
Reproducibility, precision, and accuracy. 0.01 M Cu^{2+}, wavelength of detection was 300 nm at flow rate of 0.5 mL/min

	Intra-day (n = 5)			Inter-day (n = 5)		
Amount Injected (M)	Observed amount (M) ± SD	CV (%)	Accuracy (%)	Observed amount (M) ± SD	CV (%)	Accuracy (%)
0.0001	$85.1 \times 10^{-6} \pm 1.5 \times 10^{-6}$	0.103	85.1	$85.5 \times 10^{-6} \pm 1.0 \times 10^{-6}$	0.156	85.5
0.01	$71.4 \times 10^{-4} \pm 1.1 \times 10^{-4}$	0.097	71.4	$78.9 \times 10^{-4} \pm 1.5 \times 10^{-4}$	0.077	78.9

adsorbent surfaces, and assumes that various types of active sites adsorb/interact simultaneously. Eq. (3) is a linear form of this isotherm;

$$\log C_{\text{ads}} = C_m + \frac{1}{n}\log C_e \tag{3}$$

where C_{ads} is a sorbed concentration (mol/g), C_m is multilayer sorption capacity, $\frac{1}{n}$ is sorption intensity, and C_e is an equilibrium concentration (mol/L). A plot of log C_{ads} vs. C_e exhibits straight line with slope $\frac{1}{n}$ and intercept C_m as shown in Fig. 5.

Fig. 5 is a plot of Freundlich sorption isotherm for Cu^{2+}. However, the values calculated from this isotherm are tabulated in Table 1.

The sorption intensity value ($1/n > 1$) refers the cooperative nature of the reaction and if ($1/n < 1$) infers the chemisorption nature of the reaction [6]. In present study, the value of $1/n$ is > 1 as shown in Table 1 suggesting the cooperative nature of the reaction. Furthermore, on the basis of regression coefficient, Freundlich isotherm shows the best fit to the experimental data.

The values of constants obtained from aforementioned isotherm are presented in Table 1.

3.4. Loading capacity of column

The loading capacity of the column was calculated from the AAS values of Cu^{2+}. From the loading capacity values, it has been noticed that the metal loaded was ~4×10^{-2} M, which shows that the resin has about more than 100 times loading capacity as compared to the simple calixarene molecule forming 1:1 ligand metal complex [6].

3.5. System suitability

When the analyte containing Cu^{2+} was sorbed by the column, the retention time (t_R) and k values for desorbed Cu^{2+} was 38.02 ± 03 min and 1.5, respectively, whereas the number of theoretical plates (N) was found to be 9,580. The reproducibility test was less than 1% of retention time for Cu^{2+}. It can be revealed from the results that the developed column could be applied for the sorption of Cu^{2+} with adequate efficiency and repeatability.

3.6. Precision and accuracy

Sorption of Cu^{2+} was assessed for inter-day and intra-day precision. Inter-day precision experiments were carried out every day for five days. Similarly, intra-day precision was carried out by running the standard solutions of all the Cu^{2+} five times a day. It can be observed from the Table 2 that all the coefficients of variation (CV) are less than 1%.

4. Conclusions

The present study provides information regarding sorption characteristics of *p*-tetranitrocalix[4]arene bonded HPLC column for the determination and purification of Cu^{2+} from water. All the experimental results were established using HPLC followed by AAS-based measurements. The sorption characteristics of the column were investigated using Langmuir and Freundlich sorption isotherms. Freundlich sorption isotherm was found to be best fit model for Cu^{2+} suggesting physical nature of sorption reaction. However, the value of R_L from Langmuir isotherm suggested the linear sorption process i.e. $R_L = 1$; whereas, the sorption intensity value ($1/n$) from Freundlich isotherm suggested cooperative nature of reaction. Consequently, the binding constant, sorption capacity, as well as the reusability of the HPLC column suggests higher probability to sorb Cu^{2+}. Furthermore, the method was also validated for its linearity, precision, and accuracy. The validated data suggested that the column has adequate efficiency, repeatability, precision, and accuracy, and could be applied successfully for the sorption of Cu^{2+} metal ions. All these observations provide a clear clue for the applicability of *p*-tetranitrocalix[4]arene embedded silica column for preconcentration, determination, and purification of Cu^{2+}. Furthermore, the validated data suggests that the column has adequate efficiency, repeatability, precision as well as accuracy, and could be applied successfully for the sorption of Cu^{2+}.

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