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Dynamically modified C_{18} silica monolithic column for the rapid determinations of lead, cadmium and mercury ions by reversed-phase high-performance liquid chromatography

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ABSTRACT

In this article, a new reversed-phase high-performance liquid chromatography (RP-HPLC) method for the rapid, selective and sequential separation of toxic heavy metal ions namely, Pb^{2+}, Hg^{2+}, and Cd^{2+} is reported. For this RP-HPLC method, a C_{18} silica monolith column (Chromolith RP-18e, 100 × 4.6 mm) has been surface modified using a laboratory-synthesized amphiphilic organic ligand namely, 1,5-dioctanoyl-1,5-diphenylcarbazide (DODPC), which acts an ion-selective receptor, for the separation of the target analytes. The eluted metal ions were subjected to post-column derivatization reaction with 4-(2-pyridylazo) resorcinol (PAR) prior to their detection using a UV–vis detector (at 520 nm). The post-column reagent (PAR; 4.7×10^{-4} M; pH 9.0; 1.5 mL/min) was mixed with the column eluate through an ingeniously designed T-connector. An ultra-fast separation of Pb^{2+}, Cd^{2+}, and Hg^{2+} ions with a retention time of 1.67, 1.88 and 3.62 min, respectively was achieved, using 0.0526 mmol of DODPC coated C_{18} monolithic column along with tartaric acid solution (1.0×10^{-2} M; pH 4.0; 1.0 mL/min), as the isocratic eluent (mobile phase). The chromatographic parameters such as linearity, accuracy, recovery, limits of detection and quantification were validated to achieve superior analytical results. The influence of various analytical parameters such as nature of mobile phase and its concentration, solution pH, flow rate, post-column reagent and its concentration were studied and optimized. The studies revealed a lower detection limits of 0.075, 0.090 and 0.120 μg/L, and a quantification limits of 0.225, 0.270 and 0.450 μg/L, for Pb^{2+}, Cd^{2+} and Hg^{2+} ions, respectively. A linear signal response in the concentration range of 0.05–50.0 μg/mL, was observed for the target metal ions, with an average r^2 value of 0.9994. The method was selective for the target metal ions, with excellent data reliability and reproducibility that accounts for an average recovery value of 99.76%, with a relative standard deviation of $\leq$1.83%.

1. Introduction

Environmental analysis of toxic heavy metal ions especially, lead, cadmium, and mercury, which are dispersed in various types of ecosystems at trace levels have been a key perspective in all modern scientific endeavors [1–4]. This is primarily associated with the inadvertent pressure on the ecosystem imposed by the modern civilization through the release of these contaminations by various commercial activities that have eventually led to deleterious effects on various life forms including humans. The sources of these toxic metal ions are mainly from mining, industrial waste effluents, municipal wastewater treatment plants, oil refineries, *etc* [5,6]. Most commonly found organic pollutants in the environment and their ill-effects are known and many new methods are emerging to degrade them effectively in the environment [7–13]. But the metal ions, enter the ecosystems as dissolved entities in water systems and comes under the category of human carcinogens, and non-biodegradable pollutants and enters the biological system mainly through the food chain. In the case of cadmium, which is spontaneously absorbed in the soil, gets incorporated into plant tissues at a much higher amount when compared to lead and mercury species [14]. However, mercury species are known for very high biomagnification factor and impact fetal developmental disorders, especially to the heart, brain and nervous systems

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[15–17]. Similarly, lead poisoning causes chronic inflammation to kidney and heart, apart from causing nervous and gastrointestinal disorders [18]. Like-wise, cadmium poisoning causes renal dysfunction, calcium metabolism disorders and also certain forms of cancer [19–23]. Thus, it becomes extremely important in framing stringent regulatory specifications in preventing the human exposure from these pollutants. In spite of these known ill-effects, it is quite unfortunate that we continue to be exposed to these toxic metal ions, due to the lack of careful handling and proper disposal practices [24], which eventually leads to various environmental and health issues. Hence, there is a growing concern for developing smart analytical methods for the constant monitoring of these toxic pollutants from various environmental and biological samples.

In the past, the use of chromatographic techniques, in particular, liquid chromatography (LC) is well known for its separation and determination of organic and inorganic analytes, with certain restrictions in terms of sample volume injection loop, slow flow rate and column back pressure. However, in the early 1990′s, the technological modifications have transformed HPLC technique into a powerful separation method in the field of environmental analysis, by improving on the selectivity and sensitivity factor. One such improved alternative is the development of RP-HPLC methodologies for the quantitative analysis of metal ions. However, RP-HPLC techniques were practiced only in certain sectors such as pharmaceutical industries, nuclear spent fuel reprocessing, *etc*. Moreover, their utility in environmental related applications, particularly for the separation of heavy metal toxins, happens to be an area of research that has not received complete attention. The major reason for this set-back is attributed to the preferential choice of solid-state enrichment techniques followed by metal ion analysis using sophisticated instruments such as ICP-OES, ICP-MS, and GT-AAS, apart from the use of ion-chromatography, which is quite non-selective. In the past, few interesting HPLC separation methods have been developed in this perspective which was based on different kinds of metal ion interactions (ion-exchange and chelation) between the stationary phase and mobile phase. Selective complexing agents that could bring out separations are one of the key aspects of successful RP-HPLC and most often, the complexing agents are included in the mobile phases comprising of organic solvents to bring out the separation of the desired metal ions. In few cases, the metal ions in the sample are first reacted with the complexing agents by a pre-column derivatization process and then injected into the HPLC column after performing an online enrichment technique. For instance, Khanmohammadi et al. reported on the simultaneous determination of Fe^{3+} and Hg^{2+} using a chelating agent *i.e.*, 1,4-bis-(4-pyridyl)-2,3-diazo-1,3-butadiene with a 0.03 M acetate buffer in 10% methanol as the mobile phase (pH 4.5), using Vertex C_{18} column as the stationary phase [25]. Likewise, Yang et al. reported on a pre-column derivatization method for the simultaneous determination of Pb^{2+}, Cd^{2+}, Hg^{2+}, Ni^{2+} and Zn^{2+} using 2-(2-quinolinylazo)-4-methyl-1,3-dihydroxybenzene as the chelating agent (column modifier), besides using 0.05 M (pH 8.5) of pyrrolidine-acetic acid buffer with 0.01 M of cetyltrimethylammonium bromide solution in 68% methanol as the mobile phase [26]. Similarly, Huang et al. reported on the HPLC determination of lead, cadmium and mercury ions in microwave-digested foodstuffs, after an online enrichment technique prior to pre-column derivatization procedure using 5,10,15,20-tetrakis(3-aminophenyl)porphyrin [27]. As indicated earlier, these methods require pre-column derivatization and enrichment procedures prior to their detection. In addition, it is important to note that to achieve metal ion separations, majority of the HPLC literature reports involves the use of organic solvents such as acetonitrile and methanol along with complexing agents such as L-cysteine, 2-mercaptoethanol, sodium diethyl carbamate, glutathione, *etc*., which leads to the generation of huge volumes of secondary organic wastes, which also creates serious disposal issues. It is also important to note that the majority of the RP-HPLC literature reports on mercury speciation studies were based on the use of sophisticated detection systems such as, LC–MS, ICP-MS, AFS, *etc*., that are both cost and labor intensive, thus making it an unaffordable method for majority of the government organizations in under-developed and developing countries that are involved in environmental analysis and risk management [28–36]. In addition to this, most of the literature reports on metal ion speciation deal with the use of ICP-MS systems for detection, where the use of a simple UV–vis detector is inefficient unless otherwise it is assisted by a selective post-column reagent for metal ion complexation prior to detection. This approach has lead to only a handful of reports on HPLC method based metal ion separation using UV–vis detector, and the use of relatively less expensive photodiode array detector taking a sizable share of reports along with the sophisticated detection systems, for metal ion studies. Besides, apart from the detection issues, most of the reported HPLC methods have employed spherical particles/beads as packed columns that are associated with low mobile phase flow rate and high column back pressure. This eventually increases the analysis time and also has a significant impact on the column parameters such as peak tailing and resolution factors.

In the recent years, the use of porous structured monolithic columns in RP-HPLC has brought in significant improvement in the field of environmental analysis. Monolithic columns are a special type of columns made from a single piece of either silica or polymer rods with continuous porous channels rather than spherical particles/beads that were previously used in HPLC, as packed columns. Monolithic columns with regular pores offer faster mobile phase diffusion through the stationary phase thus ensuring faster separations even at higher flow rates, without increasing the column back pressure [37]. This feature increases the rapidity of analysis and also improves the peak resolutions, as these columns are associated with greater number of theoretical plates. Based on these aspects, the current work reports on the development of an RP-HPLC method using an indigenously synthesized amphiphilic ionophore (DODPC), as the column modifier. It is known that 1,5-diphenyl carbazide (DPC), is an effective complexing agent, which is used for the determination of a wide range of metal ions, thereby serving as the potential option for column modification. DPC can form complexes with lead, cadmium and mercury ions through ligation *via* N—H functional group adjacent to the phenyl moiety. Aboufazeli et al. have reported on the use of vinyl functionalized DPC for Pb^{2+} complexation and Behbahani et al. have prepared a diphenylcarbazide-siliceous mesocellular foam for the preconcentration and detection of Cd^{2+} ions [38,39]. However, considering the fact that the DPC-metal ion complexes are water soluble and also to ensure the uniform immobilization (coating) of the ligand onto the C_{18} monolithic column *via.* Vanderwaals' force of attraction, two octanoyl groups have been introduced thereby increasing the hydrophobic nature of the DPC ligand. This approach ensures that the resulting DODPC molecules are not leached out of the column during the chromatographic run. It has been observed that the coated DODPC ligand acts as an excellent complexing agent through a preferential (selective) complexation of the target metal ions from a mixture of metal ion species thereby inducing different retention behavior for the metal ion species on the ligand modified column. Besides, the present work does not involve any pre-column derivatization and enrichment process, in addition to its environmentally benign approach by making use of a mobile phase system that does not involve any organic solvents, apart from the use of cost-effective UV–vis detection system. The investigation of various analytical parameters using the DODPC modified C_{18} silica monolith column revealed interesting chromatographic properties for the quantitative separation of the target metal ions. The modified

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