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FULL PAPER

Estimation of the total amounts of manganese ions in some of the medicinal plants leaves using flow injection technique combined with photometric detection method

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For decades, one of the most required methodologies is to develop a new, simple and sensitive analytical method for the determination of trace amounts of elements in plants. Therefore, a spectrophotometric method based on continuous flow injection technique combined photometric detector has been developed and validated for the rapid determination of trace with amounts of manganese ions in plant leaves. The developed method is based on the converting of Mn^{+2} ions to permanganate using hydrogen peroxide in an alkaline medium (KOH). The coloured violet product was measured using homemade photometric detector at 450 nm. Under optimum conditions, a flow rate of 1.8 mL. min, hydrogen peroxide concentration and potassium hydroxide, the linearity of the developed method was in the range of 0.1-36 mg L^{-1} with 0.03 mg L^{-1} and 1.8% as the detection limits and RSD% respectively. 10 mg is the required weight of the powdered sample for the determination of the manganese. Using four different types of medicinal plants (Catharanthus roseus, Taraxacum officinale, Vicia faba and Pinus nigra), the developed method was applied successfully to determine the total amounts of total Mn^{+2} in medicinal plants leaves. To ensure the validity of the developed method, the obtained results from the developed method and from those obtained from the reported method were statistically compared using paired t-test at 95% confidence interval. The statistical analysis has shown no significant difference between the methods. Therefore, the developed method can be used as an alternative analytical method for the determination of the total amounts of manganese in some of medicinal plants.

KEYWORDS

Medicinal plants; manganese; photometric method; flow injection analysis; trace elements.

Introduction

The chemical composition of medicinal plants plays a critical role to determine the therapeutic effect of these plants for the treatment of different diseases. Some of these compounds are organic and a majority of them have biological activity, however, these compounds cannot act independently in plant matrix. Lots of analytical methods have reported that medicinal plants are rich in many useful chemical elements and compounds which can be considered as the important factor that can determine the

curative effects of these medicinal plants [1-3]. The problems with these trace elements are found in a simple salt and bound, complexed with organic compounds. Besides, these elements exist in different states which means different absorption rates, functions and toxicity by the human body. Manganese is the 12th more common element on the earth [4,5]; it is found in more than five valence states, with several common states of Mn in the environment, however, the most common forms of Mn are in the oxidized states which are MnO_2 or Mn_3O_4 [6-8]. Manganese is an essential trace element for the function of nervous system and normal bone growth. Manganese works as a cofactor for many of enzymes, such as superoxide dismutase, arginase and glutamine synthetase [9-11]. In plants, this element plays an essential role in plant growth, ultrastructural changes in chloroplasts, oxidative stress and proteomic alterations in rice [12]. Both excess and lack of (Mn) in the body inspire serious deterioration of biochemical processes [13]. In plants, (Mn) is considered as one of the most essential micronutrients for development and growth. In photosynthetic machinery, (Mn) acts as a cofactor for oxygen-evolving complex [14-17]. On another hand, the deficiency of manganese in plants often occurs due to a latent disorder without any type of visual symptoms. This deficiency always affects the quantity of the crops, decreases biomass and impairs growth by the decrease in chlorophyll content, lower net photosynthetic efficiency and lower numbers of chloroplasts [18, 19]. Mainly, millions of people around the world are using medicinal plants; these medicinal plants are primarily used in healthcare by most people. Therefore, the main objective of the study is to investigate the presence of essential elements such as manganese in these plants. The trace elements in the infusions and decoctions of medicinal plants coexist with numerous chemical compounds and in complexed agents, therefore, the concentrations free of these elements in medicinal plants are very low. In recent years, there are lots of analytical methods designed to determine the total concentrations of manganese ions, however, these methods do not differentiate between the bound and free states of these ions [20,21], also, a series of preliminary separation steps are required in these methods [22]. In addition, a variety of analytical methods have been reported for the determination of total amounts of manganese in plant leaves such as inductively coupled plasma-mass spectrometry (ICP-MS) [23], graphite furnace atomic absorption spectrometry (GF-AAS) [24], neutron activation analysis [15] and spectrophotometric method [25]. However, the majority of the above techniques are time consuming, expensive, required lots of separation steps and using organic solvents. To the best of our knowledge, there are no sensitive or selective methods that allow determining the total amount of specific state (+2) of manganese in the medicinal plants. In this work, for the first time, hydrogen peroxide is employed to determine Mn^{+2}. The catalytic effect of the hydrogen peroxide on the oxidation of Mn^{+2} in the presence of potassium hydroxide was studied. A new photometric method based on the flow injection technique was developed. The developed method was successfully applied for the determination of the total amount of Mn^{2+} in medicinal plant leaves without using any organic solvents or needing complex separation techniques.

Experimental

Chemicals

Manganese (II) chloride tetrahydrate, potassium hydroxide, ammonium hydrochloride, ferric chloride, zinc chloride, magnesium sulphate, nickel chloride and calcium chloride were procured from Sigma Aldrich with purity higher than 99%. While the hydrochloric acid (35% w/w, 1.19 g/mL) and Hydrogen peroxide (30%) were purchased from BDH and Spectrum chemical

MFG Corp respectively. For all of the dilutions and preparation of working solutions, double distilled water was used. The medicinal plants (Catharanthus roseus, Taraxacum officinale, Vicia faba and Pinus nigra) were supplied from the local markets.

Apparatus

All absorbances measurements for the colour product were conducted using the manifold system. This system contains a flow injection unit and the photometric detection unit. The flow unit consisted of double lines peristaltic pump (Ismatec- Switzerland), 6-ways injection valve, Y-junction point (mixing area, homemade from methyl methacrylate) and teflon tubes which were used to join all the manifold parts as shown in Figure 1. While the atomic absorption spectroscopy (AAS) (model AA-7000, Shimadzu-Japan) was used to perform measuring the concentration of manganese ions of the reference method.

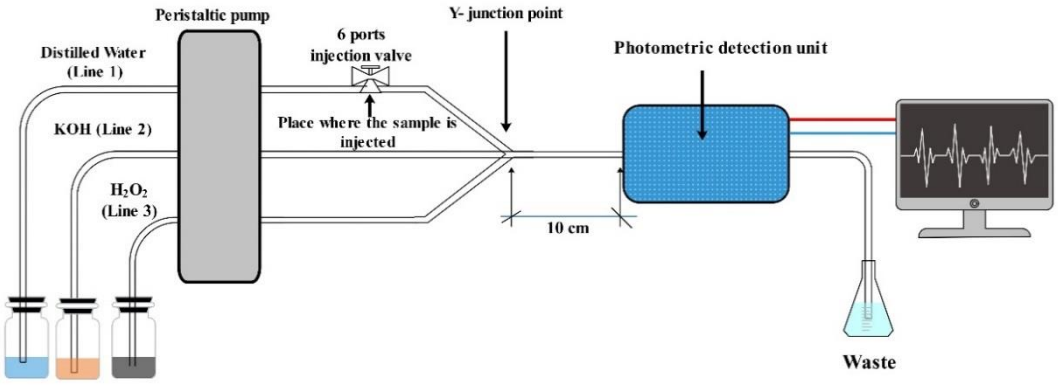

FIGURE 1 The whole manifold system with the photometric detector used for determination of Mn^{+2}

General procedure

The manifold flow system which was used for the determination of the total Mn ions in medicinal plants is shown in Figure 2. As it shows, the system has three lines, the first line is used to carry the injected sample, the second line is for the supplies of the reaction with potassium hydroxide and the final line is responsible for transporting the hydrogen peroxide. These three lines propel the solutions at 1.8 mL. min of flow rate and their compositions are mixed at Y-junction point. First, 100 µL of the extracted sample is injected into the flow system using the 6-ports injection valve; this sample is carried to the mixing point (Y-junction point) where the sample is reacted with hydrogen peroxide (30%) in the presence of potassium hydroxide. The result of that reaction is to form a violet product which is thought to be potassium permanganate as shown in Figure 2. Finally, the violet product is transported by the carrier stream to the photometric detection unit for measuring.

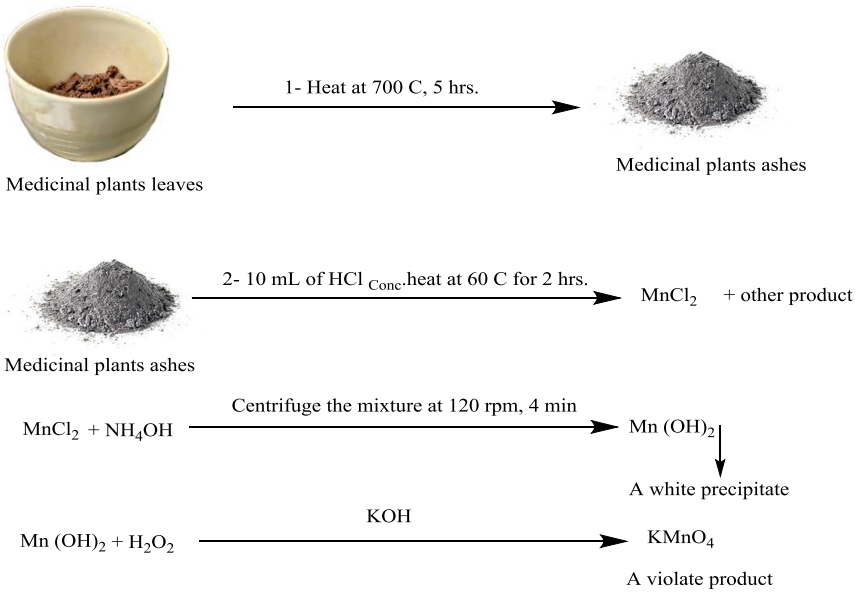

FIGURE 2 The extraction steps and the proposed reaction for the photometric determination of Mn^{2+}

Extraction and analysis of Mn^{+2} from the plants leaves

The contents of leaves were drying then heating to about 700 C° until changed to ash and placed in 250 mL flask, 10 mL concentrated HCl was added with heating to 60 C°, and $MnCl_2$ was precipitated in this stage. In order to extract Mn^{+2} from the plants leaves, 10 mL of 2 mmol/L ammonium hydroxide was added to the 10 gm of plants leaves after it was smashed into the fine powder using mortal. White precipitated compound $Mn(OH)_2$ was obtained which was separated using centrifuge at 120 rpm, 4 minutes. Then obtained white product was dissolved using concentrated HCl and the pH of the solution was adjected to 6 using the same acid. The extracted samples were injected into the manifold system using the 6 ports injection valve, while the first line is designed for the carrier stream and the second line for the hydrogen peroxide. The three lines are mixed together at the Y-junction point where the extracted $Mn(OH)_2$ is reacted with hydrogen peroxide to form a violate product which is possible due to formation of $KMnO_4$. The coloured product was measured at 450 nm. The exact amount of Mn^{+2} ion was calculated based on the calibration curve equation.

Results and discussion

Optimization of the chemical parameters

The primary experiments were conducted in order to determine the optimum concentration of the potassium hydroxide and hydrogen peroxide. These experiments were performed using 1.8 mL. min of flow rates for the three lines and 100 µL sample volume of $MnCl_2$. 4 H_2O (0.5 mg L^{-1}). Therefore, a series of hydrogen peroxide concentrations (0.1, 1, 10, 100 and 1000 mg L^{-1}) were prepared. The results have shown that there was an increase in the responses up to 100 mg L^{-1} of hydrogen peroxide concentration, using higher than 100 mg L^{-1} of hydrogen peroxide led to a decrease in the response which may be due to dissociation of the colour product. Therefore, the 100 mg L^{-1} was chosen to be the optimum concentration and used for a further experiment as shown in Figure 3. Using the above experimental conditions, 1.8 mL. min flow rate, 100 mg L^{-1} of hydrogen peroxide and 100 µL sample volume of $MnCl_2$. 4 H_2O (0.5 mg L^{-1}), the experiments for optimization of the

potassium hydroxide concentration experiments were conducted. Thus, a series of potassium hydroxide concentrations (10, 30, 50 and 70 mgL^{-1}) were prepared. The obtained results have shown that, at 50 mg L^{-1}, the highest response was obtained. Therefore, 50 mg L^{-1} was chosen as the optimum concentration and it was used in the further experiments as shown in Figure 4.

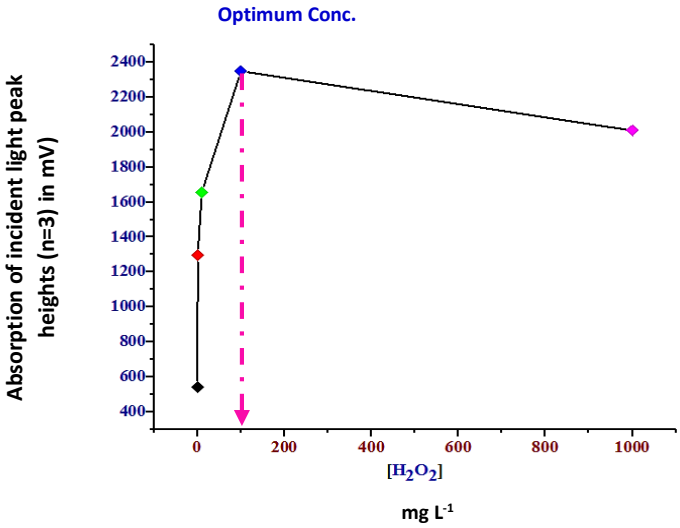

FIGURE 3 The optimum concentration of H_2O_2 under using all the experimental conditions for the determination of Mn^{+2} by flow injection combined photometric method

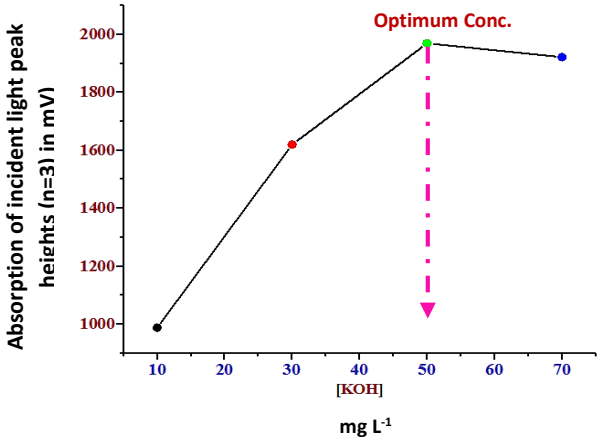

FIGURE 4 The optimum concentration of KOH under using all the experimental conditions for the determination of Mn^{+2} by Flow injection combined photometric method

Validation of the developed method

The Australian Pesticides & Veterinary Medicines Authority (APVMA-Guidelines), an international organization for the analysis of the total element in medicinal plants has been followed [26]. Therefore, linearity, LOQ, LOD and interferences study were applied.

Calibration curve, limit of detection and limit of quantification

Under optimization of all the above conditions, the flow injection system combined photometric detection method has shown linearity in the response ranging from 0.1-36 mg L^{-1} for Mn^{+2} ions with 0.12 mg L^{-1} limit of detection and 0.25 mg L^{-1} limit of

quantification. The statistical analysis of the linear equation [27] has given a high value of (r), low value for both intercept and slope, all of which are tabulated in Table 1, while the RSD% of the method has a value less than 1.8% which is within the acceptance limit. Both limits of detection and quantification have been calculated based on ICH Q2 (R1) recommendation.

TABLE 1 The statistical summary data of the linear equation for the photometric determination of Mn^{2+}

Parameter	Mn^{2+}
Linearity (mg L^{-1})	0.1-36
Regression equation	8.8225±0.6322+132.6774±6.5453c[a]
Slope	1325.6774±17.5453
Intercept	31.8225±5.1322
r [b]	0.9971
r^2 [c]	0.9944
LOD (mg L^{-1})[d]	0.03
LOQ (mg L^{-1})[e]	0.12
% RSD [f]	1.8

[a]The detector signal in mV for C of sample concentration mg L^{-1}
[b]Correlation coefficient
[c]Coefficient of determination
[d]Limit of detection
[e]Limit of quantification
[f]Relative standard deviation (*n=3*)

Precision and accuracy

The precision of the developed method has been determined using the relative standard deviation, while the accuracy of the developed method was expressed as a recovery percentage. The inter and intra-day precision for three serial concentrations 10, 20 and 30 mg. L^{-1} of Mn^{+2} showed RSD% of 1.2, 1.6, 1.8 and 1.3, 1.5, 1.65 for intra-day and inter-day respectively, while the accuracy of the developed method as a recovery percentage showed 100.55, 99.98 and 102.33 respectively as shown in Table 2.

TABLE 2 The repeatability and the reproducibility of the proposed method which expressed as the precision and accuracy respectively

Sample concentration (mg L^{-1})	Intra-day precision			Inter-day precision		
	Concentration found (mg L^{-1})	Accuracy (Rec%)	Precision (RSD%)	Concentration found (mg L^{-1})	Accuracy (Rec%)	Precision (RSD%)
10	10.12	101.20±0.2	1.2	10.21	102.10±0.4	1.3
20	20.09	100.45±1.2	1.6	19.98	99.90±0.5	1.5
30	30.11	100.36±0.9	1.8	30.24	100.80±0.7	1.65

Interferences

Ferric chloride, Zinc chloride, Magnesium sulphate, Nickel chloride and Calcium chloride were frequently added to extracted sample. In the current work, these elements were added to the sample solution that contains 0.5 mg L^{-1} Mn^{2+} ions. Then, the solution was analysed using the developed method. The obtained results have shown that the concentrations of these interferences ions do not cause significant error with less than ±1.6% (The recovery error of Mn^{2+} ions 0.5 mg L^{-1})

Determination of Mn^{2+} in real sample

The determination of Mn^{+2} ions in medicinal plants leaves were conducted using four

different plants (Catharanthus roseus, Taraxacum officinale, Vicia faba and Pinus nigra). The first step of the developed method is performed by treatment of the collected sample by ammonium hydroxide and separation of the precipitated product from the solution. After that, the white product was dissolved using hydrochloric acid and the pH of the solution was then adjected to 6. The final results show that the treated solution was then injected into the manifold system which was reacted at Y-junction point with hydrogen peroxide in the presence of potassium hydroxide to form a violate product which was measured using the photometric detector. All the obtained results are shown in Table 3. In order to ensure the validity of the developed method, the Mn^{+2} ions in plants leaves were measured using reported method based on atomic absorption technique. The obtained results from the reported method were shown in Table 3. The results of this analysis were then compared with the data obtained from using atomic absorption spectrophotometer technique in determination of the concentrations Mn^{+2} in the leaves of the same plants; the results showed in Table 3. The recently developed embraced methodology in this study was put into a t-test (the tool comparison) for the aim of accepting it as an alternate technique for test and estimation of Mn^{+2} in medicinal plants with standard utilized strategy or dismissing it as an alternative technique. On this premise, three assumptions are statistically made. There is no significant difference between the means of all four utilized techniques (i.e.; undistinguishable differences between the methods) as shown in Table 3.

TABLE 3 Summary results for the determination of Mn^{+2} by the developed and the official methods

Types of plants	*Found (n=3)* Developed method (mg L^{-1})	RSD%	Official method using AAS mg L^{-1}) [28]	*t-test at 95%*	*F*
Catharanthus roseus	22.22	1.8	22.17	0.15	1.325
Taraxacum officinale	16.55	1.22	16.22	0.11	1.425
Vicia faba	11.21	1.09	11.22	0.09	1.122
Pinus nigra	5.66	1.52	5.67	0.08	1.442

Conclusion

In conclusion, it is very obvious that the flow injection analysis technique can be modified perfectly with another detection technique and can be used as an alternative technique for the determination of trace amounts of elements in different plant leaves. The majority of the reported methods require lots of equipment, materials and it is time consuming, therefore, using the flow injection technique can led to obtain simple, rapid, low cost and short time analysis. The obtained results from the developed method were compared with the reported standard technique as mentioned previously. The results have shown that there was no significant difference between the results that obtained from the methods at 95% confidence (α = 0.05/2 two tailed). Also, in the current study, it is the first time that the hydrogen peroxide is used as an oxidizer which is never used before, this reaction was considered as a new reaction which can be used in photometric detection methods.

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