Determination of Manganese in Some Medicinal Plants and their Water Extracts by a Kinetic Spectrophotometric Method

K. L. MUTAFTCHIEV

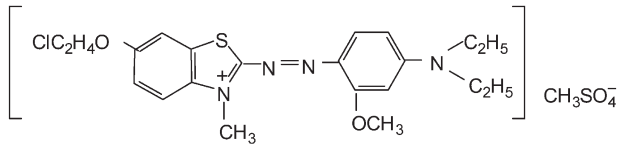
Department of Chemistry and Biochemistry, Higher Medical Institute, Pleven 5800, Bulgaria
e-mail: vmilibrpl@mbox.digsys.bg

Received 25 June 2001

The catalytic effect of manganese(II) on the oxidation of the dye 3-methyl-6-(2-chloroethoxy)-2-[2-methoxy-4-(*N*, *N*-diethylamino)phenylazo]benzothiazolium methyl sulfate with potassium periodate in the presence of 1,10-phenanthroline in weakly acidic media is investigated. The reaction is followed spectrophotometrically by measuring the decrease in the absorbance of the dye at $\lambda = 550$ nm. Under the optimum conditions (c_i: 4×10^{-5} mol dm^{-3} (dye), 6×10^{-4} mol dm^{-3} (potassium periodate), 1×10^{-4} mol dm^{-3} (1,10-phenanthroline), 0.1 mol dm^{-3} (buffer – pH 3.0), 70 °C, 8 min) ρ(Mn(II)) in the range 0.1—5 ng cm^{-3} can be determined by the fixed-time method with the detection limit of 0.03 ng cm^{-3}. The developed method is highly sensitive, selective, and simple. The method was applied successfully to analyze decoctions (fruits of one-styled hawthorn and dog-rose) and infusions (blossom of German chamomile and leaves of spearmint) for trace amounts of free manganese(II) without separation and total manganese. Total manganese was also determined in these plants.

The therapeutic effect of medicinal plants for the treatment of various diseases is based on the chemical compounds in these plants. The major components are organic compounds, some of which have biological activity, but none act independently and cannot replace the functions of the medicinal plant as a whole. Analyses have revealed that medicinal plants are rich in many trace elements, and it is suggested that this is an important factor in the curative effect of these plants [1—3]. The chemical states in which trace elements are found are organically bound, complexed, and free. Besides, different states have different functions, toxicity, and absorption rates by the body. Trace elements co-exist with numerous organic compounds (many of which are complex agents) in the decoctions and the infusions of medicinal plants [4], and most will be bound to organic compounds. Therefore, the concentration of the free trace elements can be very low.

There are many sensitive methods to determine the total concentration of the trace elements present, but these do not differentiate between free and bound states [5—8]. In order to determine the concentration of the respective state, preliminary separation is required for these methods [9]. We did not find a description of a suitable sensitive and selective method in literature, which would allow the determination of trace amounts of free manganese(II) without separation in decoctions and infusions of the medicinal plants. No data were found regarding total manganese in the medicinal plants we investigated.



In this work, the azo dye 3-methyl-6-(2-chloroethoxy)-2-[2-methoxy-4-(*N*, *N*-diethylamino)phenylazo]-benzothiazolium methyl sulfate (SPABM) is employed for the first time in a kinetic system for the determination of manganese(II). The catalytic effect of manganese(II) on the oxidation of SPABM with potassium periodate in the presence of 1,10-phenanthroline (Phen) was investigated. A new kinetic spectrophotometric method for the determination of manganese(II) was developed. It can be used for the determination of manganese(II) in the ρ range 0.1—5 ng cm^{-3} by the fixed-time method with a detection limit of 0.03 ng cm^{-3}. The method was applied successfully to determine the free state of manganese(II) without separation and total manganese in decoctions and infusions of some medicinal plants (one-styled hawthorn, dog-rose, German chamomile, and spearmint). It was also used to determine the levels of total manganese in these plants. Besides, the procedure for the determination of free manganese(II) in decoctions and infusions

after separation was developed to compare the results obtained by the procedure without separation.

EXPERIMENTAL

All chemicals, except SPABM, were of anal. grade. The benzothiazolic cationic azo dye was synthesized according to the procedure described by *Deligeorgiev* and *Simov* [10], and was purified by double recrystallization from ethanol—diethyl ether (φ_r = 4:1). All solutions were prepared with doubly distilled water. The concentrations of the stock solutions were: c(SPABM) 5 × 10^{-4} mol dm^{-3}; c(potassium periodate) 0.01 mol dm^{-3}; c(Phen) 0.01 mol dm^{-3}; c(manganese(II) sulfate) 1.82 × 10^{-2} mol dm^{-3}. c(Acetic acid—potassium dihydrogen orthophosphate (φ_r = 2.33:1)) 0.15 mol dm^{-3} (buffer PHOP of pH 3.0); c(acetic acid—boric acid—orthophosphoric acid) 0.04 mol dm^{-3} and c(sodium hydroxide) 0.2 mol dm^{-3} (buffer UB in volume proportions respective to the needed pH [11]).

Absorption spectra were recorded on a Specord UV VIS spectrophotometer, using quartz cells (l = 1 cm). Absorbance measurements were made on a Specol 11 spectrophotometer in a glass cell (l = 1 cm). A NBE ultrathermostat was used to control the temperature. Decomposition of sample solutions was carried out in a Perkin—Elmer autoclave. For comparison measurement, a model Perkin—Elmer Zeeman 5000 atomic absorption spectrophotometer was used. The separation of free manganese(II) was carried out using a Dowex 50W-X8 cation-exchange column (10 cm × 8 mm i.d.) in the H^+ form.

Decomposition of Samples of Medicinal Plants

0.1 g of the medicinal plant was taken and treated with 5 cm^3 of concentrated HNO_3 and H_2SO_4 mixture (φ_r = 5:2) for 30 min at 160 ℃ in autoclave. Then the solution was neutralized with 2 cm^3 of 2 mol dm^{-3} sodium hydroxide and the digest was diluted to the mark in a calibrated flask (V = 100 cm^3). This solution was used as a sample solution.

Preparing the Sample Solutions of Decoctions and Infusions

2 g of one-styled hawthorn or dog-rose were placed in a beaker, 50 cm^3 of hot doubly distilled water were added and decocted for 15 min. Then the solution was filtered, diluted to the mark in a calibrated flask (V = 50 cm^3) and the filtrate was used as a sample solution (decoction). 2 g of German chamomile or spearmint were placed in a beaker, and 50 cm^3 of hot doubly distilled water were added. After 60 min the solution was filtered, diluted to the mark in a calibrated flask (V = 50 cm^3) and the filtrate was used as a sample solution (infusion).

Calibration Procedure

0.80 cm^3 of SPABM solution and 1 cm^3 of standard solution containing 1—50 ng of manganese(II) were placed in a calibrated flask (V = 10 cm^3). Then the solution was diluted to the mark with a mixture of Phen, potassium periodate, and PHOP buffer solution (prepared just before use in volume proportions φ_r = 1:6:75) and the flask was placed into the thermostat at 70 ℃ for 8 min. After that it was quickly cooled with ice water (to terminate the reaction), the solution was transferred into the spectrophotometer cell, and the absorbance (A) at λ = 550 nm was measured against a reagent blank. The calibration graph of absorbance *vs.* concentration of manganese(II) was prepared. The reagent blank was prepared in a similar manner, but 1 cm^3 of standard solution was replaced with 1 cm^3 of doubly distilled water. Both flasks were placed simultaneously in the thermostat.

Determination of Total Manganese in Medicinal Plants

1 cm^3 of the sample solution (instead of the standard solution) was used as in the calibration procedure, and the absorbance (A) at λ = 550 nm was measured against a reagent blank. The amount of total manganese was calculated from the calibration graph.

Determination of Free Manganese(II) in Decoctions and Infusions

1 cm^3 of the sample solution (instead of the standard solution) was used as in the calibration procedure, and the absorbance (A) at λ = 550 nm was measured against a reagent blank. This procedure was repeated, but the potassium periodate was replaced with doubly distilled water and the absorbance (A') at λ = 550 nm was measured against a reagent blank. The free amount of manganese(II) was calculated from the calibration graph according to the difference in absorbance ($A - A'$).

Determination of Free Manganese(II) in Decoctions and Infusions after Separation

25 cm^3 of sample solution (decoction and infusion) were passed through a cation-exchange column to retain the metal ions. The flow rate through the column was 1 cm^3 min^{-1}. After washing the column with 125 cm^3 of doubly distilled water, all the compounds which were not bound to the column (including the organic compounds and complexes of manganese) were eluted. Finally, the metal ions were eluted with 5 cm^3 of 1 mol dm^{-3} nitric acid. The eluate was neutralized with 4.5 cm^3 of 1 mol dm^{-3} sodium hydroxide and diluted to the mark in a calibrated flask (V = 25 cm^3). The free

manganese(II) was determined by the above described procedure.

Determination of Total Manganese in Decoctions and Infusions

1 cm^3 of the sample solution was taken and treated with 5 cm^3 of concentrated HNO_3 and H_2SO_4 mixture ($\varphi_r = 5:2$) for 20 min at 150 ℃ in autoclave. Then the solution was neutralized with 2 cm^3 of 2 mol dm^{-3} sodium hydroxide and diluted to the mark in a calibrated flask (V = 25 cm^3). 1 cm^3 of this solution (instead of the standard solution) was used as in the calibration procedure, and the absorbance (A) at λ = 550 nm was measured against a reagent blank. The amount of total manganese was calculated from the calibration graph.

RESULTS AND DISCUSSION

The oxidation of SPABM by potassium periodate in weakly acidic media results in the decoloration of the solution. Fig. 1 shows the absorption spectra of solutions of SPABM, SPABM + KIO_4, SPABM + KIO_4 + Phen, SPABM + KIO_4 + Mn(II), and SPABM + KIO_4 + Phen + Mn(II) after heating at 70 ℃ for 8 min according to the general procedure and indicates that the oxidation of SPABM by potassium periodate is catalyzed by the presence of small amounts of manganese(II). The oxidation reaction was also accelerated by Phen, and the acceleration effect was greater when manganese(II) was present in the system. The mechanism of this accelerative reaction is not clear. SPABM has an absorption maximum at λ = 550 nm, which was chosen for subsequent studies.

The rate of the oxidation of SPABM was influenced by the concentrations of SPABM, potassium periodate, Phen, and buffer, and by the buffer pH, and temperature. The effect of each of these on the catalyzed and uncatalyzed reactions was studied for the purpose of obtaining optimum condition for the manganese(II) determination (a maximum difference between the catalyzed and uncatalyzed reactions).

The effect of the SPABM concentration was investigated in the range 8×10^{-6} mol dm^{-3}—1×10^{-4} mol dm^{-3}. The results showed that the absorbance of the blank was very high when significantly more SPABM was used ($c > 7 \times 10^{-5}$ mol dm^{-3}), and the linear range for manganese was too narrow if a small amount of SPABM was used ($c < 2 \times 10^{-5}$ mol dm^{-3}). Considering these two factors, $c = 4 \times 10^{-5}$ mol dm^{-3} was chosen, while the absorbance of the blank was less than 1.0.

The effect of the concentration of potassium periodate was investigated in the range 1×10^{-4} mol dm^{-3}—1×10^{-3} mol dm^{-3}. Concentration of 6×10^{-4} mol dm^{-3} was chosen as the optimum, because the value of absorbance (A) was the highest.

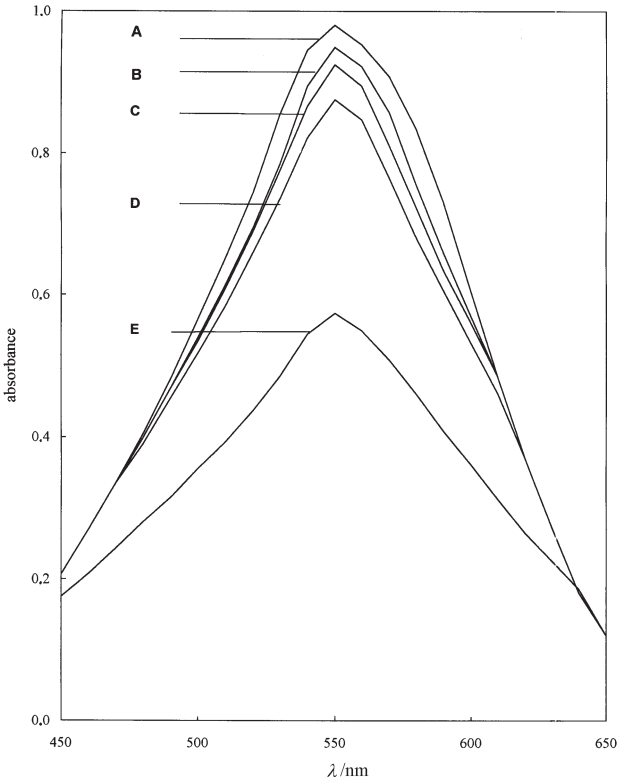


Fig. 1. Absorption spectra against water: A – SPABM; B – SPABM + KIO_4; C – SPABM + KIO_4 + Phen; D – SPABM + KIO_4 + Mn(II); E – SPABM + KIO_4 + Phen + Mn(II). c(SPABM) = 4×10^{-5} mol dm^{-3}; $c(KIO_4) = 6 \times 10^{-4}$ mol dm^{-3}; ρ(Mn(II)) = 2 ng cm^{-3}; c(Phen) = 1×10^{-4} mol dm^{-3}; pH = 3.0; θ = 70 ℃; t = 8 min.

The effect of the Phen concentration was investigated in the range 4×10^{-5} mol dm^{-3}—2×10^{-4} mol dm^{-3}. Concentration of 1×10^{-4} mol dm^{-3} was chosen as the optimum, because the value of absorbance (A) was the highest.

The influence of pH was studied in the range 2.6—5.0 by adjusting the pH with acetic acid—boric acid—orthophosphoric acid—sodium hydroxide, and a pH value of 3.0 was chosen as the optimum. The influence of several buffer solutions at pH 3.0 was tested. The slope of the calibration graph decreased when sodium citrate—hydrochloric acid or potassium hydrogen phthalate—hydrochloric acid buffer solutions were used. The slope increased when UB or PHOP were used, but with the latter better results were obtained and its preparation is easier. The effect of the PHOP concentration was investigated in the range 0.05 mol dm^{-3}—0.2 mol dm^{-3}. A concentration of 0.1 mol dm^{-3} was chosen as the optimum, because the slope of the calibration graph was maximum.

The dependence of the reaction rate on temperature was investigated between 30 ℃ and 80 ℃. A temperature of 70 ℃ was chosen as the optimum.

Table 1. Effect of Foreign Ions on the Determination of ρ(Mn(II)) = 2 ng cm^{-3}

Tolerated ratio m(ion)/ng:m(Mn(II))/ng	Foreign ion
$> 10^5$	Na^+, K^+, NO_3^-, SO_4^{2-}, CO_3^{2-}, BO_3^{3-}, PO_4^{3-}, acetate
3×10^4	Ca^{2+}, Mg^{2+}, F^-, Cl^-, Br^-
2×10^3	Cd^{2+}, Al^{3+}, $C_2O_4^{2-}$, citrate, tartrate
1000	Pb^{2+}, Ni^{2+}, Cr^{3+}, Cr(VI)
500	Cu^{2+}, Zn^{2+}
200	Fe^{3+}, Co^{2+}

Table 2. Determination of Manganese in Some Medicinal Plants

Sample	ρ(Manganese found*)/(μg g^{-1})	
	Proposed method	AAS
Fruits of one-styled hawthorn *Crataegus monogyna* Jacq.	23.71 ± 0.57	23.25 ± 0.65
Fruits of dog-rose *Rosa canina* L.	24.12 ± 0.61	23.76 ± 0.64
Blossom of German chamomile *Matricaria chamomilla* L.	36.19 ± 0.94	36.93 ± 0.92
Leaves of spearmint *Mentha spicata* L.	42.18 ± 1.05	41.35 ± 1.03

*Average values of 7 separate determinations and their standard deviations.

The effect of reaction time was studied in the range 5—10 min. The optimum reaction time was 8 min. If the reaction time was shorter or longer, the slope of the calibration graph was lower.

Under the optimum conditions, a linear calibration graph was obtained for manganese(II) from 0.1 to 5 ng cm^{-3}, and the slope of the graph was the highest. The regression equation of the calibration graph was $A = 0.01 + 0.17\rho$, where ρ is concentration (in ng cm^{-3}) and the correlation coefficient was 0.998. The proposed method yields a relative standard deviation of 2.4 % for 10 determinations of 2 ng cm^{-3} of manganese(II). The detection limit was 0.03 ng cm^{-3}, calculated as three times the standard deviation of the blank divided by the slope of the calibration graph.

The influence of foreign ions interferences on the determination of manganese(II) was investigated and the tolerated limits for the ions assayed are shown in Table 1 (with relative errors less than 5 %). There are various organic compounds in the decoctions and the infusions of the medicinal plants analyzed. If these compounds react with SPABM or potassium periodate and form new compounds that absorb at λ = 550 nm, they will interfere. In the procedure for the determination of free manganese(II), (A – A') subtract the interference from the reaction between the compounds in the decoction or the infusion and the SPABM. The fact that there was no difference in absorbance when omitting SPABM, and when omitting both SPABM and the potassium periodate, confirmed that there was no reaction between compounds in the decoctions or the infusions and the potassium periodate, which would produce an absorbance at λ = 550 nm. Therefore, there was no interference in the determination of free manganese(II) while using this procedure.

The proposed method was used to determine the levels of total manganese in some medicinal plants and the results obtained are shown in Table 2. The method was also used to determine the free manganese(II), and total manganese in decoctions and infusions of these medicinal plants and the results obtained are shown in Table 3. These results show that most of the manganese in the decoctions and the infusions exists in a bound state, and only very low concentrations are in a free state. The results of total manganese are compared with those obtained by atomic absorption spectrophotometry, and the results of free manganese(II) determined without separation are compared with those obtained after separation. The assessment by Student's t-test did not show a statistically significant difference between the methods used ($P > 0.05$).

CONCLUSION

The method proposed is highly sensitive, selective, and simple, and the precision is very acceptable for the determination of low ranges of manganese(II). Free manganese(II) without separation and total manganese were determined for the first time in decoctions of one-styled hawthorn fruits and dog-rose fruits, and in infusions of German chamomile blossom and spearmint leaves. Total manganese was also determined in these plants. The method might be suitable for the determination of free manganese(II) and total manganese in various other medicinal plants and complex systems.

Table 3. Determination of Manganese in Decoctions and Infusions of Some Medicinal Plants

Sample	State	ρ(Manganese found*)/(μg g^{-1})	
		Proposed method	AAS
Decoctions			
Fruits of one-styled hawthorn	Total	0.4268 ± 0.0102	0.4161 ± 0.0116
Crataegus monogyna Jacq.	Free	0.0047 ± 0.0001	–
	Free (s)	0.0048 ± 0.0001	–
Fruits of dog-rose	Total	0.4438 ± 0.0111	0.4567 ± 0.0123
Rosa canina L.	Free	0.0062 ± 0.0002	–
	Free (s)	0.0064 ± 0.0002	–
Infusions			
Blossom of German chamomile	Total	0.7093 ± 0.0184	0.7299 ± 0.0182
Matricaria chamomilla L.	Free	0.0085 ± 0.0003	–
	Free (s)	0.0088 ± 0.0003	–
Leaves of spearmint	Total	0.8605 ± 0.0215	0.8433 ± 0.0211
Mentha spicata L.	Free	0.0069 ± 0.0002	–
	Free (s)	0.0071 ± 0.0002	–

*Average values of 7 separate determinations and their standard deviations. Free – without separation; Free (s) – after separation.

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