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Catalytic Spectrophotometric Determination of Manganese in Some Medicinal Plants and Their Infusions

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The catalytic effect of manganese (II) on the oxidation of the dye 1,3-Dimethyl- 2 [4-N (N,N-dimethylamino) phenylazo] imidazolium perchlorate (BR) with potassium periodate in the presence of 1,10-phenanthroline was investigated. The reaction was followed spectrophotometrically by measuring the decrease in the absorbance of the dye at 540 nm. Under optimum conditions (5×10^{-5} mol dm^{-3} BR, 2×10^{-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, 5 min) manganese (II) in the range 0.1–4.5 ng cm^{-3} can be determined by the fixed-time method with a detection limit of 0.03 ng cm^{-3}. The developed method is highly sensitive, selective, and simple. The method was applied successfully to analyse infusions of some medicinal plants (common balm, creeping thyme, common lungwort, and colt's-foot) for trace amounts of total manganese and free manganese (II) ions without separation. The results showed good agreement with those obtained by atomic absorption spectrophotometry.

Key Words: manganese determination, catalytic spectrophotometric method, medicinal plants, infusions.

Introduction

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 they 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 states in which trace elements may be found in the plant matrix are organically bound, complexed and free. It is well known that different states and forms can have different functions, bio-toxicological activity and percentage absorption by the body[4–7]. Trace elements co-exist with numerous organic compounds (many of which are complex agents) in the infusions of medicinal plants[8], and probably most are bound to organic compounds. Therefore, the concentration of the free trace elements should be very low.

There are many sensitive methods to determine the total concentration of the trace elements present (ETAAS, ICP-AES, ICP-MS, etc.), but these do not differentiate between free and bound states[9–12]. In these methods, preliminary separation is required in order to determine the concentration of the free state[13]. To our knowledge, there are no suitable sensitive and selective methods described in literature which would permit the determination of trace amounts of free manganese (II) in infusions of medicinal plants without prior separation, nor was it possible to find data regarding total manganese in the medicinal plants investigated in this study.

This paper presents a catalytic spectrophotometric method to determine the free state of manganese (II) without separation and total manganese in infusions of common balm, creeping thyme, common lungwort and colt's-foot. The method is based on the catalytic effect of manganese (II) on the oxidation of the dye 1,3-Dimethyl-2 [4-N (N,N-dimethylamino) phenylazo] imidazolium perchlorate (BR), with potassium periodate in the presence of 1,10-phenanthroline (Phen). It can be used for the determination of manganese (II) in the range 0.1–4.5 ng cm^{-3} by the fixed-time method with a detection limit of 0.03 ng cm^{-3}. The method was also applied to determine the levels of total manganese in these plants. This is the first attempt to determine manganese in these plants and their infusions.

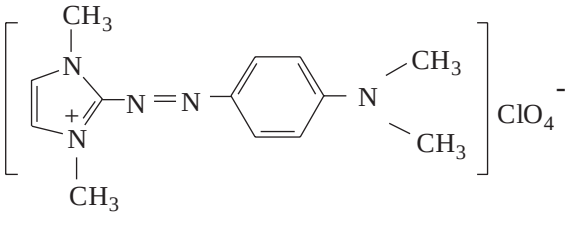
Experimental

Synthesis of the dye

This imidazolic cationic azo dye was synthesised according to the procedure described by Deligeorgiev et al.[14]. 4-Chloro- or 4-fluoroaniline (0.02 mol) was dissolved in acetic acid (2 cm^3), hydrochloric acid (5 cm^3) and water (25 cm^3) and the liquor was cooled in an ice-salt bath to 0 °C. The diazotisation was carried out with 0.02 mol of sodium nitrite dissolved in 5 cm^3 water.

The imidazole (0.02 mol) was dissolved in 25 cm^3 water, and the diazo liquor was added to the solution. Dichloroethane (16 cm^3), sodium acetate (0.06 mol) and dimethylsulphate (0.08 mol) were then added to the reaction mixture and the liquor was warmed to 70 °C. At this temperature, quaternisation proceeded over 1 min. The replacement of the halogen substituent by the dimethylamino group was complete at 50-60 °C, over 30-40 min. After cooling to room temperature, the dye was precipitated as perchlorate with sodium perchlorate. The dye was then filtered, washed with diethyl ether and air-dried. The dye was purified by double recrystallisation from acetone and subsequent passage through a silica chromatographic column, using diethyl ether + ethanol (15 + 1 v/v) as eluent.

This dye is also produced by BASF (Germany) as Basacryl Red X-BL [15].



1,3-Dimethyl-2 [4-N (N,N-dimethylamino) phenylazo] imidazolium perchlorate.

Reagents

All chemicals, except BR, were of analytical-reagent grade and the solutions were prepared with doubly distilled water. The concentrations of the stock solutions were: BR, 1×10^{-3} mol dm^{-3}; potassium periodate, 0.01 mol dm^{-3}; Phen, 0.01 mol dm^{-3}; manganese (II) sulphate, 1.82×10^{-2} mol dm^{-3}. Buffer solutions: 0.15 mol dm^{-3} acetic acid - potassium dihydrogen orthophosphate (2.33 + 1 v/v) buffer (PHOP) of pH 3.0; 0.04 mol dm^{-3} acetic acid - boric acid - orthophosphoric acid and 0.2 mol dm^{-3} sodium hydroxide (UB) in volume proportions respective to the needed pH [16].

Apparatus

Absorption spectra were recorded on a Specord-UV-Vis spectrophotometer (Carl Zeiss Jena Germany), using 1 cm quartz cells. Absorbance measurements were made on a Specol 11 spectrophotometer (Carl Zeiss Jena) in a 1 cm glass cell. A NBE ultrathermostat (VEB Prüfgeräte-Werk, Medingen, Germany) was used to control the temperature. Decomposition of the samples was carried out in a Perkin-Elmer autoclave-3 (Überlingen, Germany). For comparison measurement, a Perkin-Elmer 5000 atomic-absorption spectrophotometer (Überlingen) was used.

Decomposition of samples of medicinal plants

A 0.1 g sample of the medicinal plant was taken and treated with 5 cm^3 of concentrated HNO_3 and H_2SO_4 mixture (5 + 2 v/v) for 30 min at 160 °C in an autoclave. Then the solution was neutralised with 2 cm^3 of 2 mol dm^{-3} sodium hydroxide and the digest was diluted to the mark in a 1000 cm^3 calibrated flask.

Preparing the sample solutions of infusions for the determination of total and free manganese (II)

A 1 g sample of the medicinal plant was placed in a beaker and 100 cm^3 of hot doubly distilled water was added. After 30 min filtering, the mixture was diluted to the mark in a 100 cm^3 calibrated flask, and the filtrate was used as a sample solution.

Calibration procedure

A 0.50 cm^3 sample of BR solution and 1 cm^3 of standard solution containing 1–45 ng of manganese (II) were placed in a 10 cm^3 calibrated flask. 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 (1 + 2 + 82). The flask was placed into the thermostat at 70 °C for 5 min. After that it was quickly cooled with ice–water (to terminate the reaction), and the absorbance (A) at 540 nm was measured against a reagent blank. Manganese (II) was determined according to absorbance A. The reagent blank was prepared in a similar manner, but the 1 cm^3 of sample solution was replaced with 1 cm^3 of doubly distilled water. The tubes were placed simultaneously in the thermostat.

Procedure for the determination of total manganese in medicinal plants

A 1 cm^3 quantity of the sample solution (instead of the standard solution) was used as in the calibration procedure, and absorbance (A) at 540 nm was measured against a reagent blank. The amount of total manganese was calculated from the calibration graph.

Procedure for the determination of free manganese (II) in infusions

A 1 cm^3 quantity of the sample solution (instead of the standard solution) was used as in the calibration procedure, and absorbance (A) at 540 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 540 nm was measured against a reagent blank. The amount of free manganese (II) was calculated from the calibration graph according to the difference in absorbance ($A - A'$).

Procedure for the determination of total manganese in infusions

A 1 cm^3 quantity of the sample solution was taken and treated with 5 cm^3 of concentrated HNO_3 and H_2SO_4 mixture (5 + 2 v/v) for 20 min at 150 °C in an autoclave. Then the solution was neutralised with 2 cm^3 of 2 mol dm^{-3} sodium hydroxide and diluted to the mark in a 25 cm^3 calibrated flask. A 1 cm^3 sample of this solution (instead of the standard solution) was used as in the calibration procedure, and the absorbance (A) at 540 nm was measured against a reagent blank. The amount of total manganese was calculated from the calibration graph.

Results and Discussion

Optimum conditions

The oxidation of BR by potassium periodate in acidic media results in the decoloration of the solution. The oxidation is catalysed by the presence of small amounts of manganese (II) and this effect is greater when Phen is present in the system. BR has an absorption maximum at 540 nm, which was chosen as the optimum. The reaction was followed spectrophotometrically by measuring the decrease in the absorbance of the solution.

The rate of the oxidation of BR was influenced by the concentrations of BR, potassium periodate, Phen and buffer, and by the pH buffer and temperature. The effect of each of these reaction variables on catalysed and uncatalysed reactions was studied for the purpose of obtaining optimum conditions for manganese (II) determination (a maximum difference between the catalysed and uncatalysed reactions). One of these variables was changed, while keeping the others constant.

The effect of BR concentration was investigated in the range 1×10^{-5} mol dm^{-3}–8×10^{-5} mol dm^{-3}. The results showed that the absorbance of the blank was very high when significantly more BR was used ($> 6 \times 10^{-5}$ mol dm^{-3}). The linear range for manganese was too narrow if a small amount of BR was used ($< 3 \times 10^{-5}$ mol dm^{-3}). Considering these two factors the concentration of BR was adjusted to 5×10^{-5} mol dm^{-3} throughout the studies. Under this condition 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}–8×10^{-4} mol dm^{-3} (Figure 1). A concentration of 2×10^{-4} mol dm^{-3} was chosen as the optimum.

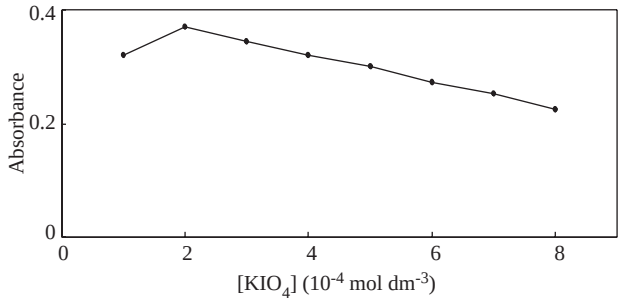


Figure 1. Effect of KIO_4 concentration. BR, 5×10^{-5} mol dm^{-3}; Phen, 1×10^{-4} mol dm^{-3}; Mn (II), 2 ng cm^{-3}; pH 3.0; 70 °C; and time 5 min.

The effect of the Phen concentration was investigated in the range 4×10^{-5} mol dm^{-3}–2×10^{-4} mol dm^{-3} and a concentration of 1×10^{-4} mol dm^{-3} was chosen as the optimum (Figure 2).

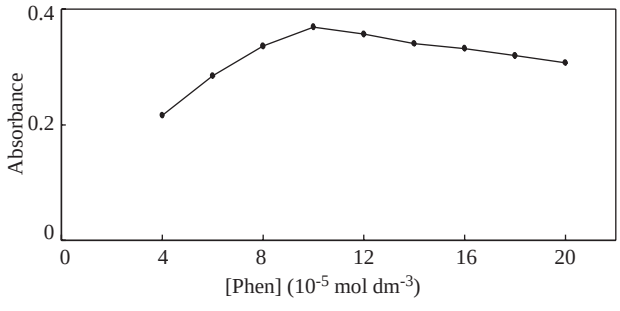


Figure 2. Effect of Phen concentration. KIO_4, 2×10^{-4} mol dm^{-3}. Other conditions as in Figure 1.

The type of buffer is also an important factor. Hence, several buffer solutions at pH 3.0 were tested. The slope of the calibration graph decreased when sodium citrate–hydrochloric acid or potassium hydrogen phthalate–hydrochloric acid were used. The slope increased when UB or PHOP were used, but better results were obtained with the latter and its preparation is easier.

The influence of pH was studied in the range 2.6-5.0 by adjusting the pH with acetic acid–boric acid–orthophosphoric acid and sodium hydroxide. The results are shown in Figure 3. A pH value of 3.0 was chosen as the optimum. 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.

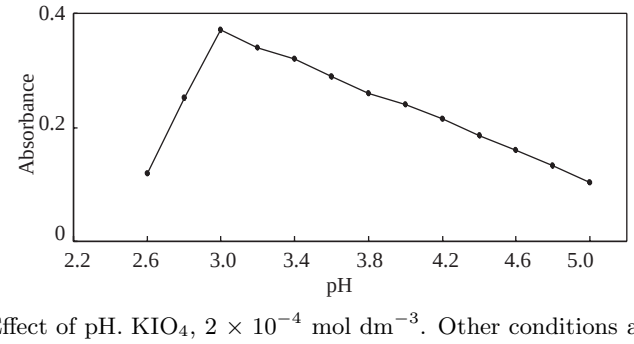


Figure 3. Effect of pH. KIO_4, 2×10^{-4} mol dm^{-3}. Other conditions as in Figure 1.

The dependence of the reaction rate on temperature was investigated between 30 and 80 °C (Figure 4). A temperature of 70 °C was chosen as the optimum.

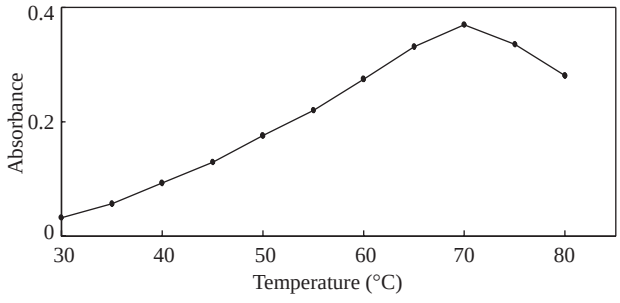


Figure 4. Effect of temperature. KIO_4, 2×10^{-4} mol dm^{-3}. Other conditions as in Figure 1.

The effect of reaction time was studied in the range 2-7 min. The optimum reaction time was 5 min. If the reaction time was shorter or longer, the slope of the calibration graph was lower.

Table 1 summarises the optimum conditions chosen for the development of the analytical method.

Table 1. Selected optimum conditions.

Reaction variable	VALUE
[BR]	5 X 10^{-5} MOL DM^{-3}
[KIO_4]	2 X 10^{-4} MOL DM^{-3}
[Phen]	1 X 10^{-4} MOL DM^{-3}
[PHOP]	0.1 MOL DM^{-3}
pH	3.0
Temperature	70 °C
Time	5 MIN

Calibration Graph

Under the optimum conditions, a linear calibration graph was obtained for manganese (II) from 0.1 to 4.5 ng cm^{-3}. The regression equation of the calibration graph was A = 0.01 + 0.18 C, where C is concentration (ng cm^{-3}) and the correlation coefficient was 0.998. The proposed method yields a relative standard deviation of 1.0% 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.

Study of interferences

The influence of 28 cations and anions on the kinetic determination of manganese (II) was investigated: 100,000-fold excesses of Na^+, K^+, NO_3^-, SO_4^{2-}, BO_3^{3-}; PO_4^{3-}, CH_3COO^-; 30,000-fold excesses of Ca^{2+}, Mg^{2+}, F^-, Cl^-; 3000-fold excesses of Cd^{2+}, Hg^{2+}, Al^{3+}, Br^-, I^-, $C_2O_4^{2-}$, citrate, tartrate; 1000-fold excesses of Pb^{2+}, Ni^{2+}, Cr^{3+}, Cr (VI), Mo (VI); 600-fold excesses of Zn^{2+}; 500-fold excesses of Cu^{2+}; 250-fold excesses of Fe^{3+}; and 120-fold excesses of Co^{2+} did not interfere with the determination of manganese (II). There are various organic compounds in infusions of medicinal plants which might react with BR or potassium periodate to form new compounds absorbing at 540 nm and thus interfering with the assay for manganese (II). In the procedure described, the term $(A - A')$ eliminates potential interference from the reactions between compounds in the infusion and the BR. Furthermore, there was no difference in absorbance when the dye was omitted compared to when both the dye and potassium periodate were omitted, thus confirming that there

was no reaction between compounds in the infusions and potassium periodate producing an absorbance at 540 nm.

Application

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 infusions of these medicinal plants and the results obtained are shown in Table 3. These results show that most of the manganese in 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 electrothermal atomic absorption spectrophotometry. The assessment by Student's t-test and Fisher's F-test did not show a statistically significant difference between the results obtained by these two methods ($P > 0.05$).

Table 2. Determination of total manganese in some medicinal plants.

Sample	Manganese found* / μg g^{-1}	
	Proposed method	ETAAS METHOD
Leaves of common balm (*Melissa officinalis* L.)	401.1 ± 6.1	392.7 ± 11.8
Stalks of creeping thyme (*Thymus serpyllum* L.)	342.8 ± 5.4	350.5 ± 8.8
Stalks of common lungwort (*Pulmonaria officinalis* L.)	207.1 ± 2.9	203.8 ± 5.7
Leaves of colt's-foot (*Tussilago farfara* L.)	108.6 ± 1.8	106.7 ± 3.0

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

Table 3. Determination of manganese in infusions of some medicinal plants.

Sample	STATE	Manganese found* / μg cm^{-3}	
		Proposed method	ETAAS METHOD
Leaves of common balm	Free	0.020	–
(*Melissa officinalis* L.)	Total	0.416 ± 0.005	0.408 ± 0.010
	Free %	4.8	–
Stalks of creeping thyme	Free	0.015	–
(*Thymus serpyllum* L.)	Total	0.520 ± 0.008	0.512 ± 0.013
	Free %	2.9	–
Stalks of common lungwort	Free	0.018	–
(*Pulmonaria officinalis* L.)	Total	0.239 ± 0.004	0.234 ± 0.006
	Free %	7.5	–
Leaves of colt's-foot	Free	0.010	–
(*Tussilago farfara* L.)	Total	0.176 ± 0.003	0.180 ± 0.005
	Free %	5.7	–

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

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). With its detection limit of 0.03 ng cm^{-3}, this method is comparable to analytical techniques with detection limits in the sub–ng cm^{-3} range (ETAAS, RNAA, ICP-AES, ICP-MS, etc.) [17]. Free manganese (II) without separation and total manganese were determined for the first time in infusions of common balm, creeping thyme, common lungwort and colt's-foot. 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.

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