

Investigation of the Reaction of Magnesium with *p*-Nitro-*o*-carboxylazochromotropic Acid and the Determination of Magnesium in Plants and in Water

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The formation of a novel complex between magnesium and *p*-nitro-*o*-carboxylazochromotropic acid (NCBAC) has been observed which is sensitive and selective at pH 10.4 in the presence of ethylenediamine and fluoride. The constants of formation of this complex have been determined by a spectral correction technique, and the complexation reaction has been applied to the spectrophotometric determination of trace magnesium in plants and in water with satisfactory results. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: Spectral correction technique; magnesium; *p*-nitro-*o*-carboxylazochromotropic acid.

INTRODUCTION

Magnesium (II) is an essential element in photosynthesis because the Mg-porphyrin complex acts as a carrier in plants, a role similar to that played by haemoglobin in the human body. Most of the Mg in a plant is present in the chlorophyll of the leaves, and the accurate, quantitative determination of Mg in chlorophyll is of importance in the study of the mechanism of photosynthesis. In natural waters, the content of Mg enables a distinction between hard and soft water. In addition, the determination of Mg is helpful to understand the exchange and transfusion of Mg among plant, soil and water. However, studies concerning the determination of trace Mg have been far fewer than those related to other metals such as Cu, Fe, Cd and Zn. Mg has properties similar to Ca and the other alkaline earths, and its classical determination has involved titrimetry (International Standards Organisation, 1984), atomic absorption spectroscopy (Bornermisza-Pausperl, 1973; International Standards Organisation, 1986), chromatography (Zenki, 1981), spectrophotometry with chromogenic agents such as catechol violet (Yu, 1988), alizarin red S (Yang and Chen, 1984), 1-(2-pyridylazo)-2-naphthol (Goto *et al.*, 1977) and beryllon-II (Qui and Zhu, 1983), and flow injection analysis (Wada *et al.*, 1983; Wada and Nakagawa, 1984). The titrimetric technique and some of the spectrophotometric methods often produce a joint value for Ca and Mg, and the detection sensitivity is typically low. Flow injection analysis is commonly difficult to carry out, whilst atomic absorption spectroscopy and chromatography cannot be used for the field analysis of a sample.

In our hands, the novel agent *p*-nitro-*o*-carboxylazochromotropic acid (NCBAC) has proven to be sensitive

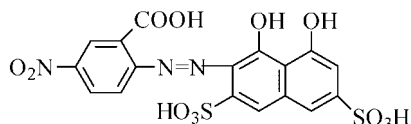
and selective for the quantitative determination of trace amounts of Mg at pH 10.4 in the presence of ethylenediamine and fluoride. In Mg-NCBAC solution, both the metal complex and free NCBAC exist simultaneously, and, owing to the high fractional absorbance of the excess NCBAC, it is impossible to use ordinary spectrophotometry to investigate the characteristic constants of the complex or to measure traces of Mg in plants with any accuracy. However, a spectral correction technique (Gao, 1995) may be applied instead of the ordinary method since it gives the absorbance of the complex and that of the free NCBAC in admixture. In this paper, 'real absorption' indicates the practical absorption in comparison with a pure water reference: 'real absorption' cannot be measured directly but calculated only by measuring the absorbance of the Mg-NCBAC solution against the NCBAC solution reference (containing no Mg). Here, we suggest that the molar absorptivity of the complex calculated from the 'real absorption' is called the 'real absorptivity' in order to distinguish it from the 'apparent absorptivity'. By means of the spectral correction technique, the characteristic constants of the Mg-NCBAC complex (e.g. the composition ratio, stepwise absorptivity and stability constant) could be determined readily. The recommended method is simpler in operation and more understandable than the classical methods such as continuous variations (Likussar, 1973), etc.

EXPERIMENTAL

General. Absorption spectra were recorded with a PGeneral (Beijing, China) model TU1901 spectrophotometer with 1.0 cm cell, and pH was measured with a Mettler-Toledo Instruments (Shanghai, China) model 320-S meter. The Mg contents of the samples were determined with a Beijing 2nd Optical Instruments

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p-nitro-*o*-carboxylazochromotropic acid

(Beijing, China) model WFX-1F2 atomic absorption spectrophotometer.

Synthesis of *p*-nitro-*o*-carboxylazochromotropic acid.

2-Amino-5-nitrobenzoic acid (4.3 g), water (30 mL) and concentrated hydrochloric acid (8 mL) were mixed in a 100 mL beaker, the whole cooled to 0–5°C, and a solution of sodium nitrite (1.6 g) in water (15 mL) slowly added. The presence of free nitric acid was determined after 15 min, and the solution was stirred at 0–5°C for 30 min. The diazonium salt was not filtered off out at this point, but rather the salt suspension was added dropwise, with stirring, to a solution containing chromotropic acid (8.4 g) in 15% sodium carbonate. The mixture was adjusted to pH 5–6 and allowed to stand for 2–3 h. After filtering, the dark-reddish precipitate was washed with 6 M hydrochloric acid and twice with ether. NCBAC was obtained in 25% yield (3.3 g): IR ν (cm⁻¹) 3464, 1688, 1614, 1581, and 1489; UV λ_{max} 530 nm.

Preparation of solutions. Standard Mg(II) stock solution (1.000 g/L) was prepared by dissolving 1.000 g of 99.5% pure Mg (Shanghai Chemicals, Shanghai, China) in 20 mL of 1 M hydrochloric acid and then diluted to 1000 mL with deionised water. A standard Mg working solution (10.00 mg/L) was prepared daily from the above standard Mg solution. The NCBAC solution (1.00 mM) was prepared by dissolving 0.2296 g of NCBAC in 500 mL of deionised water. This solution must be stored in a dark bottle and at a temperature of <5°C. The pH 10.4 buffer solution was prepared from sodium borate and sodium hydroxide solution and was used to adjust the acidity of the sample solutions. The masking agent, used to mask the transition metals, was prepared by dissolving 2 g of AR grade sodium fluoride (Shanghai Third Reagents, Shanghai, China) in 100 mL of deionised water and adding 2 mL of AR grade ethylenediamine (Huainan Chemicals, Huainan, China).

Plant materials. The Mg content of leaf material of *Canna indica* L., *Taraxacum mongolicum* Hand.-Mazz. and wheat (*Triticum aestivum*) was determined. Washed leaf material was cut into small pieces and a sample (1.000 g) treated with 2 mL of concentrated sulphuric acid. The resulting solution was diluted to ca. 50 mL with deionised water and neutralised to pH 6–7 with 5 M sodium hydroxide. The solution was finally diluted to 100 mL with deionised water.

Determination of Mg. A known volume of sample solution containing less than 50.0 μg of Mg was taken in a 25 mL volumetric flask to which was added 2.5 mL of pH 10.4 buffer solution, 1 mL of masking agent and 3.0 mL of NCBAC solution. The contents of the flask were diluted to 25 mL and mixed well. After 10 min the absorbance values at 505 nm and at 582 nm were

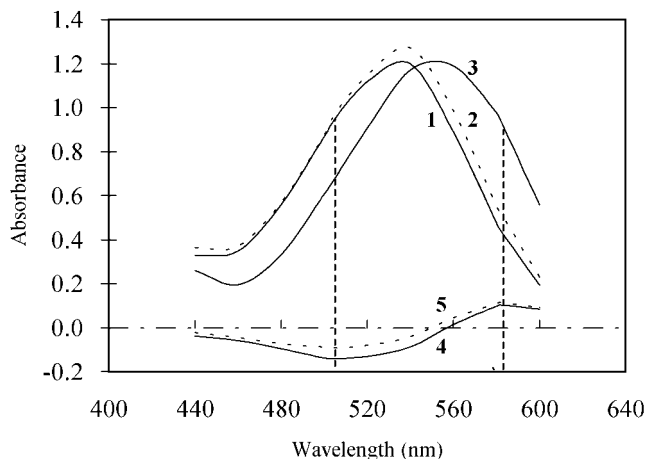


Figure 1. Absorption spectra of NCBAC and Mg-NCBAC solutions at pH 10.4. Trace 1, 1.00 μmol NCBAC; trace 2, 1.00 μmol NCBAC containing ethylenediamine and fluoride; trace 3, complex solution containing Mg (40 $\mu\text{g}/\text{mL}$) and NCBAC (1.00 μmol); trace 4, complex solution containing Mg (1.00 $\mu\text{g}/\text{mL}$) and NCBAC (1.00 μmol); trace 5, complex solution containing Mg (1.00 $\mu\text{g}/\text{mL}$) and NCBAC (1.00 μmol) together with ethylenediamine and fluoride. Traces 1–3 were recorded against a water blank, traces 4 and 5 against a reagent blank.

determined against a reagent blank. The 'real absorbance' (A_c) of the complex was calculated by the relation (Gao, 1995):

$$A_c = \frac{\Delta A - \beta \Delta A'}{1 - \alpha \beta}$$

where ΔA and $\Delta A'$ are the absorbance values of the solution at 582 and 505 nm, respectively, against the reagent blank, and α and β are correction constants obtained by measuring directly NCBAC and its Mg complex (Gao and Yu, 2000).

RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra of NCBAC and its Mg complex solution at pH 10.4. From traces 1 and 3 it may be observed that the maximum absorption of the NCBAC solution is at 530 nm whilst that of the Mg-NCBAC complex solution is at 560 nm. By comparing trace 1 with trace 2, it is seen that the presence of ethylenediamine changes the spectrum of NCBAC giving rise to a change in the correction coefficient β . From trace 4, it is clear that two wavelengths, 505 nm (valley) and 582 nm (peak) should be employed. The coefficient β for the NCBAC solution was calculated to be 0.463 from trace 1, and to be 0.586 from trace 2. Using the same method, the coefficient α for the Mg-NCBAC complex was calculated to be 0.726 from trace 3. The two expressions for A_c are thus: $A_c = 1.51 (\Delta A - 0.463 \Delta A')$ in the absence of masking reagent, and $A_c = 1.74 (\Delta A - 0.586 \Delta A')$ in the presence of the masking reagent. The former expression is used to obtain the characteristic constants of the Mg-NCBAC complex and the latter for the quantitative determination of trace amounts of Mg in plant and water samples.

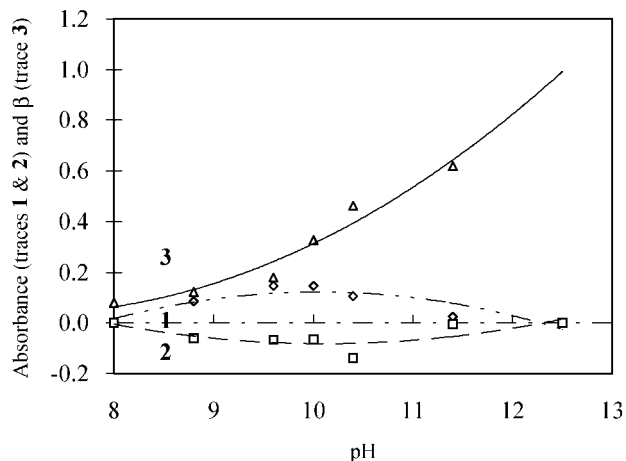


Figure 2. Effect of pH on the absorbance [trace 1 at 582 nm (Δ); trace 2 at 505 nm (Δ)] and β variation (trace 3) of a solution containing Mg (1.00 $\mu\text{g/mL}$) and NCBAC (1.0 μmol).

Effect on absorption of the Mg-NCBAC complex by changes in experimental conditions

The effect on the absorption of Mg-NCBAC solution by varying the pH of the solution is shown in Fig. 2 (traces 1 and 2): [$\Delta A - \Delta A'$] reached a maximum between pH 9.6 and 11. From trace 3 (Fig. 2), it may be observed that the coefficient β increases with an increase in pH, especially when the pH is >9.6 . Consequently, a pH 10.4 buffer solution was used for the determination of Mg.

Figure 3 shows the effect on the absorption of a Mg-NCBAC solution from the addition of NCBAC. Trace 1 shows that it is difficult to calculate the ratio of NCBAC to Mg in the complex by the molar ratio method because its inflexion point cannot be found precisely. From trace 3, the addition of 3 μmol of NCBAC solution was used. The effective fraction (η) of NCBAC and the molar ratio (γ') of effective NCBAC to Mg may be calculated according to the following relations (Gao *et al.*, 2001):

$$\eta = \frac{\alpha \Delta A - \Delta A'}{(1 - \alpha\beta)A_0'}$$

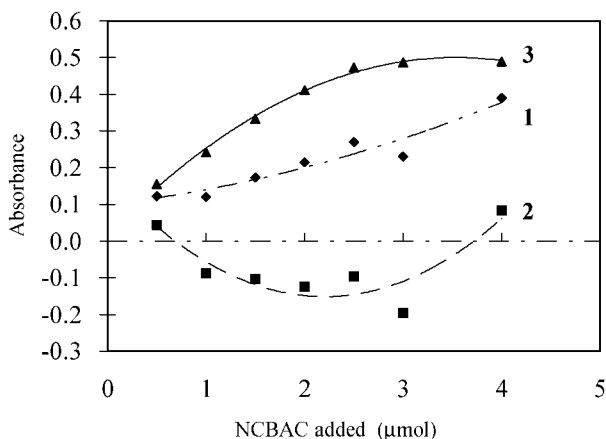


Figure 3. Effect of addition of NCBAC on the absorbance (trace 1 at 582 nm; trace 2 at 505 nm) and 'real absorbance' (A_0') at 582 nm (trace 3) of a solution containing Mg (1.00 $\mu\text{g/mL}$) and NCBAC.

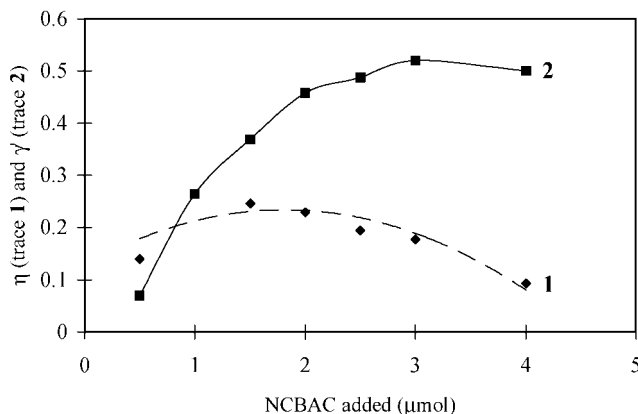


Figure 4. Effect of addition of NCBAC on the effective fraction of NCBAC (η ; trace 1) and on the molar ratio of effective NCBAC (γ' ; trace 2) of a solution containing Mg (1.00 $\mu\text{g/mL}$) and NCBAC.

where

$$\gamma' = \eta \times \frac{C_L}{C_M}$$

and C_M and C_L indicate the initial molarity of Mg and NCBAC, respectively; A_0' is the absorbance of the blank reagent measured at 505 nm against water. Graphs of η and γ' vs. the addition of NCBAC are shown in Fig. 4 (traces 1 and 2, respectively). Trace 2 shows that γ' approaches a maximum of 0.5 when the addition of NCBAC exceeds 2.5 μmol , showing the complex to be $\text{Mg}_2(\text{NCBAC})$. From curve 1 (Fig. 4) the effective fraction of NCBAC is only 16% at 3 μmol addition of NCBAC. The excess NCBAC approaches 84% so as to support the complexation equilibrium. The interference of the free NCBAC is inevitable in the measurement of absorbance of the complex.

The stepwise stability constant (K_n), cumulative stability constant (K) and stepwise absorptivity (ϵ) of the complex can be calculated from the relations (Gao,

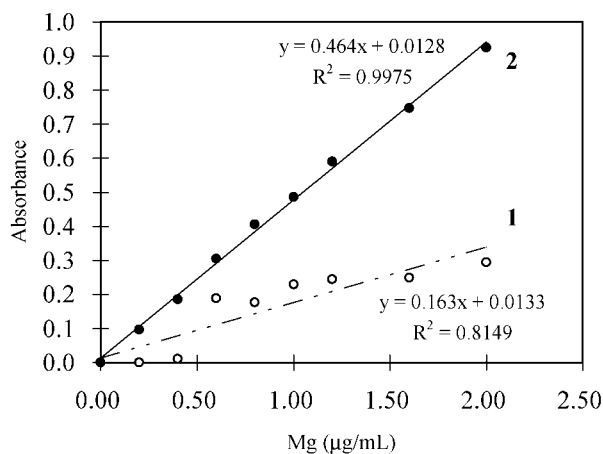


Figure 5. Calibration curves for the quantitative determination of Mg using NCBAC as the chromogenic agent at pH 10.4: line 1, employing ΔA measured at 582 nm; and trace 2, employing 'real absorbance' (A_0').

Table 1. Determination of magnesium in natural waters and plant leaf material using *p*-nitro-*o*-carboxylazo chromotropic acid (NCBAC)

Sample	Mg added	Mg determined	Average recovery (%)
Drinking water ^a	0.00	7.24 ± 0.27 (7.18) ^b RSD 3.7%	108
	10.0	18.1 ± 0.72	
River water ^a	0.00	2.99 ± 0.13 (2.87) ^b RSD 4.3%	95.7
	3.00	5.86 ± 0.40	
Wheat leaf ^c	0.00	0.212 ± 0.012 (0.218) ^b RSD 5.7%	101
	0.200	0.414 ± 0.015	
<i>Canna indica</i> leaf ^c	0.00	0.356 ± 0.010 (0.347) ^b RSD 2.8%	94.5
	0.200	0.545 ± 0.016	
<i>Taraxacum mongolicum</i> leaf ^c	0.00	0.335 ± 0.013 (0.331) ^b RSD 3.8%	98.5
	0.200	0.532 ± 0.017	

^a Mg concentration in mg/L.

^b Mean of determinations by atomic absorption spectroscopy ($n = 2$).

^c Mg concentration in mg/g.

2000):

$$K_n = \frac{\gamma' + 1 - n}{(n - \gamma')(C_L - \gamma' C_M)}$$

$$K = \prod_{n=1}^{\gamma} K_n$$

$$\varepsilon_{ML_n}^{\lambda_2} = \frac{A_c}{\delta C_M (\gamma' + 1 - n)} - \frac{n - \gamma'}{\gamma' + 1 - n} \varepsilon_{ML_{n-1}}^{\lambda_2}$$

where n indicates the n th complex and δ the thickness of the cell. The molar ratio γ' must be between $n - 1$ and n by preparing an appropriate solution. For this purpose, the solutions were prepared containing 24.3 μg of Mg plus 1.00 μmol of NCBAC. Five repetitive determinations of the property constants of the complex gave $K_{\text{Mg}_2(\text{NCBAC})} = 1.92 \times 10^4$ and $\varepsilon_{\text{Mg}_2(\text{NCBAC})} = 2.25 \times 10^4$ L/mol cm at 582 nm.

Determination of Mg

A series of standard solutions of Mg (0–2.00 $\mu\text{g}/\text{mL}$) was prepared, the absorbance of each was measured and A_c for each solution was obtained. Calibration curves are shown in Fig. 5. It is clear that the recommended method (trace 2) permits better accuracy and higher sensitivity than does ordinary spectrophotometry (trace 1). Repetitive determinations of a solution containing 0.200 $\mu\text{g}/\text{mL}$ Mg gave the relative standard deviation (RSD) as 2.0%, whilst the RSD was 22% using ordinary spectrophotometry. For an A_c of 0.010, the detection limit of Mg was calculated to be 0.02 mg/L. Solutions containing 25.0 μg

of Mg(II) and each of the following ions: 1 mg of Cl^- , SO_4^{2-} , NO_3^- , F^- , PO_4^{3-} , NH_4^+ , Ca(II); 0.2 mg of V(V), Ti(II), Al(III), In(III), Sn(II), Cr(III), Mo(II), 0.1 mg of Ni(II), Fe(II), Zn(II), Mn(II), Cu(II), 0.05 mg of Hg(II), Cd(II) and 0.02 mg of Co(II) were prepared, their absorbance values measured at 582 and 505 nm and A_c values calculated. By comparing A_c values, all relative errors were less than 10% so the recommended method can eliminate interference associated with the co-presence of considerable amounts of Ca(II) (ca. an amount equivalent to 40 times the level of Mg present) and chloride (up to an equivalent of 200 times the level of Mg) and of a number of other metal ions. The complexation is highly selective.

Using the recommended method, Mg was determined in drinking water, river water and leaf material from wheat, *Canna indica* L. and *Taraxacum mongolicum*. the results are given in Table 1. The RSD was less than 5.7% and the recovery rate of standard Mg was between 94.5 and 108%. In order to examine the accuracy of detection, samples were also determined by atomic absorption spectroscopy and the results (Table 1) show high comparability.

In the present work, the formation of the complex $\text{Mg}_2(\text{NCBAC})$ has been studied, and its stability constant and molar absorptivity have been found to be 1.92×10^4 and 2.25×10^4 L/mol cm, respectively, at 582 nm. The analyses of plant and water samples gave satisfactory recovery and precision for the determination of Mg using the recommended method. The method also removes interference caused by Ca which commonly co-occurs with Mg. The method is suitable for use in the field determination of Mg through the use of a portable, high-sensitivity photometer.

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