## Spectrophotometric Determination of Magnesium Oxide Content in Supplements of Magnesium

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#### Abstract

Magnesium is an essential element and the intracellular divalent cation involved in many biochemical functions. People with magnesium deficiency must increase their intake of magnesium, usually in the form of various supplements. A common form of magnesium supplement widely available in pharmacies is magnesium oxide (MgO). In this work, the content of MgO was determined in pharmaceutical supplementations using spectrophotometry, based on the reaction between magnesium ions and eriochrome black T at a wavelength of 535 nm. The analysed content of MgO ranged from 360.5 to 386.5 mg MgO, which corresponds to the daily Mg recommended values (300 to 400 mg).

### Keywords

Magnesium, magnesium oxide, supplementation

## **1** Introduction

Magnesium is a major cation in the human body involved in many chemical and physiological functions, including synthesis of fatty acids and proteins, metabolism of diet, and transmission of nerve impulses. It is a cofactor for more than 300 metabolic reactions in the body. These processes include protein synthesis, cellular energy production and storage, cell growth and reproduction, DNA and RNA synthesis, and stabilization of mitochondrial membranes. Therefore, magnesium plays an important role in disease prevention and human health.<sup>1–7</sup>

Low levels of magnesium, i.e. hypomagnesemia, have been associated with a number of chronic diseases including migraine headaches, Alzheimer's disease, stroke, cardiovascular disease, and Type 2 diabetes. People with magnesium deficiency must increase their intake of magnesium, usually in the form of various pharmaceutical supplements.<sup>8–13</sup> Magnesium oxide is a common form of magnesium supplement that is widely available in pharmacies because it is less expensive than other forms (i.e. citrate, chloride, and amino acid gluconate). Moreover, magnesium oxide contains about 60 % elemental magnesium. Another reason could be the differences in the bioavailability between different magnesium salts. Good solubility of magnesium salts in water is important for its bioavailability. The studies demonstrate that organic Mg salts have a higher solubility than inorganic salts.<sup>14,15</sup>

Several reagents in colorimetric as well as spectrophotometric methods for the determination of magnesium have been described in the literature, such as eriochrome black T (EBT), titan yellow (TY), ammonium phosphate method, ethylenediaminetetraacetic acid (EDTA), paratolyl-2-thenohydroxamic acid and quinalizarin, 4-methyl-3-((1-H-Indol-3-iyl)(phenyl)methyl)-1-H-Indol (MPBIM), and trizma-chloranilate.<sup>16-21</sup> All of these procedures require a true solution of a coloured magnesium complex in which the colour intensity is directly proportional to the magnesium concentration.

The aim of this work was to determine the total content of magnesium oxide in supplements from different manufacturers available in pharmacies in Sarajevo using modified spectrophotometric method. Modification was aimed at shortening the time of colour development by addition of solid EBT instead of its solution. Thus, the problems associated with EBT in the form of an unstable solution were prevented. This modification changes none of the parameters of standard eriochrome black T method.

## 2 Experimental

The magnesium supplements (a common form of magnesium as magnesium oxide, MgO) of various manufacturers were bought from pharmacies in Sarajevo, as follows: Maxi Mag-magnezijum 375 mg+Vitamin B6 (NATUR PRODUCT PHARM), Magnezium-Lek<sup>®</sup> Direkt (SANDOZ), Magn*Easy*um Forte (MINERALI ZDRAVLJA) with declared contents of magnesium in tablets as 375 mg, 300 mg, and 400 mg, respectively. For analysis, the supplements were marked as *Sample I, Sample II*, and *Sample III*.

Total content of magnesium oxide (MgO) of each supplement was determined by spectrophotometry, in triplicate, using minor modified eriochrome black T (EBT) method.<sup>17</sup>

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## 2.1 Sample preparation

Samples of tested MgO supplements (0.30 g each) were dissolved in 1.0 ml of 6 M hydrochloric acid, and diluted with distilled water to mark (50 ml). Working solutions of all samples (5.0 ml) were prepared in triplicate, and measured with a "Spectronic Genesys 2" spectrophotometer (Thermo Electron Corporation, Madison, USA) after complexation of Mg<sup>2+</sup> ions with EBT (Sodium 1-[1-Hydroxynaphthylazo]-6-nitro-2-naphthol-4-sulfonate) in an ammonia buffer.

## 2.2 Reagent and standard solutions preparation

All reagents were of analytical grade (p.a.), while distilled water (Milli-Q, Millipore) was used for samples dilution and labware washing. Solutions of eriochrome black T method, EBT (Merck, Germany) are unstable even when freshly prepared and thus useless for complexation and formation of analyte dye (with Mg<sup>2+</sup> ions). Therefore, EBT was added directly to the analysed and standard solutions, precisely weighed to 0.1 g of salt during analysis and construction of calibration curve. Standard stock solution ( $\gamma$ (Mg<sup>2+</sup>) = 1 mgml<sup>-1</sup>) of magnesium (II) ions was prepared by dissolving 0.4180 g of magnesium (II) chloride (Sigma-Aldrich Co. LLC) in a few mL of 6 M hydrochloric acid (Merck, Darmstadt, Germany), and diluted with deionized water in a volumetric flask (50 ml).

The calibration solutions were prepared by pipetting aliquots of 0.05, 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, and 2.50 ml, respectively, of the stock standard solution into volumetric flasks (10 ml), then, to each volumetric flask, adding 1.00 mL of ammonia buffer, 0.1 g of EBT and distilled water to obtain a concentration range of Mg from 0.0383 to 0.2049 mmol ml<sup>-1</sup>. Ammonia buffer solution was prepared by dissolving 54.0 g of ammonia chloride in 200 mL of water, and adding 350 mL of 10 M ammonia, then diluting it with water to 1000 ml.

The absorbance of each working solution was measured at absorption maximum of 535 nm using 10 mm quartz cuvette (Fig. 1).



#### 2.3 Total MgO content determination

For determination of magnesium oxide content in selected commercially available pharmaceutical supplements of magnesium, working solutions of all nine samples were treated with EBT and ammonia buffer as mentioned above for standard stock solutions of Mg<sup>2+</sup> ions. Reaction between magnesium ions and eriochrome black T is favourable in basic medium (pH = 10) and room temperature; therefore, an ammonia buffer was added, and measurement was performed on a wavelength of 535 nm. Working wavelength was chosen by analysing the spectrogram obtained by measuring absorbance A in function of wavelength,  $\lambda$ as the maximum wavelength gives the best sensitivity and accuracy. The optimal wavelength ( $\lambda_{max}$ ) for measuring absorbance is the wavelength that is most absorbed by the analyte in question. Practically, measurements made by scanning the absorbance of standard solution colour intensity enabled us to observe the maximum peak at which the highest absorbance was recorded.

In neutral or somewhat basic solutions (*i.e.*, pH = 10), eriochrome black T is a double dissociated ion,  $HIn^{2-}$ , which is blue in colour. When this free form of EBT reacts with magnesium ions, a pink coloured complex is formed (Fig. 2).



free indicator  $(H_2In^-)$ 



Mg<sup>2+</sup>indicator complex (MgIn<sup>-</sup>)

pink

*Fig.* 2 – Reaction between magnesium ions and eriochrome black T *Slika* 2 – Reakcija između magnezijevih iona i eriokrom crnog T

## 3 Results and discussion

Spectrophotometric analysis was carried out with EBT as an easily available and low cost indicator. In addition, we simplified the colouration of analysed samples and stability of Mg-EBT complex by adding solid salt and avoiding longterm addition of freshly prepared EBT solution to each individual solution of working and analysed samples.

The daily intake requirement in adults is about 400 mg, but when used as a treatment, magnesium is often recommended at doses of 250 to 600 mg for adults. Comparison of the recommended intake doses with the MgO content declared and measured in selected commercially available samples, showed good agreement. The analysed content of MgO in the three supplements of various manufacturers available in pharmacies in Sarajevo ranged from 290.4 to 398.5 mg MgO. Results are presented in Table 1 as mean  $\pm$  SD for *Sample I, Sample II,* and *Sample III*, respectively.

*Table 1* – Total content of MgO in analysed supplements *Tablica 1* – Ukupni sadržaj MgO u analiziranim pripravcima

Components Komponente	Analysed content of MgO*	Declared content of MgO
Sample I, mg MgO	$374.7 \pm 5.5$	375
Sample II, mg MgO	$290.4 \pm 1.3$	300
Sample III, mg MgO	$398.5 \pm 5.3$	400

 $^*$  Data presented as mean  $\pm$ SD

As may be seen from Table 1, the difference between declared and true MgO content was lowest in Sample III. Although the other two measured samples showed no significant deviations, all three analysed samples in a single capsule dose contain approximately the recommended daily dose of magnesium in the form of magnesium oxide.

Nowadays, magnesium in the form of magnesium oxide is used as a dietary supplement for various disorders and medical conditions, such as neurological (migraine, insomnia, Alzheimer disease, etc.), vascular disease (hypertension, stroke, CVD, CAD), diabetes and prediabetes, during pregnancy, etc.<sup>1,4,22-26</sup>

## 4 Conclusion

Commercially available Mg-supplements in pharmacies in Sarajevo were tested with the purpose of determining the MgO content. Based on the obtained results, the conclusions can be drawn as follows: the analysed supplements of MgO commonly used, can be considered as a good source of magnesium in pharmaceutical products, as they showed that the declared value of MgO corresponds with recommended daily doses. In addition, sample preparation, complexation of magnesium ions with solid EBT, and spectrophotometric measurement are simple, not time consuming and applicable for determination of MgO in commercial supplements.

### List of abbreviations and symbols Popis kratica i simbola

- EBT eriochrome black T – eriokrom crnog T
- TY titan yellow
  - indikator titan žuto
- EDTA ethylenediaminetetraacetic acid – etilendiamintetraoctena kiselina
- MPBIM- 4-methyl-3-((1-H-Indol-3-iyl)(phenyl)methyl)-1-H--Indol
  - 4-metil-3-((1-H-indol-3-il)(fenil)metil)-1-H-indol

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## SAŽETAK

# Spektrofotometrijsko određivanje sadržaja magnezijeva oksida u pripravcima s magnezijem

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Magnezij je esencijalni element i intrastanični dvovalentni kation uključen u više biokemijskih funkcija. Osobe s manjkom magnezija moraju ga dodatno unositi i to često u obliku različitih pripravaka. Magnezijev oksid (MgO) najčešći je oblik pripravka s magnezijem koji je široko dostupan u ljekarnama. U ovom radu spektrofotometrijom je određen sadržaj MgO u farmaceutskim dodatcima temeljen na reakciji između magnezijevih iona i eriokrom crnog T na valnoj duljini od 535 nm. Analizirani sadržaj MgO kretao se u rasponu od 360,5 do 386,5 mg MgO, što odgovara dnevnim preporučenim vrijednostima unosa Mg (300 do 400 mg).

#### Ključne riječi

Magnezij, magnezijev oksid, suplementacija

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