Indonesian Journal of Medical Laboratory Science and Technology

Open Access

p-ISSN 2684-6748 | e-ISSN 2656-9825

IJMLST

Chemometric Analysis of Serum Magnesium Calculations Using Mg-Xylidyl Blue-I Method Based on Molar Absorptivity

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Received: January 7th, 2021
Revised: March 27th, 2021
Accepted: April 2nd, 2021
Published: April 28th, 2021

DOI: 10.33086/ijmlst.v3i1.1876

Abstract

The concentration of magnesium is determined based on the absorbance of the Mg-Xylydil Blue-I complex solution use spectrophotometer. Based on the Lambert-Beer rule, the calculation of sample concentration is based on the formula A = Ɛ. b. C. Generally, the thickness of the cuvette (b) and the molar absorptivity (Ɛ) factor will be ignored because it is considered to have a fixed value, therefore the sample concentration is measured based on the ratio of the absorbance of the sample against the standard solution. However, the standard solution contains pure magnesium and has a different matrix than the sample matrix, so this condition can give analytical errors and lead to misinterpretation of the results. The purpose of this study was to determine the accuracy and the precision of serum magnesium calculation by the principle of the Mg- Xylydil Blue-I complex reaction based on molar absorptivity compared to the general method. This research uses comparative study design methods. The serum sample used was the patient's serum specimen who has a normal magnesium level. The results showed that the significance value of the paired t-test statistical was 0.000 ($p < 0.05$). The accuracy value (d%) of the calculation formula uses Ɛ is 0.00 and the precision value (CV%) is 0.53. While the accuracy value (d%) of the calculation formula without Ɛ is 0.00 and the precision value (CV%) is 0.38. Calculations based on molar absorptivity (Ɛ) can measure more significant serum magnesium than those calculated based on standard magnesium solutions.

Keywords
Epsilon, Magnesium, Mg-Xylydil Blue-I Complex Reaction, Serum, UV-Vis Spectrophotometry.

INTRODUCTION

Long-term deficiency of magnesium (Mg) can cause hypomagnesemia with clinical manifestations including numbness, tingling, muscle cramps, seizures, personality changes, and abnormal heart rhythm. On the other hand, excessive consumption of magnesium from drugs containing Mg (laxatives or antacids) led to hypermagnesemia, hyperthyroidism, kidney failure, and liver failure. Therefore, it is important to maintain the stability of the balanced micronutrient magnesium content in the body (1,2).

Magnesium is an essential element that composes the coenzyme for signal transfer in neurons and enzymes for cardiac contraction. In assition, magnesium is required for the metabolism of carbohydrates, fats, and amino acids as micronutrients. Magnesium has medicinal value as a general laxative, antacid (e.g. milk of magnesia), and to stabilize abnormal nerve excitation or spasm of blood vessels in conditions such as eclampsia (2).

An analytical method (qualitative and quantitative analysis), e.g. chemometric analysis method, is needed to determine the content of magnesium. Chemometrics work by combining statistical values with chemistry, especially analytical chemistry. The chemometric analysis uses statistical principles to design; select an optimal analytical procedure and experiment, and provide maximum and relevant chemical information through chemical data analysis (4,5).

The spectrophotometer is widely used in quantitative measurements of magnesium because the amount of light absorbed by the particles in the solution depends on the type and number of particles (6,7). The photometer spectrum is based on the Lambert-Beer law. Lambert-Beer law states that the concentration of the standard solution is directly proportional to the value of light absorption (absorbance) (8). This law applies to monochromatic rays e.g. light with a single wavelength or that has an adjacent wavelength band. To calculate the absorbance of a sample, it is necessary to know the molar absorption denoted by Ɛ (epsilon), which is a molecule or ion that absorbs a solvent with a certain wavelength but does not depend on a particular concentration and wavelength or through radiation (9).

The Ɛ (epsilon) cannot be removed because it is affected by solvents. The solvent determines the addition of the electron transition energy in the compound, thus the wavelength that becomes the energy will be absorbed in a certain color. If the solvent has many mixtures, it will require a large amount of energy due to the interaction of the solvent (energy π-π* can be smaller or greater). Therefore, mathematically, the Ɛ factor in the calculation equation cannot be ignored.

The use of different measuring formulas of magnesium content may provide different interpretations of the results. Comparative analysis of results is needed to compare the accuracy, precision, and statistical difference between the measurement results of the two formulas.

MATERIALS AND METHODS

This research used comparative study design methods. The sample used in this study was serum of healthy patients with normal magnesium levels in June 2020. The equipment used was a spectrophotometer (GenesysTM 10S). Reagents used in this study are Magnesium XL FS from DiaSys Diagnostic Systems (Germany) contains ethanolamine pH 11.0 750 mmol/L, Glycoletherdiamine-*tetraacetic acid* (GEDTA) 60 µmol/L, Xylydil Blue-I (248266 Sigma Aldrich, CAS Number 14936-97-1) 110 µmol/L and 2 mg/dL standard. The sample was measured by a photometer at a wavelength of 520 nm (11). Samples that met the inclusion criteria were 3 samples. The inclusion criteria in this study were patients who were willing to become respondents by filling out an *informed consent* sheet and the sample volume had to reach 3 mL. The sampling technique used was *random sampling* with the criteria of non-lysed, non-icteric, and non-lipemic blood sample examination (11).

The samples obtained were centrifuged at 3.000 rpm for 15 minutes. Total samples were analyzed for Magnesium levels with the photometric test method using Xylydil Blue-I. The magnesium ion forms a purple complex with Xylydil Blue-I in an alkaline condition. In the presence of the calcium ion complex GEDTA, the reaction is specific. The intensity of the purple color is proportional to the concentration of magnesium (11).

After the data on serum magnesium absorbance are collected, the magnesium content was calculated based on two formulas: Formula type A and Formula type B.

Calculation A

$$Cspl = \frac{Aspl \times Cstd}{Astd} \quad \text{.....1}$$

Cspl: Sample Concentration (mg/dL)

Aspl: Sample Absorbance

Cstd: Standard Concentration (mg/dL)

Astd: Standard Absorbance

Calculation B

$$Cspl = \frac{Aspl \times MW}{\varepsilon_{1cm}^{1\%}} \quad \text{.....2}$$

Cspl: Sample Concentration (mg/dL)

Aspl: Sample Absorbance

MW: Molecular Weigth of Xylydil Blue-I (513,5 g/mol)

$\varepsilon_{1cm}^{1\%}$: Molar Absorptivity of Xylydil Blue-I (49.000 L $M^{-1}cm^{-1}$)

The calculation results of the two formulas were analyzed using the Paired T-

test. The accuracy and precision analysis are calculated based on the deviation from repeated measurements of 3 times.

RESULTS

This research begins by creating a calibration curve to get the liner value. Standard curve determination was performed with several concentrations (2, 4, 6, 8 mg/dL).

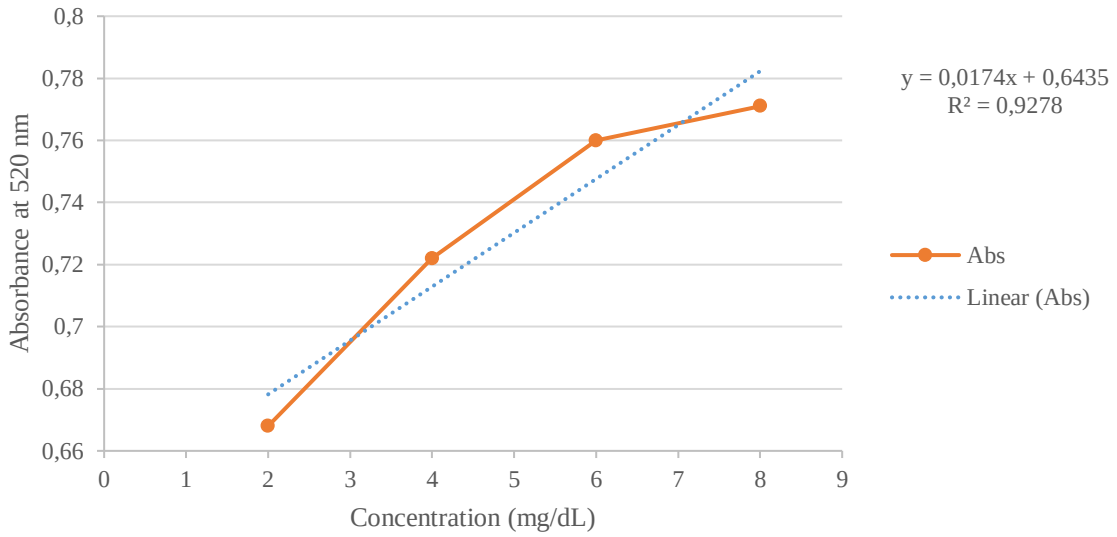

Figure 1. Curve calibration of magnesium standard solution

The calibration data shows that R = 0.9278, which means that the data shows a linear correlation between the concentration of magnesium and absorbance value.

The data collected was analyzed by statistical tests following certain conditions. Data on 20 healthy patients were obtained with a mean serum magnesium level of 3.077 mg/dL, the lowest value was 1.387 mg/dL, and the highest value was 5.581 mg/dL. All patients had normal serum magnesium levels (one day before sampling) and did fasting for 10 – 12 hours to minimize the influence of food and activity. The accuracy and precision of the Magnesium level of patients was determined by statistical analysis (Table 1).

Table 1. Quality test data

No	Parameter	Quality
1	Mean	3.077
2	Standard Deviation (SD)	1.712
3	Coefficient of Variation (CV)	0.556
4	Accuracy (D%)	0.057
5	Total Error (TE)	1.17 %
6	Total Error allowable (TEa)	4%

*based on magnesium test

Normal data distribution is obligatory before paired t-test conducted. The data normality test results show in Table 2. Result shows that all variable is normality distributed (p-value < 0.05).

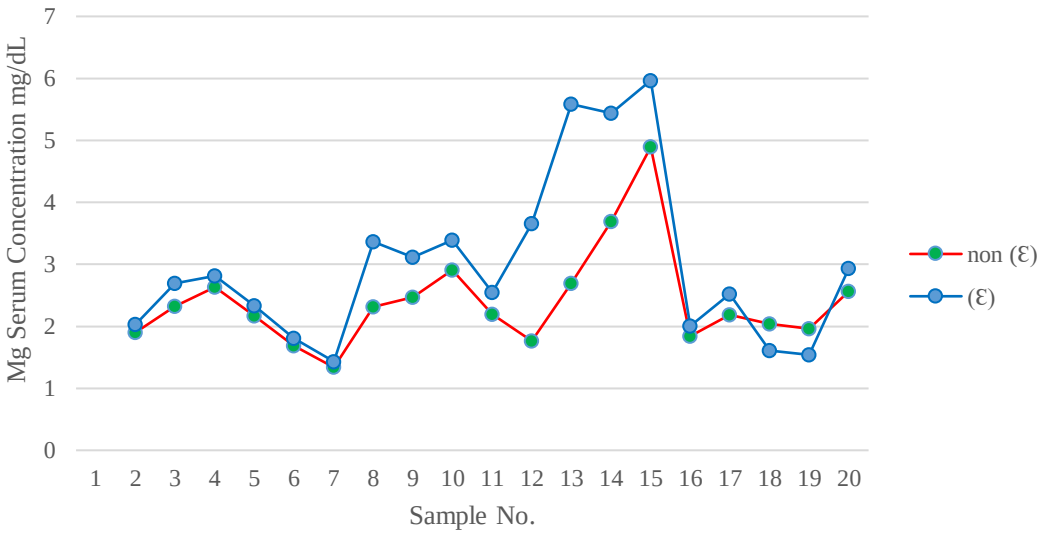

Figure 2. Serum magnesium level curve with 2 formulas: non (Ɛ) and (Ɛ)

Table 2. Variable test of normality test results for each sample

	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
Kit_Insert	0.198	16	0.093	0.898	16	0.076
Epsilon	0.116	16	0.200	0.925	16	0.205

The sample size is lower than 50, therefore the Shapiro-Wilk normality test was used. A significance value lower than 0.05 means the data is normally distributed. The significance value of Epsilon is 0.205 ($p < 0.05$) and the significance value of the Insert Kit is 0.076 ($p < 0.05$) so that the next test uses the paired T-test.

Table 3. Results of t-test statistics – paired one-sample test

	Test Value = 0					
	t	**Df**	**Sig. (2-tailed)**	**Mean Difference**	**95% Confidence Interval of the Difference**	
					Lower	**Upper**
Kit_Insert	10.40	15	0.000	2.42	1.92	2.91
Epsilon	7.52	15	0.000	2.88	2.06	3.69

Statistical test (Table 3) shows that the significance value is 0.000 ($p < 0.05$) for both the formula using the kit-insert and epsilon, therefore there is a significant difference between the calculation formula for the results of the chemometric analysis with Ɛ and analysis without Ɛ.

DISCUSSION

Spectrophotometric analysis work by white light or radiation passed through a colored solution, then radiation with a certain wavelength will be absorbed and other will be transmitted. The absorbance value depends on the content of the substances, the more molecules absorb light, the greater the absorption value. Therefore, the absorbance value will be directly proportional to the concentration of the substance contained in a sample (9). Curve calibration of magnesium standard solution have slope 0.0174 and intercept 0.6435 with equation y = 0.0174x + 0.6435. The correlation between analyte level (x) and instrument response (y) is expressed as the correlation coefficient (r = 0.9278) (Figure 1). Ideally, the intercept is zero. It is expected that no instrument response will occur when analyte free water or blanks are measured. But in this research, we find instrument response occurs due to small interaction, interference, noise, contamination or other sources of bias. Therefore, the intercept (a) in this calibration curve can be considered as the signal from the blank. While the slope (b) is a measure of the sensitivity of a test method. We have greater the value of b, so this method provides a higher sensitivity or the instrument's response is strong enough to change the magnesium existing levels. Based on the correlation coefficient obtained, it shows a linear relationship between magnesium concentration and absorbance. The linear relationship that occurs is positive and strong.

In this study, curves of serum magnesium levels in 20 serum samples with 2 formulas (non (Ɛ) and (Ɛ)) showed an average difference in yield of 15.27%, where serum magnesium levels calculated using Ɛ had higher levels (Figure 2). At magnesium levels <2.5 mg/dL, the difference in calculations is not too far away, but at magnesium levels >2.5 mg/dL, there is a very large difference. This shows that the calculation of serum magnesium using the molar absorption of Xylydil Blue-I can bind magnesium ions more than calculated compared to the absorbance of standard magnesium solutions.

The molecule that receives visible light at the appropriate frequency will experience a transfer of energy to a higher level (transfer of electrons from the ground state to the excited state). This electron transition (μ-μ*) absorbs specific energy and can be detected at certain wavelengths. The specificity and quantity of absorbed light energy are determined based on solubility. The more dissolved a compound, the more energy it will absorb. Conversely, the harder it is to dissolve, the lighter energy is transmitted and this will give false results of the measured number of molecules (8).

The calculation formula used to measure magnesium in serum is as follows formula 3:

$$Cspl = \frac{Aspl \times Cstd}{Astd} \quad \text{.....3}$$

This calculation formula comes from:

1. $Aspl1 = b1\ x\ Ɛ1x\ Cspl1$
2. $Aspl2 = b2\ x\ Ɛ2\ x\ Cspl2$

If there are two similar solutions measured, the fixed factor in the formula can be removed and combine:

1. $Aspl1 = \cancel{b}\ x\ \cancel{Ɛ}\ x\ Cspl1$
2. $Aspl2 = \cancel{b}\ x\ \cancel{Ɛ}\ x\ Cspl2$

$$\text{Cspl1} = \frac{\text{Aspl x Cspl2}}{\text{Aspl2}}$$

$Cspl_1$: Sample Concentration

$Aspl_1$: Sample absorbance (sample 1)

$Cspl_2$: Standard Concentration

$Aspl_2$: Standard absorbance (sample 2)

In this formula, the concentration of the magnesium in the standard solution (pure solvent) is used as a reference for calculating the level of magnesium in serum. The serum is a matrix containing solutes (enzyme, protein, clotting agents, immune system); body essentials such as vitamins and hormone; and dispersed cell components with a pH between 7.35 – 7.45. The solubility of magnesium ions in the serum matrix differs from the solubility of magnesium in pure solvents which do not contain other solutes. A standard solution of magnesium dissolved in water at a pH of 9.0. The difference in pH of this solution reduces the reaction of magnesium with Xylydil Blue-I the magnesium ion forms a purple complex with Xylydil Blue-I in an alkaline condition. In the presence of the calcium ion complex GEDTA, the reaction is specific. The intensity of the purple color is proportional to the magnesium concentration (8).

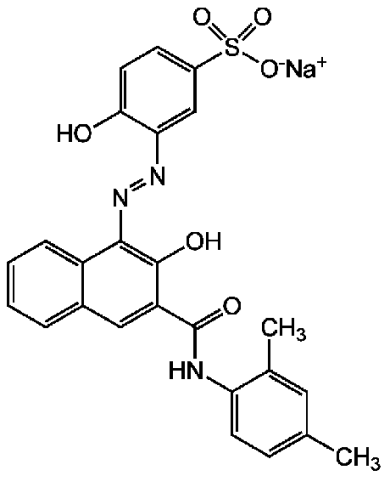

Figure 3. Chemical Structure of Xylidyl blue-I ($C_{25}H_{20}N_3NaO_6S$) (7).

Figure 3 show the structure of Xylydil Blue-I. IUPAC name of Xylidyl Blue-I is sodium; 3-[[3-[(2,4-dimethylphenyl) carbamoyl] -2-hydroxynaphthalen-1-yl] diazenyl]-4-hydroxy benzene sulfonate. Xylydil Blue-I is a synthetic colorimetric reagent for Mg detection with a molecular weight of 513.5. The aqueous solution of XB-1 is red and turns reddish-violet in the presence of Mg at pH 9 (maximum wavelength: 510 nm, molar absorptivity: 49,000, detection range 0.02-0.4 ppm. Xylydil Blue-I can react specifically with magnesium ions in the presence of a glycoletherdiamine tetraacetic acid

(GEDTA). GEDTA is a substance to bind and control metal ions because it can remove water hardness (chelating agent). That is the greater affinity of chelating ligands for a magnesium ion than that of similar nonchelating (monodentate) ligands for the same metal (6).

Xylydil Blue-I has a very good molar absorption (Ɛ = 49,000) value in polar solvents therefore it can be used for the detection of compounds in polar matrices. When Xylydil Blue-I reacts with magnesium ions at pH < 9, the stoichiometry of the reaction will shift to the left and the complex mg-Xylydil Blue-I products formed are getting less. Polar sulfonyl groups (SO_3^-) can interact favorably with similar water molecules. Therefore, the short-chain hydroxyl-benzene is soluble in water. However, since the organic portion (more C atoms) gets bigger (the longer chain in this case), this interaction is less effective and water solubility decreases (8,6).

Serum pH does not support the Mg-Xylydil Blue-I reaction because the electrons in the sulfonyl group do not have enough energy to bind protons to the magnesium ion at pH 7. On the other hand, the standard solution of magnesium has a pH of 9. If this difference is calculated using formula type A, the magnesium test results will be inconsistent, thus the calculation of serum magnesium levels must be divided by the molar absorptivity of xylidyl blue-I.

$$Cspl = \frac{Aspl \times MW}{\varepsilon_{1cm}^{1\%}} \quad \text{.....4}$$

Type B formula (Lambert-Beer) involves the molecular weight and molar absorptivity of Xylydil Blue-I. Consequently, the measured Mg-Xylydil Blue-I complex reaction corresponds to the actual reaction product. The pH changes and the number of magnesium ions in the serum matrix will not affect the detected reaction product suitability. Calculation of serum magnesium using this formula has units of mole/Liter, therefore it must be converted to mg/dL. This research was done in the patient who had normal magnesium levels. More comprehensive studies must be conducted in patients with pathological conditions that allow the maximum amount of magnesium ions that can form bonds with Xylydil Blue-I.

CONCLUSIONS

The serum magnesium level formula that include molar absorptivity factor have a significant difference result ($\alpha<0.05$) compared to formula without molar absorptivity factor. There is a significant difference between serum magnesium levels using the absorbance calculation formula with formula A (without Ɛ factor) and formula B (with Ɛ factor). Calculations based on molar absorptivity can measure more significant serum magnesium than those

calculated based on standard magnesium solutions.

AUTHOR CONTRIBUTIONS

Ally Kafesa: conceptualization, methodology, writing-original draft, visualization, supervision, funding acquisition. Cep wahyu: methodology, supervison. Nadira Nur Hajah Lutfi: formal analysis, investigation, resources.

ACKNOWLADGEMENTS

The authors express their sincere gratitude to the Rajawali Institute of Health, Bandung, Indonesia.

CONFLICT OF INTEREST

We declare that we do not have any commercial or associative in-terest that represents a conflict of interest in connection with the worksubmitted.

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