The parameters influencing the magnesium speciation in analysis of blood serum

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Zusammenfassung

Ultrafiltration als Trenntechnik, Potentiometrie mit ionenselektiven Elektroden und Atom-Absorption-Spektrometrie wurden zur Bestimmung von ionisiertem Magnesium benutzt. Die wichtigsten Parameter bei der Ultrafiltration sind die Temperatur und der partielle Druck von Kohlendioxid, der durch Sättigung der Probe mit CO_2 kontrolliert werden sollte. Die Korrelation zwischen ionisiertem, gesamten und ultrafiltriertem Magnesium ist immer noch umstritten.

Summary

Ultrafiltration as a separation technique, ion-selective electrode potentiometry and atomic absorption spectrometry were used for determination of ionized magnesium in blood serum. The paramcters of prinipal importance during ultrafiltration process are the temperature and the partial pressure of carbon dioxide, which should be controlled by saturation the sample with gaseous carbon dioxide. The correlation between ionized, total and ultrafiltrable magnesium is still questionable.

Introduction

Magnesium plays a significant role in metabolic and physiological processes in human organism and its deficiency is responsible for numerous diseases. Magnesium is present in the body in various forms. However, only the free magnesium exhibits biological activity and information about free magnesium is needed for better understanding of magnesium transport and factors controlling its metabolism [4, 6]. The knowledge of total magnesium concentration in blood serum is not sufficient and a determination of its ionized form is also often performed. The direct measuring using potentiometric clinical analyzer with magnesium ion-selective electrode has been compared with results of ultrafiltration [1].
In this paper we compare our results obtained using potentiometric clinical analyzer and ultrafiltration with previous ones.

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Material and methods

Apparatus

Atomic absorption spectrometer PU 9100 X (Philips Scientific, UK) with deuterium background correction equipped with electrothermal atomizer PU 9390 X and autosampler FS 90 was used.
Potentiometric magnesium measurements were performed using the clinical analyzer, Microlyte 6 (KONE, Finland).
Serum ultrafiltrate was prepared using covered ultrafiltration units Ultrafree-RCL (Millipore, Japan) fitted with polysulfone membrane having a nominal molecular weight limit (NMWL) of 10 000. Centrifugation was performed with high speed centrifuge Type 310 (Mechanika Precyzyjna, Poland).

Reagents and solutions

Standard magnesium nitrate solution (Wzormat, Poland) containing 1 mg ml^{-1} was used. Standard magnesium solution for preliminary experiments and serum analysis was prepared by mixing aqueous standard magnesium solution with 5% human albumin solution (Humanalbin®, Behring).
Working magnesium standards were prepared weekly by dilution with water. Reference serum was Quality Serum KONE, Diagnostics Lot 4009. Pooled human serum and plasma were used for preliminary experiments.
MilliQ-water was used to prepare all solutions. All polyethylene laboratory-ware was soaked for at least 24 hours in 2 mol l^{-1} HNO_3 and finally rinsed several times with MilliQ-water.

Ultrafiltration procedure

To eliminate contamination of magnesium originating from membranes the polysulfone membranes were cleaned by filtering 2 ml of 0.1 mol l^{-1} NaOH solution and next 10 ml of MilliQ-water. It was ascertained that such treatment does not change the membrane characteristics. Directly before ultrafiltration of the sample each ultrafiltration unit was checked for contamination by electrothermal atomic absorption spectrometry and if the absorbance of filtered water was above 0.03 the tube was rejected.
Ultrafiltration was carried out at room temperature (20°C). In each ultrafiltration experiment 1 ml of sample was transferred into the filtration cell. The first 50 µl of ultrafiltrate was discarded and the following 50 – 100 µl was collected for analysis. The centrifugation time of serum samples and magnesium standards was 20 – 35 min (table 1). After each filtration the cell and

Tab. 1: Centrifugation time, precision of ultrafiltration and magnesium recovery on polysulfone membranes of NMWL 10000

	Mg standard	Mg standard in albumin	Serum
Centrifugation time, min	20–25	25–30	30–35
Precision, RSD % (n=12)	2.8	3.6	3.9
Recovery, % (n=12)	96	81	
n-number of parallel ultrafiltration experiments			

membrane were thoroughly rinsed with water and the decontamination procedure was repeated.

Magnesium determination

Atomic Absorption Spectrometry – Total and ultrafiltrable magnesium in serum were determined by electrothermal atomic absorption spectrometry (ETAAS) technique [10]. Magnesium absorbance was measured at 202.8 nm. The spectral bandwidth was set at 0.5 nm and the magnesium hollow cathode lamp (Photron) current at 10 mA. All measurements were done using standard pyrolytically coated graphite tubes. The peak height absorbance of each sample was measured at least four times and an average was calculated.

Ion-Selective Electrode Potentiometry – Ionized magnesium in serum samples and in the ultrafiltrate was measured using a clinical analyzer Microlyte 6 [13, 14].

Analytical performance

The limit of detection (LD) of magnesium determined by ETAAS was calculated as a sum of triple standard deviation (SD) of the blank and the noise level. For Mg standard in albumin and serum LD was evaluated as 15 and 34 pg, respectively (10 µl of sample in the graphite furnace).

The precision of ultrafiltration procedure was calculated as RSD (in %) of 5 replicate ultrafiltration experiments of the magnesium standard in albumin (20 µl ml^{-1}) and pooled serum, performed on the same day. The recovery was assessed by filtering 1 ml of 20 µg ml^{-1} magnesium solution in albumin. It can be seen that the addition of compounds that are known to complex magnesium affect recovery (table 1).

Results and discussion

The main problem during ultrafiltration procedure is to keep pH constant to avoid the changes of complexing equilibrium. The change of physiological pH value of serum due to the change in the CO_2 saturation, obviously influences the speciation of magnesium. Different data have been published on the change of serum pH during ultrafiltration process. Some authors do not observe any change [2], others in order to minimize loss of CO_2 cover serum with a mineral oil layer [5], liquid paraffin [18] or bubble CO_2 through the sample [1]. A time dependence of the relation between pH and Mg^{2+} was also observed [11]. In our experiments concentration of total and ionized magnesium in the sample of pooled serum was determined. Then the serum sample was bubbled with CO_2 for 15 min and the probe for the ultrafiltration was tightly covered.

During the ultrafiltration pH of samples increased by 0.3 to 0.4. The concentration of total and ionized magnesium was determined in ultrafiltrate. The obtained results are given in table 2. The results of ionized Mg in serum and in serum ultrafiltrate are the same within the range of measurement error. The concentration of low molecular mass complexes is generally assumed at the level of a few percent [9, 18], which is in agreement with our results. The meaningful difference between our's and *Altura's* results [1] in concentration of complexed magnesium in serum is seen. The reason of this could be the difference of temperature during ultrafiltration (20°C-our, 4°C-*Altura's*) that influenced equilibrium. The difference of CO_2 pressure could be the reason of different concentration of carbonates in ultrafiltrate.

There are different reports in the literature on the correlation between ionized, ultrafiltrable and total magnesium in body fluids [3, 8, 15-18]. *Prasad* et al. [16] have reported that the increased level of ultrafiltrable magnesium was always associated with increased total magnesium. *Papadea* et al. [15] have found weak correlation between ultrafiltrable and total magnesium (r = 0.707), but have not found association between ultrafiltrable and

Tab. 2: Content of various forms of magnesium in pooled human serum of healthy objects

	Magnesium fraction (in % of total Mg)				
References	**Ultrafiltrable Mg**	**Ionized Mg**	**Ionized Mg in ultrafiltrable**	**Protein-bound Mg**	**Complexed Mg**
This paper	71	65	66	29	5
Walser 1961 [19]		55		32	13
D'Costa 1983 [5]	71				
Speich et al. 1985 [18]	66.3	60.8		33.7	5.5
Altura et al. 1994 [1]	80.6	66.6	65.5	19.1	14.4
Huijgen et al. 1996 [9]		65		27	8

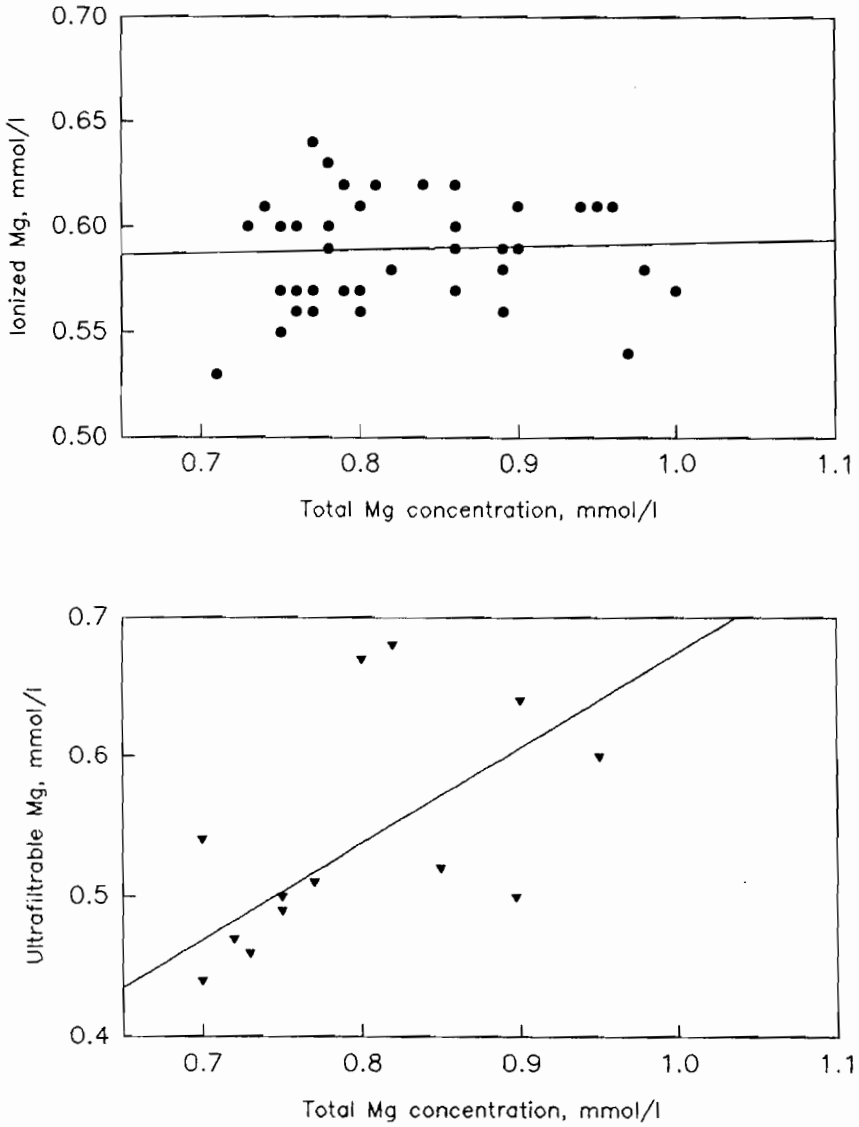

Fig. 1 Total magnesium vs. ionized magnesium (a), r = 0.05, and total magnesium vs. ultrafiltrable magnesium (b), r = 0.71, in human serum samples examined

ionized magnesium in serum (r = 0.572). However, *Deulofeu* et al. [3] reported that ionized magnesium showed a good correlation with ultrafiltered magnesium measured by AAS (r = 0.939, p = 0.018). The reports on the correlation between ionized and total magnesium are also different (r = 0.976 [17] or r = 0.480 [8]). The dependence between ultrafiltrable and total magnesium concentration in serum obtained in our experiment is shown in figure 1. The linear regression was used to establish the correlation. The significant correlation between ultrafiltrable and total magnesium in serum (r = 0.71, $p < 0.05$) and the poor correlation between ionized and total magnesium (r = 0.05) in serum were observed. Is also possible, that the range of observed Mg concentration in investigated samples of healthy objects is too narrow to see the true correlation.

Conclusions

The experimental parameters influencing the speciation of magnesium should be rigorously controlled during the whole analytical process. The effect of pH is of primary importance. The temperature during ultrafiltration also influences results. Determination of ionized magnesium in blood serum using potentiometric clinical analyser is more convenient than ultrafiltration.

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References

[1] *Altura, B.T.; Altura, B.M.:* A method of distinguishing ionized, complexed and protein-bound Mg in normal and diseased subjects. Scand. J. Clin. Lab. Invest. **54** (Suppl 217) (1994) 83–87.

[2] *D'Costa, M.; Cheng, P.T.:* Ultrafiltrable calcium and magnesium in ultrafiltrates of serum prepared with the Amicon MPS-1 system. Clin. Chem. **29** (1983) 519–522.

[3] *Deulofeu, R.; Gimenez, N.; Marin, J.L.; Bedini, J.; Mas, E.; Ballesta, A.M.:* Clinical evaluation of an automatic instrument to measure ionized magnesium by ion-selective electrode (Microlyte 6, KONE, Finland). Abstracts of 7th International Symposium on Magnesium, Magnesium Res. **7** (Suppl. 1) (1994) 7.

[4] *Durlach, J.:* Le magnesium en pratique clinique, Bailiere JB. E M inter, Paris 1985.

[5] *Eckeldt, J.H.; Koehler, D.F.:* Measurement of ultrafiltrable calcium in serum with the use of the Worthington Ultrafree Anticonvulsant Drug Filter. Clin. Chem. **26** (1980) 1871–1873.

[6] *Elin, R.J.:* Assessment of magnesium status. Clin. Chem. **33** (1985) 1965–1970.

[7] *Elin, R.J.; Rychson, T.W.; Rosenstein, D.L.; Rubinow, D.R.; Niemela, J.E.; Balabab, R.S.:* Intracellular ionized magnesium concentration compared with blood total magnesium parameters in normal subjects. Clin. Chem. **40** (1994) 1094.

[8] *Greenway, D.C.; Hindmarsh, J.T.; Wang, J.; Khodadeen, J.A.; Hebert, P.C.:* Reference interval for whole-blood ionized magnesium in a healthy population and the stability of ionized magnesium under varied laboratory conditions. Clin. Biochem. **29** (1996) 515–520.

[9] *Huijgen, H.J.; Vaningen, H.E.; Kok, W.T.; Sanders, G.T.B.:* Magnesium fractions in serum of healthy – individuals and CAPD patients, measured by an ion-selective electrode and ultrafiltration. Clin. Biochem. **29** (1996) 261–266.

[10] *Hulanicki, A.; Godlewska, B.; Brzóska, M.:* Determination of total magnesium in biological samples using electrothermal atomic absorption spectrometry. Spectrochim. Acta Part B **50** (1995) 1717-1724.

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[11] *Ising, H.; Bertschat, F.; Gunther, T.; Jeremias, E.; Jeremias, A.:* Measurement of free magnesium in blood, serum and plasma with an ion-selective electrode. Eur. J. Clin. Biochem. **33** (1995) 365–371.

[12] *Külpmann, W.R.; Gerlach, M.:* Relationship between ionized and total magnesium in serum. Autorenreferate, Deutsch-Österreichisch-Schweizerisches Magnesium Symposium Nr. 15, Bad Neuenahr, Sept. 1993, M 15.

[13] *Maj-Zurawska, M.:* Clinical findings on human blood with KONE ISE for Mg^{2+}. Scand. J. Clin. Lab. Invest. **54** (Suppl. 217) (1994) 69–76.

[14] *Maj-Zurawska, M.; Lewenstam, A.:* Fully automated potentiometric determination of ionized magnesium in blood serum. Anal. Chim. Acta **236** (1991) 331–335.

[15] *Papadea, Ch.; Filzen, A.G.; Gadsden, R.H.Sr.; Cate, J.C.:* Ionized magnesium measured in plasma and whole blood using Nova 8 Analyzer. Clin. Chem. **40** (1994) 1097.

[16] *Prasad, A.S.; Flink, E.B.; McCollister, R.:* Ultrafiltration studies on serum magnesium in normal and diseased states. J.Lab. Clin. Med. **58** (1961) 531–541.

[17] *Saha, H.; Harmoinen, A.; Pietila, K.; Morsky, P.; Pasternack, A.:* Measurement of serum ionized versus total levels of magnesium and calcium in hemodialysis patients. Clin. Nephrol. **46** (1996) 326–331.

[18] *Speich, M.; Bousquet, B.; Nicolas, G.:* Reference values for ionized, complexed and protein-bound plasma magnesium in men and women. Clin.Chem. **27** (1981) 246–248.

[19] *Walser, M.:* Ion Association VI. Interaction between calcium, magnesium, inorganic phosphate, citrate and protein in normal human plasma. J. Clin. Invest. **40** (1961) 723–730.

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