

MONOENZYMATIC DETERMINATION OF SERUM MAGNESIUM, AN ALTERNATIVE

St. V. Hobai, L. Popoviciu, B. Aşgian, I. Nicolaescu, S. Tolan,
Minodora Iuga

Introduction

The clinical interest and generally the bio-medical interest in knowing magnesemia has stimulated the elaboration of a rather accurate method of assaying serum magnesium. One of the older colourimetric methods, used by clinical laboratories even at present, is Mann & Yoe's method /1/. This is based on the shift from blue to red which Xylidil Blue reagent undergoes when associated with the magnesium ion.

The first monoenzymatic method of the free ionic magnesium determination was proposed by Baum & Czock /2/. It was not successful for lack of specificity. The specificity by which magnesium activates glucose phosphorylation catalysed by hexokinase and the fact that the chemical methods. A comparison between the magnesemia of an epileptic patient nonspecific have stimulated the elaboration of an enzymatic method based on hexokinase. In the bienzymatic method /3/ hexokinase and glucose-6-phosphate dehydrogenase are made use of. The latter enzyme is used to eliminate the inconvenience brought about by the inhibitory effect that glucose-6-phosphate has upon hexokinase.

The current literature /3, 4/ criticizes the Mann & Yoe's method by comparing it with atomic absorption spectroscopy and the bienzymatic methods. A comparison between the magnesemia of an epileptic patient group and controls was the object of a recent work /5/. The epileptic group differs significantly from the other groups as to the values obtained by a method based on hexokinase.

Our aim was to work out an automatic monoenzymatic method based on hexokinase, as an alternative to the bienzymatic method. This choice was determined by the desire to simplify the system of reaction, to reduce the number of variables in order to control the reaction more effectively.

Principle, material & method

Our method consists in determined the kinetics of the glucocose phosphorylation reaction by adenosine-5'-triphosphate- Na_2 , catalysed by hexokinase, by measuring the rate of pH variation in the medium of the reaction. The measurements were made on samples in which the capacity of acid-alkaline buffering was previously measured.

Our equipment includes a small enzymatic reactor connected with a digital pH-meter, the data of which are processed on-line by a personal computer, some of the processing stages being displayed on the mo-

monitor and the bulletin of analysis can be obtained by printing. The system of acquiring the data has been carried out on the structure of the computer. The analogous signal provided by the pH electrode is amplified and transformed in binary code data (BCD) by means of the pH-meter (fig. no. 1).

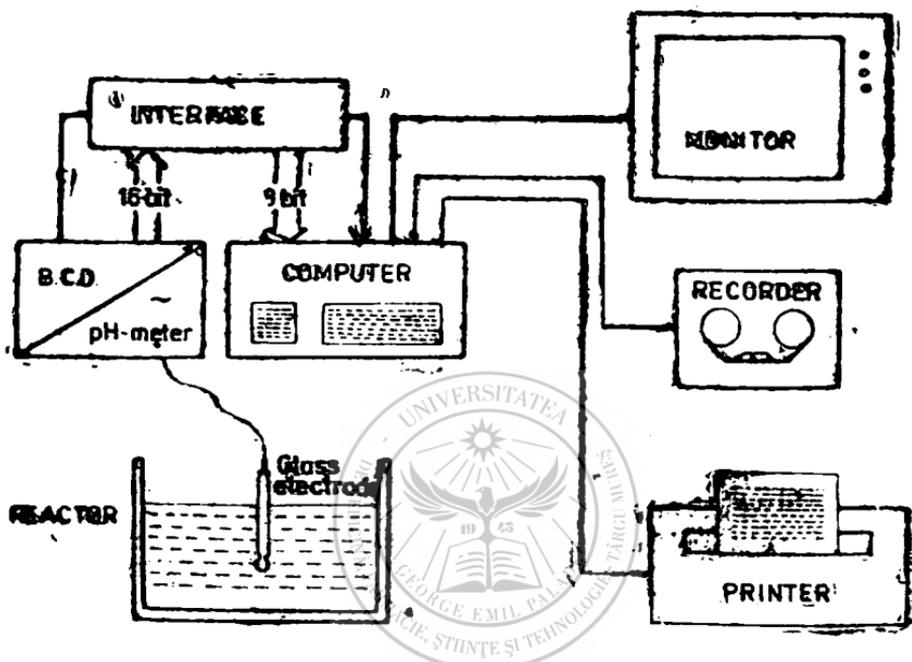


Figure no. 1. The equipment used for enzymatic determination of serum magnesium. The pH-meter transforms the analogous signal provided by the glass electrode (reference electrode is not represented) in Binary Code Data (BCD). The interface adapts the BCD to computer. The computer is connected to monitor, printer and recorder.

The interface makes it possible to feed the data into the computer. It has been made of a multiplexory group (MX 2:1) and a specialized circuit by type INTEL (CI, i 8212) (fig. no. 2).

As the rate of acquiring has to be high, in order to achieve the desired accuracy, the programme itself has been carried out in machine code and the processor is used in the working mode Non-Mascable-Interact (NMI). The processings of the results are being in BASIC language.

The enzymatic reaction is initiated by dispersing of 0.2 mL hexokinase solution in the reactor containing glucose and adenosine-5'-triphosphate-Na₂ at michaelian saturation (10 mM and 4 mM, respectively) as well as 100.0 microL of the serum. Hexokinase was bought from Amerifirm SIGMA (Cat. 1989/H 5125) or was purified by us from brewer's yeast up to a precrystalline degree of purity /6/. The data of the kinetics

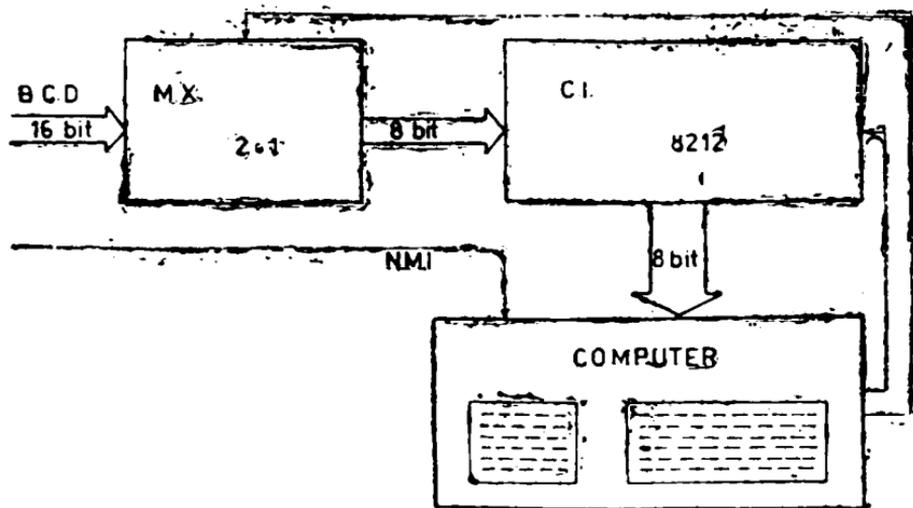


Figure no. 2. The interface. It adapts the pH-meter to computer by means of a multiplexory group (MX, 2:1) and of a circuit CI, i 8212.

of the reaction, activated by magnesium in standard solution of magnesium chloride, are processed by the computer through the linear regression method in this manner obtaining a calibration of the apparatus. The data corresponding to the sera have been automatically compared with the calibration and the results were displayed and printed.

Results & discussion

Table 1 shows the mean (M) and the standard deviation (SD) of the individual values of the intraassay determinations. The SD value of the monoenzymatic method was in agreement with the value obtained by

Table 1.

Statistical characteristics of magnesium determination by the monoenzymatic and Mann & Yoe's method.

METHOD	Individual measurements		Calibration	
	Mean (mM)	SD (mM)	RSD	CC
Monienzymatic (n=4)	0.83	0.020	5%	0.999
Mann & Yoe (n=5)	0.67	0.087	7%	0.993

atomic absorption spectrophotometry and far better than that obtained by Mann & Yoe's method. Some statistical characteristics of calibration, as the relative standard deviation (RSD) and the correlation coefficient (CC), were better in monoenzymatic method than in Man & Yoe's method.

References

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KW: ^{setu} Magnesium; bio-medicine; monoenzymatic method;

