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SHORT COMMUNICATION

Serum Water Determination by Means of Microwave Evaporation

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Summary: A novel method is described for the determination of serum water using a microwave oven. The sources of experimental errors were analysed. Serum samples from two hundred patients were analysed for sodium, water and protein, and the data were used to calculate serum sodium molalities. A possible correlation was investigated between serum water content and protein concentration. The results were compared with those in the literature.

Introduction

In most clinical chemistry laboratories serum water is not routinely determined in the samples offered for analysis. However, there are circumstances where a knowledge of the water content is indispensable, for instance in order to differentiate between essential and pseudo-hyponatraemia. In this paper a new method for the determination of serum water is described and its accuracy is analysed. It was also investigated whether the serum protein concentration can be used to estimate the serum water content, as has been proposed in the literature (1).

Serum water can be determined by evaporation, which is a lengthy process when performed in an oven at normal or reduced pressure. The operational time can be shortened considerably when the sample is placed in an infrared dryer, as shown recently (2). Another method would be to use a commercially available microwave oven for this purpose. This method has the advantage that it is quick (it takes less than 5 minutes to dry the sample to constant weight) and, depending on the volume and power generated in the oven, a considerable number of samples can be dried in one session.

Material and Methods

Evaporation was performed in a commercially available microwave oven (Philips AVM. 706/PH 1.3 kW, 2450 MHz).

Samples were weighed with an electronic analytical balance (Sartorius H 51).

The determination of the water content was performed as follows:

Blood samples obtained from the clinic were centrifuged. The serum was removed by aspiration and transferred to stoppered test tubes to avoid evaporation. From these samples one ml was applied with an Eppendorf pipette to a preweighed glass petri dish. Immediately hereafter the petri dish was weighed again. To avoid foaming 400 µl of ethanol was added to the serum. The samples were placed in the microwave oven and irradiated for five minutes. The samples are then weighed again and the water content calculated by subtraction.

The serum remaining in the test tubes was used to determine the Na^+ and protein concentrations on a multichannel continuous-flow analyser (SMAC from Technicon Corp.; Tarrytown, NY, USA).

Sodium was measured with an ion selective electrode after prior dilution of the serum sample. The calibration was performed with two serum calibrators (2-point calibration) in which the Na concentrations had been established with a reference method that ensures accurate values in human serum specimens. The typical standard deviation was 1 mmol/l (the coefficient of variation is then 0.7%).

Total protein was measured with the biuret procedure.

The calibration was performed with a single serum calibrator. The value assigned to the calibrator ensures accurate results in human serum specimens (1-point calibration).

The typical standard deviation was 0.5–0.6 g/l (coefficient of variation 0.08%).

Results

As the differences in serum water content between normal subjects and patients with an abnormal hydratation status are small, the errors occurring in the weighing, pipetting and drying of the samples should be even smaller. We therefore started by determining the errors involved in the estimation of the water content of serum samples.

Tab. 1. Two batches of pooled serum called A and B were used to obtain two series of fifteen numbered samples.
The water content of the samples from series A were first determined with the magnetron oven. The dry samples were then put in an oven for 24 h at 120 °C and the water content calculated again.
The samples from series B were first placed in the oven to determine the water content. Next the dry samples were treated in the magnetron oven and water content calculated again.

	Water content series A n = 15		Relative error (I−II)/I	Water content series B n = 15		Relative error (I−II)/I
	I Magnetron	II Oven		I Oven	II Magnetron	
Mean	0.9217 kg · l^{-1}	0.9229 kg · l^{-1}	−0.13%	0.9289 kg · l^{-1}	0.9282 kg · l^{-1}	+ 0.08%
SD	0.0044 kg · l^{-1}	0.0044 kg · l^{-1}	0.05%	0.0041 kg · l^{-1}	0.0038 kg · l^{-1}	0.05%

Systematic errors

Two possible errors can occur in the drying of a serum sample in a microwave oven. First the possibility exsists that a small part of the water remains attached to the dried protein. This can happen not only when the sample is dried in a microwave oven, but also when the drying takes place in a conventional oven at 100 °C. In both cases the result would be an underestimation of the water content of the sample. Further drying by application of microwave energy could result in pyrolysis of the remaining proteins, which would lead to an overestimation of the water and an underestimation of the protein content. To determine whether a systematic error is introduced by the microwave method, thirty glass petri dishes were marked and 1 ml of pooled serum was added to each of them. Fifteen dishes, called group A, were put in the microwave oven and dried for 5 minutes. After weighing they were placed in a conventional oven and heated for twenty four hours and weighed again. For the remaining dishes, belonging to group B, this train of events was reversed. The results of the measurements for both groups are indicated in table 1. From this table we conclude that a negligible decrease in dry weight occurs from extra heating in the microwave oven of samples that were previously dried in the conventional oven (weight loss would occur on pyrolysis). In the samples that were treated by microwave radiation prior to heating in the oven at 100 °C, a very small loss of weight was observed. This shows that a negligible amount of "hidden" water (even if present) is removed by the conventional treatment.

A second possible systematic error can be induced by the technique of sampling. It is important to keep the serum samples in closed test tubes to avoid an underestimation of the water content due to evaporation.

Pipetting error

Pooled serum was pipetted into twenty five petri dishes and weighed. This resulted in a mean weight of 0.9702 g and a standard deviation of 0.0153 g, giving a coefficient of variation of 1.6%.

Error as a result of drying and weighing

Of the forementioned twenty five pooled samples the residual weights were divided by the sample wet weights to exclude pipetting errors. When this was done, a standard deviation of 0.0005 g was observed, due to errors occurring at the drying and weighing stages. The weight of the water in the sample is given by the following formula, including the errors:

$$\text{Weight} = (\text{sg} \times \text{vol} - \text{dry weight}) \pm \text{sg} \times \Delta v \pm 2 \times \Delta w,$$

where sg: specific gravity = density, vol: volume of sample, Δv: pipetting error, and Δw: weighing error.

Total error is equal to 0.0153 g + 0.0005 g giving a relative error of 1.63%.

As a simple calculation demonstrates, the ratio of dry weight to wet weight equals the ratio of dry weight per ml to the serum density. The density is constant when the serum samples used are from the same pool. A variation in the first ratio is therefore a result of the variation of dry weight per ml and this variation is the result of the error introduced by the drying and weighing process.

Our next goal was to assess the biological variation of the sodium molality and compare it with the experimental error. To this end two hundred serum samples of hospitalised patients were analysed for sodium, total protein and water. The mean values and coefficients of variation are shown in table 2. The water contents of the samples were then used to calculate the sodium molalities (results given in tab. 2).

Tab. 2. Comparison between the experimental error calculated for the estimation of sodium, water and protein and the biological variation observed in 200 serum samples of hospital patients.
pop.: Total standard deviation observed due to biological variation and variation induced by the experimental error.
det.: Standard deviation of the used analytical method.

	Mean	pop.	det.
Sodium molarity (mmol/l)	142	2.8%	0.7%
Total protein concentration (g/l)	72	10.0%	0.8%
Water content (kg/l)	0.9302	2.0%	1.6%
Sodium molality (mmol/kg)	152	3.5%	2.3%

Eisenman et al. (3) claimed that serum water can be estimated from the concentration of protein, when the total lipid concentration is within normal limits.

The following formula is given by these authors.
$W = 0.984 - 0.00073\ P$
(W: serum water [kg · l^{-1}];
P: protein concentration [g · l^{-1}]).

This formula is obtained by a theoretical argument and has found limited use in clinical practice (1, 4). It predicts a water content of 0.9310 kg/l for healthy individuals. this value was also obtained by titrimetric methods (5). To test the usefullness of this equation we subjected the results obtained in our experiments to a linear regression. The following result was obtained: $W = 0.9839 - 0.00090\ P$ (correlation coefficient $r = -0.5290$). This line does not match the theoretical one, although both cross at the point of normal water content and protein concentration.

Conclusions

It appears that the serum water content can be easily determined by evaporation in a microwave oven in a fraction of the time necessary for the conventional method, and a large number of samples can be dried at the same time. It was shown that the accuracy of the method is good. Although microwave evaporation leaves a negligible amount of water in the samples, and it appears that prolonged irradiation produces some pyrolysis, the errors introduced are very small and of different sign, and hardly have any influence on the total error observed. Preliminary results obtained with a group of hospital patients showed a mean water content in agreement with the literature (6). It also appeared that only a rough estimation of water content can be obtained with formulae from the literature (3). Possibly a differentiation between the various proteins and lipids would render the equation more accurate, but it would be more realistic to determine the serum water content directly. The biological variation of the sodium molality calculated within a group of hospital patients is composed of the true variation within that group and the standard error of the determination. When normal distributions are assumed, this true variation is about 2.5%.

We therefore conclude that the estimation of the serum water content by microwave evaporation is accurate, easy to perform and could give a valuable parameter for the physician in assessing water and electrolyte balance.

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