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Multielement Analysis of Human Blood Serum by Neutron Activation and Controlled Potential Electrolysis

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The combination of neutron activation analysis and controlled potential electrolysis has proved itself to be a useful multielement method for the determination of 14 elements in a 500-μL sample of human blood serum. After freeze-drying and irradiation, the samples are decomposed and electrolyzed for 3 h. The radioactive species deposited on the mercury cathode allow simultaneous determination of eight elements (Ag, Au, Cd, Co, Fe, Hg, Sb, Zn). Another six elements (Br, Ca, Cs, Na, Rb, Sc) are determined by measuring the activities in the residual solution. Data illustrating the precision of the method are given. The determination limits are of the order 10^{-1}–10^{-3} μg/L for the nine trace elements investigated.

According to the World Health Organization (*1*) 14 trace elements, i.e., Co, Cr, Cu, F, Fe, I, Mn, Mo, Ni, Se, Si, Sn, V, and Zn, are considered to be essential to the human organism. Several other elements (e.g., Br, Ba, Sr) are supposed to be added to the list as more knowledge about their metabolic functions is obtained.

The concentrations of the trace elements in body tissues and fluids are frequently in the parts-per-billion range or lower. The metabolism of a healthy individual maintains the concentrations of trace elements within narrow limits. In addition, the concentration ranges between essential and toxic levels for different chemical forms of elements are rather narrow. Moreover, species of several elements (e.g., As, Cd, Hg, Pb) are assumed to be hazardous even in very low concentrations. Thus, investigation of trace elements in human tissues and body fluids has become a field of great emphasis in recent years, and attempts have been made to relate changes in concentration of specific elements to various diseases. In some cases such relationships are firmly established (*2–6*).

Human blood serum supplies all parts of the body with necessary components, and it can be obtained easily and frequently in routine procedures. Thus, analysis of human blood serum is very useful in studies of trace elements in man (*5–8*).

However, the number of useful methods for multielement analysis of human blood serum is rather limited, due to the low concentrations involved and to interfering effects of the matrix.

Application of instrumental neutron activation analysis (INAA) to whole blood and serum has recently been reported (*8–12*). After irradiation with thermal neutrons, whole blood and blood serum show a γ-spectrum in which the predominant nuclide 15.0 h ^{24}Na prevents determination of other elements. The background activity is also increased by the presence of 35.4 h ^{82}Br and 14.3 days ^{32}P (bremsstrahlung).

Most applications are therefore hampered by these effects and the resulting necessity of singling out groups of elements. This requires many samples, a variety of irradiation times (10 s–10 days) and decay times (10 s–6 months), or introduction of radiochemical separation procedures. In this way only, a number of elements can be determined by neutron activation analysis; however, it is expensive and time-consuming.

The elimination of Na, Br, and P without affecting the concentration of activation products of other elements of interest would not only permit simultaneous determination of a greater number of trace elements but also reduce the irradiation time and avoid the long decay times mentioned above.

The present paper describes a technique based on a combination of neutron activation and controlled potential electrolysis for the simultaneous determination of eight elements (Ag, Au, Cd, Co, Fe, Hg, Sb, Zn) in human blood serum 3 days after irradiation. During the electrolysis, reducible species in solution will be deposited on the cathode, while the interfering elements Na, Br, and P will remain in solution. In addition, another six elements (Br, Ca, Cs, Na, Rb, Sc) may be determined in the residual solution of the sample by means of their activation products, 3–4 weeks later.

Table I. Elemental Content of Seronorm, Batch No. 139

element	certified values (*13*)	this work
Ag (μg/L)		3.64 ± 0.07
Au (μg/L)		0.72 ± 0.02
Br (mg/L)		1.48 ± 0.04
Ca (mg/L)	109	108 ± 3
Cd (μg/L)		9.2 ± 0.3
Co (μg/L)		1.39 ± 0.03
Cs (μg/L)		2.68 ± 0.09
Fe (mg/L)	1.54	1.54 ± 0.05
Hg (μg/L)		2.67 ± 0.08
Na (g/L)	3.06	3.06 ± 0.03
Rb (μg/L)		350 ± 7
Sb (μg/L)		0.85 ± 0.02
Sc (μg/L)		0.053 ± 0.003
Zn (μg/L)	804	803 ± 9

METHOD OF ANALYSIS

Reference Standard. Seronorm (batch no. 139, standard reference material from NYCO, Oslo (*13*)) with certified values for Fe, Zn, Ca, and Na was used as a standard. On the basis of

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Table II. Elements Determined by Neutron Activation and Controlled Potential Electrolysis of Human Blood Serum

element	nuclide	half-life	most abundant γ-ray, keV
	Deposited on the Mercury Electrode		
Ag	^{110m}Ag	250.4 days	657.7
Au	^{198}Au	2.7 days	411.8
Cd	^{115}Cd	2.2 days	527.9
Co	^{60}Co	5.26 years	1332.5
Fe	^{59}Fe	44.6 days	1099.3
Hg	^{203}Hg	46.6 days	279.2
Sb	^{122}Sb	2.7 days	564.1
Zn	^{65}Zn	243.8 days	1115.5
	Left in Solution		
Br	^{82}Br	35.4 h	554.3
Ca	^{47}Ca-^{47}Sc	4.54 + 3.40 days	159.4
Cs	^{134}Cs	2.05 years	604.7
Na	^{24}Na	15.0 h	1368.6
Rb	^{86}Rb	18.6 days	1078.8
Sc	^{46}Sc	83.9 days	889.3

standard solutions, the Seronorm concentrations of the above mentioned four elements, and of Ag, Au, Br, Cd, Co, Cs, Hg, Rb, Sb, and Sc, were determined, using the present method and INAA. As seen from Table I, the values obtained for Fe, Zn, Ca, and Na are in full agreement with their certified values. Thus, this batch of Seronorm serves as a multielement standard for all the elements in question.

Sample Preparation. Samples of human blood serum and Seronorm (500 μL) are pipetted into quartz ampules, using Eppendorf pipets, as described by Medina et al. (*14*). The samples are freeze-dried for 5 h under a pressure of 0.05 torr, which has been shown to be a convenient drying procedure for biological samples, including blood serum (*15*). The ampules are then heat-sealed.

Irradiation. Samples of human blood serum and of Seronorm are irradiated for 3 days at a fixed position in the reactor JEEP II, Institute for Energy Technology, Kjeller, Norway, at a thermal neutron flux of 1.5×10^{13} neutrons cm^{-2} s^{-1}.

Controlled Potential Electrolysis. After a decay time of 3 days, the freeze-dried samples of human blood serum and Seronorm are decomposed by 0.5 mL of concentrated H_2SO_4 and 1 mL of concentrated HNO_3, and diluted to 20 mL with distilled water. Tests have shown that the usual addition of carrier is unnecessary in the present procedure.

The electrolysis used is a modification of the method reported by Jørstad and Salbu (*16*) for the determination of 28 elements in seawater. The main modification is that the magnetic stirrer is replaced by a synchronous electric stirrer, to avoid the deviation from a first-order kinetic expression when minute concentrations are reached during the electrolysis (*16*).

Controlled potential electrolysis is performed by means of a potentiostat especially contructed for this purpose (*17*). Mercury is used as cathode material (area 8 cm^2), a platinum coil serves as counterelectrode, and the reference electrode is a saturated Ag/AgCl electrode. The applied potential is −1.5 V, the volume of electrolyte 20 mL, and the temperature 22 ± 2 °C. For further details, reference is given to Jørstad and Salbu (*16*).

Each sample of human blood serum and of Seronorm is electrolyzed for 3 h, which is shown to be sufficient for maximum deposition of the electroactive forms of the elements in question. The mercury cathode is then transferred into an especially constructed container for measurement of the radionuclides deposited on the mercury electrode. The residual solution is transferred to a counting vial in order to measure the nonelectroactive species.

Counting and Data Processing. In the present study, the radioactivity measurements were based on Ge(Li) γ-spectrometry (efficiency of 20.7% and resolution of 1.89 keV, defined by means of the 1332.5-keV γ from ^{60}Co). The elements determined, their corresponding radionuclides, and the γ energies used in the measurements are given in Table II. Peak location and calculation of peak areas were performed by means of GAMANL, a computer program developed by Gunnink et al. (*18*) and adjusted to the CDC-CYBER 74 computer at the University of Oslo by Scheidemann (*19*).

The concentrations of Ag, Au, Cd, Co, Fe, Hg, Sb, and Zn were determined by measuring the γ-spectrum of the mercury electrode. As seen from Figure 1, which shows a typical spectrum, matrix elements (i.e., ^{24}Na, ^{82}Br, ^{32}P) are no longer interfering. Thus, a simultaneous determination of the above mentioned elements is obtained.

Determination of Br, Ca, Cs, Na, Rb, and Sc was carried out by γ-spectrometry of the residual solution 1 week (Br, Na) and 3–4 weeks (Ca, Cs, Rb, Sc) after irradiation.

RESULTS AND DISCUSSION

The described method has been tested and applied to serum samples from individuals in a healthy state.

The results obtained for the 14 elements are in good agreement with literature values compiled by Iyengar et al. (*20*) and results for Norwegian individuals reported by Blekastad et al. (*21*) (Table III).

In order to investigate the reproducibility of the method, we analyzed four aliquots of human blood serum. The results obtained, together with the maximum deviation from the mean value for each element, are listed in Table IV. The precision varies from 3% (Ag, Fe, Br, Ca, Cs, Na) to 7% (Sc).

The determination limits (n_Q) (Table V) of the present method are calculated by using the GAMANL (*18*, *19*) criterion of the least possible registerable peak area, as described by Salbu et al. (*22*). The determination limits are of the order 10^{-1}–10^{-3} μg/L for the nine trace elements investigated. The

Table III. Content of Some Elements in Human Blood Serum

element	this work		Iyengar et al. (*20*)		Blekastad et al. (*21*)
	mean value	range	mean value	range	mean value
		Deposited on the Mercury Electrode			
Ag (μg/L)	6.60	5.17-7.84		2.9-200	
Au (μg/L)	0.32	0.17-0.44	0.08		
Cd (μg/L)	10.07	8.11-12.91		2.3-12	
Co (μg/L)	0.67	0.32-1.04		0.22-14	
Fe (mg/L)	1.35	1.04-1.67	1.09	0.87-1.87	1.7 ± 1.4
Hg (μg/L)	8.38	7.18-9.74	12		1.3 ± 0.8
Sb (μg/L)	2.82	2.43-3.33		2.5-3.2	⩽0.3 ± 0.2
Zn (mg/L)	1.38	1.02-1.72	1.15	0.67-1.83	0.84 ± 0.14
		Left in Solution			
Br (mg/L)	2.24	1.92-2.73	3.9	1.04-7.5	2.4 ± 0.8
Ca (mg/L)	106.01	98.16-113.41	97	92-109	
Cs (μg/L)	1.60	1.48-1.74		1.33-60	1.5 ± 0.9
Na (g/L)	3.11	2.97-3.21	3.251	3.130-3.70	3.21 ± 0.12
Rb (μg/L)	169.76	159.13-192.64	200	40-580	250 ± 60
Sc (μg/L)	0.074	0.052-0.096	0.15		

Table IV. Data Obtained by Analysis of Four Aliquots of Human Blood Serum

element	parallel 1	parallel 2	parallel 3	parallel 4	mean value	max dev from mean value, %
			Deposited on the Mercury Electrode			
Ag (μg/L)	5.33	5.31	5.11	5.37	5.28	3
Au (μg/L)	0.42	0.42	0.39	0.41	0.41	5
Cd (μg/L)	12.58	13.22	12.73	11.87	12.60	6
Co (μg/L)	0.56	0.60	0.61	0.59	0.59	5
Fe (mg/L)	1.22	1.16	1.20	1.17	1.19	3
Hg (μg/L)	7.96	7.73	8.17	8.34	8.05	4
Sb (μg/L)	2.71	2.53	2.47	2.65	2.59	5
Zn (mg/L)	1.06	1.05	0.99	0.97	1.02	5
			Left in Solution			
Br (mg/L)	1.98	2.04	1.95	2.07	2.01	3
Ca (mg/L)	115.52	111.31	117.26	115.43	114.88	3
Cs (μg/L)	1.38	1.45	1.47	1.47	1.43	3
Na (g/L)	3.08	2.94	2.98	3.12	3.03	3
Rb (μg/L)	161.15	175.63	182.19	165.63	171.15	6
Sc (μg/L)	0.060	0.057	0.054	0.059	0.058	7

Table V. Determination Limits of the Present Method Applied to Samples of Human Blood Serum

element	determination limit, n_Q, μg/L	background level, counts/channel
	Deposited on the Mercury Electrode	
Ag	0.81	70
Au	0.004	200
Cd	0.25	120
Co	0.004	40
Fe	36.30	80
Hg	0.012	500
Sb	0.014	90
Zn	40.90	80
	Left in Solution	
Br	2.33	5000
Ca	54.79	240
Cs	0.20	40
Na	46.73	1700
Rb	0.89	10
Sc	0.0023	20

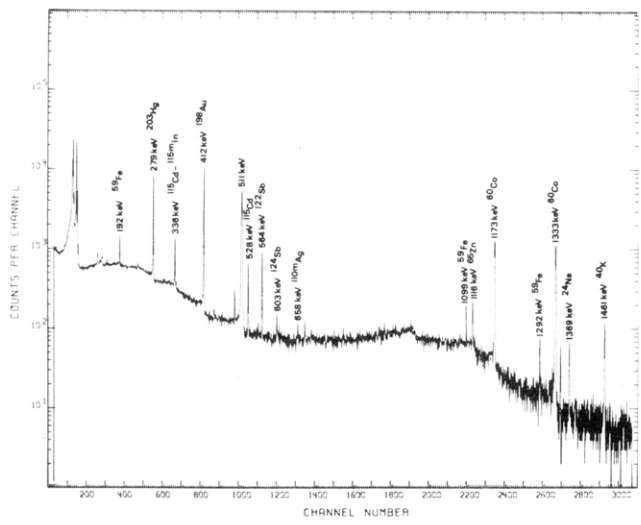

Figure 1. γ-spectrum of the mercury electrode after electrolyzing human blood serum for 3 h.

remaining five elements (Fe, Zn, Br, Ca, Na) occur in human blood serum as major elements.

A comparison between the results obtained by the present method and by conventional instrumental neutron activation analysis (INAA) is given in Table VI. Ag, Au, Cd, and Sb cannot be determined by INAA. The results obtained by using

Table VI. Elements Determined by Controlled Potential Electrolysis and by INAA

element	controlled potential electrolysis[a]	INAA[a]
	Deposited on the Mercury Electrode	
Co (μg/L)	0.59 ± 0.03	0.63 ± 0.04
Fe (mg/L)	1.19 ± 0.03	1.13 ± 0.05
Hg (μg/L)	8.1 ± 0.3	7.8 ± 0.4
Zn (mg/L)	1.02 ± 0.05	1.09 ± 0.06
	Left in Solution	
Br (mg/L)	2.01 ± 0.06	2.02 ± 0.05
Ca (mg/L)	115 ± 4	119 ± 6
Cs (μg/L)	1.43 ± 0.05	1.50 ± 0.08
Na (g/L)	3.03 ± 0.09	2.94 ± 0.06
Rb (μg/L)	171 ± 11	180 ± 14
Sc (μg/L)	0.058 ± 0.004	0.056 ± 0.006

[a] Mean values based on analysis of four aliquots.

Table VII. The Fractional Deposition of Some Elements after Electrolyzing for 3 h

element	% deposition: human blood serum	% deposition: Seronorm
Co	92.3	92.1
Fe	73.1	72.6
Hg	81.2	80.9
Zn	88.1	88.8

controlled potential electrolysis as here described are in good agreement with those obtained by using INAA. However, a decay time of 3–4 weeks is required when using INAA, as compared to 3 days when using the present method. Hence, introduction of controlled potential electrolysis as a chemical separation step not only eliminates the long decay time required by INAA but moreover allows determination of a greater number of elements, without affecting the concentration of the elements remaining in solution after the electrolysis.

Measurements of the residual solution allow determination of the fractions of Co, Fe, Hg, and Zn not deposited on the mercury electrode during electrolysis. The fractional deposition of these four elements in human blood serum and Seronorm is given in Table VII. As no further deposition takes place during 12 h of electrolysis, small amounts of these elements most likely occur in nonelectroactive forms. This may be caused by an incomplete decomposition of the irradiated sample. However, tests including different destruction

procedures show no change in the nonelectroactive fractions.

Emphasis should also be placed on the fact that no contamination problems occur during electrolysis, as only radioactive species are being measured, deposited on the electrode, or left in the solution.

CONCLUSIONS

The present paper shows that the combination of neutron activation and controlled potential electrolysis is a very convenient analytical method for determination of 14 elements in a 500-μL sample of human blood serum. The method may also be used in multielement analysis of other human body tissues and fluids having high content of interfering elements.

Biologically active trace elements in body fluids are often associated with particular compounds (e.g., organic ligands). Thus, in combination with biochemical separation techniques, trace element analysis as described in the present paper may elucidate associations to fractions of proteins, and information of chemical states of different trace elements are obtained.

As measurable changes in the trace element content in body fluids and tissues occur with certain diseases, determination of trace elements and their chemical states may give valuable information for diagnostic work and in treatment planning.

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Model for Treatment of Selectivity Coefficients for Solid-State Ion-Selective Electrodes

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The model presented provides an explanation of the observed variability among selectivity coefficients of the solid-state ion-selective electrodes (ISE). This model considers the mechanism of metathetic reactions at the membrane surface and describes the sensed surface ion concentrations in terms of the bulk solution concentrations. The influence of various parameters such as bulk concentrations, time, temperature, and stirring rate on determined selectivity coefficients is discussed. Currently existing models are shown to be limiting cases of the presented model. The $AgCl|Br^-$, $AgBr|Cl^-$, and $LaF_3|OH^-$ systems were chosen as representatives of the solid-state ISEs.

The selectivity coefficient for an ion-selective electrode (ISE) is of great interest from both theoretical and empirical points of view. Theoretical interpretations of this parameter first appeared in the works of Dole (*1*) and Nikolsky (*2*). More empirical approaches have been reviewed recently (*3*).

It is well-known that empirically determined values of selectivity coefficients may be different, depending on the method of determination (separate or mixed solutions), the measurement technique (the time elapsed for observing potential, stirring, etc.) chemical conditions in the solution (composition, levels of ion concentration), the character and kinetics of electrode reaction, the influence of interfering processes besides ion-exchange like leaching, corrosion, redox processes, adsorption, and electrode history (all phenomena acting to create the present state of the membrane surface).

This complexity of factors not only results in a divergence of empirical selectivity coefficients but also creates some difficulties in theoretically describing ISE action and interpreting and predicting values for the selectivity coefficients.

Existing and generally accepted models usually predict definite, discrete values of the selectivity coefficients. The coefficient is usually expressed in the case of solid membrane electrodes as the ratio of solubility products of the respective salts of the main and interfering ions. This assumes that mobility of interfering ions in the membrane phase equals zero. If this is not so, the selectivity coefficient includes mobility terms (*4–6*). The selectivity coefficients predicted on this basis are occasionally not equal to those measured experimentally. This fact was pointed out in some papers (Table I) but until

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