Determination of protein-bound trace elements in biological material by gel filtration and neutron activation analysis

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struction materials of the freeze dryer itself are minimized. The entire new system, with nine chambers for samples is shown in Figure 1. An expanded diagram of one of the units is shown in Figure 2. Since this freeze drier was installed in a Class 100 clean room facility, contamination of the dry residue from laboratory air particulates is minimized (*15*). Under normal laboratory conditions, it is recommended that air which is bled through inlet valves be filtered.

It was previously stated that one of the advantages of this method of freeze drying water was the elimination of the need to transfer the dry residue. The bag containing the residue after freeze drying may be heat sealed, and then folded to make a small sample (1 cm × 2 cm × 0.5 cm) ready for irradiation and counting.

The results of a typical analysis of dissolved species in water by INAA following the described preconcentration procedure appear in Table IV. This sample was taken from the Back River, just east of Baltimore, Md. The ratio of trace element content in the sample to the average trace element content in a polyethylene bag is sufficiently high for most elements to result in reliable data, particularly considering errors due to the sampling procedure alone. Work carried out in this laboratory to determine the variations in trace element concentration attributable to sampling techniques, suggest that such errors may easily exceed ±20%.

The use of neutron activation for the analysis of trace elements in the aquatic environment is now well established. The multielement analyses of fresh to moderately saline water is greatly aided by the ability to handle relatively large samples with a minimum of pre-irradiation manipulation, as reported here. In addition, the use of freeze drying does not preclude subsequent post-irradiation chemical separations for elements that cannot be observed instrumentally.

LITERATURE CITED

(1) R. H. Filby, W. A. Haller, and K. R. Shah, *J. Radioanal. Chem.*, **5**, 277 (1970).
(2) H. D. Buenafama, (Conf-721069-3), 1972, 10 pp. Dep NTIS.
(3) W. H. Zoller and G. E. Gordon, *Anal. Chem.*, **42**, 257 (1970).
(4) A. J. De Groot, K. H. Zschuppe, M. De Bruin, J. P. W. Houtman, and P. Amin Singgih, "Modern Trends in Activation Analysis", J. R. DeVoe and P. D. LaFleur, Ed., Vol. I, 1969, p 62.
(5) E. L. Kanabrocki, L. F. Case, L. Graham, T. Fields, Y. T. Oester, and E. Kaplan, *J. Nucl. Med.*, **9**, 478 (1968).
(6) T. Mamuro, Y. Matsuda, A. Mizohata, T. Matsunami, and T. Takeuchi, "Annual Report of the Radiation Center, Osaka Prefecture", Vol. II, 1970, p 23.
(7) D. E. Robertson, "Pacific Northwest Laboratory Annual Report for 1972 to the USAEC Division of Biomedical and Environmental Research", Vol. II, part 2, p 24.
(8) D. E. Becknell, R. H. Marsh, and W. Allie, Jr., *Anal. Chem.*, **43**, 1230 (1971).
(9) S. Nagatsuka, H. Suzuki, and K. Nakajima, *Radioisotopes*, **20**, 305 (1971).
(10) T. Fujinaga, Y. Kusaka, M. Koyama, H. Tsuji, T. Mitsuji, S. Imai, J. Okuda, T. Takamatsu, and T. Ozaki, *J. Radioanal. Chem.*, **13**, 301 (1973).
(11) D. F. Schutz and K. K. Turekian, *Geochim. Cosmochim. Acta*, **29**, 259 (1965).
(12) A. P. Grimanis, G. Pantazis, C. Papadopoulos, and N. Tsanas, "Third United Nations International Conference on the Peaceful Uses of Atomic Energy", 1964.
(13) P. Schramel and K. Samshal, *Int. J. Environ. Studies*, **5**, 37 (1973).
(14) W. F. Merritt, "Proceedings of International Conference on Identification and Measurement of Environmental Pollutants", 1971, p 358.
(15) T. J. Murphy, "Proceedings of 7th Materials Research Symposium", National Bureau of Standards, 1974.

RECEIVED for review November 21, 1974. Accepted March 26, 1975. Certain commercial equipment, instruments, or materials are identified in this paper in order to adequately specify the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

Determination of Protein-Bound Trace Elements in Biological Material by Gel Filtration and Neutron Activation Analysis

Gunnar Norheim[1]

Institute of Forensic Medicine, Rikshopitalet, University of Oslo, Oslo, Norway

Eiliv Steinnes

Institutt for Atomenergi, Isotope Laboratories, Kjeller, Norway

Neutron activation analysis is a powerful technique for the simultaneous determination of a number of elements in biological material, especially when combined with radiochemical separations (*1–4*). With a very few exceptions, the neutron activation studies carried out in this area so far have involved the determination of total concentrations of the elements concerned, with no regard to the chemical forms in which the elements occur. In order to study the metabolism of trace metals in the human body, it is useful to determine the extent of protein binding and, if possible, the association with different molecular weight protein fractions. Activation analysis was employed by Fritze and Robertson (*5*) for the determination of protein bound metals in human blood serum using filtration on Bio-Gel P6 for a bulk separation of proteins prior to activation. It seemed reasonable to assume that a similar technique could be advantageously used for more detailed studies concerning the association of trace metals with particular protein fractions. The present communication describes a method involving isolation of liver proteins followed by fractionation using gel filtration and subsequent determination of the elements Zn, Cd, Hg, Cu, As, and Se in the individual elution fractions by neutron activation.

[1] Present address, National Veterinary Institute, Oslo, Norway.

EXPERIMENTAL

Isolation and Separation of Liver Proteins. Liver samples, collected at autopsy and stored at −20 °C before the analysis, were homogenized in a Sorvall Omnimixer in stainless steel bottles with an equal amount of 0.01*N* Tris buffer (pH 8.0 at 4 °C) containing 0.05*N* sodium chloride and 100 mg sodium azide per liter to avoid bacterial growth. The homogenates were centrifuged refrigerated (4 °C) at 20.000 × g for 1 hour. Five ml of the supernatants were separated on a Sephadex G-75 superfine (Pharmacia) column and eluted with the above Tris buffer. The column was 2.6 × 40 cm (Pharmacia K-26). The separations were performed in a thermostatically controlled room at 4 °C and 10-ml fractions were collected. The flow rate was about 10 ml/hour. The absorption of each

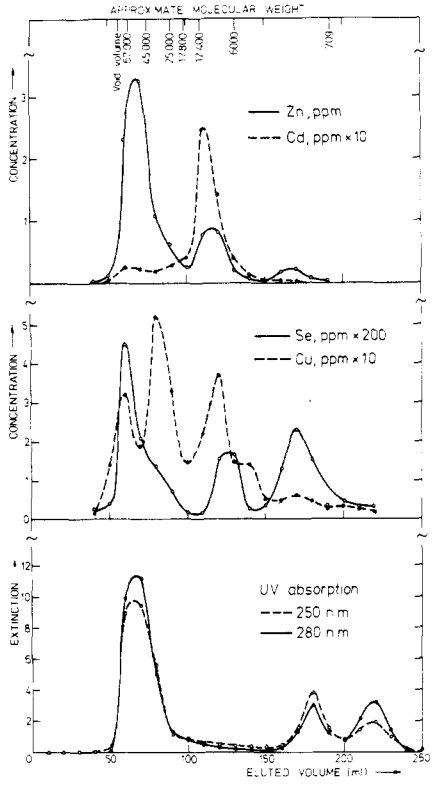

Figure 1. Separation of human liver proteins on Sephadex G-75.

Concentrations of Cu, Zn, Cd, and Se in the eluate, and UV absorption at 250 nm and 280 nm, plotted vs. eluted volume

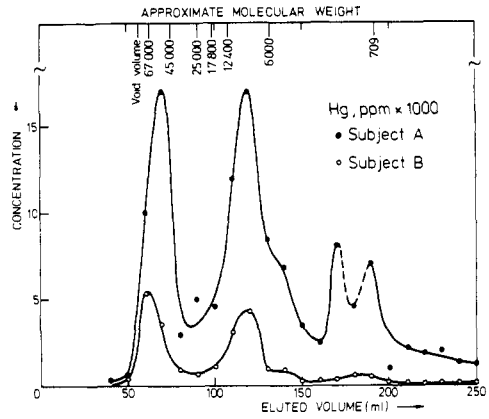

Figure 2. Separation of proteins from two different human livers on Sephadex G-75.

Concentration of Hg in the eluates plotted vs. eluted volume

fraction at 25 and 280 nm was read in a Zeiss PMQ II spectrophotometer. All fractions were stored at −20 °C until the trace elements could be determined by neutron activation analysis.

To determine the molecular weights collected in the various fractions, a series of substances with known molecular weights were separated on the Sephadex column. The following substances were used; albumin bovine (mol wt 67.000), albumin chicken (mol wt 45.000), chymotrypsinogen A (mol wt 25.000), myoglobin horse (mol wt 17,.800), cytochrom C (mol wt 12.400), insulin (mol wt 6.000) and β-DPN reduced form (mol wt 709). Three-ml fractions were collected at a flow rate of 10 ml/hour. The void volume was determined with Blue dextran 2000 (Pharmacia).

Neutron Activation Analysis. Samples of 3 ml of eluate were sealed in quartz ampoules and irradiated for 3 days at a thermal neutron flux of about 5×10^{12} n $cm^{-2}sec^{-1}$ in the JEEP-II reactor (Kjeller, Norway). Standards were irradiated simultaneously, either in 0.1*M* nitric acid solution in a quartz ampoule (Hg), or prepared by evaporation of 100-μl aliquots of appropriate standard solutions on 3 × 3 cm sheets of aluminum foil and folding (Cu, Zn, Cd, As, Se).

After 3 days' delay in order to reduce the ^{24}Na activity from the buffer solution used in the elution procedure, the sample ampoules were subjected to careful surface cleaning with strong nitric acid and subsequent freezing at −20 °C. After crushing, the ampoule with its contents was then transferred to the flask of a distillation apparatus similar to that used by Sjöstrand (*6*), containing 2 ml concentrated H_2SO_4, 1 ml 30% H_2O_2 and carriers (Se, 5 mg; As, 2 mg; Hg, 5 mg; Cu, Zn, Cd, 0.5 mg). Heating was then performed with an electrothermal burner to incipient fumes of SO_3. If a dark color occurred another few drops of 30% H_2O_2 were added. The distillate was discarded.

After cooling, 5 ml concentrated hydrochloric acid and 5 ml concentrated hydrobromic acid were added to the flask, and the mixture was heated to strong fumes of SO_3. The distillate was diluted to 150 ml with water, and a mixed-sulfide precipitation (As, Se, Hg) was carried out by means of thioacetamide by heating. If necessary, more water was added until the dark color of HgS occurred. The precipitate was collected on a membrane filter (0.45 μm), heat-sealed between two sheets of polyethylene, and then subjected to γ-spectrometry.

The residue in the distillation flask was carefully heated with 10 ml of 9*M* HCl to dissolve possible precipitate and then added to the top of an anion exchange column (Dowex 1-X8, 100–200 mesh, 80-mm height, 10-mm diameter, pre-equilibrated with 9*M* HCl). The column was washed with 3 × 5 ml 9*M* HCl. Elution with 2 × 10 ml 1*M* HCl was then performed (Cu). After adjusting the eluate to pH ~3–5 with ammonia solution, copper was precipitated as CuS and prepared for counting in the same manner as described above.

Further elution was then performed with 3 × 10 ml 1*M* NH_4OH (Zn, Cd). The standards evaporated on Al-foil were released by gentle heating with 1:1 HNO_3 and subsequent dilution to 25 ml, whereafter appropriate amounts of each standard were combined in a distillation flask and run in the same manner as the samples.

The gamma-activities of the isolated fractions were recorded by a Ge(Li) detector connected to a multichannel analyzer based on a small digital computer. The following nuclides and corresponding γ-energies were used: ^{76}As (558 keV), ^{75}Se (264 keV), ^{197}Hg (69 + 78 keV), ^{64}Cu (511 keV), ^{65}Zn (1114 keV), ^{115}Cd–^{115m}In (335 keV). The copper fractions were subjected to measurement on the day of isolation, while the other measurements were postponed to the next day.

Peak areas were calculated according to the methods of Covell (*7*) or Sterlinski (*8*).

Chemical yields were determined by reactivation (As, Hg, Se, Cu) or atomic absorption spectrophotometry (Zn, Cd). For the reactivation, the small plastic envelopes containing the filters representing samples and standards were irradiated for 30 sec, after decay of ^{64}Cu, ^{76}As, and ^{197}Hg. After 30 minutes' delay, the γ-peaks of ^{76}As, ^{197}Hg, ^{81m}Se (103 keV) and ^{64}Cu were recorded. For the atomic absorption measurements, the Zn–Cd fractions were diluted to 500 ml with 1*M* NH_4OH and analyzed with a Perkin-Elmer 305 instrument equipped with a three-slot burner. Except for Hg, in which case the yields were typically 40–60%, the chemical yields were in general of the order of 80% or higher.

RESULTS AND DISCUSSION

The method was tested on liver samples from healthy subjects who had been killed in road accidents. Fractions corresponding to 40–260 ml elution volume from the Sephadex column were analyzed. In Figure 1 are shown distribution curves as a function of eluted volume for the UV absorption at 250 and 280 nm, representing approximately the protein concentration, and the concentrations of Cu, Zn, Cd, and Se, in the eluate from the same liver sample. The approximate molecular weights as determined by the model experiments are also indicated. In Figure 2, similar curves for Hg in eluates from two different liver samples are given. For As, no curves are presented, since this element, in contrast to the other elements studied, did not exhibit any significant distribution trend with regard to molecular size.

Integrated values for the content of the five elements in the eluates from two samples A and B, expressed in ng/ml sorption solution, are given in Table I. The values have been corrected for blank contribution determined from el-

Table I. Concentration of Zn, Cd, Hg, Cu, Se, and As in Sample Solutions from Two Livers, Compared with Integrated Values from Eluated from the Sephadex Column after Separation of the Same Samples, Recalculated to Sample Solution

Element	Zn		Cd		Hg		Cu		Se		As	
Subject	A	B	A	B	A	B	A	B	A	B	A	B
ng/ml of original sample solution	23400	20200	520	1240	130	50	3800	3000	170	102	5	8
ng/ml of separated sample, integrated over entire eluate	22940	21000	740	1180	198	36	...	4500	186	96	...	...
Total system blank value (ng/ml eluate)	<20	<20	<5	<5	1.0	0.3	...	30	<1	~2		~1
Limit of detection (ng/ml eluate)	20		5		0.2		2		1		0.1	

uate fractions collected <50 and >250 ml, the magnitudes of which are also given in the Table. The integrated values are compared with results obtained by direct analysis of fractions of the sorption solutions. The agreement is satisfactory, indicating that the accuracy of the method is good provided that essentially the total amount of element in the sample was protein-bound, and that blank problems were under control during the present work. The practical limits of detection in these experiments are sufficiently low for studies of the elements in question in samples of human liver.

An interpretation of the results with regard to the extent of binding for each element to different molecular weight fractions of proteins will not be attempted in this paper because of the limited amount of data presented. It should be mentioned, however, that the trend observed for cadmium, which tends to be associated with a component of mol wt ~10000 not matching the major protein components, is in accordance with that observed in a similar study (*9*) where the distribution of Cd among protein fractions of mouse liver was studied.

As indicated from the examples given in Figures 1 and 2, the combination of gel filtration and activation analysis should be a powerful tool in studies related to trace element binding to proteins in general. By sampling from the column at shorter intervals, it may be possible to reveal further details in the distribution patterns. The method as described in this paper is fairly laborious if as many as 25 fractions from each sample are to be analyzed separately. If other buffer systems without sodium as a major constituent are introduced, however, it may be possible to reduce considerably the chemical separation efforts necessary to remove interfering radionuclides, and thus allow a substantial increase in capacity.

LITERATURE CITED

(1) K. Samsahl, P. O. Wester, and O. Landström, *Anal. Chem.*, **40**, 181 (1968).
(2) G. H. Morrison and N. M. Potter, *Anal. Chem.*, **44**, 839 (1972).
(3) J. Schumacher and W. Maier-Borst, *Kerntechnik*, **14**, 165 (1972).
(4) J. A. Velandia and A. K. Perkons, *J. Radioanal. Chem.*, **20**, 473 (1974).
(5) K. Fritze and R. Robertson, *J. Radioanal. Chem.*, **1**, 463 (1968).
(6) B. Sjöstrand, *Anal. Chem.*, **36**, 814 (1964).
(7) D. F. Covell, *Anal. Chem.*, **31**, 1785 (1959).
(8) S. Sterlinski, *Anal. Chem.*, **41**, 1995 (1968).
(9) G. F. Nordberg, M. Piscator, and B. Lind, *Acta Pharmacol. Toxicol.*, **29**, 456 (1971).

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Determination of Ozone in the 5-ppb to 100-ppb Range by a Modified Saltzman Technique

Steven R. Goates, Jerald S. Bradshaw, and Nolan F. Mangelson

Chemistry Department, Brigham Young University, Provo, Utah 84602

During a study of indoor air quality in a storage complex for documents and microfilm (*1*), it was necessary to calibrate ozone meters which were used to measure ozone concentrations in the 5-ppb to 100-ppb range. The method developed by Saltzman and Gilbert (*2*) is considered to be a reliable method for ozone determinations (*3*), but it gives values about 100 ppb low at ozone concentrations below 2 ppm (*2*).

The Saltzman technique involves a spectrophotometric determination of iodine produced as a result of the oxidation of iodide by ozone when air is bubbled through a potassium iodide solution. It occurred to us that the sensitivity of this method could be improved by adding a few drops of a starch solution and measuring the absorbance of the darker starch–iodine color. We hoped also that the error in the iodide analysis would become small at very low ozone concentrations. With the suggested modification, ozone concentrations in the parts-per-billion range could indeed be measured with an accuracy of about 5 ppb.

EXPERIMENTAL

Ozone was determined using the Saltzman and Gilbert technique (*2*), outlined by Katz (*3*), modified by the addition of a starch solution just before measuring absorbance. Ten milliliters of