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Evaluation of some procedures relevant to the determination of trace elemental components in biological materials by destructive neutron activation analysis

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I. INTRODUCTION

A. Trace Elements in Biological Systems

Advances in medical technology have demonstrated that the proper biological functioning of an organism is critically dependent on the presence of elements other than the basic organic building blocks: carbon, hydrogen, oxygen, nitrogen, and phosphorus (1-3). If methods of the proper sensitivity are employed, most elements in the geochemical environment can be detected in plant and animal tissue (4). In biological systems, these elements are present at either bulk or trace concentration levels. The definition of what constitutes a trace amount varies somewhat throughout the literature. From a biological or physiological perspective, Schroeder and Nason specify that an element exists at the trace level in the human body if that element comprises less than .01% of the body's total mass (1). More generally, Varcoe (5) states that it is commonly accepted that 100 parts-per-million (ppm) in any sample is the upper limit of a trace amount. Whichever definition is used, to date, approximately fifty elements have been detected and identified as "trace elements" in the human body.

Trace elements are in turn divided into two classes, essential and nonessential. Essential elements are those

which are required for the normal functioning of the body. Nonessential elements are, conversely, those trace elements detected in the human body which have not been found to serve in any physiological capacity. It should be noted that in 1971 there were roughly twelve trace elements recognized as essential (1), and presently some sixteen trace elements are so classified (5). With the development of more sensitive analytical techniques, the number of essential trace elements is expected to increase.

The body regulates the levels of the various elements, both essential and nonessential, within specific limits. Abnormalities associated with the essential trace elements may be caused by a specific deficiency (from dietary inadequacies, imbalances, or the result of other diseases) or accumulation of innately toxic concentrations of trace elements from the environment. While disease states due to the deficiency, as opposed to the excess of an essential trace element, are more prevalent, practically all concern with nonessential trace elements is with respect to the physiological effects of accumulating excesses. Since excursions in the concentrations of trace elements outside of normal ranges are theoretically detectable by an appropriately sensitive technique, the diagnosis and treatment of human disorders based on changes in the levels of trace elements in the body is feasible.

1. Trace elements identified

Table 1 lists the relative amounts of essential and nonessential trace elements in the body and blood of "Reference Man" (6). A listing of the concentrations of essential and nonessential elements which have been found in the serum of normal human subjects is provided in Table 2. The values listed in Table 2 can be accepted only as an indication of the levels of traces elements which can be found in the human body (1, 7-9). In reality, the actual bodily concentrations of trace components will certainly be a function of the subject's environment. Sophisticated techniques are needed which can accurately distinguish these environmental differences, thus enabling one to establish a basis for the "normal" concentration.

2. Function of trace elements

Trace elements are involved in many biological functions. Evidence supports the role of trace elements as active components in enzyme systems, transport mechanics, tissue synthesis, and bone formation (10). Trace elements are probably significant with respect to longevity (11). Correlation of trace elemental variations with diseases began as soon as their importance in metabolism was recognized.

Most trace elements are not found in the body as free ions, but exist as complexes with various stabilities.

Table 1. Trace element content of reference man[a]

Element	Body		Blood	Plasma	RBC	Remarks
	mg	µg/g	mg total			
I. Essential[b] Trace Elements						
Iron	4200	60	2500	3.6	2400	70.5% in hemoglobin
Fluorine	2600	37	0.95	0.87	0.17	98.9% in bone
Zinc	2300	33	34	5.6	2.8	65.2% in muscle
Strontium	320	4.6	0.18	0.17	0.008	99.0% in bone
Copper	72	1.0	5.6	3.5	2.2	34.7% in muscle
Selenium	13	0.2	1.1	---	---	38.3% in muscle
Manganese	12	0.2	0.14	0.025	0.12	43.4% in bone
Iodine	11	0.2	0.29	2.6	0.35	87.4% in thyroid
Molybdenum	9.3	0.1	0.083	---	---	19.0% in liver
Chromium	1.7	0.02	0.14	0.074	0.044	37.0% in skin
Cobalt	1.5	0.02	0.0017	0.0014	0.00034	18.6% in bone (marrow)
Possibly essential						
Nickel	10	0.1	0.16	0.09	0.07	18.0% in skin
Vanadium	<18	0.3	0.088	0.031	0.057	>90.0% in fat
II. Nonessential Trace Elements						
Rubidium	320	4.6	14	2.2	12	Follows potassium
Bromine	200	2.9	24	17	7.5	60.0% in muscle
Lead	120	1.7	1.4	0.14	1.2	91.6% in bone
Aluminum	61	0.9	1.9	1.3	0.14	19.7% in lung, 34.5% in bone
Cadmium	50	0.7	0.036	---	---	27.8% in kidney and liver
Boron	<48	0.7	0.52	---	---	Essential for plants
Barium	22	0.3	<1.0	<0.62	---	91.0% in bone
Tin	<17	0.2	0.68	0.10	0.55	25.0% in fat and skin

Mercury	13	0.2	0.026	0.009	0.017	69.2% in fat and muscle
Titanium	9	0.1	0.14	0.12	0.08	49.1% in lung and lymph nodes
Gold	<10	0.1	0.00021	---	---	52.0% in bone
Antimony	? 7.9	0.1	2.024	0.16	---	25.0% in bone
Cesium	1.5	0.02	0.015	---	---	Follows potassium
Uranium	0.09	0.001	0.0046	---	---	65.5% in bone
Beryllium	0.036	---	<0.00052	---	---	75.0% in bone
Arsenic	?18	0.3	2.5	<0.093	0.59	Follows phosphorus
Lithium	2.2	0.03	0.10	0.093	0.061	50.0% in muscle
Zirconium	420	6.0	13	1.2	12	67.0% in fat
Niobium	?110	1.6	13	<0.25	13	26.0% in fat
Tellurium	? 8.2	0.1	0.18	0.09	0.078	Probably in bone

[a]Data of Bowen (3) and Tipton (6).

[b]As of 1971.

Table 2. Experimentally determined elements in human blood serum

Element	Serum Concentration µg/ml	Reference
Essential Bulk		
Ca	2.8	(7)
Cl	1,000	(7)
K	1,700	(7)
Mg	13	(7)
Na	3,250	(7)
P	0.50	(7)
S	5,400	(8)
Essential Trace		
Co	0.00018	(1)
Cr	0.02-0.05	(9)
Cu	1.16	(1)
F	0.028	(1)
Fe	1.14	(1)
I	0.0029-0.11	(1)
Mn	0.0083	(1)
Mo	0.4	(1)
Ni	0.0042	(1)
Se	1.1	(1)
Si	7.0	(8)
Sn	0.033	(1)
V	1.0	(1)
Zn	0.98	(1)

Table 2. (Continued)

Element	Serum Concentration µg/ml	Reference
Nonessential		
Ag	0.28	(7)
Al	5	(8)
As	0.19	(1)
Au	0.03	(7)
B	2	(8)
Ba	0.059-0.066	(7)
Be	<0.004	(1)
Br	830	(7)
Cd	<0.1	(1)
Cs	0.05	(8)
Hg	0.0027-4.0	(7)
La	18	(7)
Li	0.031	(1)
Pb	0.046	(1)
Rb	14	(8)
Sb	0.054	(1)
Sc	0.04	(7)
Sr	0.057	(1)
Th	0.4	(8)
U	0.048-0.072	(7)

Through these stability constants, control is maintained over the concentrations of the trace elemental components in the fluids circulating throughout the body. Analyses for diagnostic purposes have been performed on both whole blood and serum - coagulated blood plasma. For elements which are largely contained within the cell (e.g., potassium, magnesium, iron, zinc, copper, manganese), the serum concentrations will not necessarily reflect the body content of those elements (1). For these elements, the concentrations in the red blood cells are a more reliable index. On the other hand, for elements which accumulate in bone (e.g., fluorine, strontium, lead, barium, beryllium) or in fat (e.g., vanadium, mercury, zirconium), both blood and plasma may be unreliable indicators of total body burden. For these elements, the analysis of a pathological specimen from the site of concentration would be optimum for the determination of total body load.

To determine general availability of trace elements to the body, analytical studies of the urine may be sufficient. If the amount of a trace element in the urine is normal, it may be presumed that the amount of that trace element available to the body is sufficient (1). In the search for a specific disorder, the use of a more specialized fluid may be indicated (e.g., spinal fluid analysis for disorders of the nervous system). Of the materials available for

diagnostic analysis, blood, which contacts all tissues and organs, is the most pervasive and would most frequently yield values of trace components reflective of total body load.

3. Disease relationships

Correlations between abnormal concentrations of trace elements and various disease states have been extensively enumerated (1,5). Usually, the trace element abnormality is a secondary effect of a disorder which may be effectively treated by correcting the concentration abnormality (5). In this particular situation, the analysis for a trace component can be of significant diagnostic value as an indicator of a disorder.

Studies implicating trace elements in cardiovascular diseases and cancer, two of the leading causes of death each year in the United States, are numerous (10-26). Whether the trace elements actually influence the course of the disease has not yet been definitely established. However, it has been shown that knowledge of trace element concentrations can be significant in understanding and diagnosing a disease. In practice, it has also been shown that the examination of the concentrations of some trace elements can be useful in evaluating the course of treatment of some cancers (10). Specifically, changes in the concentration of copper in patients under treatment for tumors may precede

by an appreciable amount of time any apparent clinical changes in the tumor. Other diseases for which trace element concentration relationships have been studied include: abnormal thyroid activity (27-34), rheumatoid arthritis (35,36), hepatitis (11, 15-24), and diabetes mellitus (11,37). A listing of disorders and conditions for which trace element analysis may aid in diagnosis is presented in Table 3.

4. Analysis methods

The literature abounds with reports on the analysis of blood and serum for trace elements. Most of these analyses are limited to elements which are present at relatively high concentrations or are particularly sensitive to a specific analysis technique (5, p. 2, 38-54). Lately, multi-elemental techniques have become more prevalent (54-57). All of these studies have illustrated that if a method is to be of value as a diagnostic tool, it must be capable of multi-elemental analysis, reproducible, relatively rapid, highly sensitive, specific, and suitable for automation.

The nature of a biological material presents a particularly troublesome problem which an analysis technique must be able to surmount: matrix interference is severe. The chosen method must be capable of multi-elemental determinations at the trace and subtrace level in the presence of several elements at macro-levels (See Table 2).

Table 3. Diseases and conditions in which analysis of trace elements may be of aid in diagnosis, or disclose abnormalities

Disease or Condition	Deficiency Present	Excess Present	Reference
Acute Myocardial Infarction (Heart tissue)	Co, Cs, K, Mo, P, Rb, Zn	Br, Cu, Ce, La, Na, Sb, S	(12)
Myocardial Infarction (Serum)	Zn	Cu, Mn, Ni, Mo, B	(13,14, 11,15)
Allergic States	Co?		(1)
Arterial Hypertension	Zn?	Cd[a]	(1)
Atherosclorosis	Cr		(1)
Ischemic symptoms	Zn		
Indolent ulcers	Zn		
Alcoholism (Chronic)	Zn, Mn		(1)
Cancer			(16,23,
Leukemia		Fe, Zn	24)
Lung		Bi, Ni, Cr(VI)	
Prostate		Cu	(11)
Breast		Cu	(25)
Hodgkins		Cu	(10)
Cartilaginous Malformations	Mn?		(1)
Chronic Infections	Zn		(1)
Cirrhosis of Liver, Adult	Zn	Cu	(1)
Juvenile		Cu, Ag	
Collagen Diseases			(1)
Disseminated lupus	Mn?		
Rheumatoid arthritis		Cu	
Hydralazine disease	Mn		
Diabetes Mellitus, juvenile	Cr		(1)
Mild, adult	Cr		

[a]Underlined elements are considered causal factors in the diseases.

Table 3. (Continued)

Disease or Condition	Deficiency Present	Excess Present	Reference
Dietary (Bantu) Siderosis		Fe, Pb	(1)
Dental Caries	Sr, Mo?, F		(1)
Excessive Proteinuria	Zn		(1)
Hepatolenticular Degeneration		Cu, Ag	(1)
Hypoproteinemia	Cu		(1)
Idiopathic Hemochromatosis		Fe, Pb	(1)
Ill-defined Asthenia		Pb	(1)
Ill-defined Neurosis		Pb	(1)
Kwashiorkor	Cr, Mn, Cu, Zn, Mo?		(1)
Malnutrition	Cu, Mn, Cu, Zn, Mo		(1)
Milkman's Syndrome (itai itai disease)		Cd	(1)
Oral Contraceptives	Zn		(1)
Parkinsonism		Mn?	(1)
Postoperative Convalescence	Zn		(1)
Pregnancy Toxemia	Zn, Mn?, Cr, Cu? Zn		(1)
Prolonged Intravenous Feeding	Zn, Mn, Cu		(1)
Psoriasis		Zn	(1)
Renal (Xanthine) Calculi	Mo		(1)

Table 3. (Continued)

Disease or Condition	Deficiency Present	Excess Present	Reference
Renal Insufficiency	Zn		(1)
Rheumatoid Arthritis	Zn, Fe, Pb	Cu, Ba, Cs, Sn, Mo	(1)
Senile Osteoporosis	Sr		(1)
Tuberculosis, Active	Zn		(1)
Wilson's Disease	Cu		(1)
Wound Healing (Slow)	Zn		(1)
Cholesterol Synthesis (Decreased)		V, Fe	(21)
Cholesterol Synthesis (Increased)		Cr, Mn	(21)
Hepatitis		Zn, Mn	(1)
Mental Retardation (Juvenile)		Pb	(1)

A discussion of some of the trace elemental techniques most frequently cited in the literature for the analysis of biological materials follows.

a. Atomic emission, atomic absorption, atomic fluorescence Atomic emission (AE), atomic absorption (AA), and atomic fluorescence (AF) are complimentary spectroscopic methods of analysis capable of determining elements at the trace level. Of the three techniques, only AE has a practical simultaneous multi-elemental capability. Intrinsically, the sensitivity of AF measurements are greater than those of AA because of the benefits gained from signal amplification and increased source intensity (58). Table 4 contains a listing of elements for which AA has a lower limit of detection than AE by at least a factor of five. Those elements for which AE has a lower limit of detection than AA by at least a factor of five are listed in Table 5. Elements for which AA and AE have comparable limits of detection are listed in Table 6. Reference to Table 2 indicates that for those trace elements in the human body which can be determined spectroscopically, the AA detection limits are equal to or lower than corresponding AE limits. This accounts for the early popularity of AA for the analysis of trace components in biological materials.

Table 4. Elements for which AA has a detection limit lower than AE by at least a factor of 5[a]

Element	Lower Limit of Detection: Flame AA, μg/ml	Reference
As	0.1	(59)
Au	0.01	(60)
Be	0.002	(59)
Bi	0.5	(59)
Cd	0.0006	(60)
Co	0.005	(59)
Fe	0.005	(59)
Ge	0.1	(60)
Hf	15	(61)
Hg	0.2	(60)
Ir	4	(61)
Mg	0.0003	(59)
Ni	0.005	(59)
Pb	0.03	(59)
Pt	0.5	(61)
Rh	0.03	(59)
Sb	0.07	(60)
Se	0.1	(59)
Si	0.1	(59)
Sn	0.02	(59)
Te	0.1	(59)
Ti	0.1	(61)
Zn	0.002	(59)
Zr	5	(61)

[a]Based on compilations by Varcoe (5).

Table 5. Elements for which AE has a detection limit lower than AA by at least a factor of 5[a]

Element	AE Detection Limit, µg/ml	Reference
Al	0.005	(62)
Ca	0.0001	(63)
Ce	10	(61)
Cs	0.008	(61)
Eu	0.003	(61)
Ga	0.01	(63)
In	0.002	(61)
La	1	(61)
Li	0.000003	(61)
Lu	0.2	(61)
Na	0.0001	(61)
Nb	1	(61)
Os	10	(61)
Sm	0.6	(61)
Sr	0.0002	(63)
Th	150	(61)

[a]Based on compilations by Varcoe (5).

Table 6. Elements for which AE and AA have comparable detection limits[a]

Element	Lower Limit of Detection, µg/ml	Technique	Reference
Ag	0.005	AA	(59)
Ba	0.03	AE	(61)
Cr	0.005	AA-AE	(59,61)
Cu	0.003	AA	(60)
Dy	0.1	AE	(61)
Er	0.2	AA	(61)
Gd	2	AE	(61)
Ho	0.1	AE	(61)
K	0.003	AE	(61)
Mn	0.002	AA	(59)
Mo	0.03	AA	(59)
Nd	1	AE	(61)
Pd	0.02	AA	(60)
Pr	2	AE	(61)
Rb	0.002	AE	(61)
Re	1	AE	(61)
Ru	0.3	AA-AE	(61)
Sc	0.07	AE	(61)
Ta	6	AA	(61)
Tb	1	AE	(61)
Tl	0.02	AA-AE	(64,65)
Tm	0.1	AA	(61)
U	10	AE	(61)
V	0.01	AE	(63)
W	3	AA	(61)
Y	0.3	AA-AE	(61)
Yb	0.04	AA	(61)

[a]Based on compilations by Varcoe (5).

The absolute detection limits of AE, AA, and AF are dependent upon the method of excitation of the analyte atoms. The available methods of excitation may be categorized as either flame or nonflame. Flames, the earliest source of excitation energy, were inexpensive and easy to operate. Compared with the other trace techniques available at the time (e.g., colorimetry, fluorimetry, etc.), flame spectroscopy was least likely to be affected by interferences. The principal interferences of flame spectroscopy are either chemical (those which prevent the atoms from reaching the free, dissociated, unionized state necessary for measurement in the flame) or spectral (bands emitted by the OH and CN radicals, which obscure atomic excitation lines). Chemical interferences are generally circumvented by the use of various masking and releasing agents. The proper choice of fuel and oxidizer, fuel/oxidizer ratios, and the position of the analytical zone in the flame could contribute to lower spectral interference.

The primary disadvantage of the flame methods is the need to keep the total salt concentration of the analyte fluid below 1%. The required sample dilutions or sample extraction procedures adversely affect trace determinations due to contamination and sample losses (66, p. 127). Recent developments in nonflame atomization and excitation sources have lowered the absolute detection limits of AA,

AE and AF considerably. The advantages of nonflame methods (e.g., Inductively Coupled Plasma Atomic Emission (ICPAE), Non-Flame Atomic Absorption (NFAA), and Non-Flame Atomic Fluorescence (NFAF)) over the corresponding flame techniques are illustrated by comparing the limits of detection listed in Table 7 with the previously given flame detection limits. (Compare Tables 4, 5 and 6).

Inductively coupled plasmas (a special type of plasma that derives its sustaining power by induction from a high frequency magnetic field) as compared to flames, provide cleaner background spectra, higher temperatures, and longer residence times in an inert environment for the analyte species. These factors lead to reduced solute vaporization interferences, nearly 100% atomization, and reduction of inter-element and matrix effects (67). Disadvantages in the use of the plasma system include: 1) the fraction of the atomized sample which enters the plasma is small; 2) the total salt concentration in most samples must be controlled - usually via dilution of the sample - to ensure efficient nebulization; and 3) the method is subject to contamination errors because most samples must be chemically processed prior to analysis (68, p. 236).

Nonflame AA and AF usually employ a form of electrically heated graphite furnace as atomizer. The principal reasons for the superior sensitivity and lower detection

Table 7. Detection limits for nonflame spectrophotometric methods

Element	ICPAES[a] µg/ml	NFAAS[b] µg/ml	NFAFS[c] µg/ml
Ag	0.004	1×10^{-7}	0.007
Al	0.002	1×10^{-6}	
As	0.04	1×10^{-6}	
Au	0.04	1×10^{-6}	
B	0.005	2×10^{-4}	
Ba	0.001	6×10^{-6}	
Be	0.0005	3×10^{-8}	
Bi	0.05	4×10^{-6}	0.01
Ca	0.00007	4×10^{-7}	
Cd	0.002	8×10^{-8}	0.001
Ca	0.007		
Co	0.003	2×10^{-6}	
Cr	0.001	2×10^{-6}	0.04
Cu	0.001	6×10^{-7}	0.002
Dy	0.004	2.2×10^{-10} g	
Er	0.001	3.7×10^{-1} g	
Cu	0.001	3×10^{-1} g	
Fe	0.005	1×10^{-5}	0.02
Ga	0.014	1×10^{-12} g	
Hg	0.2	2×10^{-5}	
Ho	0.01	3.3×10^{-10} g	
In	0.03	4×10^{-7}	0.01
La	0.003	0.1 µg/ml	
Mg	0.0007	4×10^{-8}	0.003

[a]Fassel and Kniseley (67).

[b]Dulka and Risby (2) and L'vov (69).

[c]Clyburn, Bartschmid and Veillon (70).

Table 7. (Continued)

Element	ICPAES[a] µg/ml	NFAAS[b] µg/ml	NFAFS[c] µg/ml
Mn	0.0007	2×10^{-7}	0.005
Mo	0.005	3×10^{-6}	
Na	0.0002	1×10^{-12} g	
Nb	0.01	12.0 µg/ml	
Ni	0.006	9×10^{-6}	0.03
Pb	0.008	2×10^{-6}	0.01
Pd	0.007	4×10^{-6}	
Pt	0.08	1×10^{-5}	
Rh	0.003	8×10^{-6}	
Sb	0.2	5×10^{-6}	
Sc	0.003		
Se	0.03	9×10^{-6}	
Si	0.01	5×10^{-8}	
Sn	0.3	2×10^{-6}	0.02
Sr	0.00002	1×10^{-6}	
Ta	0.07	7.0 µg/ml	
Te	0.08	1×10^{-6}	
Ti	0.003	4×10^{-5}	
Tl	0.2	1×10^{-6}	
V	0.006	3×10^{-6}	
W	0.002	1.0	
Y	0.0002	10.0	
Zn	0.002	3×10^{-8}	0.005
Zr	0.005	5.0	

limits achieved by the graphite tube atomizers are: 1) a nonreactive atmosphere is used; 2) the carbon provides a strongly reducing medium; 3) the analyte atoms are confined to a relatively small space for a relatively long time; and 4) the furnace gases have a low background emission (71, p. 256). The primary disadvantages of the graphite furnace are: 1) sample sizes are usually limited to between 1 and 100 microliters; 2) reproducibility is strongly dependent on sample placement in the furnace and the surface condition of the graphite; and 3) matrix effects are more troublesome (71, p. 256).

Combination atomization and excitation sources associated with AE are the electrical arcs and sparks. With the high temperature arc, the analytical sensitivity is high, and low limits of detection result. The wandering nature of the arc on the electrode surface during the discharge is such that the amount of radiation reaching the detector is not reproducible from sample to sample. Reproducibilities better than about ±10% are difficult to achieve (71, p. 136). Because limits of detection in arc spectroscopy depend on so many variables, only approximate ranges are given in Table 8. The usefulness of the DC arc source in qualitative and semiquantitative work is evident: virtually every metal in the periodic table can be detected at or below concentration levels of 100 ppm (0.01%).

Table 8. Approximate detection limits for elements detectable by DC arc sources[a]

Approximate Detection Limit[b]	Elements
Below 10^{-4}%	Li, Na, Cu, Ag
10^{-4} - 10^{-3}%	K, Rb, Cs, Be, Mg, Ca, Sr, Ba, Sc, Y, La, Ti, Zr, V, Cr, Mo, Mn, Fe, Ru, Co, Rh, Ni, Pd, Au, Zn, Cd, B, Al, Ga, In, Tl, Ge, Sn, Pb, Pr, Nd, Eu, Tb, Dy, Ho, Er, Tm, Yb, Lu
10^{-3} - 10^{-2}%	Hf, Nb, Ta, W, Re, Os, Ir, Pt, Hg, Si, P, As, Sb, Bi, F, Th, U
10^{-2} - 10^{-1}%	Se, Te, Ce, Sm, Gd

[a]Data of Veillon (71, p. 137).

[b]10^{-4}% = 1 microgram analyte per 1 gram solid sample.

The DC spark has an effective excitation temperature in the discharge many times that in the DC arc, which means that virtually any element can be ionized, resulting in more complicated spectra. Detection limits are poorer than the DC arc, primarily because of the small amount of sample consumed. For the fifty or so elements most frequently analyzed by spark source spectroscopy, detection limits ranging from 10^{-7} to 10^{-2}% (by weight) with most in the 10^{-4} to 10^{-2}% range are typical (71, p. 139).

b. Colorimetry and fluorimetry Colorimetry and fluorimetry are two of the least expensive methods capable of determining elements at the trace level. However, the use of either method is severely limited by inter-element interferences. The organic reagents, with which the analyte element must form complexes in order to achieve the appropriate colored or fluorescent species, are generally not specific for one element which consequently leads to interference problems. For complex samples, such as blood, extensive separations are needed to isolate the desired element. As suggested by the need for separation schemes, neither technique has a practical multi-elemental capacity. Included in Table 9 are some of the lower limits of colorimetric detection for several elements in clinical samples (1). Where specified in micrograms rather than micrograms per gram, the limit of detection represents the

Table 9. Colorimetric lower detection limits[a]

Element	Limit (µg/g)
Essential	
Cr	0.006
Mn	0.01 µg
Mo	1.0 µg
Ni	0.05
Sn	5.0 µg
V	0.5 µg
Nonessential	
As	0.5 µg
B	0.5
Ge	0.1
Nb	0.04
Pb	0.05 µg
Ti	0.25
Zr	0.8

[a]Compiled by Schroeder and Nason (1).

smallest amount detectable by differences in two solutions, both with known amounts of the element added.

The enhanced signal-to-noise ratio resulting from the optical geometry used in fluorimetric measurements results in lower detection limits than inorganic absorption spectrophotometry (72). The detection limits of several elements as noted by Winefordner et al. for an interference free media (Table 10) illustrate that under ideal conditions, fluorimetry can be comparable to the modern, sophisticated methods of trace analysis.

c. X-ray methods Recent developments in excitation sources, energy dispersive detectors and associated electronics have significantly increased the sensitivity and efficiency of X-ray fluorescence measurements. Under vacuum conditions, X-ray fluorescence is capable of determining all elements with atomic number 9 or greater at the ppm level (73, p. 352). However, the quantification of complex samples is complicated by the necessity to empirically correct for inter-element and matrix absorption effects. Even though these corrections, involving relatively large systems of simultaneous equations, have become routine with the introduction of mini-computers into fluorescence analysis systems, the precision and accuracy of the results are still significantly dependent on the nature of the sample. Methods for

Table 10. Fluorimetric lower detection limits[a]

Element	Limit, µg/ml	Element	Limit, µg/ml
Ag	0.004	Lu	100
Al	0.0008	Mg	0.00001
As	7	Mn	0.002
Au	0.5	Mo	0.1
B	0.0005	Nb	0.1
Be	0.0004	Nd	5
Bi	0.5	Ni	0.00006
Ca	0.01	Os	0.05
Cd	0.02	P	0.0000006
Ce	0.05	Pb	5
Cl	0.05	Pr	0.5
Co	0.0001	Ru	1
Cu	0.0002	Sb	0.05
Dy	0.01	Sc	0.01
Er	10	Se	0.005
Eu	0.005	Si	0.08
F	0.001	Sm	0.5
Fe	0.0008	Sn	0.1
Ga	0.001	Tb	0.1
Gd	10	Te	0.2
Ge	0.004	Th	0.02
Hf	0.1	Tl	0.02
Hg	0.002	Tm	10
Ho	100	U	0.01
I	0.6	V	2
In	0.04	W	0.04
Ir	2.0	Y	0.02
Li	0.2	Zn	0.002
		Zr	0.02

[a]Compiled by Winefordner, et al. (72).

the separation and concentration of the analyte species have been employed, but all are complicated by the necessity of carrying out the procedures without contaminating the sample. The tediousness and expense of using clean room facilities and ultra-pure reagents will be encountered if the full sensitivity of X-ray fluorescence is to be exploited.

The limit of detection of X-ray fluorescence for a given element is a function of the intensity of the fluorescence reaching the detector. Therefore, the limits are greatly affected by the form and type of matrix. In general, analyses of samples from about 1 microgram of analyte per gram (0.0001% by weight) to essentially 100% analyte are possible (73, p. 363). Limits of detection have been reported in the range of 1 to 50 micrograms per gram and from 0.1 to 1 microgram for all elements with atomic number greater than 15 (phosphorus) (73, p. 363). A listing of some reported detection limits for X-ray fluorescence is provided in Table 11. Despite the high sensitivities, the multi-elemental capacity, and the versatility with respect to the types of samples acceptable for analysis, the uncertainty of the corrections for inter-element and absorption effects have limited the use of X-ray fluorescence primarily to the analysis for components present at or greater than 1% by weight (73, p. 363).

Table 11. X-ray fluorescence detection limits[a]

Element	Limit (μg)	Element	Limit (μg)
Ag	1.2	Nd	0.30
Al	5.0	Ni	0.06
As	0.11	P	0.001
Au	0.001/cm^2	Pb	0.0003
Ba	0.12	Rb	0.0075
Bi	0.61	Rh	103/ml
Ca	0.100	Sc	0.38
Cd	0.40	Se	0.020/cm^2
Ce	0.17	Si	170 ppm
Co	0.15	Sm	4.1/ml
Cr	0.00006	Sn	3.9 ppm
Cs	0.15	Sr	0.00007
Cu	0.00002	Tb	159/ml
Eu	0.66	Te	0.12
Fe	0.0085	Th	6.5/ml
Ga	0.01	Ti	0.001
Hg	0.24	U (as UO_2)	0.72
In	1.1	U	0.00002
K	0.52	Y	0.22
La	0.12	Yb	6.8/ml
Mn	0.00015	Zn	0.00004
Mo	0.072	Zr	0.00002

[a]Data from Dulka and Risby (2, p. 44).

Electron Microprobe and Laser Probe X-Ray Microanalysis are two additional X-ray methods which have been applied to the analysis of blood samples. However, these methods allow only small areas of excitation and shallow penetration of the excitation radiation resulting in lower sensitivities than the X-ray fluorescence method. Only in special situations can analyses in the parts-per-million range be accomplished (73, p. 381).

d. Mass spectroscopy Mass spectroscopy offers the capability of determining the greatest number of elements with some of the lowest attainable limits of detection. The utility of mass spectroscopy is dependent upon the type of ionization and detection systems employed. Spark Source Mass Spectroscopy (SSMS) offers the greatest versatility with respect to the usable matrices and overall sensitivity. Consideration of equipment and maintenance costs, sensitivity of operation, and overall difficulty in obtaining quantitative results, explains why SSMS has not found widespread use as an analytical tool.

The detection limits of elements by SSMS are highly dependent upon the matrix and the detection systems. The infeasibility of spectrum scanning, due to the instability of the spark in the ionization system, and, the resultant time variation in the ion currents at the detector, limits the technique to using photographic plates as detectors (5).

Precision is thus limited to the homogeneity of the photographic emulsion of a single plate. Inhomogeneity in emulsions between plates and between emulsion lots are of significant magnitude and result in generally poor precision. The overall precision, according to de Galan, is limited to approximately 25% (66, p. 127).

A theoretical approach to limits of detection based on signal levels has been described by Werner (74). A listing of detection limits as calculated by Werner for some elements in an iron matrix is given in Table 12. Morrison has determined limits of detection for elements based on the photographic plate as detector (75) (See Table 13).

Some elements after chelation with the proper organic reagents are susceptible to analysis by Chemical Ionization Mass Spectroscopy (CIMS). As indicated by the limits of detection listed in Table 14, CIMS offers an advantage over SSMS for some of the elements for which the technique is viable.

Better quantitative agreement can be obtained in SSMS if isotope dilution techniques are employed. Isotope dilution allows precise quantitative determinations because only isotopic ratios for analyte lines recorded under identical matrix, exposure, development, and emulsion conditions are measured. Though blood analysis by Isotope

Table 12. SSMS calculated limits of detection for some elements in an iron matrix[a]

Element	Q_{rel}	Cm, lim (ppm)
^{27}Al	15.4	0.005
^{48}Ti	8	0.01
^{28}Si	3.6	0.02
^{43}Ca	0.35	0.3
^{181}Ta	0.2	0.4
^{208}Pb	0.04	3
^{197}Au	0.02	4

Q_{rel} = Current for ion M^+ relative to internal standard ion, R^+.

Conditions: Primary ion current = 10^{-6}A
Ion current for isotope M = 10^{-19}A
Reference ion current = 10^{-12}A
Matrix isotope (reference) = ^{56}Fe

[a]Data of Werner (74).

Table 13. Estimated detection limits for SSMS with photographic plates as detectors[a]

Element	Absolute (ng)	Element	Absolute (ng)
Ar	0.03	Mn	0.05
Ag	0.2	Mo	0.3
Al	0.02	N	0.01
As	0.06	Na	0.02
Au	0.2	Ne	0.02
B	0.01	Nb	0.08
Ba	0.2	Nd	0.4
Be	0.008	Ni	0.07
Bi	0.2	O	0.01
Br	0.1	Os	0.4
C	0.01	P	0.03
Ca	0.03	Pb	0.3
Cd	0.3	Pd	0.3
Ce	0.1	Pr	0.1
Cl	0.04	Pt	0.5
Co	0.05	Rb	0.1
Cr	0.05	Re	0.2
Cs	0.1	Rh	0.09
Cu	0.08	Ru	0.03
Dy	0.5	S	0.03
Er	0.5	Sb	0.2
Eu	0.2	Sc	0.04
F	0.02	Se	0.1
Fe	0.05	Si	0.03
Ga	0.09	Sm	0.5
Gd	0.5	Sn	0.3
Ge	0.2	Sr	0.09
H	0.0008	Ta	0.2
Ho	0.003	Tb	0.1
Hf	0.4	Te	0.2
Hg	0.6	Ti	0.2
He	0.1	Tl	0.1
I	0.1	Tm	0.2
Ir	0.3	V	0.04
K	0.03	W	0.5
Kr	0.1	Xe	0.4
La	0.1	Y	0.07
Li	0.006	Yb	0.5
Lu	0.1	Zn	0.1
Mg	0.03	Zr	0.1

[a]Data compiled by Morrison (75).

Table 14. Sensitivities for elements by chelation coupled with CIMS[a]

Element	Chelates	Element	Chelates
Mn	$Mn(acac)_2$, $Mn(tfa)_2$ $Mn(thd)_3$	La	$La(thd)_3$, $La(fod)_3$
Ni	$Ni(acac)_2$, $Ni(tfa)_2$ $Ni(thd)_2$	Pr	$Pr(thd)_3$, $Pr(fod)_3$
Cu	$Cu(acac)_2$, $Cu(tfa)_2$ $Cu(thd)_2$	Nd	$Nd(thd)_3$, $Nd(fod)_3$
Zn	$Zn(acac)_2$, $Zn(tfa)_2$ $Zn(thd)_2$	Sm	$Sm(thd)_3$, $Sm(fod)_3$
VO	$VO(tfa)_2$, $VO(thd)_2$	Eu	$Eu(thd)_3$, $Eu(fod)_3$
Cr	$Cr(acac)_3$, $Cr(tfa)_3$ $Cr(thd)_3$	Gd	$Gd(thd)_3$, $Gd(fod)_3$
Fe	$Fe(acac)_3$, $Fe(tha)_3$ $Fe(thd)_3$	Tb	$Tb(thd)_3$, $Tb(fod)_3$
Co	$Co(acac)_3$, $Co(tfa)_3$ $Co(thd)_3$	Dy	$Dy(thd)_3$, $Dy(fod)_3$
Ru	$Ru(acac)_3$, $Ru(tfa)_3$	Ho	$Ho(thd)_3$, $Ho(fod)_3$
Rh	$Rh(acac)_3$, $Rh(tfa)_3$	Er	$Er(thd)_3$, $Er(fod)_3$
Pd	$Pd(tfa)_2$, $Pd(thd)_3$	Tm	$Tm(thd)_3$, $Tm(fod)_3$
Pt	$Pt(tfa)_2$, $Pt(thd)_3$	Yb	$Yb(thd)_3$, $Yb(fod)_3$

acac: 2,4-pentanedionate
tfa: 1,1,1-trifluoro-2,4-pentanedionate
fod: 1,1,-2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate
thd: 2,2,6,6-tetramethyl-3,5-heptanedionate

[a]Dulka and Risby (2, p. 31).

Dilution Mass Spectroscopy has been reported (76), as with SSMS, the equipment expense and, particularly, the tediousness of the technique limits its popularity.

e. Electrochemical methods Potentiometric methods have been the mainstay of electrochemical methods applied to trace elemental analysis in a biological matrix. Gochman and Young have extensively reviewed those potentiometric methods which have been reported in the literature for use on biological matrices (77). The potentiometric analysis with ion selective electrodes of both *in situ* and *in vivo* blood serum has been reported (78-81). Coulometry has received limited use in biological trace element analysis. Selenium in urine (81) and chlorine (82), as little as 0.2 micrograms, in serum are examples of the coulometric technique. Some literature values for limits of detection for Anodic Stripping Voltammetry and Differential Pulse Polarography, two of the most sensitive electrochemical techniques, are listed in Table 15. Though sensitive, these methods, as with most electrochemical methods, are particularly susceptible to interferences. For example, with anodic stripping, multi-elemental blood trace analysis is not possible for elements with similar unmasked electrodeposition potentials. Between the cathodic deposition potentials and the anodic water-salt breakdown potential limit, there is only a 1.5 volt anodic dissolution window,

Table 15. Detection limits with anodic stripping voltammetry and differential pulse polarography[a]

Element	ASV	DPP
Ag	0.25 ppb	
Au	1.0 ppb	
Bi	0.01 ng/ml	0.2 ng/ml
Cd	0.005 ng/ml	0.98 ng/ml
Co		0.01 ng/ml
Cu	0.005 ng/ml	13.7 ng/ml
Eu		1.2×10^{-4} M
Fe		1 µg/ml
Ga	0.4 ng/ml	2×10^{-8} M
Hg	4.0×10^{-9} M	
In	0.1 ng/ml	8×10^{-9} M
K	1×10^{-5} M	
Mn		0.03 µg/ml
Ni	0.1 g/ml	0.78 ng/ml
Pb	0.01 ng/ml	1.5 g/ml
Pd		5.0×10^{-8} g
Pt	1×10^{-9} M	
Rh	0.1 ng/ml	
Sn	2.0 ng/ml	1×10^{-3} M
Tl	0.01 ng/ml	4×10^{-8} M
U		0.18 µg/ml
V		0.08 µg/ml
Zn	0.04 ng/ml	

[a]Tabulated by Dulka and Risby (2).

which prevents obtaining separate and distinct dissolution peaks for the large number of different ions present in blood (82).

f. Gas chromatography Gas chromatography (GC) with an appropriate detection system offers a sensitive means for determining some of the trace elemental components in a biological matrix. The intrinsic separations occurring in the GC process can, in some cases, remove most of the inter-element and matrix interferences. GC is limited to those elements capable of forming the complexes which are stable under the environment of the GC column. Table 16 includes a listing of some elements which have been separated by GC, but Sievers et al. (83) suggest that due to thermal instabilities, not all of the complexes listed are suitable for quantitative analysis.

Varcoe examined the use of Neutron Activation Analysis (NAA) in conjunction with GC as a method for the analysis of serum for trace elemental components (5). NAA with gamma ray detection provides an excellent means for the identification of the various components separated by GC. However, due to the lack of quantitative chelation and extraction of the complexes from the matrix, the difficulty of obtaining quantitative GC recoveries, and the general sensitivity of the GC step to column conditions, high precision quantitative analysis is possible only in special situations (5).

Table 16. Metal chelates separated by GC[a]

Element	Chelating Agent[b]	Element	Chelating Agent
Be	acac, tfa, hfa, fod	In	tfa
Al	acac, tfa, hfa, fod	La	thd
Sc	acac, tfa, fod, thd	Pr	fod, thd
V	acac, tfa	Nd	fod, thd
Cr	acac, tfa, hfa, fod	Sm	fod, thd
Mn	tfa	Eu	fod, thd
Fe	tfa, hfa, fod	Gd	fod, thd
Co	tfa, hfa, thd	Tb	fod, thd
Ni	hfa, thd, fod	Dy	fod, thd
Cu	acac, tfa, hfa, fod	Ho	fod, thd
Zn	tfa, hfa, thd	Er	fod, thd
Ga	tfa	Tm	fod, thd
Zr	tfa, thd	Yb	fod, thd
Nb	hfa	La	fod, thd
Ru	tfa, hfa	Hf	tfd
Rh	tfa, hfa	Ta	tfa

[a]Tabulated by Dulka and Risby (2).

[b]See Table 14 for abbreviations.

g. Neutron activation analysis With its extreme sensitivity, Neutron Activation Analysis (NAA) has become one of the most important methods for trace element analysis. Theoretical lower detection limits for NAA are given in Table 17. These values are based on the measurement of forty disintegrations per second in a sample irradiated at 5×10^{13} neutrons cm^{-2} sec^{-1} for an optimum time period. Many reactors operate routinely at fluxes of 10^{13} and some at 10^{14} ns cm^{-2} sec^{-1}, thus indicating that most elements can be analyzed within the range of 10^{-9} to 10^{-14} grams, a sensitivity not routinely attainable on a broad scale with other methods (80).

The advantages of multi-element capability, low limits of detection for many elements, and a wide dynamic range has lead to the extensive use of NAA for the analysis of biological matrices, including blood and serum. NAA, in addition, offers two advantages which are unique to trace element methods. Errors typically introduced into an analysis by blank values of reagents used in processing the sample are avoidable in NAA by employing only post-irradiation chemical manipulations of the sample. Following irradiation of the sample, the analyst does not have to be concerned with contamination by radioactively stable nuclides since the contaminants will not register on a radiation detection system.

Table 17. NAA detection limits[a]

Element	ppm	Element	ppm	Element	ppm
Al	0.0002	In	0.000005	Ru	0.0008
Sb	0.0014	I	0.00008	Sm	0.0008
As	0.0016	Ir	0.000001	Sc	0.00006
Ba	0.004	Fe	0.1	Se	0.00006
Bi	6	La	0.0004	Si	0.1
Br	0.00004	Pb	0.1	Ag	0.000006
Cd	0.01	Lu	0.00008	Na	0.001
Ca	0.01	Mn	0.00004	S	0.1
Ce	0.18	Mg	0.01	Sr	0.016
Cs	0.006	Hg	0.002	Ta	0.0012
Cl	0.0008	Mo	0.008	Te	0.004
Cr	0.02	Nd	0.006	Tb	0.002
Co	0.000006	Ni	0.04	Tl	0.004
Cu	0.001	Nb	0.00012	Th	0.00008
Dy	0.000001	Os	0.002	Tm	0.016
Er	0.008	Pd	0.002	Sn	0.014
Eu	0.00001	P	0.08	Ti	0.012
Gd	0.0012	Pt	0.0018	W	0.0018
Ga	0.002	K	0.04	U	0.0004
Ge	0.006	Pr	0.0012	V	0.000016
Au	0.0004	Re	0.0004	Yb	0.002
Hf	0.006	Rh	0.000001	Y	0.018
Ho	0.0004	Rb	0.004	Zn	0.0012
				Zr	0.1

[a]Data of Leddicotte (7).

The freedom from considerations of post-irradiation contamination suggests the second unique advantage of NAA. By adding relatively large amounts of the stable forms of radioactive elements found in the irradiated sample to the sample prior to chemical processing, the analyst has the capability of determining traces of elements while working with micro or semi-micro quantities. The larger quantities of material used for processing alleviates the difficulties associated with quantitatively reacting, separating, and recovering trace quantities. If necessary, corrections for the incomplete recovery of material can be calculated by using radioactive or stable carriers to determine the chemical yield for a particular step or procedure.

Interference-free sensitivities, such as those given in Table 17, can differ significantly from the sensitivities observed during a real analysis. Compton continuum or photopeak overlap can partially or completely obscure a small photopeak from a trace constituent. In addition, sensitivity is determined by both fixed nuclear parameters (e.g., half-life, cross section, and natural abundance), and by variables which differ considerably with each installation (e.g., neutron flux, detector efficiencies, and measurement geometries). If the half-lives of the analyte nuclides and the interfering activities are sufficiently different, a judicious choice of the durations

of the neutron irradiation and decay periods with detection by high resolution gamma-ray spectroscopy may allow quantitative analysis. For severe interferences or unfavorable nuclear characteristics, a chemical separation may be performed to remove the interferences.

Compton background due to 15-hr ^{24}Na is the principal detriment to NAA analysis of biological materials. The parent nuclide, ^{23}Na, has a large natural abundance, a high thermal neutron cross section, and is present at concentrations from 10^3 to 10^5 times greater than the trace elements of interest. These factors contribute to the production of large amounts of high energy gamma radiation. The resultant Compton background obscures the low energy regions of the gamma spectra, preventing the measurement of small amounts of radionuclides in many biological matrices. Yule (84) has published a list of instrumental detection limits for NAA in various matrices, including blood (Table 18). Note that according to Table 2, the amounts of trace elements which have been previously determined in blood are present at levels too low to be detected by NAA without first removing the sodium. Reference to the interference-free sensitivities (Table 17) indicates that combining chemical separations with NAA can result in considerably improved detection limits.

Table 18. Elemental detection limits in blood with instrumental NAA[a]

Element	ppm	Element	ppm	Element	ppm
Ag	0.6	Ho	0.4	Re	4
Al	---	I	4	Rh	4
As	4	In	0.1	Ru	2
Au	0.002	Ir	0.006	S	6000
Ba	12	K	1000	Sb	0.08
Br	---	Kr	21	Sc	0.04
Ca	200	La	0.05	Se	4
Cd	500	Lu	0.006	Si	500
Ce	2	Mg	800	Sm	0.04
Cl	---	Mn	0.9	Sn	300
Co	---	Mo	2	Sr	40
Cr	3	N	---	Ta	0.1
Cs	0.4	Na	---	Tb	0.2
Cu	40	Nb	1000	Te	3
Dy	0.06	Nd	6	Ti	200
Er	20	Ne	500	Tl	300,000
Eu	1	Ni	8000	Tm	2
F	30	Os	30	V	0.8
Fe	300	P	1000	W	2
Ga	300	Pb	200,000	Xe	8
Gd	60	Pd	3	Y	10
Ge	50	Pr	8	Yb	0.2
Hf	0.2	Pt	3	Zn	2000
Hg	20	Rb	7	Zr	20,000

[a]Data from Yule (84).

None of the previously considered methods meet all of the criteria for the ideal method (Table 19) in the analysis biological matrices. Undoubtedly, the most formidable prohibition to NAA is the cost. In situations where the extreme sensitivity, specificity, and multi-elemental capabilities are necessary, the applicability of the method largely offsets the cost. Thus, overall, NAA with chemical separations and high resolution gamma ray spectroscopy represents the method of choice for the trace elemental analysis of biological materials.

Table 19. Criteria for an ideal analytical method[a]

1.	Extremely sensitive for many elements.
2.	Highly specific, but capable of simultaneous multielemental determinations.
3.	Nondestructive for solids, liquids, and gases.
4.	Independent of matrix effects and chemical interferences.
5.	Free from contamination problems.
6.	Inexpensive.
7.	Simple to operate.
8.	Capable of automatic operation.
9.	Capable of giving absolute values independent of standards.
10.	Highly precise and accurate.
11.	Reasonably independent of analyst error.

[a]Zief and Mitchell (68, p. 241).

II. LITERATURE REVIEW

A. Destructive Neutron Activation Analysis

Of primary importance in the determination of elements at the trace level in biological samples is the prevention of sample contamination and the loss of sample components during processing. Analyses of trace elements *via* destructive neutron activation analysis (DNAA) are particularly susceptible to sample contamination and component losses during five specific stages of processing: 1) pre-irradiation treatment; 2) sample irradiation; 3) removal of the organic matrix; 4) removal of interfering radio-activities; and 5) concentration and separation of analyte activities.

Individual studies of the difficulties associated with DNAA have a tendency to concentrate on a specific area of processing, resulting in solutions which eliminate problems in that area without regard to the effect of that solution on other steps in the overall procedure. Thus, it is not unusual to find an author expounding on the benefits of using concentrated hydrochloric acid as an eluent during a sodium decontamination step, while another author dealing with the removal of organic matrices warns of expected losses if the sample is processes in concentrated hydrochloric acid. In developing a complete

procedure for the routine use of DNAA in the analysis of biological samples, an attempt to synthesize a viable procedure from some of the existing but incompatible "solutions" could be a valid approach. To this end, a review of the methods presently employed to circumvent problems associated with the individual stages in analyses of biological material by DNAA is appropriate.

B. Pre-Irradiation Treatment

1. Sample drying

In their natural state, biological materials which are generally of diagnostic value contain a significant amount of water (e.g., whole blood, plasma, urine, soft tissue). For pathological samples of different types, the amount of water entrained with the sample can vary with sampling and storage techniques, leading to difficulties in interpreting the results on the basis of "wet" weights. In addition, the thermal and radiolysis decomposition products generated by the effects of high temperature and large neutron and gamma ray fluxes on water can create pressures which make the irradiation of samples containing appreciable quantities of water particularly hazardous. The most obvious, and perhaps the most practical, approach is to remove the water from the sample.

Classically, the application of heat to cause evaporation has been used to remove gross amounts of water from a sample. However, the loss of elemental components, particularly volatile ones such as mercury and selenium, has been demonstrated for biological samples heated to moderate temperatures (85-87).

Lyophilization or freeze-drying - the removal of water by sublimation and evaporation at low temperature and pressure - has become the most popular method for drying biological samples (85-91). However, the suitability of freeze-drying to biological samples is subject to considerable debate. Most of the uncertainty in lyophilization arises with respect to the behavior of the more volatile components, particularly mercury, during processing. LaFleur (91) reported quantitative retention of organomercury compounds in biological materials following lyophilization, while Pillay et al. (85), and Fourie and Peisach (86) under similar conditions incurred substantial losses. Fourie also indicated the loss of selenium. Harrison et al. reported essentially quantitative recovery of all elements tested except inorganic mercury from aqueous solutions (87). On the other hand, Litman et al. (90) experienced up to 80% losses; Foldzinska and Zmijewski (89) obtained mixed results; and Friedman et al. (92) reported no loss of mercury from aqueous solutions during freeze-drying.

Several avenues for the reported loss of volatiles have been proposed. Most of the pathways designate vaporization, either directly due to inherent vapor pressure of the free element or an indigenous compound, or indirectly after the formation of a volatile component during processing as the principal mechanism for losses. The vapor pressure of some free elements (e.g., mercury, iodine, bromine, and selenium) and their respective compounds in a sample will be greater than the vapor partial pressure of the respective component in the environment of the sample during freeze-drying, and losses will occur through normal vaporization (85,87,91). Fourie and co-workers suggested that with real biological samples the loss of mercury and selenium is predominately through direct vaporization of organo complexes produced by physiological metabolism (86). The gross loss of mercury and possibly other elements through evaporation are also pursued by Litman et al. (90). However, in their model, the conditions of lyophilization lead to the reduction of the element to its zero oxidation state prior to vaporization. Calculations based on the Nernst equation support their model. MacKenzie, while not specific, indicates that some chemical reactions unlikely by thermodynamic considerations are possible in a lyophilized sample wherein finite water activities cannot be demonstrated (89). In his model, trapped residual water

or another species (notably oxygen) can react with nearest neighbors on a molecular basis, thus opening the possibilities for oxidation - reduction reactions which could lead to losses. In support of vaporization, Litman and co-workers have demonstrated that the primary loss of volatiles cannot be attributed to entrainment of those components in the withdrawn water vapor.

Whatever the mechanism for the elemental losses, a number of factors affecting experimental recoveries have emerged: 1) in "spiking" experiments, where tracers are added to a sample, inorganic and organo-metallic tracers behave differently. Typically, the lower volatility of the inorganic compounds usually results in higher recoveries (85,90,91); 2) the results of "spiking" experiments, utilizing either inorganic or organo-metallic compounds, cannot be extended to experiments employing physiologically incorporated tracers (86); 3) many parameters such as total ion content, concentration of a specific element, percent of water initially present, and acidity can influence the retention of components during freeze-drying (90,91); and 4) the presence of some functional groups can affect the behavior of an element in a matrix (89,91,92).

With respect to the final point, the presence of sulfhydryl groups as found in proteins correlated with improved recoveries of mercury from biological samples (89, 91). This explains in part the differing results of

"spiking" tracer experiments and biologically incorporated tracer experiments. Indications are that in a natural environment, an element will be contained in an organism in both metabolized and nonmetabolized (absorbed) forms, resulting in variable freeze-drying results. More importantly, the results suggest that samples of differing origins within an organism will behave differently during freeze-drying. Quantitative recoveries of mercury from blood but significant losses from urine samples may be explained in this manner.

In considering the merits of lyophilization, the analyst is in a difficult position. Most of the information concerning lyophilization was obtained through work with the volatiles mercury and selenium, and the results cannot necessarily be applied to work with the more refractory elements. Although some workers have included data for other elements, the variety of species used for incorporation, the different techniques of incorporation, and the number of matrices employed in the testing make the comparison of results from different experiments dubious. In the face of the discussion concerning lyophilization, the National Bureau of Standards insists that biological Standard Reference Materials should be lyophilized before use (93).

In order to fully exploit the attribute of neutron activation analysis which renders contamination by reagent

blanks negligible, no other operations are usually performed on the samples prior to irradiation.

2. Containment during irradiation

The choice of a proper vessel for the containment of biological samples during the irradiation phase of DNAA is critical for a successful analysis. First of all, the vessel must be able to contain the original sample and all radiolysis and thermal degradation products generated at the reactor operating temperature with large radiation fluxes. The environment within the reactor requires that the vessel material maintain structural integrity at temperatures in excess of 100°F in an integrated neutron flux of 10^{17} to 10^{18} neutrons per cm^2, which is approximately equivalent to a 15 hour irradiation at a neutron flux of 3×10^{13} neutrons per cm^2 per second. (The irradiation time and flux intensity are typical of those reported in the literature, and are quite reasonable parameters for the average research reactor.) Since some of the degradation products are gases, and relatively volatile components are known to be initially present in most samples, the irradiation vessel must also be impervious to gaseous diffusion.

The irradiation process drastically alters the sample, and a weighed aliquot of the irradiated residue

will in most cases not be representative of the original sample. Thus, for the purposes of DNAA, the ideal irradiation vessel should allow complete recovery of any material placed into it. Normally, charring of the organic material and recoil reaction from decaying nuclei cause the adherence or embedding of sample components into the walls of the irradiation container. The embedded materials are particularly resistant to ordinary decantation methods.

Traditionally, polyethylene has been a favorite material for constructing irradiation vessels (94,95). Though available in a variety of forms with a fairly low contamination blank, the polyethylene can be further "cleaned" by treatment with nitric acid (96). A number of authors have investigated losses of mercury during sample irradiation in plastic containers (94, 97-99). The recommendations for controlling losses during irradiation (i.e., addition of strong mineral acids, oxidizing agents, or hydrogen sulfide) suggest that the mechanism of loss involves vaporization of either a high volatility mercury compound or elemental mercury, followed by diffusion of that component through the plastic. Loss of halogens during irradiation has also been reported (100). At the Ames Laboratory, failure to quantitatively remove some nuclides

(e.g., mercury, arsenic, antimony) from polyethylene vials following irradiation has been experienced (101). Extensive structural damage and a concomitant loss of containment integrity have been experienced with polyethylene vessels when irradiated for 15 hrs in a neutron flux of approximately 3×10^{13} at an ambient reactor temperature of 130°F (102).

Some authors have recommended sealing biological samples into quartz ampoules (103-106). They have shown that quartz capsules are able to withstand the reactor environment during extended radiations if certain packing restrictions and considerations for shock during transport are followed. The elemental blank for natural quartz is generally too large for most applications, but a number of specially fabricated quartz materials, such as Quartex fused quartz and Spectrosil fused silica, with elemental blanks that are appreciably lower than either natural quartz or polyethylene, are commercially available and have been evaluated (106). Further reductions of the indigenous blanks of the synthetic quartz materials have also been accomplished by treatment with nitric acid and etching with hydrofluoric acid (106). Even after treatment, however, the levels of some elements in the synthetic materials are still comparable to the levels of these elements in blood serum.

With respect to the total removal of the sample from the irradiation vessel, quartz could represent a significant advantage over polyethylene. Since it has been shown that strong acid oxidizing conditions (i.e., the medium used for "cleaning" the irradiation capsules) can remove elemental components from both polyethylene and quartz, the conditions typically used for the destruction of organic matter in the later stage of a DNAA procedure could remove both the adhering charred organic matter and the recoil - embedded material from the irradiation containers. However, since polyethylene is an organic material, partial or complete destruction of the container would add a significant blank from the polyethylene to the sample. In the case of quartz, the blank contribution should be minimized since the bulk of the material will not be affected and any contamination will be limited to contributions from the surface. The surface contribution should be low if previously described clean-up procedures are employed. Of course, the extent of the surface contribution will have to be examined to ascertain its significance relative to the content of the sample.

C. Destruction of Organic Matter

Destructive neutron activation analysis implies that the bulk of the sample along with any components which may be detrimental to determination of the trace elements is removed prior to the measurement of the analyte species.

The mechanism of most chemically oriented separation processes requires the atoms to be present in an ionic form. Furthermore, the effectiveness of the separation procedures is dependent on the specific ionic states which are present. Thus, an analysis of a biological material for trace elements which may be incorporated into large organic complexes requires the destruction of the organic matrix, subsequently freeing the trace elements as ions. Methods for the destruction of organic material are categorized as either "wet" or "dry" ashing. Wet ashing, generally referred to as "digestion", typically requires an acid - oxidizing medium under reflux, whereas dry ashing usually requires only the addition of heat. Although the literature decisively favors wet digestion to dry ashing of biological samples, disagreement over the applicability of different wet digestion systems to biological materials is rampant.

1. Dry ashing

Dry ashing proceeds with the heating of a sample contained in either a porcelain or platinum crucible to a maximum temperature between 450 and 700°C for periods ranging from a few minutes to a couple of days. Under these conditions, the organic matrix is theoretically oxidized primarily to carbon dioxide and gaseous water, which are removed from the system leaving behind a mineral product which should be soluble in an appropriate solvent, typically

an aqueous acid. There is general agreement that such a procedure results in the loss of the more volatile components (i.e., arsenic, selenium, mercury, and halogens) from biological samples (107-109). Evidence suggests that for some elements, the volatile forms indigenous to the samples can be converted to less volatile mineralized forms via the application of ashing aids, such as magnesium nitrate and sulfuric acid, and low levels of heat (pre-ashing) prior to the imposition of the harsher conditions required for ashing (109-123). Pre-ashing has been accomplished through the use of a temperature programmed furnace and by starting with a cold furnace which allows a slow increase to ashing temperature (110-112). One of the most successful applications of the pre-ashing technique was reported by LeBlanc and Jackson who obtained quantitative recoveries of arsenic from fish tissue by pre-ashing with an infra-red lamp, followed by ashing at 500°C in a muffle furnace (112). Generally, high temperature dry ashing has been successful only in analyses for the more refractory elements or elements which form refractory compounds. Gorsuch has compiled an extensive collection of data in which elements at the trace level can be successfully determined after dry ashing (109,123).

Though the losses of trace elements due to volatility seem to have attracted the most scrutiny, Koirtyohann and

Hopkins reported that adherence of components to the ashing vessel was the most frequently encountered factor limiting quantitative recoveries (110). Gorsuch and Hamilton indicated that the extent of adherence-type losses was a function of age and history of the containers (108-109). Their work suggests that recovery of the embedded material was not possible.

Orvini et al. employed the volatility of specific elements during dry ashing as a means to separate trace components from organic matrices (114). At a final temperature of 1180°C, they were able to volatilize selenium, arsenic, zinc, cadmium, and mercury, and quantitatively collect the components downstream in a cold trap. This procedure should work for any elemental components of sufficient volatility, but the charred material remaining in the combustion vessel is not amenable to collection and further analysis of the less volatile components.

Low temperature dry ashing which utilizes the energy of an oxygen plasma excited by a radio-frequency generator offers one definite advantage over the standard dry ashing procedure: the ambient operating temperature of the plasma is typically less than 120°C. The lower ashing temperature should mitigate the loss of volatiles. Since Gleit and Holland (115) introduced the method with reported favorable results for the determination of volatile components in

spiked blood, a number of contradictory findings have been reported. Mulford has shown that the loss of arsenic and selenium from biological samples during low temperature dry ashing in a plasma system is a function of the applied radio-frequency power (116). Hamilton et al. referred to the low but consistent loss of seventeen elements from dried ox blood (108). Following a series of experiments with spiked and natural fish tissue, Pilly et al. pronounced low temperature ashing to be unsuitable for the preparation of fish samples for neutron activation analysis (85). To further confuse the problem, Lutz et al. have confirmed the original work of Gleit and Holland, indicating no appreciable losses of volatiles during low temperature ashing with an oxygen plasma (117).

Other difficulties with low temperature oxygen plasma ashing have been reported. Varcoe has stated that ashing times are quite long and that a large surface area of the sample must be exposed if ashing times are to remain practical (5, p. 52). One group of authors has implied that there is some difficulty in ensuring that all of the sample material is removed from the apparatus following ashing (117). In that work, the authors found it necessary to use "nonvolatile" tracers to determine sample yield from the ashing chamber. Hamilton et al. have reported that the plasma does not adequately penetrate some

samples (e.g., powdered bone) (118), which necessitates stirring the sample to ensure completeness of ashing operation. Overall, the literature suggests that low temperature plasma ashing can be of value for processing biological samples. However, the results are dependent on the particular matrix to which the process is applied.

2. Wet ashing

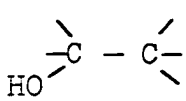
Wet ashing is the most commonly used method for the destruction of organic matter (109). Applicable at significantly lower temperatures than dry ashing and usually proceeding under reflux conditions, wet ashing or digestion is a natural choice whenever there is a possibility of loss of components through volatilization. Gorsuch has extensively investigated and reviewed wet digestions with respect to the loss of trace elements and its applicability to various matrices (107,109).

The majority of wet ashing methods are variations of the singular or mixed application of nitric, perchloric, and sulfuric acids to organic materials (107,109,113, 120-124). Special low temperature wet digestions requiring the addition of strong oxidizing agents to the basic three acid media have been reported. Malaiyandi and Barrette have described the advantages of lower digestion temperatures and shorter processing times gained by the addition of vanadium pentoxide as an oxidizer/catalyst to the

nitric - sulfuric acid digestion system (125-127). Other catalysts proposed for general use in variations of the three acid system include ammonium metavanadate (128), sodium molybdate (129), and chromic acid (130).

Since its introduction by Polley and Miller (131), 50% hydrogen peroxide has gained wide usage as an oxidizer in variations of the nitric - perchloric - sulfuric (NPS) acid digestion systems. Although originally employed as a secondary of "clean-up" oxidizer, Down and Gorsuch (132) investigated the feasibility of using 50% hydrogen peroxide as a primary oxidant for organic materials. In systems where volatile components are the object of the investigation, milder digestion conditions employing potassium permanganate have become popular (122, 133-135).

Some insight into the versatility of the basic NPS acid digestion system and its variants is contained in the currently accepted description of the wet ashing process. Shemyakin and Shchukina contend that most wet oxidations are oxidative-hydrolytic processes, with insertion of oxygenated substituents by the oxidizing agent serving to facilitate hydrolysis of the organic material (136). Studies with substituted compounds led them to conclude that the occurrence of general hydrolysis coincides with the existence or the potential existence of the grouping



The formation of this grouping from polarized double bonds is a function of the degree of polarization, and, hence, related to the nature of the substituents on the α and β carbon atoms. The presence of electron donating substituents on the α-carbon, and electron accepting substituents on the β-carbon greatly ease hydrolysis, where the presence of similar substituents on both carbons clearly neutralizes the effect. The use of the NPS acid mixtures as digestion medium accords well with these principles if the following points are considered: 1) sulfuric acid is a dehydrating agent which can readily produce double bonds; 2) nitro groups are strong electron acceptors; and 3) hydrogen peroxide readily introduces hydroxyl groups into many molecules.

Even after years of usage, there is still controversy in the literature regarding the loss of trace components from biological samples treated with combinations of the NPS acid-oxidation digestion system. The original procedure required refluxing the sample in a mixture of the three acids, followed by increased heating to drive off the nitric acid and to allow the stronger oxidizing properties of the perchloric acid to act on the sample. In some cases, intense heat was applied with only sulfuric acid to aid the destruction of the sample by charring the organics prior to introduction of the nitric and perchloric acids.

The following points have been identified as significant with respect to achieving quantitative retention of trace components during a wet digestion involving the NPS systems (107,109, 119-130): 1) the temperature for refluxing the acid mixtures (approx. 120°C) is sufficient to cause losses through the volatilization of some components. 2. Charring the organic materials in sulfuric results in the loss of material because of volatilization of components at the high charring temperatures (approx. 200°C), and through the reduction of some elemental components (e.g., selenium and mercury) to the more volatile zero oxidation state.
3. Halogens, even in the amounts present in analytical grade perchloric acid, should be excluded from the digestion medium due to the possibility of the formation of volatile halides. (Table 20 contains a listing of boiling points for some commonly occurring halides.) 4. With some samples, the amount of sulfuric acid should be limited to prevent the precipitation of insoluble sulfates and the subsequent loss of trace components through co-precipitation.
5. Perchloric acid in the presence of certain organic materials represents a definite explosion hazard. Charring of organic materials in the presence of perchloric acid and evaporation of perchloric acid mixtures to dryness should be avoided. It is recommended that perchloric acid should always be used in the presence of sulfuric acid to prevent accidental evaporation to dryness.

Table 20. Boiling points of halides which could form during wet ashing

Element	Halide	Boiling Point °C
Ti	$TiCl_4$	135.8
V	VCl_4	152
	$VOCl_3$	127
Cr	CrO_2Cl_2	117
Ga	Ga_2Cl_6	200
Ge	$GeCl_4$	83.1
As	$AsCl_3$	130
Sn	$SnCl_4$	113
Sb	$SbCl_3$	221
	$SbCl_5$	140

The many variants of the basic NPS acid digestion system evolved as analysts employed combinations to selectively avoid the particular factors listed above. Thus, Chernoff (123) used all three acids in a controlled temperature procedure to digest fish tissue; Isaac and Johnson (113), Feldman (121), and Abu-Samra (124) utilized only nitric and perchloric acids in a controlled temperature process for ashing various biological materials; and Malaiyandi and Barrette (125-127) employed nitric and sulfuric acids along with a catalyst to achieve destruction of biological matrices.

As different modifications on the NPS systems gained in popularity, disputes arose about the effectiveness of some of the milder digestion systems. For example, Portmann and Riley claimed that the use of sulfuric acid in digestion systems resulted in the loss of arsenic through volatilization (137). They suggested that nitric acid alone is sufficient for tissue samples, and would allow quantitative recovery of arsenic. On the other hand, Sach et al. claimed that a long period of fuming in sulfuric acid was necessary to destroy carbon-arsenic bonds. Furthermore, they demonstrated that arsenic was not lost in that environment (138). One point of agreement concerning the milder variants is that those systems not employing perchloric acid or a strong heating step will not achieve complete destruction of the lipids (125).

The sulfuric acid-hydrogen peroxide ashing system offers a method with a potentially low blank if pre-irradiation treatment is necessary, and is a procedure which does not require external heating (109). The products of the digestion medium should consist primarily of water and oxygen, which should not interfere with any further chemical processing. Also, in this system removal of excess oxidizer could be accomplished by refluxing the mixture. Gorsuch and Down examined the recovery of trace elements in the sulfuric acid-hydrogen peroxide system during the destruction of organic materials (132). They reported incomplete recovery of germanium, ruthenium, arsenic, and selenium. No one reason could be given to account for all observed losses. Germanium and ruthenium losses appeared to result from volatilization of the higher oxides, selenium apparently volatilized as the hydride and simple alkyls following charring of the organics in the sulfuric acid, and the loss of arsenic was attributed to the formation of high volatility chlorides. Since the reported losses appear to be matrix related, these results cannot be extended to different biological matrices.

The permanganate ashing systems have been developed to avoid volatilization problems, excessive oxidation conditions, and general hazards associated with NPS acid systems (130,132,133). In a typical procedure, the sample

is initially treated with only sulfuric acid, followed by extended treatment with an excess of potassium permanganate. This procedure has two disadvantages: 1) the method is limited to samples which will not precipitate sulfates; and 2) permanganate is not an effective oxidizer for large quantities of organic material (134,139, p. 840). To ensure complete digestion, some authors have employed potassium persulfate as a stronger oxidizer following the permanganate step (140). Even with the second oxidizer, permanganate digestions typically require significantly longer time periods than other wet digestion methods. There exists a genuine lack of data concerning the behavior of trace elements in these systems because they have been employed almost exclusively in determinations of mercury and selenium.

The acid pressure bomb has not been extensively used for the digestion of biological materials; however, its basic operation indicates that it could perform adequately. Losses of high volatility components should be minimized since the digestions occur in an impermeable container which can be cooled to condense out the volatiles prior to opening (68, p. 165). A decrease by a factor of eight in the time necessary for acid pressure bomb vs. NPS digestion of fish tissue has been reported (68, p. 165). Also, quantitative recovery of most cations has been reported

(141). These digestions are usually performed prior to irradiation, thus requiring ultra-pure reagents to avoid contamination.

The advantages of faster destruction, freedom from gross losses of trace elements, and relative simplicity of equipment indicated that wet digestions are probably the most effective means for the destruction of organic matrices. The extensive data already available, and the versatility of the system, suggest that a variant of the NPS acid-oxidizer system can be developed to handle a number of different matrices prior to follow-up chemical processing as part of an overall procedure for DNAA analysis of biological materials.

D. Decontamination and Concentration

The advantages, represented as higher sensitivities and lower detection limits, of DNAA over instrumental neutron activation analysis (INAA) are derived from the capability to remove contaminating activities and to concentrate the analyte activities prior to assay by gamma ray spectroscopy. In the analysis of biological materials by DNAA and INAA, the primary sources of radioactivities which interfere with gamma ray spectroscopy are the radioactive isotopes of sodium, bromine, phosphorus, and potassium. The occurrence of ^{24}Na in irradiated samples

poses the most serious detriment to accurate, precise spectroscopic assay. The large natural abundance, sizable cross-section for thermal neutron capture, and pervasiveness of stable sodium (^{23}Na) in biological systems, coupled with the moderate half-life of ^{24}Na, cause the gamma peaks associated with ^{24}Na to be the major activities present following irradiations under all but the most extreme conditions.

The principal gamma rays of ^{24}Na have energies of 2753 and 1368 KeV. In gamma ray spectroscopy, the gamma ray is "detected" as the absorption of its energy by the detector material, which is lithium drifted germanium for high precision work. However, the absorption of the gamma ray energy is a multiple event process wherein a portion of the total gamma ray energy is absorbed with each interaction with the detector until the total energy available is absorbed or the remnant gamma ray escapes from the detector. If all of the interactions generated by the entrance of a single gamma into the detector occur within the detector, the gamma ray will be sensed as a photopeak at an energy represented by the sum of the energies absorbed in each interaction. Alternatively, if some of the secondary radiation escapes from the detector, the energy imparted by the partial absorptions of the gamma ray will register as distinct gamma ray peaks. Since the absorption

processes are not quantized, and all absorptions occurring within the detector crystal are summed, the virtual photopeaks can occur at all energies below the energy of the full or total gamma peak. As the intensity of the true photopeak increases, the virtual peaks will be manifested as a high background extending up to the full energy peak, but will be particularly evident on the low energy side of the photopeak as a severe distortion of the theoretical Gaussian shape of that photopeak. This background, called Compton background, can obscure lower intensity analytical peaks, and can cause distortions in the shapes of the photopeaks. As the distortion of the peak shape from the idealized gaussian function increases, so does the inaccuracy in the peak areas as determined by peak fitting computer programs.

Radioactive bromine, the second most observable component in the gamma spectra of irradiated biological materials following moderate irradiation periods, produces the same type of Compton interference as ^{24}Na. In addition, the primary activity, ^{82}Br, has approximately 18 gamma photopeaks which increases the possibility that a bromine photopeak will interfere with or obscure a gamma peak of analytical interest.

Unlike sodium and bromine, the principle activity produced by the irradiation of stable phosphorus, ^{32}P,

decays as beta particles (negatrons) rather than by gamma emission. The negatrons usually do not reach the detector because they are absorbed by air or by polyethylene absorbers placed expressly for this purpose before the detectors. However, the capture of the negatron by the absorber releases electromagnetic radiation, bremsstrahlung, which can reach the detector. The release of negatrons by decaying atoms, the absorption of negatrons by the absorber and the subsequent release of radiation, and the final absorption of the bremsstrahlung by the detector are not discrete processes. Consequently, the bremsstrahlung radiation which reaches the detector will appear as a background superimposed over the gamma ray spectrum for the sample. The background theoretically extends up to 1.71 MeV, the maximum energy possessed by a negatron emitted by a decaying atom of ^{32}P, but is typically most apparent as a maximum below 400 KeV. Beside the quantities of stable phosphorus present in biological materials, ^{32}P can also be produced by the interaction of fast neutrons with stable forms of chlorine and sulfur.

Irradiation of potassium by thermal neutrons produces ^{42}K, which is a gamma emitter. Consequently, the same type of interference as produced by ^{24}Na and ^{82}Br is encountered. It is interesting to note that the interferences from potassium activities are generally not noticeable in

irradiated biological samples until after removal of the sodium.

Successful analyses of biological materials have been accomplished without chemical separation of the interfering elements (142). The half-lives of the spectral contaminants, ^{24}Na and ^{42}K, are sufficiently short (i.e., 15 and 12.4 hours, respectively) that an appropriate delay before spectroscopic assay can effectively eliminate interferences associated with these components. However, during the delay, the radiation intensity from analyte species having half-lives comparable or less than the contaminants will also diminish. In effect, information on a number of biologically important elements (e.g., copper, molybdenum and arsenic) can be lost. The half-lives of the bromine and phosphorus contaminants (35 hours and 14.3 days, respectively) are too long for short decay periods to be effective.

1. Sodium and potassium decontamination

Sodium was recognized early as a detriment to analyses by nonnuclear techniques, and many procedures have been developed for its separation from various matrices. The earliest sodium decontamination procedures employed precipitation or crystallization as isolation methods. In 1927, Kolthoff reported the reduction of sodium content in mineral samples to 10^{-3} moles/liter by precipitating the

sodium with uranylacetate (143). The radioactivity from the fissioning uranium which remained in the solution made the procedure incompatible with assay by gamma ray spectroscopy. Ricq successfully precipitated sodium as sodium chloride by the use of concentrated hydrochloric acid (144). The method was designed for removing sodium from samples which had been previously processed by fusion techniques (i.e., with sodium carbonate, caustic soda, or sodium peroxide), and does not effectively remove less than milligram quantities. Menon and Wainerdi also precipitated sodium in the chloride form, but did so from a medium of hydrochloric acid and butanol. According to Torok and Diehl, the procedure must be performed repeatedly on a sample in order to achieve the necessary efficiency (145). Banks and Richards and Bock-Werthmann precipitated sodium in the form of organic salts of α-methoxyphenylacetic acid and S-benzamino-anthraquinone-sulphonic acid (146,147). In the latter case, reduction of the sodium concentration to 10^{-6} moles/liter was achieved.

The precipitation methods suffer from the disadvantage of co-precipitation of trace components. In his precipitation procedure, Bock-Werthmann reported that precautions are taken to avoid co-precipitation of trace elements of interest (147). The organic medium used in several of the precipitation procedures also presents difficulties if further separation or concentration methods are required.

This is particularly critical because precipitation methods always dilute the sample during processing requiring, in some cases, removal of the excess solvent prior to assay.

Ion exchange in various forms has been exploited in sodium decontamination processes. In 1963, Spronk reported a low sensitivity anion exchange procedure for sodium removal (148). Tera et al. used precipitation ion exchange in concentrated hydrochloric acid to remove sodium in the chloride form (149,150). The high salt concentration suppressed the distribution coefficients of the trace elements effectively, avoiding co-precipitation. Morrison has referred to another procedure by Tera which removed radioactive sodium by isotopic exchange on a cation exchange column which had been saturated with stable sodium (147). Tang and Maletskos first reported the isotopic separation of radio-sodium (^{24}Na) on a column of finely divided sodium chloride in an acetone-hydrochloric acid medium (151). Bowen and Cook later confirmed this work and extended the procedure to use columns of sodium perchlorate (152). The latter work pointed out the sensitivity of the method to the conditions of the column and to the preparatory history of the salt forming the column. Bowen also demonstrated that other elements in addition to sodium were susceptible to absorption on the salt columns.

At first glance, hydrated antimony pentaoxide (HAP) seems to possess ideal characteristics for inclusion in a procedure for removing sodium from biological samples following an acid-oxidation digestion. While surveying precipitates of various oxides, Girardi and Sabbioni found that HAP could selectively and quantitatively remove sodium from solutions which were 12M with respect to hydrochloric acid (153). Of the 60 elements studied for absorption, tantalum was the only other element found to be quantitatively absorbed from the 12M hydrochloric acid solutions. Other reported properties which favored the use of HAP for sodium decontamination include: 1) a batch equilibrium total capacity for sodium of 30 mg/g-HAP; 2) a column breakthrough capacity for sodium of 12 - 24 mg/g-HAP; and 3) a column equilibrium sodium decontamination factor of 10^{10} from 12M hydrochloric acid.

Although a specific mechanism of operation for HAP was not given, the authors did identify a number of favorable factors with respect to the mechanism. 1) The sodium was irreversibly bonded to HAP. Rinses with more eluents and other alkali metals could not displace the sodium. 2) High loading of the columns did not change the distribution coefficient of sodium, indicating that any amount of sodium could be removed as long as enough HAP was present. 3) The adsorption of sodium by a specific amount of HAP reached a

plateau which did not increase with time. This indicated that the absorption of sodium was a surface process and the sodium did not diffuse into the bulk of the material.

Since the initial report, HAP has been extensively studied, and inconsistencies and disputations abound (145, 153-161). Krishnan and Crapper, while trying to extend the work of Girardi and Sabbioni to different concentrations of hydrochloric acid and different acids, found that scandium, silver, and potassium were also adsorbed to a limited extent from 12M hydrochloric acid solutions (156). The partial adsorption of scandium on HAP was also supported by Gills et al. (154). In direct contradiction to Girardi and Sabbioni is a report which stated that sodium could be eluted from HAP with either nitric acid or ammonium nitrate (155). Konecny and Hartl reported a significantly lower selectivity of HAP for sodium over the other alkali metals (158). The high capacity of HAP for sodium has been verified by Caletka et al. (155), but has been contradicted by Baestle and Huys (159), and Ralston and Sato (157). Baestle and Huys have proposed and shown experimentally that, based on a structure which they determined for HAP and on their mechanism for its operation, the theoretical maximum for adsorption of sodium on HAP is 5.05 mg/g-HAP. Ralston and Sato reported that with treatment by HAP in hydrochloric acid, they were only able to reduce the sodium

content of real samples by factors ranging from 0.013 for rocks to 3.8×10^{-5} for protein samples.

Similar disagreements are apparent over the mechanism which accounts for the selectivity of HAP. As mentioned previously, Girardi and Sabbioni in their original work did not propose a mechanism for operation of HAP (153), but they did produce experimental results which ruled out simple ion exchange, counter-ion exchange, and isotopic exchange as the singular mode of operation. However, Konecny and Hartl, and Caletka et al. then proceeded to propose explanations for the properties of HAP based solely on ion exchange phenomena (155,158). Their results were only partially successful in describing the behavior of ions on HAP columns. Baestle and Huys showed that the behavior of ions which were not particularly susceptible to absorption on HAP could be accounted for on the basis of an ion exchange mechanism (159). Nagy et al. although not proposing a mechanism, demonstrated that an ion exchange mechanism was valid for absorptions from low acidity solutions but invalid at high acidities (i.e., 12M HCl) (160).

The present state of knowledge of the properties and operation of HAP is probably not as bad as a cursory glance at the literature seems to indicate. One reason for the apparent contradictions is that many of the experiments and, therefore, findings are not directly comparable. The

difficulty in comparing arises because the authors were probably not working with the same materials. The HAP used in the original work by Girardi and Sabbioni was produced by Carlo Erba of Milan, Italy, via a proprietary process (153). As reported, the synthesis involved the hydrolysis of antimony pentachloride. The studies done by Caletka, Konecny and co-workers used "HAP" synthesized by the hydrolysis of hexachloroantimonic acid (155,158,161). Baestle and Huys produced "HAP" by three different methods, each having the same apparent structure but vastly different absorption properties for sodium in nitric acid (159). Torok and Diehl always used antimony(III) oxide dissolved in aqua regia as the starting material in the synthesis of "HAP" (145). They also showed that variations in the synthesis parameters, such as time for hydrolysis, and time and temperature of drying, would produce chemically similar products with significantly different absorption properties. Even those researchers who had access to HAP prepared by Carlo Erba reported inhomogeneities and varied results with different batches (145,154,156,157). Though there is some ambiguity with respect to overall selectivity, capacity, and mechanism of operation, not one author disputed the fact that HAP quantitatively removed sodium from all of the acid systems which were tested.

After the removal of radio-sodium, researchers realized that radio-potassium also contributed significant interference to assays by gamma ray spectroscopy. For this reason, methods for potassium removal first appeared as side products of sodium removal procedures. The previously described method of Tang and Maletkos, utilizing finely divided sodium chloride and an organic eluent (acetone-HCl) for the removal of sodium was adaptable to the removal of potassium (151). The disadvantages of an organic solvent and the sensitivity of the columns to preparation methods were still operative with the lower acid concentrations needed for potassium separations. In the same paper where they disclosed the properties of HAP for sodium decontamination, Girardi and Sabbioni demonstrated that potassium could also be removed from acid solutions if the concentration of the acid was decreased (153). At the reduced acid molarities a marked decrease in the selectivity of HAP was encountered.

Massart studied a number of inorganic exchangers for use as a stationary phase in the removal of radio-potassium from acid solutions (162). Both potassium phosphomolybdate (PPM) and potassium phosphotungstate (PPT) removed potassium from acid solutions. The latter material, because of its superior characteristics for preparing columns, was chosen for more intensive study. Radio-tracer

studies showed that sodium, arsenic, and selenium were absorbed to varying extents with potassium on PPT from 9M hydrochloric acid (162). A critical disadvantage of PPT for routine applications is the small grain size of the precipitate, which prevents an adequate flow through columns without a pressurized setup.

Titanium phosphate (TIP) is another inorganic exchanger used in the removal of radio-potassium from digested biological samples (163,164). Nagy et al. have provided a basis for the synthesis of TIP with an enumeration of the many factors which must be controlled during synthesis (164). The actual synthesis parameters for the optimum material are not given. Considerable care must be exercised to ensure repeated synthesis of the same product. Although no information is provided concerning the selectivity and capacity of TIP for potassium, a scheme employing the material for the removal of potassium from digested biological samples was presented (163,164).

2. Phosphorus decontamination

Following acid digestion of biological materials under oxidizing conditions, phosphorus is usually present as the phosphate anion (165). The methods which have been employed for the removal of phosphate concentrated on using inorganic exchangers. Tin dioxide (TDO), hydrated manganese dioxide (HMD), acid aluminum oxide (AAO), and zirconium phosphate

(ZRP) have been studied for selectivity, capacity, and decontamination factors in the removal of phosphate from acid solutions (163-167). Following studies of TDO, HMD, and AAO, in various eluents, Sabbioni et al. chose AAO as the most promising material for phosphate decontamination (165). TDO was eliminated because of a lower overall capacity for potassium, and HMD was effective only in perchloric acid solutions. The properties of AAO were evaluated in acid molarities up to 7M, which is considerably below the level at which HAP has been found to be most effective for selective removal of sodium. This indicates a need for evaluation of AAO at higher acid concentrations prior to it coupling with a HAP column in a combined sodium and phosphorus decontamination step. Particularly detrimental to the potential coupling of HAP and AAO is the inability of AAO to absorb phosphorus from solutions of hydrochloric acid, which is the best eluent for the operation of a HAP column (165). AAO has also been shown to absorb arsenic, molybdenum, tungsten and antimony at all molarities tested, necessitating the determination of yield factors or a different method for those elements (165,167).

Most of the work involving zirconium phosphate (ZRP) has been detailed by Nagy, Torok, and co-workers (163,164). Girardi et al. have published data concerning the selectivity of ZRP for other elements (167). The synthesis

procedure detailed by Nagy et al. is typical of methods for the production of hydrated amorphous precipitates. The properties of the resulting material are dependent on the synthesis conditions to the extent that the consistent production of a satisfactory product becomes an art (164). As with AAO, the characteristics of ZRP have been reported for solutions up to 7M in acid, and the absorption of trace elements by ZRP is clearly a function of the acid molarity of the solution (167).

3. Extraction decontamination methods

The basic philosophy in the application of extraction chromatography to the problem of removing spectral interferences from irradiated biological samples is just the opposite of the approach followed in those procedures employing inorganic exchangers. The objective with inorganic exchangers was to develop a material which would selectively remove the interfering species from the sample solution prior to assay by gamma ray spectroscopy. With extraction chromatography, the decontamination is usually based on the removal or extraction of an element or, more likely, a group of elements from the digested sample, leaving behind the spectral contaminants (168-176). As such, on an operation basis, column absorption of contaminants on inorganic exchangers would be more efficient than extraction methods. Fewer operations on the columns would

be necessary to obtain data on a maximum number of elements. Solvent extraction techniques are rarely quantitative, and are typically effective only within a narrow range of solution parameters. For this reason, yield determinations for the extraction steps must be made. The conditions for solvent extractions employing organic complexing agents with narrow functional pH ranges are incompatible with conditions following an acid digestion step, which requires drastic alteration of the sample prior to extraction with the inherent possibilities for losses and contamination.

4. Column chromatography

Ion exchange techniques based on column equilibria have been utilized for separating spectrally interfering activities from digested biological samples (177). Decontamination factors of greater than 1000 for the radioactive forms of sodium, potassium and phosphorus have been reported. Similar to solvent extraction techniques, the procedures have concentrated on conditions which would remove the components of interest from the digested solutions, allowing the interferents to be eluted away. Unlike solvent extractions, the isolation process concentrates the analyte species which are separated. Further separations of the isolated species are usually achieved by simply altering the eluting solution.

Individual decontamination processes for specific components are practically useless if conditions cannot be arranged so that a number of the procedures can be applied to a sample without drastic changes in conditions between applications. In their survey of the literature of the 15 years following 1960 concerning radiochemical separations, Girardi and Pietra noted that approximately 70 separation schemes had been published (178). Of those schemes which were reported for specific application to biological materials, some showed apparent attempts at compatibility between processing operations, while in others, drastic changes in the samples were accommodated in order to use a specific separation technique (179-186). One of the simplest schemes involved the determination of 11 elements as three groups and required a distillation step, a drying-off step, and three columns, HAP and two anion exchangers (179,180). A point of dispute concerning this scheme is the low hydrochloric acid concentration (7M) used on the HAP column, which does not guarantee the high selectivity for the elements as indicated by the authors (153,156, 167). One of the more complicated schemes requires an evaporation step, a drying step, five columns, and 15 eluent changes for the determination of 40 elements as 13 groups. Points of disputation concern the claim of quantitative recovery of selenium and arsenic on HAP columns (156), and

the reliability of quantitative recoveries in the drying and evaporation steps, especially since the evaporation step occurs in the presence of halides, a condition previously noted for the loss of trace elemental components (107, p. 143). Overall, the separation schemes appear to be overly complicated, even though most were intended for automation. The number and nature of the required operations are detrimental to consistent quantitative recoveries (187). The degree of separation attempted in most schemes is more than conceptually necessary for obtaining diagnostic information from a biological specimen. Based on the available literature data, simpler, more concise schemes which would provide adequate decontamination and separations should be possible.

III. EXPERIMENTAL

A. Physical Facilities and Equipment

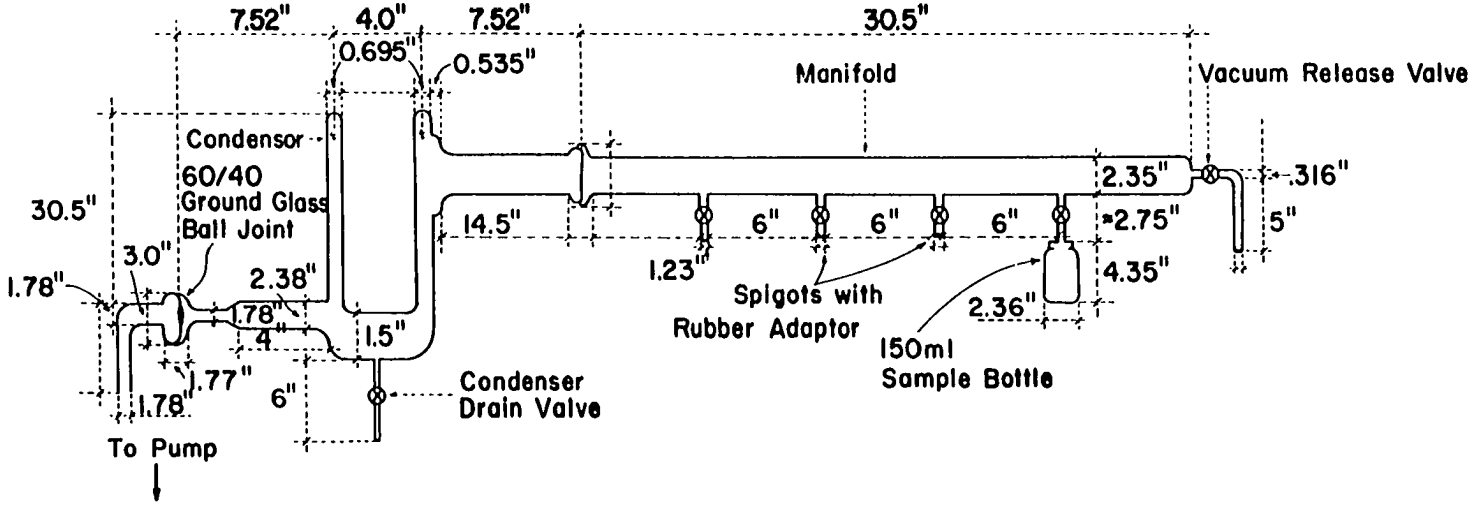
1. Nuclear reactor

All irradiations were accomplished in the Ames Laboratory Research Reactor (ALRR), Ames, Iowa. The ALRR is administered by Iowa State University under contract with the Department of Energy. It is a large scale enriched uranium-heavy water reactor designed for operation at power levels in excess of 10 megawatts (MW) but normally operated at 5 MW. Two pneumatic tube transfer systems called R3 and R5 were used for long and short irradiations, respectively. Both transfer systems approach the same high neutron flux area immediately below the reactor core from opposite directions. The most recent measurements of the radiation profiles at the R3 and R5 positions while operating at 5 MW indicated the following: a thermal neutron flux in R3 of 2.8×10^{13} neutrons/cm^2/sec, a thermal neutron flux in R5 of 2.3×10^{13} neutrons/cm^2/sec, cadmium ratio in both R3 and R5 between 19 and 20 to 1, and a gamma dose rate at both positions of approximately 6.5×10^5 R/Hr.

2. Freeze-dryer

The freeze drying apparatus (Figure 1) consisted of a Pyrex glass manifold (fabricated by the Ames Laboratory Glass Shop) with ports for the attachment of four sample

Figure 1. Freeze-dryer


7.52"
4.0"
7.52"
30.5"
0.695"
0.535"
Manifold
Vacuum Release Valve
Condensor
60/40
Ground Glass
Ball Joint
30.5"
3.0"
1.78"
2.38"
1.78"
4"
1.5"
14.5"
1.23"
6"
6"
6"
2.35"
.316"
2.75"
5"
4.35"
Spigots with
Rubber Adaptor
2.36"
150ml
Sample Bottle
1.77"
1.78"
6"
Condenser
Drain Valve
To Pump

bottles, a commercially available condenser (Kontes Glass Co., Vineland, New Jersey), and a Duo-Seal Single Stage Vacuum Pump (W. M. Welch Manufacturing Co., Chicago, Illinois). The condenser has a volume of approximately 0.5 liters, and can be used with either liquid nitrogen or Dry Ice-solvent mixtures. The commercially available sample bottles are 150-200 milliliters in volume, and are supplied with rubber O-ring fitted caps for an air-tight seal. Each cap has a replaceable fritted glass filter in the opening between the bottle and the manifold spigot.

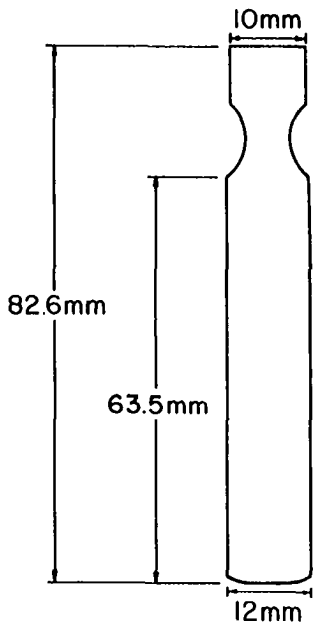
3. Capsulation

The system shown in Figure 2 was developed for sealing a biological sample into a quartz vial without thermal decomposition of the sample from the high temperature used in softening the quartz. The liquid nitrogen reservoir is a commercially available 750-ml Dewar Flask, capped with a shaped styrofoam plug which is protected from heat by a covering of household aluminum foil. The cold-finger which extends through the center of the styrofoam plug is a solid piece of brass which was machined to specifications in the ALRR Machine Shop. A styrofoam cup is provided as a support for the cold-finger and as protection for the glass walls of the dewar.

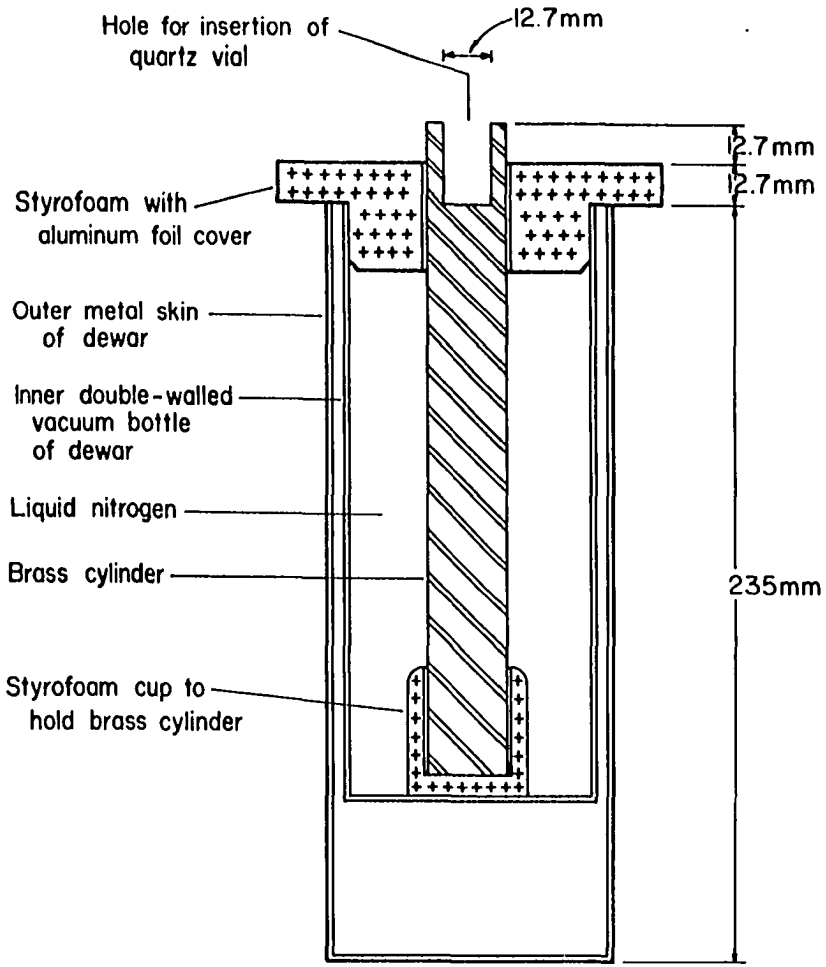
The quartz vials (Figure 2a) used for containment of samples during irradiation were fabricated in the Ames

Figure 2. System for the capsulation of heat sensitive biological materials

a) Quartz vial for containment of the sample
b) Cold-finger device used during sealing of the quartz sample vials



A) QUARTZ VIAL



B) SEALING DEWAR

Laboratory Glass Shop from Supercil CFQ T21 Tubing (Amercil: Fused Quartz and Silica, Hillside, New Jersey). The original tubing possessed an outer diameter of 12 mm and an inner diameter of 10 mm.

4. Wet-ashing apparatus

During the course of this work, two wet-ashing devices were employed. The first (Figure 3) was a modification purposed by Gorsuch (113) of an original design by Bethge (188). The second device (Figure 4) represents a further modification of the Bethge device which should ensure against the escape of volatile components from the system. The primary modifications are the introduction of a thistle section between the reaction flask and the air condenser for easier introduction of reagents during a digestion, and the inclusion of the liquid nitrogen cold-finger condenser atop the air condenser. Included in the air condenser of both devices is a three-way stopcock which allows for a cyclic reflux-distillation mode of operation.

5. Support columns

The support columns (Figure 5) for chromatographic studies were fabricated in the Ames Laboratory Glass Shop. The reservoir of each column was formed from a 24/40 ground glass (g.g.) outer joint, and had a maximum volume of 30 ml. The column section was formed from standard wall Pyrex tubing with an outer diameter of 12 mm.

Figure 3. Bethge apparatus as modified by Gorsuch for the controlled destruction of organic materials

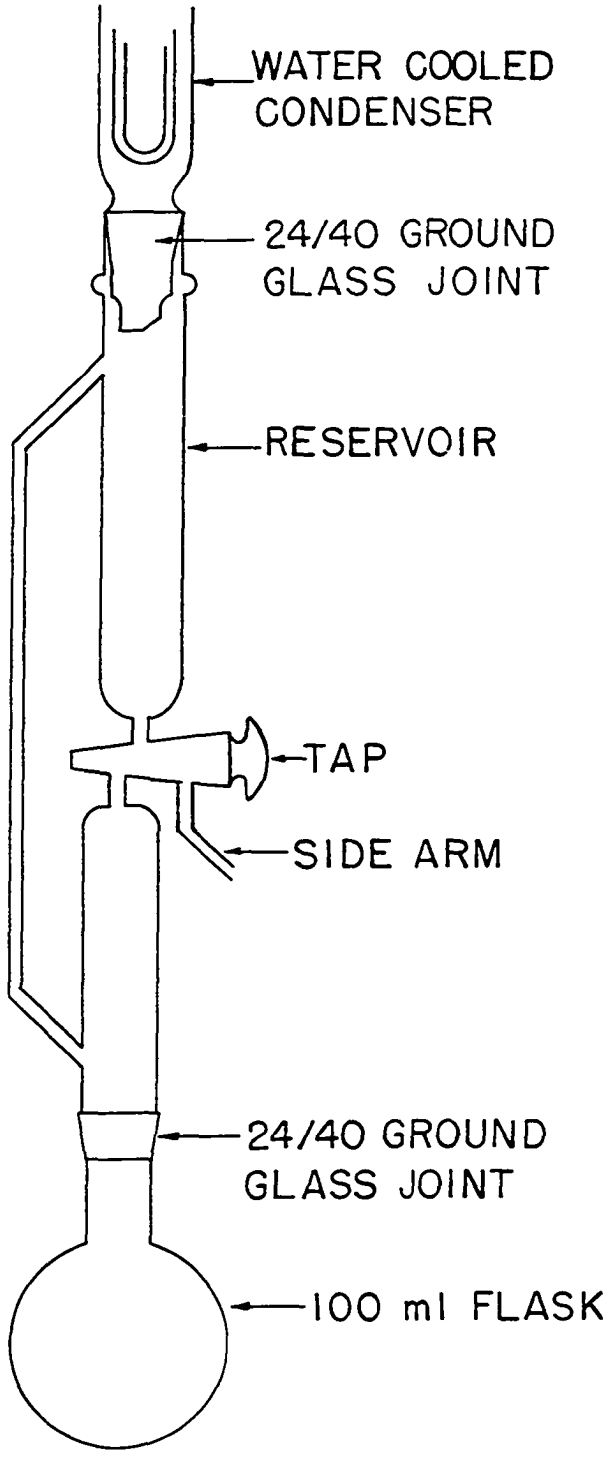

WATER COOLED
CONDENSER
24/40 GROUND
GLASS JOINT
RESERVOIR
TAP
SIDE ARM
24/40 GROUND
GLASS JOINT
100 ml FLASK

Figure 4. Gorsuch apparatus as modified to enhance the retention of trace components and to facilitate the introduction of reagents during the "wet-ashing" of organic matrices

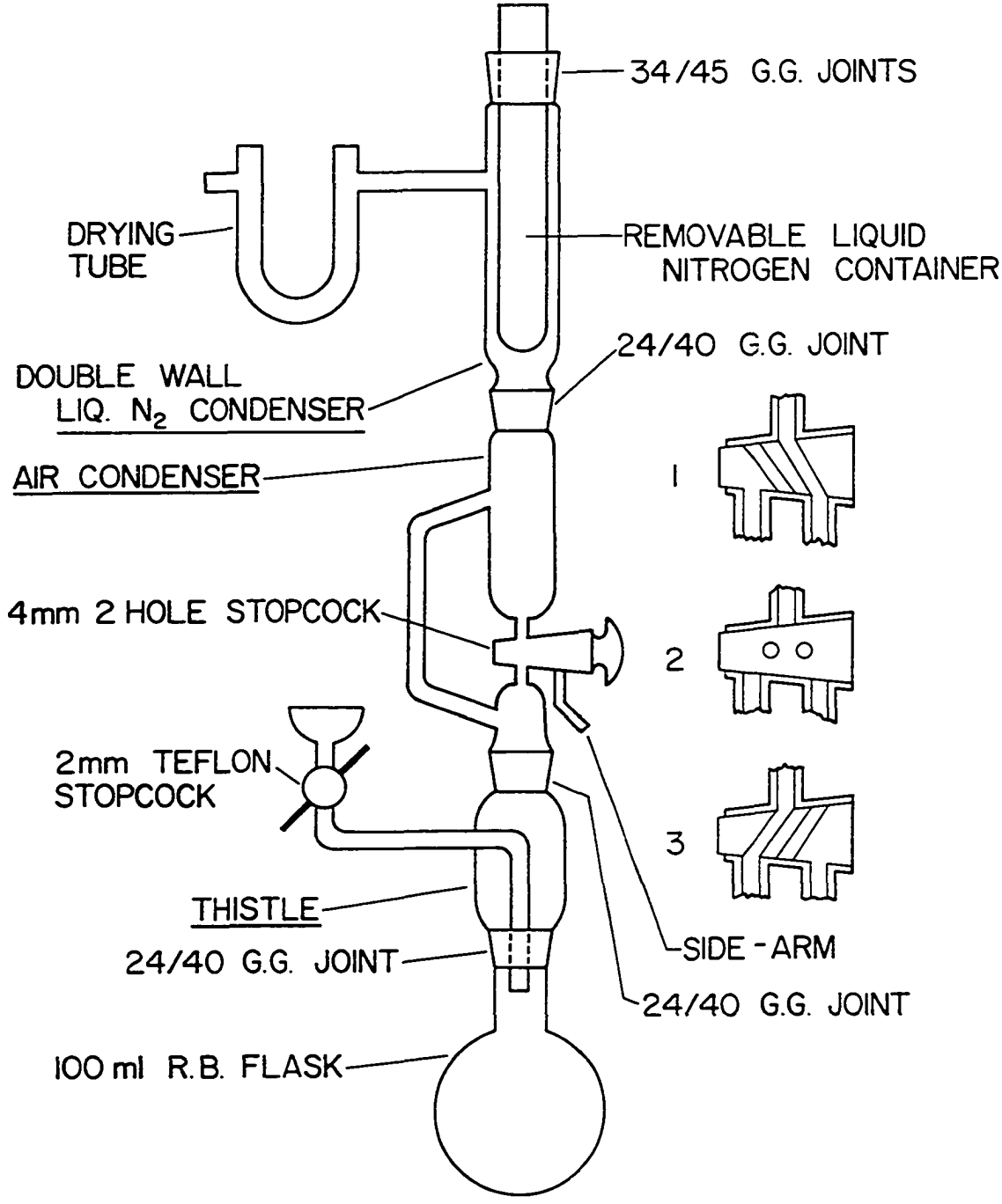
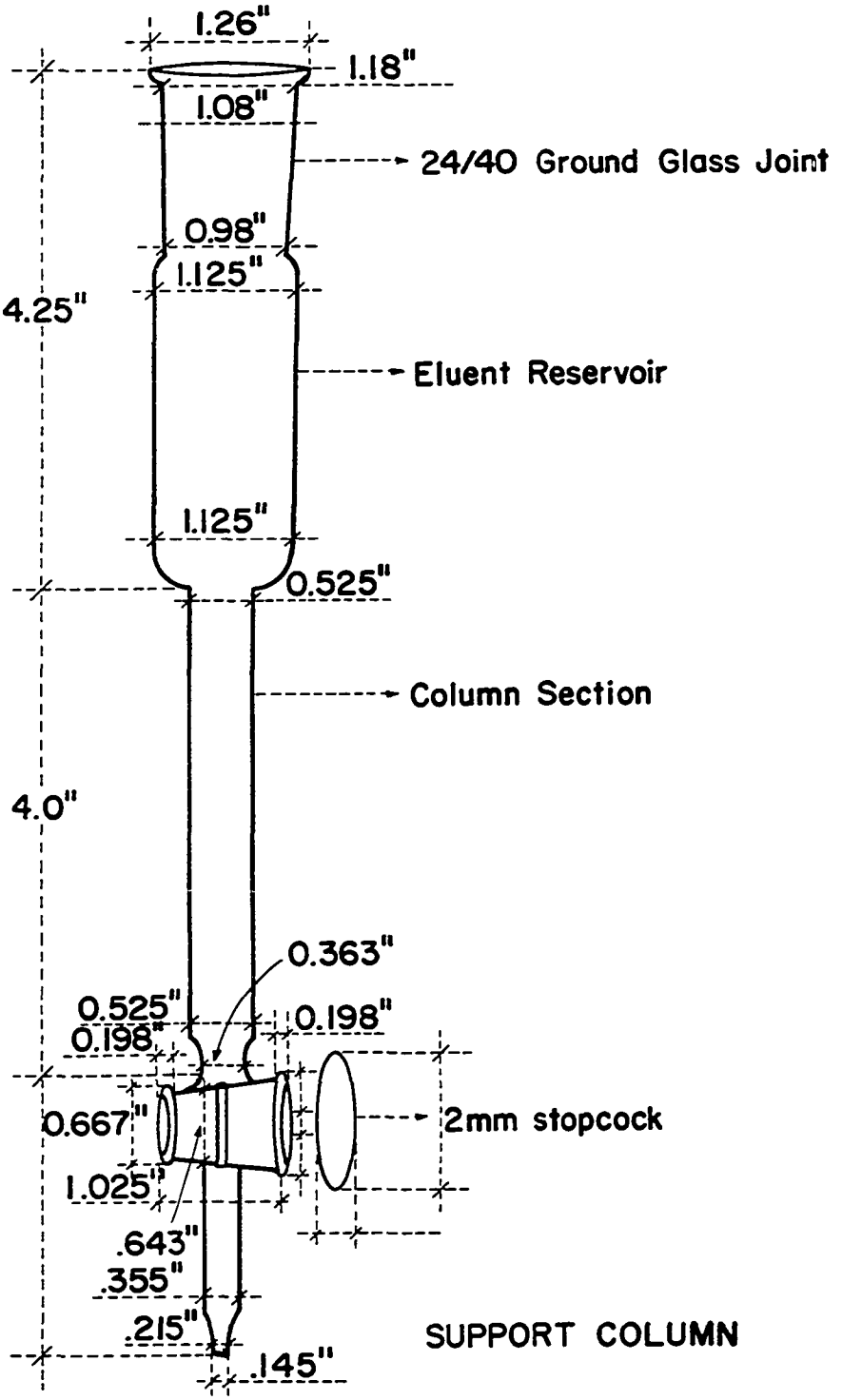

34/45 G.G. JOINTS
DRYING TUBE
REMOVABLE LIQUID NITROGEN CONTAINER
24/40 G.G. JOINT
DOUBLE WALL LIQ. N_2 CONDENSER
AIR CONDENSER
1
4mm 2 HOLE STOPCOCK
2
2mm TEFLON STOPCOCK
3
THISTLE
SIDE-ARM
24/40 G.G. JOINT
24/40 G.G. JOINT
100 ml R.B. FLASK

Figure 5. Chromatographic support column


1.26"
1.18"
1.08"
24/40 Ground Glass Joint
0.98"
1.125"
4.25"
Eluent Reservoir
1.125"
0.525"
Column Section
4.0"
0.363"
0.525"
0.198"
0.198"
0.667"
2mm stopcock
1.025"
.643"
.355"
.215"
.145"
SUPPORT COLUMN

6. Ovens

Experimental requirements necessitated modification of a commercially available muffle furnace and a convective drying oven. For more stable temperature control, a Blue M Electric Furnace (Blue M Electric Co., Chicago, Illinois) was operated with the time controller of the heater out of the circuit, and with the heater temperature control in the "High" position. The current to the heaters and therefore the temperature of the oven was controlled with a Type 136 Powerstat Variable Autotransformer (The Superior Electric Co., Bristol, Connecticut). This system was used to obtain temperatures in excess of 400°C.

An 850 watt, convection-type Sargent Drying Oven (E. H. Sargent and Co., Chicago, Illinois) was modified for forced air drying of samples at temperatures approaching 400°C. The modifications were as follows: 1) the heated volume of the oven was restricted by placing a sheet of asbestos on an appropriately positioned shelf within the oven. The asbestos sheet was cut to allow circulation of air. 2) The forced air was obtained from a laboratory air spigot equipped with moisture and particulate filters. Rubber tubing coupled the air jet to a glass tube which was inserted through a partially blocked vent atop the oven. The tube extended into the oven cavity through the asbestos sheet, and into a region below the heating coils. 3) The

thermostat control of the oven was bypassed and a Powerstat Variable Autotransformer was connected for temperature control. The air flow rate was restricted to approximately 30 liters per minute.

7. Reagents

Commercially available reagents and chemicals were used without further purification in the production of carrier solutions. Most irradiation standards were produced from special high purity metals refined at the Ames Laboratory. If the high purity form of an element was not available from the laboratory, a commercially available electronic or analytical grade was used without further purification.

High purity water was obtained by passing the effluent of the laboratory deionized water taps through a Barnstead Bantam Demineralizer equipped with a bed of high capacity mixed ion-exchange resins. Only water which demonstrated a specific resistance greater than 10^6 ohms/cm^3 as measured with a Barnstead direct reading conductivity meter was used for making standards and for special cleaning purposes.

8. Biological samples

Two biological standards were used throughout this work: NBS Bovine Liver SRM 1577 and U.K. Human Blood Mastermix. Bovine Liver was obtained from the National Bureau of Standards, Department of Commerce, Washington,

D.C., as part of the Standard Reference Materials program. A sample of the United Kingdom Human Blood Mastermix was obtained from Dr. E. I. Hamilton of the Institute for Marine Environmental Research, England.

Human blood samples obtained through the courtesy of Dr. John Fenton formerly of Southeastern Massachusetts University, North Dartmouth, Massachusetts, were used in some of the preliminary work.

B. Counting and Data Processing Equipment

An Ortec WIN[15] Series (Ortec, Oakridge, Tennessee) coaxial lithium-drifted germanium {Ge(Li)} detector was used for high resolution gamma ray spectroscopy. The detector specifications included a measured resolution equal to 2.1 KeV full width at half maximum for the 1.33 MeV photopeak of ^{60}Co, a peak-to-Compton ratio of 38 to 1, and an efficiency warranted by the manufacturer to be 20% of a 3 x 3 inch sodium iodide detector at 1.33 MeV. A specially designed battery pack (Ames Laboratory Electronic Shop) provided a +4800 volt bias to the detector. An Ortec preamplifier was supplied as an integral part of the detector.

As a method of correcting spectra collection times for the analyzer dead time, a 60 Hz pulse was fed to the pre-amplifier during the collection of spectra. The pulses

were formed by a BNC Tail Pulse Generator Model RP-1 (Berkeley Nucleonics, Berkeley, California) which was gated at 60 Hz by a RIDL Model 47-2 Pulser (Radiation Instrument Development Laboratory, Chicago, Illinois). Primary amplification of sample and timer pulses was obtained with a CI Model 1417B Spectroscopy Amplifier (Canberra Industries, Meridan, California) prior to digitilization and storage with a Nuclear Data 50/50 Analyzer System (Nuclear Data, Inc., Chicago, Illinois) which included a PDP-8/L Computer (Digital Equipment Co., Maynard, Massachusetts) as an integral component. A Nuclear Data Series 2200 ADC, capable of 4096 channel resolution at a conversion frequency of 50 MHz, performed the analog-to-digital conversions in the 50/50 analyzer system. The PDP-8/L is a 12-bit machine with 4K core storage. The interface between the ADC and the computer allowed for independent operation of either device.

Input and output devices employed with the analyzer system included a Model ASR-33 teletype (Teletype, Skokie, Illinois), a Nuclear Data Series 2200 BRPE Punch/Reader, and a PEC Model 6X40 Synchronous Read After Write Tape Transport (Peripheral Equipment Corp., Chatsworth, California). A visual display of the ADC memory was available on a Tektronix Type 602 Display Unit (Tektronix, Portland, Oregon).

Software was available with the 50/50 Analyzer which allowed data processing of gamma ray spectra stored in the memory of the ADC. The need for operator intervention for isolating each photopeak made the analysis procedure excessively tedious and time consuming when processing complete 4096 channel spectra. The simplicity of some data processing routines, particularly the peak area calculation and background correction algorithms, could result in significant errors and low precision when processing complicated spectra (i.e., spectra containing partially or completely overlapping photopeaks). Therefore, data reduction of the high resolution gamma ray spectra was usually accomplished on one of the large ISU computer systems, either an IBM 360-65/IBM 370-158 or an IBM 360-65/ITEL AS-5 tandem.

After collection, each gamma spectrum was transferred from the 50/50 Analyzer to magnetic tape for temporary storage. All data tapes written by the PEC tape drive were initially processed by the computer program DOGGIT (189). The PEC tape drive wrote each spectrum on tape as a stream of 24 bit words. However, the large computers were designed to operate on 32 bit words. DOGGIT read the PEC generated tapes and converted the data to 32 bit words which were compatible with the IBM and ITEL computers.

The identification of photopeaks in gamma ray spectra was performed by ICPXGT, a Fortran-coded peak finding routine. After ICPXGT detected potential photopeaks by analysis of the smoothed second derivative of the gamma spectrum, an attempt was made to fit a Gaussian curve to each of the peaks. The Gaussian fitting routine, which was essentially the one described by Heath et al. in AEC-Report IDO-17017, evaluated three parameters for the top 75% of the peak, a region which has been found empirically to consistently yield the best results by minimizing errors from sources such as poor background corrections, non-Gaussian peaks, etc. Background corrections were made by fitting a least-squares straight line through five channels on either side of the peak and subtracting the result from the region to be fitted. Printed output for each peak identified in the preliminary search included the exact channel location, standard deviation of the location, peak width, peak height, peak area, standard deviation of the area, the energy calculated from a quadratic calibration line, and a code indicating whether the Gaussian fitting routine converged. Optionally, the same information could be obtained on punched cards, magnetic tape, or a direct access device like a disk pack.

As an option, ICPXGT provided semi-log graphical representations of selected spectra. SIMPLOTTER (190), a high

level plotting system maintained as an on-line procedure by the I.S.U. Computation Center, created the plots from data passed by ICPXGT. The finished plots were available on a line printer or an incremental plotter. For most work, the plots were produced on a Calcomp Digital Incremental Plotter with a resolution of 0.01 inches.

ICPXGT was designed to identify and resolve multiplets into their component photopeaks. However, when a multiplet consisted of photopeaks with significantly different intensities, ICPGXT would not "see" the smaller peak even though the graphical output showed that it existed. In those few cases where ICPXGT was inadequate, SKEWGAUS (191) was used to resolve the multiplet. SKEWGAUS, unlike ICPXGT, was not designed to search gamma ray spectra for peaks. The user had to supply SKEWGAUS with the locations of the multiplet and of each component suspected of being present in the multiplet. With this information, SKEWGAUS estimated seven parameters for each potential component and attempted to fit each peak as part of the multiplet. The name SKEWGAUS is applicable because the fitting routine was not based on a true Gaussian function. The fit was made to a Gaussian function with the "skewness" term added to account for the distortion in the tail on the low energy side of a photopeak when measured with a semiconductor detector.

After the gamma spectra were reduced by ICPXGT to a series of peak areas, locations, etc., the Fortran program ANALYZ3 (102) was executed to obtain information on an elemental basis. The versatility of ANALYZ3 allowed the user to obtain a listing of the elements present in a sample along with their respective count rates, a comparison of the decay rates of elements in different spectra, or a listing of the elements present in a spectrum along with its concentration (ppm).

Each of the previously described computer programs, except for SKEWGAUS, was designed to provide information in a form suitable to input to that program which would logically follow in a data reduction scheme. Thus, in one computer pass, it was possible to go from the raw spectral data on magnetic tape to a listing of the elements and respective concentrations in a sample.

A thallium-activated sodium iodide [NaI(Tl)] scintillation detection system was utilized for low resolution gamma ray spectroscopy. The system consisted of a 4 x 4 inch well-type Harshaw NaI(Tl) Matched Window Scintillation Detector with an optically coupled, photomultiplier tube (Nuclear Chicago Corp., Des Plaines, Illinois), a RIDL Model 30-19 Linear Amplifier and Discriminator, a RIDL Model 33-10B Anti-walk single Channel Analyzer, a RIDL Preset Counter Model 49-43, and a RIDL

Model 40-12B High Voltage Power Supply. The samples assayed by low resolution gamma spectroscopy were of high radiochemical purity and contained species with moderately long half-lives. Operating the single channel analyzer in the "differential" mode with the proper discriminator settings allowed only impulses pertinent to the species of interest to reach the scaler. This meant that the data necessary for determining the area of the photo-peaks could be obtained directly from the counter without an analysis of the complete gamma spectra.

C. Experimental Procedures

1. Special cleaning

All quartz, glass, and plastic articles used in the preparation or storage of irradiation standards were cleaned to minimize the contamination blank from the surfaces of those articles. Initially, the labware was washed in a solution of Alconox detergent and water. After a thorough rinsing in deionized water, the articles were placed in a bath of analytical grade nitric acid, and kept at an elevated temperature for at least 48 hours. If plastic items were involved, the temperature of the bath was not allowed to exceed 80°C. For all other materials, the temperature was maintained between 110 and 120°C. After removal from the acid, the articles were rinsed in

high purity deionized water and then placed into a bath of high purity water to soak for at least 24 hours. Finally, the labware was air dried in a protective enclosure to prevent contamination by settling airborne particulates.

2. Standards and carriers

During the course of this work, the standard and carrier solutions had to be remade a number of times. Initially, the procedures for making the carriers and standards differed significantly. The carriers were originally produced with available analytical grade materials, while extra efforts were made to obtain special high purity components for the irradiation standards. Because of the large number of solutions this procedure generated, the production methods for the solutions were changed. During the later stages of this work, single standards of unusually high concentrations (typically in the range 5-50 mg/ml) were made from the highest purity materials available. After appropriate dilutions with high purity water, these solutions were usable as either carriers or standards. In addition, this method of production reduced the frequency at which the stock solutions had to be remade, because the high concentrations negated any adsorption or desorption effects with the walls of the storage containers.

Table 21 provides information concerning the materials used in the production of both standards and carriers in the later stages of this work. Unless otherwise noted, the dissolutions were accomplished in water with nitric acid applied as needed. When necessary, all stock solutions, carriers, and standards were adjusted to pH 1 with nitric acid.

3. Freeze-drying

Freeze-drying was used to dry all biological materials employed in this work. In each case, the sample was transferred from its original container to a sample bottle which had undergone the special surface cleaning procedure. Liquid nitrogen was used to freeze the material in the sample bottle prior to connecting the bottle to the one of the ports of the freeze dryer (Figure 1). Only one sample was dried at a time. Samples of NBS Bovine Liver and U. K. Master Mix were dried for 24 hours with no special arrangements to prevent defrosting after the initial freezing. During the drying of a whole blood sample, it was necessary to refreeze the sample at least seven times during the first 24 hours to prevent material losses from excessive frothing and sputtering. After the first 24 hours, the mass of blood in the sample bottle was broken up with a Teflon coated spatula, refrozen in liquid nitrogen, and freeze-dried for an additional 24 hours.

Table 21. Stock solutions used in the production of carriers and standards

Element	Compound	Concentration (mg/ml)	Notes
As	As_2O_3	10.00	Initially dissolved in small amount of NH_4OH
Au	$HAuCl_4 \cdot 3H_2O$	9.887	Very deliquescent
Co	Co metal	50.95	---
Cr	CrO_3	25.15	Slightly deliquescent
Cu	Cu metal	49.96	---
Fe	Fe wire	51.43	---
Hg	HgO	50.04	---
Mo	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	25.38	Does not form stable solutions at low pH
Sb	Sb metal	5.190	Dissolved in conc. H_2SO_4. Cleared solution with Aqua Regia
Sc	Sc_2O_3	0.7920	Heated at 800°C for 4 hrs before weighing. Dissolved slowly in conc. HNO_3
Se	Se metal	50.08	---
Rb	Rb_2CO_3	31.57	Heated at 800°C for 4 hrs before weighing
W	W metal	49.97	Dissolved in minimum HF
Zn	Zn metal	50.65	---

A mixture of either Dry Ice/acetone or Dry Ice/propanol was used to charge the water vapor trap in the freeze drying unit. The pressure in the evacuated system was not measured, but the pump specifications indicated a capability to pull a vacuum of less than 5 microns of mercury.

4. Supercil quartz

The following procedures were developed to determine the extent to which proposed digestion systems would result in contamination of biological samples by species indigenous to quartz of which the irradiation vials were fabricated. This study was necessary to determine whether the radioactive species from the irradiation vial during a post-irradiation digestion procedure would be significant relative to the corresponding species originally present in the sample. Two approaches were used in this evaluation. In the first, a crushed quartz vial and a biological sample were individually capsulated in quartz vials and irradiated in facility R-3 of the ALRR for 15 hours. The containers were cut open and a weighed portion of the contents of each vessel along with 200 mg of bovine liver was transferred to the previously described digestion apparatus (Figure 3). Digestion procedure DG-2 (See section entitled "Digestion schemes".) was performed on each sample. The specific activities of radionuclides detectable in the digests of both samples were compared.

The second approach was based on approximately the amount of material actually extracted from a crushed quartz vial during a post-irradiation wet ashing operation. An empty, weighed, quartz vial and a quartz vial containing 1 ml of a mixed elemental standard solution were irradiated for 15 hours in facility R-3 of the ALRR. The vials were individually frozen in liquid nitrogen, wrapped in a small Kimwipe, crushed, and placed into separate digestion apparatus along with carriers and 200 mg of bovine liver. Digestion procedure DG-3J was performed on each sample. The digests were collected and assayed by gamma ray spectroscopy. Calculation of the elemental concentrations extracted from the quartz was possible after the activities present in the standard were corrected for the respective activities detected in the quartz sample.

5. Capsulation system

A series of experiments was executed to study the loss of sample components during the three stages of processing which directly involved the capsulation system: 1) pre-irradiation sealing of a sample into sample containers, 2) irradiation of sample/container system, and 3) post-irradiation removal of a sample from the container. In order to avoid the difficulties associated with the intense backgrounds in the spectra, the evaluation was accomplished

through the use of tracer solutions with an oven to simulate the thermal conditions within a reactor.

The tracer solution was produced from multi-element standards which were irradiated in the ALRR. Table 22 contains a listing of the elements which were investigated, and the approximate amounts of elements present in each vial during testing. (Stable carrier nuclides were not added to the test vials.) Concentrated sulfuric acid was added to 0.5-g samples of bovine liver and blood to form a slurry. Three test vials were assembled as follows: 1) 400 µl of tracer solution and 400 µl of bovine liver slurry; 2) 400 µl of tracer solution and 400 µl of blood slurry; and 3) 400 µl of tracer solution, 200 µl of bovine liver slurry, and 200 µl of blood slurry. Each vial was capped with a sheet of aluminum foil to prevent the loss of components through vaporization. The radioactive species in each vial were measured with the high resolution gamma spectroscopy system.

Vial #3, containing the blood and liver slurries, was set aside for reference. The cold-finger system (Figure 2b) and a gas-oxygen torch were used to seal the remaining vials. The gamma spectra of the three vials were collected. Vials #1 and #2 were placed into a Sargent drying oven for at least 24 hours at 150°C. After cooling to room temperature, the gamma spectrum of each of the three vials was

Table 22. Elements comprising tracer solution used in capsulation experiments

Element	Amount (μg)
Co	0.03
Cr	0.08
Fe	2.27
Hg	0.05
Mo	0.14
Rb	0.08
Sb	0.03
Sc	0.003
Se	0.19
Zn	0.76

again collected. Each vial was frozen in liquid nitrogen, wrapped in a plastic glove and crushed. The vial fragments and contents were rinsed into 10 dram plastic containers and assayed for gamma activities. Data reduction proceeded as previously described.

6. Digestion schemes

A biological matrix, usually bovine liver, spiked with a solution of radionuclides was used to test wet ashing schemes. The principal variant between schemes was the specific oxidizing medium and required modifications for its application. Each digestion system was evaluated for optimum ashing conditions, completeness of ashing, and susceptibility of the ashing system to losses of elemental components.

In the component recovery tests, an aliquot of a radio-tracer solution was diluted to a specific volume for use as a reference solution, while an identical aliquot of the tracer solution was added to 200 mg of a biological matrix and subjected to a wet ashing procedure. Carrier nuclides (Table 23) were added to the reference solution and to the digest mixture to reduce losses associated with low component concentrations. After diluting the ashed material to a specific volume, a comparison was made between aliquots to the reference and ashed solutions to determine material losses.

a. Nitric acid (DG-1)

1. In the reaction flask of a Bethge digestion apparatus (Figure 3) place 400 mg of bovine liver, 5 ml of concentrated nitric acid, an aliquot of a radio-tracer solution, and an aliquot of a carrier solution of stable nuclides.

Table 23. Composition of typical carrier solutions used throughout the project

Carrier	Element	Concentration (mg/ml)
A	As	3.00
	Cu	5.00
	Mo	2.54
	Se	5.01
	W	5.00
	Zn	5.07
B	Co	5.10
	Cr	2.52
	Fe	5.14
	Hg	5.00
	Rb	3.14
	Sb	3.04
	Sc	0.16

2. Swirl the flask to wet the matrix and reconnect the flask to the apparatus.

3. Place digestion apparatus on a hot plate and heat to boiling. With the three-way stopcock in the reflux position (Figure 3, position 3), reflux the solution until it clears.

4. Allow the apparatus to cool. Collect the ashed sample and all rinses of the apparatus in a 50-ml volumetric flask and dilute to volume.

b. Nitric and perchloric acids (DG-2)

1. Place 400 mg of a biological matrix, 5 ml of concentrated nitric acid, an aliquot of a radio-tracer solution, and an aliquot of a carrier solution into the reaction flask of the Bethge digestion apparatus. Swirl flask to wet the matrix. Reassemble the apparatus and place it on a hot plate.

2. With the three-way stopcock in the reflux position, bring the digestion medium to a boil. Allow the mixture to reflux for 15 min.

3. Remove from heat and allow the sample to cool. Slowly add 8 ml of 70% perchloric acid to the reaction flask through the condenser system.

4. Return the apparatus to the hot plate and bring to a boil. Allow the mixture to reflux for 10 min. Close the three-way stopcock and collect the distilling nitric acid

in the air condenser. After most of the nitric acid has collected in the air condenser, carefully reopen the three-way stopcock and allow the nitric acid to return to the reaction flask. An explosion hazard exists if the acid mixture is distilled to dryness.

5. If necessary, repeat step 4 until the solution clears.

6. Remove the sample from the heat and allow to cool. Collect the digest solution in a 50-ml volumetric flask. Rinse the apparatus with water and combine all rinses with the ashed sample in the volumetric flask. Dilute the sample to volume with water.

c. Sulfuric acid and hydrogen peroxide (DG-3)

Evaluation of the sulfuric acid/hydrogen peroxide digestion medium was begun in the Bethge apparatus as shown in Figure 3. Quite early in the evaluation, the Bethge apparatus modified with liquid nitrogen cold-finger and thistle section (Figure 4) replaced the original system. The following procedure was employed with the earlier version of the Bethge apparatus in the initial work with the sulfuric acid/hydrogen peroxide system:

1. Place 400 mg of the biological material into the reaction vessel of the Bethge apparatus (Figure 3). While cooling the flask in an ice bath, slowly cover the material with 5 ml of concentrated sulfuric acid and swirl or use a

magnetic stirring bar to mix. Add aliquots of radio-tracer and carrier solutions.

2. Remove the flask from the ice bath and connect it to the digestion device. Warm the flask and contents by gently heating with a Bunsen burner. After a few minutes, intensify the heating until fumes of sulfuric acid are produced.

3. Allow the flask to cool. Very carefully add 10 ml of 90% hydrogen peroxide through the condenser system to the reaction vessel. Additions must be made incrementally with sufficient times between additions to allow the reaction to subside.

4. Collect the digest and rinses of the apparatus in a 50-ml volumetric flask and dilute to volume with water.

A number of variations on the above procedure (DG-3) were tested. The differences between the variations and DG-3 are listed below.

DG-3A: All steps are as indicated in DG-3, except an oil both regulated at a temperature between 70 and 90°C is used for all heating purposes. Naturally, the ashing medium is not heated to fumes of sulfuric acid.

DG-3B: All steps are as in DG-3A except the modified Bethge apparatus (Figure 4) is used.

DG-3C: The procedure is identical to DG-3B except heat is supplied by a Bunsen burner and the sample is heated to fumes of sulfuric acid.

DG-3D: The procedure follows the outline of DG-3B. However, after the final addition of hydrogen peroxide, the temperature of the oil bath is raised to 100°C and the mixture is stirred for 15 minutes before proceeding.

DG-3E: The procedure follows DG-3B except after the final addition of hydrogen peroxide, granular manganese dioxide is washed with water through the thistle section into the reaction vessel until effervescence subsides before proceeding.

DG-3F: Proceed as in DG-3E except 1.5 to 3.0 ml of a saturated solution of ferrous sulfate is added instead of manganese dioxide.

DG-3G: Proceed as in DG-3E except 2.0 ml each of carrier solutions "A" and "B" (Table 23) are added instead of the manganese dioxide.

DG-3H: Proceed as in DG-3G except 50-100 mg of chromic acid is included in the reagents initially added to the sample in the reaction flask.

DG-3I: Proceed as in DG-3H except 50-100 mg of vanadium pentaoxide in sulfuric acid is added to the reaction vessel instead of chromic acid.

DG-3J: Proceed as in DG-3G except a 9:1 mixture of sulfuric and nitric acid is used in the initial treatment of the sample in the reaction flask.

7. Synthesis of hydrated antimony pentaoxide (HAP)

Hydrated antimony pentaoxide was selected for use in the removal of radio-sodium from solutions of ashed biological materials. In order to avoid shipping delays and reported inconsistencies in the commercial preparation, HAP for use in this project was synthesized locally. The false starts and failures which marked the attempts to synthesize a second batch of HAP eventually evolved into a study of how various parameters of HAP syntheses affected the characteristics of the final products.

a. HAP 1A and 1B The synthesis procedures for HAP materials 1A and 1B are based on the information provided in the original article by Girardi and Sabbioni (153) on the properties of hydrated antimony pentaoxide in sodium decontamination. Under an evacuating hood, the contents of a 1-lb bottle of antimony pentachloride ($SbCl_5$) were slowly added with stirring to 500 ml of deionized water

in a 2-l. beaker. The vigorous, exothermic hydrolysis yielded a fine, white precipitate. As excess $SbCl_5$ was added, the precipitate turned yellow and redissolved. With the addition of more water, the white precipitate returned. As the hydrolysis continued, water was added as necessary to reverse the formation of the yellow coloration. The final volume of $SbCl_5$ and water was approximately 1.8 liters. The precipitate had a light green tint and the odor of hydrogen chloride over the beaker was strong. After allowing the precipitate to settle, the supernatant was decanted, and more water was added to the precipitate. The three step operation was repeated three more times. After the final addition of water, the precipitate was stirred overnight. During filtration through a Buchner with aspiration, the precipitate formed an apparently dry mass. However, the material would liquify if pressure, as when pressing or stirring with a spatula, was applied. After liquification, more water could be removed with the Buchner funnel. The final precipitate was pasty, white and without the odor of hydrogen chloride. The pH of the supernatant was always less than 1. In order to test the drying procedure the precipitate was split into two unequal portions.

The smaller portion of the precipitate was placed in a drying oven for 5 hours at 150°C to remove excess moisture,

then transferred to a muffle furnace for 5 hours at a temperature which varied between 270 and 290°C. During the high temperature treatment an uneven yellow color developed in the precipitate which by this time had formed large chunks. To ensure a homogeneous transformation, the material was broken up with a mortar and pestle and returned to the muffle furnace to complete the five hour treatment. The final material was pale yellow and crystalline. After noting the results of this procedure on the second portion of the precipitate, this material was returned to the muffle furnace for five additional hours at 270°C. The only apparent change was a darkening of the yellow color. This material was designated HAP 1A.

The second portion of the synthesis product was placed in a drying oven at 130°C for 1.0 hour prior to transferring it to the muffle furnace at 270°C for 5.0 hours. During the high temperature treatment, the sample was fractured as with HAP 1A. The final product of this operation (HAP 1B) was a yellow material with a powdery texture. The color was darker than the initial HAP 1A product, which is the reason 1A was reheated.

The total production of HAP material was approximately 200 g. Before use, however, the material was sized and the fraction between 150 and 250 microns was kept for testing. This operation reduced the amount of usable material by half.

b. HAP 2 The second attempt to synthesize HAP was a failure, but the method is included because the final material was used in an attempt to gain information about the composition of hydrated antimony pentaoxide. The synthesis proceeded as described in the synthesis of HAP 1B. However, during the high temperature drying step, temperature control in the muffle furnace failed. Instead of drying for 5 hours at 270°C, the material was exposed to a temperature between 650 and 700°C for an indeterminate amount of time. The final product (HAP 2) was a powdery, grayish-brown material. Approximately 190 g of material was recovered. A portion of this material was fractioned ($150 \leq X \leq 420$ microns) and tested for sodium decontamination and selectivity.

c. HAP 3 This synthesis of HAP followed the same sequence of operations described in the synthesis of HAP 1 products. A noted deviation from the HAP 1 routine was the presence of the pungent odor of hydrogen chloride following the last filtration step. The odor became much stronger and white fumes developed as the precipitate was treated at 270°C in the muffle furnace. The material at the end of the heat treatment was translucent, white and crystalline. Additional drying at 270°C did not produce the expected yellow coloration. The material was subjected to a series of water additions and subsequent dryings at

270°C until a yellowish-white color developed. When further treatment did not increase the extent or intensity of the color, the treatments were stopped. The final yellowish-white crystalline material was designated HAP 3. After sizing, a portion was subjected to decontamination and selectivity tests.

d. HAP 4A and 4B The work of Torok and Diehl (145) provided the basis for the following synthesis procedure. The contents of a 1-lb bottle of $SbCl_5$ were slowly mixed with 1.0 liter of a 10% NH_3 solution. The interaction was more violent than hydrolyses with plain water. After the white precipitate formed, it was allowed to remain in contact with the mother liquor for 4 hours. The mother liquor was then filtered off, and the precipitate was redispersed in 1.0 liter of deionized water and left to stir overnight. After the water was filtered off, the precipitate, unlike HAP 1 at the same stage of processing, was very tacky and tended to form lumps. Also, the odor of hydrogen chloride was distinctly present. After two washes with the NH_3 solution, the odor subsided, but the consistency of the precipitate did not change. The precipitate was dried initially at 100°C for 1.0 hour in a Sargent drying oven, then transferred to a muffle furnace to dry for 5 hours at 270°C. The resulting product (HAP 4A) was light yellow with a powdery texture.

Approximately one-half of product 4A was returned to the oven for an additional five hours. The resulting material (HAP 4B) was crystalline and had a deeper yellow coloration than HAP 4A.

e. HAP 5A and 5B HAP 5A was produced by following as closely as possible the procedure described by Torok and Diehl (145). Five ml of $SbCl_5$ was hydrolyzed with 25 ml of 1% NH_4OH. The resulting precipitate was pasty and lumpy with a slight odor of hydrogen chloride. After standing four hours in contact with the mother liquor, the precipitate was filtered and redispersed in 500 ml of deionized water for 12 hours. After collection, the material was washed twice, then transferred to a muffle furnace to dry at 270°C for five hours. Approximately 2.1 g of a pale yellow, powdery precipitate (HAP 5A) was recovered.

HAP 5B was produced with an abbreviated form of the procedure used in producing the HAP 1 variants. Five ml of $SbCl_5$ was hydrolyzed with 100 ml of deionized water. Another 200 ml of water were added after the addition of $SbCl_5$ was completed, and the mixture was allowed to stir overnight. The precipitate in solution was finely dispersed and had a very strong odor of hydrogen chloride. Following filtration and a single washing (the pungent odor was not removed), the precipitate was dried in a muffle furnace for five hours at 270°C. The resulting

product (HAP 5B) was crystalline with a yellow color which became off-white and translucent as the material cooled.

f. HAP 6A and 6B A dripper system with a 250-ml separatory funnel as the reservoir was employed to add 185 ml of $SbCl_5$ to 1.0 liter of a stirred 1% NH_4OH solution over a period of 2.3 hours. After four hours of stirring in contact with the mother liquor, the precipitate was removed. The mother liquor possessed a slight green tint and a pungent odor. Treatment of the mother liquor with more of the NH_4OH solution yielded more precipitate. All of the precipitates were gathered and dispersed into 1.0 liter of deionized water for 12 hours. After collection, the precipitate was dried at 270°C for five hours in a modified Sargent drying oven. The resulting material was in fairly large chunks which accounted for the uneven yellow and white coloration. After an additional 13 hours in the oven, no more of the material changed to yellow. The material was cooled, crushed and sized. The fraction between 155 and 450 microns was collected and designated HAP 6A. A portion of 6A was returned to the oven for 10 hours at 300°C. A golden material (HAP 6B) which lightened to golden-yellow while cooling in a desiccator was obtained.

g. HAP 7 HAP 7 was synthesized according to the same general procedure used in the preparation of the HAP 6 products. The deviations were the inclusion of more NH_4OH

solution in the initial hydrolysis, and a prolonged drying period (18 hours) at 300°C in a Sargent drying oven modified for forced air flow. The final product was light yellow with a powdery texture. The total recovery of material was 200.9 g, of which 127.1 g was usable after sizing to between 150 and 250 microns.

8. Sodium decontamination with HAP

The products of each synthesis attempt were tested for sodium adsorption from either hydrochloric or nitric acid. The basic objective of the decontamination test was to compare the activities of ^{24}Na which were present in an untreated aliquot of a reference solution with the activities which were present in an identical aliquot of the reference solution which had been processed through a column of HAP. In the earliest tests, a radio-sodium reference solution was prepared to provide sufficient activity for counting purposes, but the total amount on the column was kept below 30 mg-Na/g of HAP which comprised the column. In later testing, an irradiated biological material was used to make the reference solution.

In the sodium decontamination test, a chromatographic column was filled with 1.3 to 1.5 g of HAP material (column height approximately 3 cm) which had been previously washed with the appropriate acid to remove fines. Pyrex wool plugs were used above and below the resin bed for

stabilization. The column material was equilibrated by rinsing with four bed volumes of the acid to be used as the eluent.

Radio-sodium was obtained by irradiating a small amount of sodium carbonate. After the irradiation, the carbonate was dissolved in acid and the sample container was rinsed with three volumes of acid. The dissolved sample and the rinses were combined. A quantity of the radio-sodium solution was transferred with an Eppendorf Automatic Pipette (either 10 or 200 µl) to a volumetric flask containing enough inactive sodium to provide a total sodium concentration of 6 mg-Na/ml after the solution was brought to volume with the appropriate acid. The amount of radio-sodium solution initially transferred was empirically determined such that a 5-ml volume of the final sodium solution would provide approximately 10^6 counts/min at approximately 10 cm from the face of the Ge(Li) detector used in the high resolution gamma spectroscopy system.

Two different procedures were used to obtain the solutions for comparison of radio-sodium activity. The first approach allowed the collection of data concerning the minimum and maximum amounts of eluents which could be used on the column. With experience, this information became unnecessary and the procedure was considerably simplified.

a. Decon test 1 The reference solution was made by pipetting 5 ml of a radio-sodium solution to a 50-ml volumetric flask and diluting to volume with the eluent acid. A 5-ml aliquot of this solution was transferred to a 10-dram plastic vial for use as the reference. Another 5-ml aliquot of the radio-sodium solution was pipetted directly onto the HAP column and allowed to flow through at about 1 ml/min. The column was then eluted with 15 ml of the appropriate acid, either 12M hydrochloric or 12-15M nitric acid. All discharges from the column were collected in a 50-ml volumetric flask and diluted to volume. Depending on the experiment, as many as 6 additional 15-ml elutions were performed with the collected eluent of each elution going into separate volumetric flasks. In all cases the volumetric flasks were diluted to volume with the eluent acid and 25 ml of the resulting solutions were transferred to 10-dram plastic vials for counting on the high resolution gamma system.

b. Decon test 2 The reference solution was prepared by pipetting a known amount of a radio-sodium solution to a 125-ml plastic bottle. The bottle was then filled to a predetermined mark with an acid solution. An identical aliquot of the radio-sodium solution was transferred directly to the HAP column and allowed to flow through at a rate of about 1 ml/min. Depending on the

experiment, the column was then eluted with up to 60 ml of acid. All of the eluent was collected in a 125-ml plastic bottle. The activity in the two bottles was measured on the high resolution gamma system.

9. Percent antimony in HAP

The percentage of antimony in most of the HAP materials was determined by Neutron Activation Analysis using the standard-comparator method. The standard consisted of a weighed quantity of potassium antimony tartrate $\{K(SbO)C_4H_4O_6 \cdot 1/2H_2O\}$ irradiated along with a sample of the synthesis product for up to 1 hour. After a decay period of 10 days, the gamma spectra of the synthesis products and the standard were obtained on the high resolution system and processed as previously described.

10. Water in HAP materials

A weighed sample of each HAP product was heated in a muffle furnace for 5 hours at 700°C. The samples were removed to a desiccator to cool prior to reweighing. Each sample was returned to the oven and left for about 10 hours at 560°C. Following removal, each sample was cooled in a desiccator and reweighed.

11. Selectivity of HAP materials

The basis of the selectivity test was identical to the method employed in the sodium decontamination tests. A comparison was made between the radioactivities present in an aliquot of a reference solution of mixed radionuclides and an identical aliquot of the reference solution processed through a column of HAP material. The reference solution contained stable carriers for each radionuclide in the solution. In addition, the concentration of stable sodium in the reference was adjusted to have approximately 30 mg of sodium on the column during the testing. Both of the elution techniques described for the sodium decontamination test were also employed in the selectivity testing. Table 24 contains data concerning those nuclides which were checked for sorption by the HAP materials. Some of the elements (i.e., Ba, La, Sm, etc.) initially included in the sorption tests were eliminated later as analyses of real biological samples failed to detect them.

12. Sodium capacity of HAP materials

As a method for further characterization of the synthesis products HAP 6 and HAP 7, the capacity of these materials for sodium was experimentally determined. Two techniques were employed. In the first, an aliquot containing a known amount of radio-sodium and stable sodium in the appropriate acid was passed through a column of the

Table 24. Elements tested for sorption on various HAP materials

Element	Element
As	La
Ba	Lu
Ce	Na
Co	Rb
Cr	Sb
Cu	Sc
Eu	Se
Fe	Sm
Hg	Yb
K	Zn

material that was being tested. The column was then eluted with three more column volumes of eluent. All eluents were combined in 50-ml plastic bottles for assay with the low resolution system. These operations were continued until more than 30 ml-Na/g-HAP in the column was added to the resin. The ^{24}Na activity of each collected sample was compared with a reference which consisted of an aliquot of the radio-sodium solution diluted to a predetermined level in a 50-ml plastic bottle.

In the second procedure, a solution of known sodium concentration containing radio-sodium was allowed to flow through an equilibrated column of a HAP material at a rate less than 1 ml/min. The eluent was collected in 5-ml volumes and counted on the low resolution system. Information concerning the amount of sodium on the column was obtained by comparing the count rates of the eluents with a 5-ml aliquot of the sodium solution which had not been processed through the HAP material.

13. HAP and wet ashing compatibility

Each HAP material which was judged suitable on the basis of sodium retention and nuclide selectivity tests was evaluated for compatibility with wet ashing methods which were potentially usable in the project. The objective of the testing was to discover whether the properties of the HAP materials were adversely affected by

the composition of the solution resulting from the ashing procedure. Aliquots of the ashed matrix solutions described in the step 4 of the digestion routes listed in the section titled "Digestion Schemes" were used as the test solutions. For the most part, compatibility testing followed the approach outlined in Decon Test 2 of the sodium decontamination tests.

14. Alumina

Chromatographic acid alumina (powder, Brockmann activity grade 1) was tested for the removal of ^{31}P in the form of phosphates from nitric acid solutions. Solutions of radiotracers were used to ascertain the specificity of alumina for phosphate over other ionic species. The basic approach of the test procedure was the same as outlined in the section concerning the sodium decontamination properties and ionic selectivity of HAP materials. The phosphorus decontamination factors were determined with irradiated biological samples rather than synthetic tracer solutions. Alumina was evaluated in 10M, 12M, and 16M nitric acid.

15. AG2-X8 anion exchanger

The chloride, bromide, and acetate forms of the anion exchanger AG2-X8 (Bio-Rad Laboratories, Richman, Calif.) were evaluated for sodium, potassium, bromine, and phosphorus decontamination. Each material was tested with a number of

eluents for selective elution of anionic species as part of a decontamination operation. All experiments involved solutions of ashed biological matrices spiked with radionuclide tracers. The selectivity and elution tests followed the same schemes as described for the HAP evaluations.

a. Chloride form The chloride form of AG2-X8 was obtained commercially in 100-200 mesh. Prior to use, the resin was rinsed in 6M HCl to remove fines. The remaining material was poured into support columns to form resin beds of 4.5 to 5.0 cm in height. The top and bottom of the resin bed was secured with plugs of Pyrex wool. The resin beds were equilibrated by elution with 3 to 4 bed volumes of 10-12M HCl prior to application of the test solutions.

b. Bromide form The bromide form of AG2-X8 was synthesized from the commercially obtained chloride form of the resin. The chloride form was initially converted to the sulfate form by elution with 0.25 M sulfuric acid until silver nitrate tests showed that chlorine was absent from the eluent. Complete conversion of a 80-ml bed volume of the chloride form to the sulfate form required approximately 1.0 liter of the acid solution at a rate of 2-4 ml per minute. Without rinsing the resin, the bed was converted to the hydroxyl form by elution with a 2.8 M NaOH solution. The conversion to the hydroxyl form, which

was facilitated by a decrease in the affinity of the resin for sulfate anions in a basic medium, was highly favorable and required only 2-3 bed volumes to ensure a complete transformation. Darkening of the resin during formation of the hydroxyl form was a good indicator of the completeness of the conversion. Without rinsing the column, the eluent was changed to 48% HBr, and the resin was eluted until the entire bed was a uniform dark brown. Two more bed volumes of HBr were eluted through the column to ensure the completeness of the transformation. After removal from the supporting column, the resin was stored in a 10% HBr solution.

c. Acetate form The acetate form of AG2-X8 was synthesized from the commercially obtained chloride form in a conversion procedure identical to the bromide conversion except for the final step. The hydroxyl form was converted to the acetate by elution with glacial acetic acid. A color change useful for following the extent of the transformation did occur. The acetate resin was stored in a 6 M acetic acid solution.

16. Resin/digestion compatibility

Each resin/eluent combination deemed suitable for inclusion in a DNAA procedure was tested for compatibility with prospective wet ashing processes. The evaluation

proceeded according to the procedures indicated for the compatibility studies with the HAP materials.

D. DNAA Procedure

The schemes developed while testing the individual operations for inclusion in a DNAA procedure had to be modified for the analysis of real biological samples. The primary alterations were due to the need for larger aliquots of the digested samples in order to obtain sufficient counting statistics without excessively long counting periods. Although the concentrations of elements in the digestion steps during the analysis of real samples and in the primary evaluations were similar, the ratio of radioactive to stable isotopes during the evaluations was kept higher in order to keep the counting times reasonable. The following is a step-by-step description of the procedure developed for the analysis of biological standards by DNAA.

1. Capsulation

Approximately 400 mg of the biological material were transferred to a tared, acid-cleaned Supercil quartz vial. Any material adhering to the walls of the vial was removed with blasts of filtered air prior to weighing the vial and its contents. After capping with a sheet of high purity aluminum foil to prevent contamination by airborne particles, the vial was clamped into the recess of the

liquid nitrogen cooled cold-finger (Figure 2). The vial was allowed to cool until frost was at least 1.9 cm above the lip of the cold-finger and on the clamp. An oxygen-gas torch was used to seal the vial.

Capsulation of the standard solutions was achieved in the same manner, except transference of the solution to the vial was accomplished with fixed-volume 10 and 200 µl Eppendorf Automatic Pipettes. The total volume of the standard solution never exceeded 1.0 ml.

2. Irradiation

The vials containing the samples and standards were wrapped in aluminum foil and sealed in styrofoam padded irradiation capsules. Since the small rabbit capsules could contain only two vials, a standard and a sample were always irradiated together. Typically, the capsules were irradiated in the R-3 facility of the ALRR for 15 hours and allowed to "cool" for 24 hours.

3. Digestion

After removal from the irradiation capsule, the sample vials were stripped of adhering packing materials and aluminum foil. Successive baths of boiling nitric acid, water, methanol, and acetone were used to clean the outer walls of the quartz vessels. Each vial was placed in a bath of liquid nitrogen until boiling at the walls

of the vials ceased. With a minimum of elapsed time and direct handling, each vial was wrapped in a small Kimwipe (average weight was 0.54 g, dimensions were 21.7 x 12.3 cm), crushed with a lead brick, and transferred to a 100 ml round-bottom flask which constituted the reaction vessel of the modified Gorsuch digestion apparatus (Figure 4). The flask was immediately immersed in liquid nitrogen until boiling subsided. Using a Pasteur pipette, the entire sample was coated with 5-10 ml of a 9:1 mixture of sulfuric and nitric acids, and 2.0 ml each of carriers "A" and "B" (Table 22). The flask was removed from the liquid nitrogen bath, connected to the apparatus, and allowed to warm until the frost cleared before immersing in an oil bath maintained at 90°C. To prevent foaming, magnetic stirring was not applied until the sample had liquified (i.e., approximately 5 minutes after placement in the bath). After at least 15 minutes of stirring, incremental additions of 90% hydrogen peroxide were initiated. The hydrogen peroxide additions were continued until the digest cleared or until 8 ml of peroxide had been added. During the peroxide additions, the three-way stopcock was in the reflux position and the cold-finger was kept charged with liquid nitrogen. After the last addition of peroxide, 2.0 ml of carriers "A" and "B" were added to the digest which was stirred until the

effervescence ceased. The apparatus was removed from the oil bath and allowed to cool. Afterwards, the contents of the reaction vessel were transferred to a 50-ml volumetric flask. All rinse water was collected and added to the volumetric flask. When the digested samples were transferred from the reaction vessel to the volumetric flask, a funnel with a small amount of Pyrex wool was used to prevent the transfer of pieces of quartz to the volumetric flask. After dilution to volume, aliquots of the digest solution were processed by the decontamination and concentration steps.

4. AG2-X8 bromide form

Depending on the experiment, 5-10 ml of the ashed sample and 2 ml of a 12% (w/v) solution of hydroxylamine hydrochloride were pipetted into 30 ml of 48% hydrobromic acid. The acid solution was eluted through a 5.0-cm column of the bromide form of the AG2-X8 resin. Prior to applying the sample solution, the column was equilibrated with 3 resin bed volumes of the 48% hydrobromic acid. Flow rates were not allowed to exceed 1 ml per minute. The eluent was collected in 125-ml plastic bottles with screw caps. After the initial sample solution was eluted, the column was rinsed with 50 ml of HBr which was combined with the initial eluent. The resin bed was rinsed with 48% hydrobromic acid from the support column into a wide-mouth 10-dram plastic vial.

5. HAP, alumina, AG2-X8 chloride form

A second 5-10 ml aliquot of the digest solution was pipetted directly onto a 3-cm bed of AG2-X8 resin in the acetate form which had been equilibrated in 0.03 M nitric acid. Following elution of the sample, the bed was rinsed with 20 ml of 10 M nitric acid. All eluents were collected and mixed with 60.0 ml of concentrated nitric acid. The acid solution was eluted through a column consisting of 3.0 cm of HAP and 1.0 cm of alumina which had been equilibrated with several bed volumes of 10 M nitric acid. After elution of the solution at a rate of less than 1 ml/min, the column was rinsed with 10 M nitric acid. All eluents were collected in a 125-ml plastic bottle with a screw cap. The resin beds were discarded.

IV. RESULTS AND DISCUSSION

A. Objectives

The overall objective of this work was to develop and evaluate the schemes utilizing destructive neutron activation analysis (DNAA) without yield determinations for the determination of trace elemental components in biological materials. More specifically, the DNAA scheme was to be evaluated in terms of its possible use as a medical diagnostic tool. This work was not only concerned with the accuracy and precision of the technique, but also with simplifying the processing operations and minimizing the time between securing the sample and obtaining results.

B. Outline of Proposed DNAA Scheme

Based on the literature review, several points concerning the proposed DNAA scheme were adopted. In order to exploit the full potential of a radiochemical technique to eliminate the problems associated with contamination of a sample by reagent blanks, pre-irradiation treatment of the sample should be limited to freeze-drying. The irradiation container should be fabricated from quartz or silica, preferably of synthetic origin. To eliminate the need for post-irradiation weighing of samples and the subsequent potential for sample losses, the irradiation vial and the sample should be included in the process used to destroy the

organic matrix. For reasons of versatility, simplicity, and speed of operation, destruction of the organic matrix should be accomplished by a wet ashing procedure. Radioactive species present after ashing which would interfere with the collection and interpretation of gamma spectra of the sample should be removed by chemical processing. Tentative decontamination systems should include hydrated antimony pentaoxide and alumina for the removal of radionuclides of potassium, sodium, and phosphorus. An anion exchange system should be included as a means to concentrate the analyte species prior to assay.

The approach employed in the development of the DNAA procedure required verification of the effectiveness of each proposed operation. Experimental investigations of the various operations were begun simultaneously in four areas: 1) capsulation, 2) wet ashing, 3) decontamination, and 4) concentration and separation.

C. Freeze-Drying

In devising the attack on the problems associated with the pre-irradiation aspects of an analysis by DNAA, a study of the behavior of the biological standards during freeze-drying was eliminated. As inferred from the cited literature, the best way to ascertain the effects of freeze-drying on a particular matrix is to compare the results of duplicate analyses performed on freeze-dried and untreated aliquots of

a sample previously dried by another method. However, the preferred way to accomplish the analyses would be a DNAA procedure valid for biological materials. Since it was the objective of the project to develop this scheme, the freeze-drying study was considered premature. No difficulties should arise if the parameters of freeze-drying operations presented in the literature were followed (92,118). This should ensure that the characteristics of the dried samples are identical to samples in previous works for which compositional data is available.

D. Capsulation

The objectives of the capsulation studies were as follows: 1) determine the relative "purity" of the container material, and consequently, the feasibility of including the container in the ashing step; 2) devise and test a system for sealing the containers without the loss of sample or sample components; and 3) devise and test a system for transferring the irradiated samples to the ashing apparatus.

Synthetic CFQ (Clear Fused Quartz) T21 Supercil tubing was tentatively selected as the container material. The imperviousness of the quartz to the ashing systems tentatively selected for inclusion in the DNAA scheme was the primary reason its selection. A synthetic quartz was chosen because of the trend for lower blank values in this type of material (106).

The "purity" tests were actually concerned with the extent to which potentially interfering species were leached from the quartz during the ashing operation. Table 25 lists elements which were identified in the digest remaining after a wet ashing procedure was executed on samples of irradiated CFQ T21 quartz. The relative amounts of elemental components common to digestion extracts of quartz and biological materials are presented in Table 26 as the ratio of their specific activities. Neutron activation analysis was used to approximate the actual amount of elemental species extracted from quartz during ashing (Table 27). The results of the specific activity measurements and the semi-quantitative analysis indicated only limited possibilities that materials leached from the irradiation containers would interfere with the analysis of a sample. Significant errors would occur only for determination of gold in blood and antimony in both the blood and bovine liver standards.

Specifics of the two experimental procedures indicated that the quantitative data was more reliable. The specific activities in the biological samples were determined without chemical processing of the samples. Consequently, all of the difficulties which normally plague the analysis of biological materials by neutron activation were present. Decay periods necessary to eliminate interferences resulted in the loss of data for shorter lived nuclides (i.e., As, Cu, etc.).

Table 25. Elements identified in digest remaining after "wet ashing" CFQ T21 quartz

Element	Element
Ag[a]	Na
As[a]	Sb
Au	
Cr	Sc
Cu	Se
Hg[a]	Sm[a]
K	W
La	Zn

[a]These elements were detected in only one of three analyses performed on the synthetic quartz.

Table 26. Ratio of specific activities of nuclides common to Amercil Quartz and biological samples[a]

Nuclide	Energy (Kev.)	Blood	Liver
^{76}As	657	---[b]	---[b]
^{60}Co	1173	.05	.05
^{51}Cr	320	---[b]	---[b]
^{24}Na	1368	.0002	.0002
^{42}K	1524	.0002	.0001
^{122}Sb	564	0.46	1.15
^{124}Sb	603	0.78	0.28
^{75}Se	264	0.03	0.01

[a] $\text{Ratio} = \frac{\text{SpAc Quartz}}{\text{SpAc Bio. Smpl.}} \times 100.$

[b] The nuclide was detected in the biological samples but poor counting statistics prevented calculation of the ratio.

Table 27. Quantitative results for elements common to CFQ T21 quartz, bovine liver, and U.K. master mix blood

Element	Energy (Kev.)	µg in Quartz[a]	µg in Liver[a]	% Ratio Qrtz/Liver	µg in Blood	% Ratio Qrtz/Blood
As	559	ND[b]	2.2×10^{-2}	---[c]	1.0×10^{-2}	---
Au	412	6.5×10^{-4}	ND	---	1.0×10^{-4}	647
Co	1173	2.6×10^{-3}	7.2×10^{-2}	3.6	ND	---
	1332	3.2×10^{-3}	7.2×10^{-2}	4.4	ND	---
Cu	1345	5.6×10^{-3}	77.2	1.4	0.01	0.40
Sb	564	1.5×10^{-2}	ND	---	4.0×10^{-3}	382
	693	1.5×10^{-2}	ND	---	4.0×10^{-3}	374
	603	1.6×10^{-2}	ND	---	4.0×10^{-3}	412

[a]The vial weighed 4.81 g. Each biological sample was approximately 400 mg. Both weights were typical of actual experimental conditions.

[b]Not detected.

[c]Not calculated.

The quantitative data was obtained by comparing the activities of the quartz extract with the activities of a standard solution. Specific interferences were thus eliminated. Even with the superior spectral characteristics, the results nevertheless represented only approximations of the amounts of materials leached from the quartz. Exact determination of the amount of quartz transferred to the ashing apparatus with the standard was not possible because of deposits left on the vials by the gas-oxygen torch. Further uncertainty resulted because the extent of the leaching process was dependent on the exposed surface area. The degree to which the vials were crushed could lead to variations in the amount of material leached from the quartz.

The trace elemental composition of CFQ T21 Supercil tubing was not determined. The elemental composition of the synthetic quartz materials, Quartex and Spectrosil, listed in Table 28 should provide an idea of the levels found in the Supercil quartz.

E. Sample Containers

The liquid nitrogen cryostat shown in Figure 2b was designed for enclosing biological samples in quartz irradiation vials (Figure 2a) without causing significant thermal damage to the sample. Three questions concerning the irradiation capsule needed to be answered: 1) Were any sample components lost sealing the vials? 2) Could the vials contain the

Table 28. Masses of elements contained in 4.81 g of two kinds of synthetic silica glass[a]

Element	Quartex (Fused Quartz)	Spectrosil (Fused Silica)
Au	4.81×10^{-2}	6.25×10^{-4}
Cl	$<4.81 \times 10^{-1}$	1.78×10^{1}
Co	6.25×10^{-3}	$<4.81 \times 10^{-3}$
Fe	6.93	<2.41
K	<4.81	<2.41
La	7.7×10^{-3}	1.11×10^{-2}
Mn	1.3×10^{-1}	1.5×10^{-1}
Sb	1.6	1.9×10^{-3}
Sc	7.2×10^{-4}	4.8×10^{-3}
Se	4.4×10^{-3}	---
Sm	1.4×10^{-3}	1.8×10^{-3}
Zn	3.5×10^{-1}	4.8×10^{-2}

[a]Based on data of Maziere, *et al.* (106).

pyrolysis and radiolysis products generated in the reactor environment? 3) Could the procedure devised for opening the vials following irradiation prevent the loss of sample components?

Excessive radiation and spectral contaminants would have complicated, if not defeated, any attempt to use irradiated biological materials in this study. Radio-tracers in combination with biological matrices were used to follow trace elemental components through the processing operations. An oven was used to simulate the heating conditions experienced by the sample in the reactor. To ensure the production of similar decomposition products, the oven temperature (150°C) was intentionally set in excess of the ambient temperature of the ALRR (65°C). The higher temperature was needed to account for internal heating of the sample which would occur in the reactor from intense gamma ray flux and induced nuclear reactions.

The data presented in Table 29 represents the recoveries of radio-tracers from vials following each operation normally associated with the vial-sample system (i.e., sealing, heating or irradiation, and opening). The results indicated that the irradiation capsules and the sealing and opening procedures were effective for manipulating biological samples without the loss of trace elemental components. Experimental results of the study were similar for the blood and bovine liver matrices. The values presented in Table 29 are the

Table 29. Summary of ampoule test results. The "pre-sealed" recoveries indicated that the same amount of each nuclide was in the test and reference vials. The remaining recoveries indicated that relative to the reference vials no material was lost from the test vials during sealing, heating and decanting

Nuclide	En	Pre-Sealed %R[a]	σ	Sealed %R	σ	Heated %R	σ	Decanted %R	σ
Co	1173	98.3	0.8	101.5	1.1	97.2	2.4	102.9	1.9
	1332	97.9	0.9	98.0	1.3	97.2	2.5	99.8	1.9
Cr	320	103.3	2.0	103.1	2.2	97.3	4.8	100.2	3.3
Fe	1099	98.6	4.2	87.2	3.4	92.0	8.0	105.5	23
Hg	279	104.6	1.1	104.3	2.0	99.2	3.3	98.6	1.9
Sb	603	97.4	1.6	93.2	2.3	97.3	2.7	105.2	1.5
Sc	889	101.1	0.7	102.9	1.0	105.6	2.2	100.6	1.0
	1120	102.1	0.8	100.2	1.0	101.7	2.3	99.4	1.7
Se	264	103.9	0.9	104.2	1.1	103.6	2.6	98.4	1.5
Zn	1115	99.1	0.9	99.0	1.2	98.8	2.7	100.2	2.0

[a]R = Recovery.

average recoveries for both matrices in three to five separate experimental observations.

The tracer solutions used in this study were similar in elemental composition to others used throughout this project. Repeated irradiations of biological matrices under time and neutron flux conditions similar to those anticipated for the final DNAA scheme showed that the elements listed in Table 29 were consistently detectable. Arsenic was included in the tracer solution, but during the delay between irradiation and use of the tracers, the count rate of the arsenic isotope decayed below spectral background levels. When some authors indicated difficulties retaining arsenic, they also expressed concern about volatile forms of mercury and selenium (113). The quantitative recoveries of mercury and selenium in this work implied that arsenic was not lost by volatilization. The apparently low recovery of iron following the sealing operation was due to spectral distortions caused by statistical fluctuations in the small number of counts accumulated for the iron isotope. The shape of the photopeak deviated significantly from a gaussian function, resulting in a negative error in the peak fitting routine. Extended counting periods in later experiments mitigated this problem.

F. Wet Ashing

In the wet ashing studies, the objectives were to select an ashing medium and develop a procedure for its application to biological samples. Each ashing system was evaluated with respect to: 1) completeness of the matrix destruction, 2) time required for the operation, 3) parameters controlling the loss of trace elemental components, and 4) compatibility of the ashing medium with other operations in the DNAA procedure.

Some restrictions were initially adopted in choosing an ashing medium. Systems requiring ashing temperatures significantly in excess of 100°C were avoided due to previously demonstrated losses of trace components at elevated temperatures (113). Ashing media containing halogens or perchloric acid were initially shunned because of studies demonstrating the loss of trace elements due to the formation of volatile halides (107,113). Sulfuric acid was not considered a viable component for an ashing system because of excessive temperatures usually associated with its use and the possibility for loss of trace components *via* co-precipitation with insoluble sulfates.

The Bethge apparatus as modified by Gorsuch (Figure 3) was used in the initial studies of ashing systems. The advantages of this type of apparatus were: all operations were performed in a "closed" system and the ashing operation

could proceed in either a reflux or distillation mode. (The distillation mode was required with systems like the perchloric-nitric acid mixtures in which the nitric acid had to be removed after an initial pre-digestion in order to utilize the superior oxidizing properties of the perchloric acid.)

The difficulty encountered when successive small additions of reagent to the reaction flask were required was the primary disadvantage of the Gorsuch version of the Bethge apparatus. As designed, the air condenser was the intended avenue for the introduction of reagents. However, this required either the removal of the water-cooled condenser or the addition of the reagent through both condensers. Removal of the water condenser violated the concept of a 'closed' system and was also a cumbersome undertaking. On the other hand, the large surface areas of the combined condensers made that path ineffective for the addition of small amounts of reagent. The modified Gorsuch version shown in Figure 4 was designed to provide: 1) a more effective condenser to trap volatiles leaving the system, and 2) a better means for introducing reagents to the reaction vessel during an ashing operation.

In the discussion which follows, the characteristics of ashing systems which were extensively studied are detailed. The progression through different systems was not haphazard,

but usually was determined by the results of compatibility tests. Specific incompatibilities will be described in a separate section. Rather than describing only the ashing system selected for inclusion in the finalized DNAA scheme, information concerning the "failures" is reported so that others may avoid futile efforts.

In accordance with the earlier described restrictions, nitric acid was tested as an ashing medium for blood and bovine liver. Althcugh adequate for destruction of the blood matrix, the nitric acid left clear globules of material floating in the digest solution of bovine liver. Extended refluxing periods did not remove this material. Even though the material was not analyzed, it was tentatively identified as lipids from the bovine liver. Authors have indicated difficulties in destroying this component in most biological matrices (107,127,134).

Efforts to find a stronger oxidizer for the ashing operation centered on perchloric acid. Use of this reagent necessitates several precautions. Gorsuch noted that free chloride found in perchloric acid could lead to the loss of trace elements via the volatilization of chlorides (109). Girardi also noted that the presence of perchloric acid severely reduced the selectivity of hydrated antimony pentaoxide, a material intended for use in a different stage of the DNAA scheme. In an attempt to mitigate these potential difficulties, the amount of perchloric acid in the

ashing procedure was minimized. The reported recoveries of radio-tracers from biological samples ashed by the devised nitric-perchloric acid system (Table 30) demonstrate the immunity of the system to losses of trace components. In order to obtain sufficient counting statistics within a reasonable time period, the amount of each radionuclide used in the ashing test was four times the amount of that nuclide thought to be indigenous to the biological matrix. However, the total concentration of each element in the test was controlled by the addition of stable carriers, and was fixed at the level expected to be used in the analysis of real samples.

Although the nitric-perchloric system was abandoned because of an unexpected incompatibility with the HAP sodium decontamination step, the ashing system possessed a number of good qualities. The ashing operation could be completed within 0.5 hours depending on the source of heat. No difficulties related to the potential explosion hazard which exists on reacting perchloric acid with biological materials were encountered. The resulting ashed solution was usually clear, and varied from colorless to a faint yellow.

Down and Gorsuch had extensively studied various ashing procedures which incorporated sulfuric acid and 50% hydrogen peroxide (132). They indicated that in many cases this mixture could replace the nitric-perchloric acid mixtures. Their procedures contained three apparent disadvantages

Table 30. Recovery of tracer nuclides following a $HNO_3/HClO_4$ wet ashing procedure

Element	Energy (KeV)	% Recovery	σ
As	559	97.1	0.4
	657	97.9	2.7
	1216	100.2	6.9
Co	1173	99.3	6.7
	1332	100.1	1.6
Cr	320	98.9	11.1
Fe	1099	106.4	3.2
Hg	297	97.2	4.7
Na	1368	101.9	1.0
Sb	564	96.2	2.3
	693	93.1	5.2
Sb	603	97.4	1.4
	1693	96.8	1.9
Sc	889	96.6	3.4
	1121	103.3	7.4
Se	264	97.5	1.2
Zn	1115	103.4	8.2

relative to the analysis of biological materials: 1) the procedures required relatively large volumes of sulfuric acid (e.g., 20 ml) and strong heating for preliminary destruction of the matrix; 2) the final volume of reagents required for the operation were large (e.g., approximately 40 ml); and 3) the total time for the ashing operation was in excess of 2 hours.

Ashing procedure DG-3 employing sulfuric acid and hydrogen peroxide was designed to eliminate most of the inadequacies in the method of Gorsuch and Down. Since the matrices for which the ashing system was developed are not as impervious as the materials examined by Down and Gorsuch (i.e., polyethylene, liquid paraffin, etc.), the sulfuric acid was reduced to the minimum amount necessary to wet the sample, and charring was eliminated. To further reduce the volume of the reactants and at the same time improve the oxidizing capacity of the mixture, 90% hydrogen peroxide was used as the primary oxidant.

Ashing procedure DG-3 gave variable results. The effectiveness of the ashing operation was very dependent on the extent of destruction caused to the matrix by the sulfuric acid. For optimum destruction, the slurry of sulfuric acid and the matrix had to remain in the oil bath over 1.5 hours. Elevating the temperature of the oil bath (Procedure DG-3A) improved the overall effectiveness of the system. Table 31 contains the results of tracer recovery experiments executed with ashing scheme DG-3A.

Table 31. Recovery of tracer nuclides following a H_2SO_4/H_2O_2 wet ashing procedure at a temperature between 70°C and 90°C

Nuclide	Energy (KeV)	Recovery (%)	σ
As	559	98.9	0.2
	657	96.6	4.4
	1216	108.4	3.0
Co	1173	98.2	2.9
	1332	100.2	2.5
Cr	320	106.0	3.5
Cu	1345	98.3	3.6
Fe	1099	114.1	5.5
	1292	102.7	5.7
Hg	279	97.1	2.9
La	1596	98.7	2.0
Na	1368	82.0	4.8
Sb	564	100.1	0.3
	693	101.1	4.2
Sb	603	99.6	2.1
	1292	95.6	6.3
Sc	889	101.6	2.1
	1120	101.1	2.3
Se	264	89.9	5.2
Sm	103	84.4	2.0
Zn	1115	101.1	3.8

Further improvement of the reaction of sulfuric acid on the matrix was sought by increasing the temperature of the oil bath to 90°C. The modified Bethge apparatus was employed to provide a more effective trap for volatiles and a better means for controlling the rate of addition of hydrogen peroxide to the mixture. As indicated in Table 32, the system successfully contained the trace elemental components. As an extreme test of the effect of elevated temperatures on the sulfuric acid-matrix interaction, and of the efficiency of the cold trap, ashing scheme DG-3C (which required heating the sample to fumes of sulfuric acid) was executed. Of the elements listed in Table 32, only selenium was not recovered quantitatively.

DG-3B represented an effective method for the destruction of the blood and bovine liver matrices, but it was not dependable. The viscosity of the sulfuric acid produced the major problem. Even with magnetic stirring, it was difficult to ensure that the entire sample was in contact with the sulfuric acid. Material which had not reacted with the sulfuric acid was not affected during the addition of hydrogen peroxide. The use of secondary or pre-oxidizer in conjunction with sulfuric acid during the initial attack on the matrix was studied. According to the literature, removal of the reducing environment during processing of the sample would also eliminate a situation conducive to the loss of selenium and mercury (132).

Table 32. Recovery of tracers from a H_2SO_4/H_2O_2 wet ashing medium and the modified Gorsuch device

Element	Energy (KeV)	Recovery (%)	σ
As	559	96.2	1.2
	657	97.7	1.8
	665	101.1	0.6
Co	1173	99.8	2.0
	1332	99.4	0.6
Cr	321	101.2	1.3
Cu	1345	97.1	1.2
Fe	1099	97.6	2.8
Hg	279	95.4	0.8
Mo	740	92.3	3.8
Sb	564	97.6	2.4
	693	100.8	4.2
Sb	603	99.8	1.0
Sc	889	98.7	0.8
	1120	96.0	0.9
Se	264	96.2	2.5
W	606	96.8	1.5
	479	95.8	0.4
Zn	1115	95.9	2.4

Chromic acid (DG-3H), vanadium pentaoxide (DG-3I), and nitric acid (DG-3J) were tested as additives during the treatment of the biological matrix with sulfuric acid. In each case, they were initially favorable. Liquification of the samples required significantly shorter time periods in the oil bath, and the amount of peroxide needed to complete the matrix destruction was significantly reduced. As indicated by the clarity of the final solutions, chromic acid and vanadium pentaoxide were more effective in destroying the matrices. The inorganic oxidizers notably increased the vigorousness of the reaction of the ashed mixture and the hydrogen peroxide. On the other hand, the ashed mixtures containing the chromium and vanadium species were also sensitive to excess hydrogen peroxide. With excess peroxide, a reddish-brown precipitate formed with either oxidizer as the ashed mixtures cooled. The precipitates were probably peroxychromates and peroxo compounds of vanadium (139, p. 840). The specifics for formation of the precipitates were not developed because incompatibility tests eliminated the procedure. As a side note, recovery tests with tracers did indicate quantitative retention of all elements in these digestion systems.

Inclusion of a small amount of nitric acid with the sulfuric acid was effective in increasing the initial attack on the matrices. However, it was evident that the nitric acid did not aid the destruction of the matrix during the

additions of peroxide. Detrimental factors previously attributed to nitric acid were avoided because the acid was converted to oxides of nitrogen by the hydrogen peroxide. Quantitative recoveries of trace elemental components were also obtained with this system.

G. Decontamination and Separation

1. HAP

Hydrated antimony pentaoxide was selected as the means for removing radio-sodium (^{24}Na) from solutions of ashed biological materials. As noted in Chapter II, considerable disagreement exists concerning the properties of this material. The disparity was traced in part to the differences in HAP produced by the experimenters and inconsistencies in HAP obtained commercially. In order to avoid this situation, the synthesis and testing of HAP to be used in this project was undertaken.

Nine different products of HAP synthesis procedures and a sample of HAP produced by C. Erba (Milan, Italy) were examined. Table 33 contains a listing of some characteristics and properties observed for the HAP materials during testing. Full synthesis procedures (i.e., starting with a 1-lb bottle of $SbCl_5$) yielded approximately 200 g of product, but sieving reduced the amount of usable material to about 100 g.

In Table 33 "Mechanical Properties" refers to the effectiveness of the material for forming a resin bed for column chromatography. The HAP materials 4A, 4B, and 5B were not extensively tested because on equilibration with an acid eluent, they formed an amorphous mass which blocked eluent flow through the column. HAP 1A, although good with respect to sodium removal and nuclide elution, was unstable in extended contact with the principal eluent, 12M HCl. With regard to correlation between observable characteristics and the effectiveness of the material for sodium removal, the best materials had a powdery texture and a yellow color. The deeper the yellow, the more stable the material was in concentrated hydrochloric acid. HAP 1B was the only crystalline material that performed satisfactorily in the selectivity and sodium retention tests.

The percent of antimony in each of the samples of HAP was determined by neutron activation analysis using the standard comparator method. Except for HAP 3, the materials are quite similar in this respect. In the original paper concerning the properties of HAP, Girardi and Sabbioni reported an antimony content of 68.3 ± 0.3% for their experimental material, which agrees with the value determined in this analysis for the C. Erba material. Since the antimony content of the products 4A, 4B, 5A, and 5B was not determined, correlations could not be drawn concerning the quality of the material and the percent of antimony.

Table 33. Characteristics of HAP materials

Material	%Sb	$n \cdot H_2O$	$O+n \cdot H_2O$	%Δ	Na Decon (%)	Mechanical Properties	Description
HAP 1A	67.0±2.0	2.2±0.3	15.4	13.5	99.87-99.94	Good	Powdery, pale yellow, unstable in conc. HCl
HAP 1B	69.8±1.5	1.4±0.2	11.9	13.5	---	Excellent	Crystalline, yellow
HAP 2	70.4±0.7	1.2±2.0	11.3	2.5	5-7	Good	Powdery, grey
HAP 3	62.2±0.6	1.6±0.2	12.8	8.9	33.5	Good	Crystalline, white w/yellow
HAP 4A	---	---	---	---	---	Very Poor	Crystalline, pale yellow
HAP 4B	---	---	---	---	---	Poor	Crystalline, brilliant yellow
HAP 5A	---	---	---	---	79.98	Good	Powdery, light yellow
HAP 5B	---	---	---	---	22.8	Good	Crystalline, white translucent
HAP 6	68.2±3.9	1.7±0.4	13.0	7.8	99.998	Excellent	Powdery, golden yellow
HAP 7	69.5±0.5	1.5±0.2	12.2	7.5	>99.99	Excellent	Powdery, light golden yellow
C. Erba	68.4±0.7	1.6±0.2	12.7	12.9	---	Good	Powdery, yellow w/orange

Confounding any correlation are HAP 3 which had a significantly lower percentage of antimony and performed poorly in sodium removal, and HAP 2 which had a reasonable percentage of antimony but was absolutely worthless for sodium decontamination. The inclusion of HAP 2 in the comparison may not be valid because there is uncertainty in the molecular formula of this material. While HAP 2 was drying, the temperature accidentally exceeded 500°C for at least 4 hours. At this temperature, Sb_2O_5 should have been converted to the tetraoxide (192), which consists of 79.0% antimony. With only 70.4% antimony, HAP 2 could not be Sb_2O_4.

Using the values for percentage of antimony, and assuming no other major constituents besides water were present (as confirmed during the analysis for antimony), the percentage of Sb_2O_5 and consequently the mole ratio (n) of water molecules to formula weights of the pentaoxide were determined for some HAP materials. For comparison, Girardi and Sabbioni (153) reported that n equalled 1.5 for their product. Torok and Diehl reported n-values from 1.1 to 2.4 for a series of HAP synthesis procedures (145). Their best product for sodium retention had a mole ratio of 2.4. Interestingly, this material was not rated among the best with respect to mechanical properties. Also, they produced another product with a similar molar water ratio (i.e., $n = 2.4$) that performed poorly in the sorption of sodium.

The extraordinary heating of HAP 2 apparently caused that material to lose more water than the others. Overall, the suitability of a precipitate for the production of chromatographic columns varied inversely with the mole ratio of formula-water to formula-pentaoxide.

As a means to further evaluate the structure of the precipitates, a sample of each HAP material was equilibrated in an oven at 700°C. The column in Table 33 labeled "O + nH_2O" lists the percentage change in weight that would be expected of each material if an oxygen atom and all of the bound water was lost from the empirical formula as previously determined. Under the heading "%Δ", the actual percentage changes which occurred following equilibration at 700°C are listed. The products HAP 1A, HAP 1B, and C. Erba lost enough weight to represent removal of an oxygen atom and "n" molecules of water. Weight losses in HAP 3, HAP 6 and HAP 7 only indicated the loss of bound water.

Table 33 also includes sodium decontamination factors which indicate the percentage of sodium the HAP material removed from a reference solution. The precision of the decontamination factors was dictated primarily by the counting statistics. In many cases, the sodium content was reduced below the spectral background and the factor was based on the maximum possible sodium activity which could have been present but not detected (i.e., twice the square root of the background count rate in the region of the

expected peak). For HAP 6 and HAP 7 similar decontamination factors were obtained in 12 M HCl and 15 M HNO_3.

Table 34 contains the results of nuclide selectivity or elution tests on several of the synthesis products and the C. Erba material. The elution tests for HAP 6 were performed in both 12 M HCl and 15 M HNO_3, while the other materials were tested in 12 M HCl or 15 M HNO_3. HAP 1A, HAP 1B, and the C. Erba material were tested at approximately the same time, and the other products were tested over a year later. Part of the improvement in the later materials was probably due to better manipulation techniques. The most important point in this data is the failure to obtain quantitative recoveries of arsenic and selenium from the materials. This directly contradicts the original claims made concerning the inorganic exchanger (153). These results also cast doubts on those DNAA procedures which claim quantitative retention of arsenic and selenium on HAP columns. Typically, the recovery of mercury from the HAP materials was not quantitative. However, it was found that the elution of mercury was dependent on the eluent flow rate. In a few specially designed experiments, mercury was quantitatively eluted when flow rates significantly below 1 ml/min were used. At least 30 ml of eluent were required to ensure quantitative elution of most elements from a 3 - 5 cm column of the HAP material. Significantly, the selectivity of the HAP materials did not vary appreciably with respect to nuclides other than sodium.

Table 34. Recovery of nuclides from several HAP materials

Element	HAP 1A		HAP 1B		C. Erba		HAP 6				HAP 7	
							HCl		HNO_3		HNO_3	
	%R[a]	δ	%R[a]	δ	%R[a]	δ	%R[a]	δ	%R[a]	δ	%R[a]	δ
As	91.8[b]	0.3	65.3	1.5	63.3[c]	1.9	0.95	0.03	---[d]	---	---	---
Ba	98.2	2.0	---	---	---	---	---	---	---	---	---	---
Co	98.1	0.7	72.2	1.2	95.8	4.2	100.2	0.3	100.0	1.2	98.9	0.4
Cr	100.5	0.7	95.5	---	97.1	4.1	99.1	0.4	97.4	0.8	99.2	0.6
Eu	85.4	5.4	---	---	---	---	---	---	---	---	---	---
Fe	98.8	1.7	94.6	---	86.1[e]	3.5	98.5	0.6	98.2	1.2	97.1	0.8
Hg	---	---	73.9[f]	2.0	90.7	7.3	99.4	0.3	98.6	0.5	5.1	0.1
La	98.9	0.5	100.7	1.0	100.1	2.0	---	---	---	---	---	---
Lu	98.9	0.5	86.6	---	89.1	6.1	---	---	---	---	---	---
Mo	---	---	---	---	---	---	96.5	1.2	---	---	---	---
Rb	---	---	---	---	---	---	85.3	0.7	99.3	2.2	100.3	2.0
Sb	99.3	0.3	99.6	1.6	93.1	1.6	98.4	0.2	2.5	0.1	0.36	0.02
Sc	97.7[g]	0.6	98.3	1.8	99.4	2.1	99.1	0.2	5.3	0.1	98.4	0.3
Se	89.4	0.6	70.3[g]	---	84.4[f]	---	92.0	0.2	99.2	0.4	---	---
Sm	100.6	0.6	100.2	---	97.7	7.0	---	---	---	---	---	---
W	---	---	---	---	---	---	93.0	1.1	11.8	0.1	75.8	0.2
Yb	100.8	0.4	101.6	1.0	102.9	2.6	---	---	---	---	---	---
Zn	103.8	1.7	103.1	---	102.6	5.4	99.7	0.4	99.9	0.6	99.6	0.4

[a]Values are percent of nuclide eluted with 30 ml conc. HCl.

[b]Using 60 ml eluent, recovery was 93.7±0.1%.

[c]Using 60 ml eluent, recovery was 68.8±1.8%.

[d]Not determined or not detected.

[e]Using 60 ml eluent, recovery was 103.5±2.5%.

[f]Using 60 ml eluent, recovery was 97.5±4.7%.

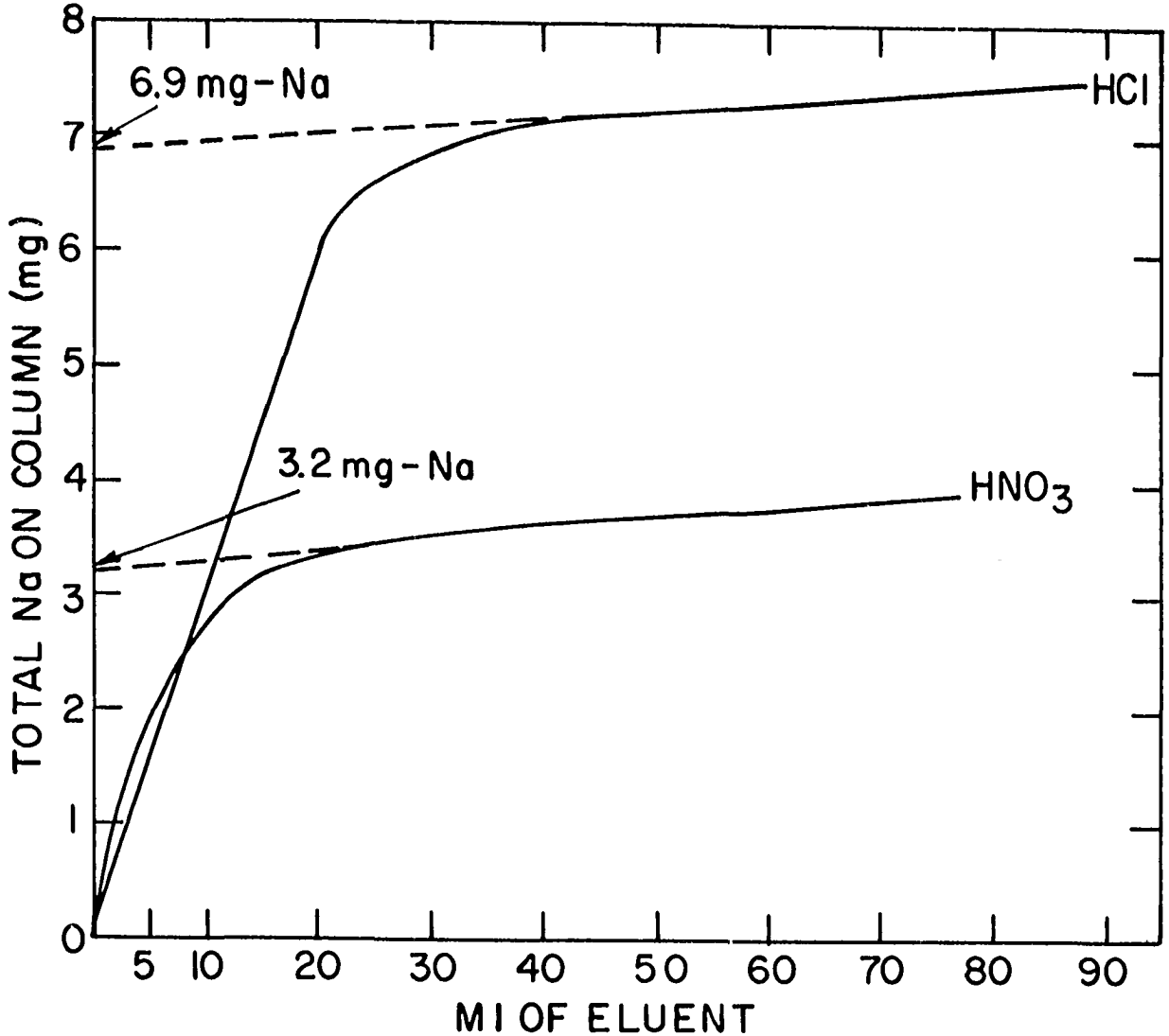
[g]Using 60 ml eluent, recovery was 90.5% for HAP 4, 79.5% for HAP 5, and 95.1% for HAP 6.

On the other hand, the nuclide selectivity of the HAP materials was dependent on the eluent system. With concentrated hydrochloric acid as the eluent, all elements tested except sodium, arsenic, selenium and mercury could be readily eluted from a HAP material. In concentrated nitric acid, additional elements were retained on the column. As the acid concentration of the eluents decreased, so did the selectivity of the HAP materials.

Following the synthesis of HAP 6 and HAP 7, a study was undertaken to determine the specific sodium capacity of these HAP materials. Figure 6 illustrates the results obtained from elution experiments with HAP 7 in nitric and hydrochloric acids. The maximum capacity was determined by extrapolating from the plateau of the curve to the ordinate (145,153). Values of 5.83, 2.12 and 1.77 mg-Na/g-HAP were determined for HAP 6 and HAP 7 in HCl, and HAP 7 in HNO_3, respectively. Girardi and Sabbioni initially reported a sodium capacity of 30 mg-Na/g-HAP (153). Torok and Diehl obtained values in the range 5 - 11 mg-Na/g-HAP.

Several facts were suggested by the synthesis and testing data. The degree of sorption of sodium ions from acid solutions was dependent on the amount of bound water in the HAP. However, the structures of the HAP materials could be different even when the mole ratios of bound water were nearly equivalent. Apparently, the temperature at

Figure 6. Integral sodium elution curve for HAP materials using hydrochloric and nitric acids


6.9 mg-Na
HCl
3.2 mg-Na
HNO3
TOTAL Na ON COLUMN (mg)
MI OF ELUENT
0
1
2
3
4
5
6
7
8
5
10
20
30
40
50
60
70
80
90

which the amorphous Sb_2O_5 was initially dried set the structure of the oxide, while the duration of drying determined the amount of bound water remaining in the compound.

The conditions within the oven were also significant in determining the structure of the oxide. If the excess water was not removed from the precipitate and final drying was attempted in a closed system (e.g., a muffle furnace) the water from the precipitate was held in contact with the precipitate and a white translucent material of no significant value was produced. The differences between the products HAP 1 and HAP 5B support this point.

As the mole ratio of water in the compounds decreased, the mechanical properties of the precipitates improved and the intensity of the yellow color increased. At the same time, the capacity of the materials for sodium apparently decreased. These factors suggested that solvation trapping (145) of sodium ions within the structure of the oxide was a significant mechanism for the irreversible sorption of sodium by HAP.

2. AG2-X8

A disadvantage in using a wet ashing step in a radiochemical procedure is the relatively large volume of the final solutions in which the analyte species are usually found. Gamma spectroscopy of species dispersed within a large volume is hampered by geometry effects produced by the

small size of the detector relative to the sample, and by self-absorption and attenuation of gamma rays by the bulk of the sample. Typically, an aliquot of a larger sample is used for gamma assay. In the case of biological samples with inherently low elemental concentrations and correspondingly low radionuclide count rates, it is particularly advantageous to include as much of the total content of the analyte species as possible.

Ion exchange was selected as the method for collecting trace components in a solution of an ashed biological matrix prior to spectroscopic analysis. Ion exchange offered the possibility of selective elution of components from the resin after collection. Selective elution would have allowed the separation of analyte species which were mutually detrimental to their quantification by gamma spectroscopy, either from unresolvable gamma rays (e.g., ^{203}Hg and ^{75}Se) or significantly overlapping photopeaks (e.g., ^{65}Zn and ^{46}Sc).

The anion exchange resin AG2-X8 was selected for use in this project because of its documented capability for removing selenium and mercury in the form of anionic complexes from acid solutions. Initially, experiments were directed towards sorption of the maximum number of different nuclides onto the resin, followed by the selective stripping of the nuclides from the resins. Tables 35 and 36 contain a summary of results obtained with radio-tracer solutions and

Table 35. Elution characteristics of AG2-X8 bromide form with various solvents

Element	Conc. HBr	.01M HBr	6M HCl +0.1% H_2O_2	NH_4OH	Conc. H_2SO_4	12M HNO_3	H_2SO_3	Na_2SO_3
			QE = Quantitatively eluted MB = Uncertain, could be quantitatively eluted PP = Partially eluted (50-90%) P = Poorly eluted (11-50%) VP = Very poorly eluted (5-10%) NE = Not eluted NT = Not tested					
As	NE	P	QE	PP	PP	PP	NT	NT
Co	NE	QE	PP	PP	QE	QE	PP	PP
Cr	QE	QE	NT	NT	NT	NT	QE	QE
Cu	NE	QE	NT	NT	NT	NT	NE	NE
Fe	NE	PP	NE	PP	MB	MB	PP	PP
Hg	NE	NE-VP	P	VP	NE	MB	VP	PP
Mo	NE	P	NE	VP	P	PP	NT	NT
Rb	NE	QE	QE	NT	NT	NT	NT	NT
Sb	NE	PP	VP	P	PP	PP	PP	PP
Sc	QE	QE	QE	NT	NT	NT	NT	NT
Se	NE	PP	MB	VP	PP	P	QE	PP
W	NE	P	NE	VP	PP	P	NT	NT
Zn	NE	P	NE	PP	P	MB	PP	MB

Table 36. Elution characteristics of AG2-X8 chloride form with various solvents

Element	Conc. HCl	8M HCl	.01M HCl	.01M HBr	2.5N NH_4OH	2N H_2SO_4	12M HNO_3	H_2SO_4	Na_2SO_3	Na_2SO_3 (Basic)
	QE = Quantitatively eluted MB = Uncertain, could be quantitatively eluted PP = Partially eluted (50-90%) P = Poorly eluted (11-50%) VP = Very poorly eluted (5-10%) NE = Not eluted NT = Not tested									
As	QE	NT	QE	NT	NT	NE	NE	VP	VP	NT
Co	NE	NE	QE	QE	MB	MB	MB	NT	NT	NT
Cr	QE	NT	QE	QE	NT	NT	NT	NT	NT	NT
Cu	PP	NT	QE	QE	NT	NT	NT	NT	NT	NT
Fe	NE	NE	MB	MB	PP	MB	MB	QE	PP	PP
Hg	NE	PP	VP	VP	PP	VP	PP	VP	P	PP
Rb	QE	NT	NT	NT	NT	NT	NT	NT	NT	NT
Sb	NE	NE	VP	VP	P	VP	P	VP	PP	PP
Sc	QE	NT	QE	QE	NT	NT	NT	QE	NT	NT
Se	PP	PP	PP	VP	VP	PP	PP	PP	PP	P
W	NE	NT	PP	NT	NE	P	PP	NT	NT	PP
Zn	NE-VP	PP	PP	PP	P-PP	P-PP	P-PP	P	P-PP	PP

a series of eluents on the bromide and chloride forms of the resin AG2-X8.

The eluent systems were chosen to identify different properties of the resin and the ionic species which affect the selectivity of the resin. Ammonium hydroxide was used to identify the properties of resin in a basic medium. The other eluents were selected to provide a variety of oxidizing and reducing conditions which allowed an assessment of the specificity of the resin to different ionic forms of a nuclide.

Of the nuclides tested, only chromium and scandium were not quantitatively collected on the bromide form of the AG2-X8 resin from a 48% hydrobromic acid solution (Table 36). After sorption, elution with up to 80 ml of 48% HBr failed to remove any of the ionic species from a 5.0 cm column of the resin. As the concentration of the acid was decreased, more elements could be quantitatively eluted. No eluent or combination of eluents was found which could strip all the tested nuclides from the resin. Except for the concentrated acid, no eluent was found which could quantitatively remove one group of nuclides and leave another group entirely on the resin. Partial success was obtained for the separation of the nuclides of selenium and mercury. After sorption from solutions of concentrated HBr, selenium was elutable with .01M HBr followed by 10M HNO_3 or 6M HCl containing 0.1% H_2O_2

to the extent of 88 - 96%. Usually less than 1% mercury was also eluted.

Complexes collected on the chloride form of the AG2-X8 resin were not as strongly held as on the bromine form. Concentrated HCl quantitatively eluted As, Cr, Rb and Sc with all of the other species in the test remaining entirely on the column. Repeated elutions with 12M HCl eventually lead to the breakthrough of Co from the column. However, Co could not be quantitatively eluted with a reasonable volume (i.e., <100 ml) of the eluent. No eluent combination was found which could quantitatively strip all test nuclides from the resin.

Elution of ashed biological materials through beds of the AG2-X8 resin also produced a decontamination effect for nuclides not of analytical interest. Evidently, the ionic species of sodium, potassium and phosphorus formed anionic species which interacted poorly with the resin, and were eluted with rinses of the concentrated acids. Elution of a sample which was sorbed on the bromide form of the resin with concentrated HBr removed radio-bromine from the column by an isotopic exchange process. Due to the higher selectivity of the resin for Br^- over Cl^-, elution of the chloride form of the resin with concentrated HCl resulted in the near quantitative retention of bromine nuclides from solutions of ashed biological materials. The ion exchange decontamination factors were not as impressive as those reported for the

inorganic exchangers (Table 37). The degree of phosphorus removal could not be measured directly.

Table 37. Percent removal of interfering activities from solutions of irradiated standard biological materials

	AG2-X8 (Br)	HAP/Al_2O_3	HAP/Al_2O_3/Acetate
Br	90.2	10.0	62.0
K	95.0	∿10.0	∿10.0
Na	98.0	>99.99	>99.99

The experiments performed with the acetate form of the AG2-X8 resin were for the expressed purpose of finding a method for removing Br^- from solutions of nitric acid. The best decontamination factor achieved with this system was approximately 67%.

3. Alumina

Of the materials employed for removing phosphorus nuclides from acid solutions, acid alumina was the most readily available and the least complicated to use. With

respect to this project, alumina was found to have the distinct disadvantage of being inoperative in hydrochloric acid solutions. The principal concern of the selectivity tests with alumina was to determine behavior of ionic forms of Cr, Sc and Rb in solutions of nitric acid of various concentrations. Elution tests were performed with alumina in 15M, 12M and 10M nitric acid. Concentrated nitric acid reacted when initially mixed with alumina, producing oxides of nitrogen. Whether or not this reaction was significant in determining the properties of the alumina was not determined. Selectivity experiments showed that the sorption of test nuclides by alumina decreased with decreased concentrations of the acid. However, since the eluent from the alumina column was to be eluted through a column of HAP, it was necessary to keep the acid concentration as high as possible to prevent the sorption of specific nuclides by the HAP. Recoveries of Co, Cr, Fe, Rb, and Sc from solutions which were 10M in nitric acid exceeded 97% for elutions through 2.0 cm columns of alumina.

The extent of phosphorus decontamination was measured semi-quantitatively by calculating the level of the background in the low energy region (i.e., 100 - 200 keV) of a high resolution gamma ray spectrum. Using irradiated biological materials, the background in the region of 320 keV (energy of the ^{51}Cr gamma ray) was reduced by approximately a

factor of 10. The primary detriment to larger decontamination factors was an apparently low specific capacity of alumina for the phosphorus nuclides and a slight but significant inverse correlation between recoveries of chromium and the amount of alumina in the column. The capacity of alumina for phosphorus was estimated to be considerably less than 1 mg-P/g-alumina. Since the primary reason for investigating the removal of phosphorus was to obtain better data on the partially obscured chromium peak, the extensive use of alumina for removing phosphorus was self-defeating. A column bed of 2.0 cm was a compromise which obtained a reduction in the background and still maintained nearly quantitative recoveries of chromium.

H. Compatibility Tests

As shown in the Literature Review, a variety of methods were usually available to accomplish the chemical processing typically required in a DNAA procedure: destruction of the organic matrix, removal of interfering radionuclides, and concentration and separation of the analyte species. The most stringent criteria limiting the inclusion of a particular operation in a DNAA procedure was the effect of the parameters of that operation on other stages of the procedure. For example, a digestion medium which allowed quantitative recoveries of all trace constituents but

dissolved the material used in the sodium decontamination step was of little use.

The presence of excessive sodium radioactivity during gamma spectroscopy is the predominant detriment to the analysis of biological materials. Therefore, development of a successful sodium decontamination step was considered to be the most crucial phase in formulating a feasible DNAA procedure. The conditions required to exploit the optimum performance characteristics of the HAP material were initially considered an invariable part of the proposed procedure. All operations in the tentative DNAA procedures were originally designed to allow execution of the sodium decontamination operation on a HAP column in a medium of concentrated hydrochloric acid. As combinations of operations were examined, it became apparent that numerous incompatibilities existed between the first-choice methods for the ashing, sodium decontamination, and concentration phases in the procedure. The nature of the incompatibilities caused compromises which resulted in a DNAA procedure based on operations executed under less than optimum conditions.

1. HAP columns

HAP exhibited maximum absorption of radio-sodium from solutions which were 12M in hydrochloric acid. The earliest wet ashing schemes involving nitric acid or nitric and perchloric acids were unacceptable because of an

oxidation-reduction reaction which occurred when the digest was diluted to volume with concentrated hydrochloric acid. This problem was foreseen in designing the operations, but was thought to be avoidable by limiting the amount of nitric acid in the digestion vessel. Tests eventually showed that the problem persisted even when the concentration of nitric acid in the aliquot of sample transferred to the column was as low as 0.2 molar. Further reduction of the amount of nitric acid added to the digestion flask resulted in an insufficient amount of medium to ensure complete ashing of the sample.

Individually, neither concentrated nitric nor hydrochloric acids detrimentally affected the HAP column. In combination, the acids caused extensive dissolution of the columns, resulting in less sodium removal. The dissolution was apparently associated with the redox reaction which was initiated when hydrochloric acid was added to the digest mixtures. Although the ratio of components was not correct, the color of the mixed acid solution and the apparent evolution of nitrogen oxides indicated a reaction similar to the formation of nitrohydrochloric acid. The generation of gas bubbles in the digest solutions reduced the accuracy of pipetting and caused disruption of the HAP columns. Volatilization of mercury halides during this processing could have accounted for the particularly low recoveries of mercury in the early HAP tests. A mechanism for the possible

loss of mercury in the zero valence state from this medium has also been described (90).

Switching to an ashing medium of sulfuric acid and hydrogen peroxide did not provide an immediately compatible digestion system. Hydrogen peroxide increased the solubility of the HAP material in concentrated hydrochloric acid and also prevented the quantitative recovery of some trace components (Table 38).

A number of methods were employed to destroy the peroxide before processing the samples through the decontamination and concentration steps. Boiling, a commonly cited means for decomposing hydrogen peroxide (139), was tested and abandoned for the following reasons: 1) in order to ensure destruction of the peroxide, excessively long periods of boiling (45 - 60 minutes) were necessary. 2) Extended boiling of the mixtures was undesirable because the condenser system was less than 100% efficient under harsh conditions.

Catalytic agents were examined for the destruction of hydrogen peroxide. Solid manganese dioxide (Digestion DG-3E) and a saturated solution of iron(II) sulfate (Digestion DG-3F) were evaluated as decomposition agents. Both materials effectively destroyed the peroxide without interfering with the quantitative recovery of trace components. The iron solution was by far the more convenient to use. The actual destruction of the peroxides was achieved through a stoichiometric rather than a catalytic mechanism. Early

experiences with the system demonstrated that minimal amounts of the reagents could have accomplished the destruction if sufficient time was allowed. Rather than wait, an excessive amount (relative to catalytic quantities) was added with a resultant reduction in the processing time. Typically, either 10 to 30 mg of manganese dioxide or 3 ml of the iron solution was sufficient to ensure destruction of the peroxide within a 5 minute period.

The addition of 2 to 3 ml of carriers "A" and "B" (Table 23) to the digest after the treatment with peroxide also eliminated the problems associated with residual hydrogen peroxide. Using the carrier solutions for the destruction of peroxide avoided the situation in which one analyte component was present at a radically higher concentration than the others. This situation was significant during the anion exchange operations. Apparently, differences in selectivity coupled with the high concentration of one component caused the elution of trace components which had previously been retained quantitatively. Color changes during the addition of the carrier and an elution phenomena discussed in the following section indicated that chromium in a lower oxidation state was involved in the mechanism governing the decomposition of the peroxide. Obviously, any other easily oxidized species could have been involved.

Table 38. Ashing and HAP recoveries from a medium containing H_2O_2

Nuclide	Ashing Recoveries		HAP Recoveries	
	%	σ	%	σ
As	97.8	4.4	48.3	0.5
Co	99.2	3.8	93.3	5.9
Cr	106.0	3.5	94.6	3.7
Cu	98.3	3.6	83.8	3.3
Eu	91.0	1.2	39.1	1.4
Fe	102.7	5.7	62.6	6.8
Hg	97.1	2.9	ND	ND
La	96.1	3.4	25.8	1.5
Sb	100.3	2.1	89.8	1.6
Sc	101.1	2.3	27.4	2.9
Se	89.9	5.2	ND	ND
Yb	95.9	3.6	93.4	5.4

2. AG2-X8 bromide form

Procedural incompatibilities affecting the anion exchange operations occurred only with the bromide form of the AG2-X8 resin. Reagents which promoted the oxidation of bromide to bromine were the central factor in all disorders. Specific problems were encountered with nitric acid, hydrogen peroxide, and chromium. Residual hydrogen peroxide and nitric acid caused the release of bromine gas from the AG2-X8 resin in the bromide form. In addition to disrupting the columns, the gaseous emissions probably resulted in the loss of trace components in the form of volatile bromides. If the concentration of peroxide on the column was reduced to approximately 0.1%, the formation of bromine subsided. The difficulties associated with hydrogen peroxide and nitric acid were countered with the methods described in the previous section.

In the presence of an ashed organic matrix, chromium reacted with the AG2-X8 resin, resulting in the release of bromine. Initial elution tests demonstrated that the oxidation-causing agent could be eluted with concentrated hydrobromic acid. Further tests with the species eluted with concentrated hydrobromic acid (Rb, Sc and Cr) indicated that chromium was the source of difficulty and suggested the oxidation of ionic species of chromium by hydrogen peroxide in the digestion step as the contributing factor. After testing a number of reagents, hydroxylamine hydrochloride was

selected as the best reducing agent for converting species of chromium to lower oxidation states. Hydroxylamine hydrochloride was selected because of its ease of use, mildness of reaction with the digestion mixture, and freedom from severe complications.

As expected, hydroxylamine hydrochloride could not be added directly to the total volume of the digestion mixture. If this were done, the digestion mixture would react on the HAP columns when eluted with nitric acid. The reducing agent was added directly to the column of the anion exchanger with an aliquot of the ashed sample. Complete suppression of the chromium reaction was accomplished with approximately 2 ml of a 12% hydroxylamine hydrochloride solution. Excess reducing agent caused the precipitation of a reddish-brown material which contained a significant amount of ^{75}Se. The precipitation of selenium and possibly mercury and copper could have occurred in a strongly reducing environment. However, the presence of radionuclides of copper and mercury was not positively detected. Further testing showed that if the amount of the reducing agent was controlled, the behavior of trace elemental components on the resin bed was not detrimentally altered.

I. Analyses of Biological Materials

The results of analyses of samples of United Kingdom Master Mix Blood and NBS Bovine Liver (SRM 1577) are

presented in Tables 39 and 40. By design, the AG2-X8 resin in the bromide form was intended to allow the determination of As, Au, Co, Cu, Fe, Hg, Mo, Sb, Se and Zn. According to earlier testing, these elements should have been quantitatively retained on the resin with a concurrent separation of sodium, potassium, bromine and phosphorus species during elution with concentrated hydrobromic acid. Figures 7 through 10 illustrate the advantages achieved with gamma spectroscopy. Approximately 90 - 96% of the sodium, 96% of the potassium, and 90% of the bromine present in the blood and liver samples were removed by this treatment.

The column of HAP, alumina, and AG2-X8 with nitric acid elutions was designed specifically for determining those elements which were not retained on the AG2-X8 resin in the bromide form (Sc, Rb and Cr). Unlike the anion exchange operation, the components of interest were to be eluted from the columns. Therefore, the decontamination column was formulated to retain the interfering species (Na, K, Br and P). Approximately 99.99% of the sodium, 80-90% of the potassium, and 60-65% of the bromine present in the blood and liver samples were retained by this treatment.

The actual reduction of the phosphorus in both materials could not be determined. All gamma rays in a multi-component sample contribute to the Compton background in the energy range where the phosphorous activities are prominent. Since the decontamination steps remove other components in addition

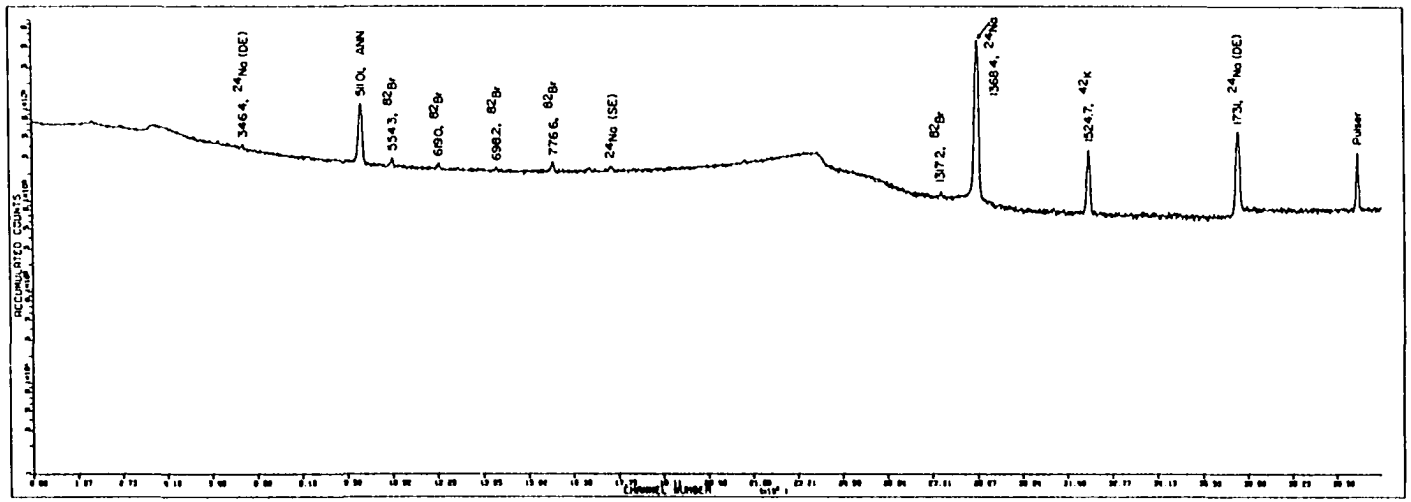


Figure 7. Spectrum of digested bovine liver sample without decontamination processing: sample decay time = 2.84 days; sample counting time = 0.50 hour(s)

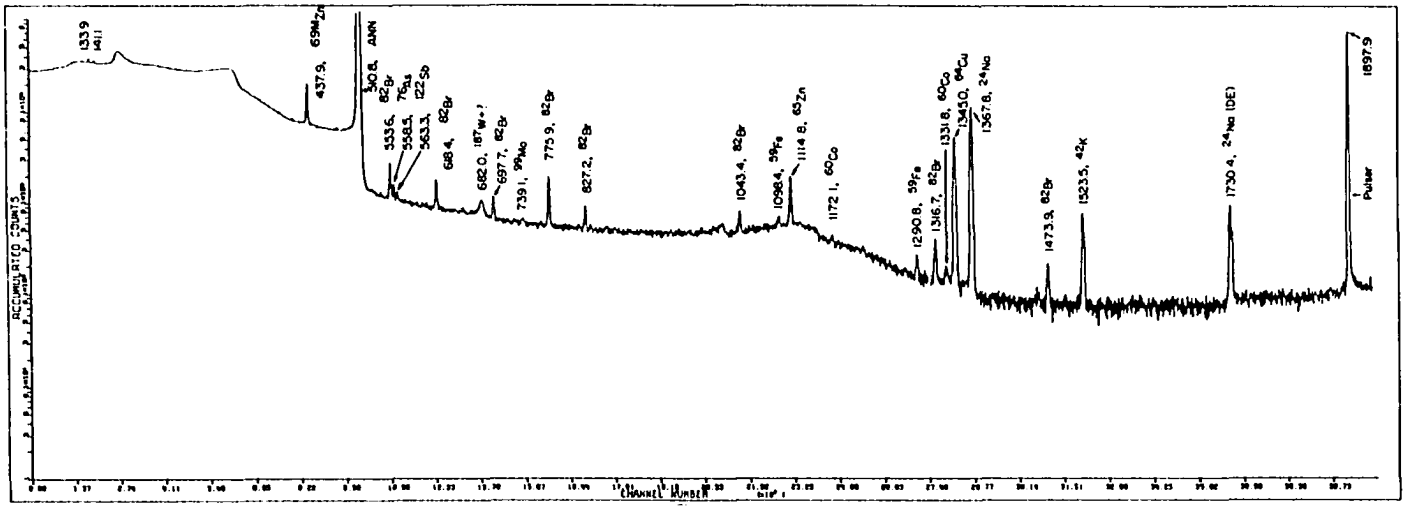


Figure 8. Spectrum of digested bovine liver decontaminated by elution through AG2-X8, in the bromide form, with concentrated hydrobromic acid eluent: sample decay time = 1.49 days; sample counting time = 1.85 hours

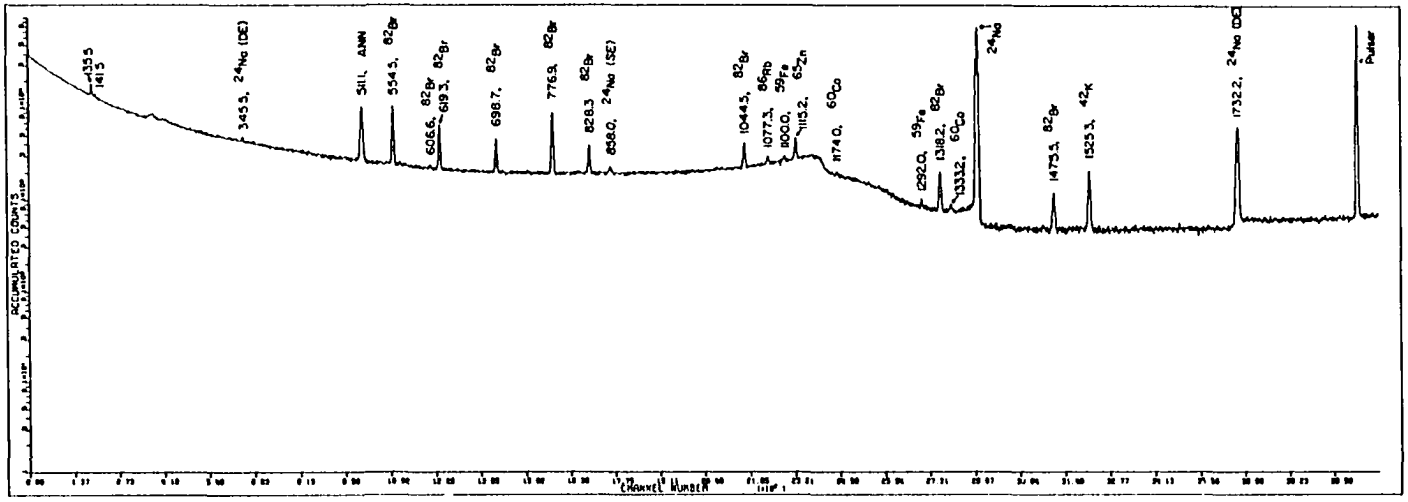


Figure 9. Spectrum of digested bovine liver sample without decontamination processing: sample decay time = 5.91 days; sample counting time = 2.00 hours

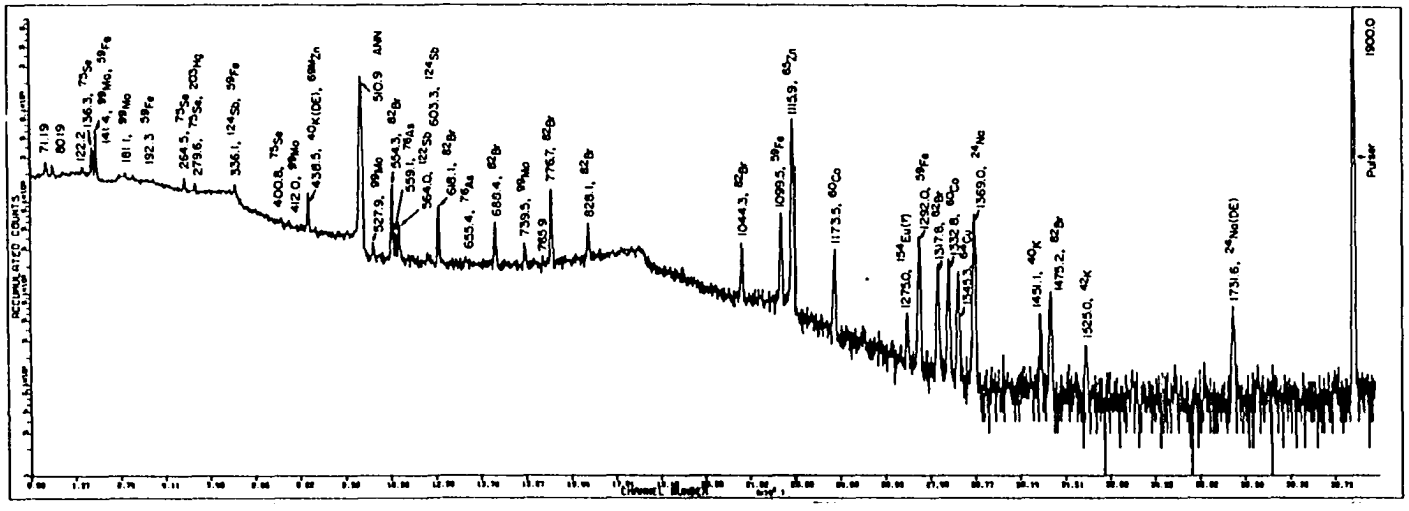


Figure 10. Spectrum of digested bovine liver decontaminated by elution through AG2-X8, in the bromine form, with concentrated hydrobromic acid eluent: sample decay time = 5.30 days; sample counting time = 3.06 hours

to phosphorus species, the reduction in the background level which was due specifically to the removal of phosphorus could not be isolated.

Tables 39 and 40 contain the results of analyses of blood and liver samples by DNAA, INAA and inductively coupled plasma emission spectroscopy (ICPES). The DNAA values and standard deviations were based on 3 to 6 determinations of the element using the procedure developed over the course of this work. The INAA values were determined along with the DNAA analyses by using aliquots of the digested blood and liver samples which were not processed further. Decay intervals ranging from 12 to 28 days were needed in order to obtain the listed data.

The ICPES data was obtained on a routine basis by personnel in the Emission Spectroscopy section of Analytical Services Group II of the Ames Laboratory at Iowa State University. The results are based on two determinations with one gram samples of the blood and one determination with a one gram sample of the bovine liver. The samples were digested by slowly heating each material in a vessel containing a 5:2 ratio of nitric-to-perchloric acid. Digestion was assumed complete when perchloric acid fumes were observed. The samples were filtered through millipore filters into 25-ml volumetrics. The sample solutions, blanks and standards were 8% perchloric acid by volume when

Table 39. Summary of analyses of United Kingdom Master Mix Blood

Element	DNAA (ppm)	NAA (ppm)	ICPES (ppm)	Literature[a] (ppm)
As	0.026±0.001	ND[b]	2.20±0.35	0.025
Au	4.2×10^{-5}[c]	ND	ND	0.00025
Co	0.04±0.01	0.024±0.007	44.3±0.3[d]	---[e]
Cr	0.027±0.002[e]	ND	---[f]	0.35±0.25
Cu	3.6±0.2	ND	4.1±0.4	3.5
Fe	2131±94	2102±91	2242±101	2452±19
Hg	0.03±0.01	0.03[e]	236.5	0.03
Mo	ND	ND	ND	0.005
Rb	13.4±1.0	12.2±0.7	ND	13.9±5.5
Sb	0.012±0.005	0.013±0.002	ND	0.010±0.003
Sc	ND	ND	ND	0.04±0.01
Se	0.34±0.01	0.40±0.01	3.7±0.9	0.4±0.2
Zr	26.4±0.5	25.9±0.3	40.9±5.8	28.86

[a]Hamilton *et al.* (118).

[b]Not detected.

[c]Two values determined. The standard deviation equalled zero, but the counting statistics for each determination indicated an uncertainty equal to 10^{-4}.

[d]Error due to intense iron background.

[e]Single determination.

[f]Not determinable.

Table 40. Summary of analyses of NBS Bovine Liver SRM 1577

Element	DNAA (ppm)	NAA (ppm)	ICPES (ppm)	Literature[a] (ppm)
As	0.045±0.005	ND	ND	0.055
Au	ND	ND	ND	---
Co	0.26±0.01	0.29±0.02	ND	0.018
Cr	ND	ND	ND	90±15[b]
Cu	200±18	ND	219	193±10
Fe	260±20	275±28	232	270±20
Hg	0.013±0.001	ND	24	0.016±0.002
Mo	3.2±0.2	ND	ND	3.2
Rb	19±2	19.4±0.02	ND	18.3±1.0
Sb	$2.2\times10\pm8\times10^{-4}$	$2.7\times10^{-3}\pm4\times10^{-4}$	ND	---
Sc	ND	ND	ND	---
Se	0.98±0.02	0.81±0.12	2.3	1.1±0.1
Zn	131±3	128.5±0.2	115	130±10

[a]NBS (93).

[b]DeKalb (193).

diluted. Values determined by ICPES were reported only for those elements for which DNAA results were available.

The literature values for the elemental concentrations found in the United Kingdom Master Mix Blood were provided in an article by Hamilton et al. (118) in which the details of producing the material were described. The concentrations were converted from a "wet" basis to a "dry" basis using values provided by the authors for the average percentage of water in the samples. Information concerning the NBS Bovine Liver was obtained from the NBS circular describing SRM 1577 (93).

DNAA provided data on more elements than INAA or ICPES for the elements of interest. On the other hand ICPES provided information on Cd and Pb, two biologically significant elements for which neutron activation techniques have limited sensitivity. Based on the literature values, DNAA provided the most accurate information. The ICPES determinations were hampered by significant interference from the iron. However, according to information provided with the analysis results, only the values for Hg and Co were affected. The agreement between the DNAA and INAA results is quite good, confirming the validity of the DNAA procedure after the digestion step. Significantly, the DNAA values were determined within 1 to 5 days after the irradiation while the INAA values could not be obtained without decay periods ranging from 12 to 28 days.

Based on the analysis of CFQ T21 Quartz, high values were expected for gold and antimony determinations. In the bovine liver, the only matrix for which a value for antimony was listed, the antimony value by DNAA is within standard error of the listed literature value. However, the value determined for gold in the blood samples was noticeably high. Two independent measurements yielded the same value for the gold concentration: 4.2×10^{-5} ppm. Based on counting statistics, the standard error in these values was of the order of 10^{-4}. An extremely low count rate for the 411 keV peak in the samples led to the large uncertainty in the peak areas.

There appear to be two different factors causing the high values for cobalt in the liver matrix and the disagreement of the DNAA and INAA values for cobalt in the blood samples. In both cases, the anomalous values were in part related to the fitting routines of the computer program and the shape of the gamma spectra. The Compton shoulder from the 1332 keV cobalt gamma ray distorted the background on the low energy side of the photo-peak. Plots of the gamma spectra indicated that the apparent background on the low energy side ranged from 10 to 100 times higher than the background on the high energy side. The 1173 keV cobalt peak was positioned on the Compton shoulder from the 1332 keV and generated its own Compton shoulder. This peak was also distorted by the proximity of nonanalytical peaks. As the

blood samples decayed, the values determined for cobalt decreased significantly, indicating that as the background leveled out and interfering activities decayed, the accuracy of the computer fitting routines improved. Compounding the situation, the low concentration of cobalt in the blood samples led to low count rates with an increased statistical fluctuation in the counting data. The combination of distorted spectra and poor counting statistics resulted in values for cobalt ranging from 0.15 to 0.03 ppm.

The cobalt values reported for the liver samples were based on the average of all determinations. The consistently high values indicated that a common source of error affected each determination. Three possible sources of error were considered: a high radio-cobalt background in the detector system, an error in calculating the concentration of the standard, and contamination of the liver sample. The contribution of background levels of ^{60}Co at 1173 and 1332 keV was determined to be negligible. The concentration of the standard solution was measured by neutron activation and was found to be accurate. No explanation was developed to account for possible contamination of the liver samples by cobalt.

The HAP, Alumina, and AG2-X8 (Acetate) columns did not achieve the desired goal with respect to chromium. A value for chromium was provided in the literature for both the blood and liver, but the chromium photopeak was detected in

gamma spectra of only one sample. The high background in the region of 320 keV could have obscured the chromium photopeak. However, the absence of a chromium photopeak in the INAA samples after a 12 to 28 day decay period suggested that the chromium peak was not present and that the single determination was based on a statistical fluctuation and not a "real" photopeak.

Analysis of individual count data indicated that decay periods of less than 2 days prior to gamma spectroscopy produced highly variable results for components present at particularly low concentrations. Even after chemical processing removed interfering species, the Compton background generated by the analyte species still presented difficulties with respect to computer analysis of the spectra. Since this problem was more evident with the AG2-X8 bromide column, it was probably accentuated by the lower decontamination efficiency of the process for concentrating the analyte species. Perhaps a high degree of sodium removal would allow valid determination after shorter decay periods.

J. Conclusions

There were two significant provisions shaping the development of the DNAA procedure in this project: 1) the procedure had to be applicable to biological samples, and 2) the procedure had to be developed with possibility for inclusion in routine analyses. To a large extent these

objectives were achieved. The simplified DNAA system developed during the course of this work was shown to be applicable to the determination of some components in biological materials (As, Cu, Fe, Hg, Mo, Rb, Sb, Se and Zn) without yield determinations. Decay periods of 2 to 4 days coupled with 15 hour irradiations and computer analyses of the results could allow a turn-around time of approximately 4 or 5 days in processing a diagnostic sample. In the case of chronic disorders as listed in Table 3, the delay would be neither unusual nor untenable.

The suitability of this DNAA procedure for routine analyses was not verified. In addition to the physical requirements for neutron activation, the procedure required the constant supervision of one knowledgeable in the chemistry involved and in the handling of highly radioactive materials. Unless a laboratory is specially designed to cope with the particular problems of neutron activation analysis, the procedure would most likely not be used.

Automation could be the key to providing a DNAA procedure favorable to routine analyses. Automating the digestion step would remove the need for much of the supervision and reduce the exposure of the operator to radiation. Although automation of the sulfuric acid - hydrogen peroxide ashing step of the present DNAA procedure is not immediately feasible, the concentration and separation steps would be particularly susceptible to automation if the analysis could

be limited to only one eluent. Eliminating the combination column (HAP, Alumina, and AG2-X8 Acetate) would result in a one eluent process with the subsequent loss of only data on rubidium. Thus, this work provides a framework on which an automated procedure for the analysis of biological samples on a routine basis can be developed.

K. Future Work

The anion exchanger AG2-X8 in the bromide form was effective for concentrating the analyte species but was not optimum for sodium decontamination. An effort should be made to develop a concentration process which would be effective in hydrochloric acid. This would enable the use of the superior sodium removal properties of HAP.

It was suggested that the factor determining the minimum decay period before gamma spectroscopy may have been the Compton background produced by the analyte species. Investigations using the DNAA procedure and a Compton suppression spectrometer could determine whether or not this is correct.

More elements (e.g., Cr) could be determined if the background below 400 keV due to phosphorus activities could be lowered. The inorganic exchanger zirconium phosphate has been used for this purpose (165,167) and should be further investigated.

Numerous possibilities exist for automating the present DNAA procedure for developing a procedure more favorable towards automation. Development of a more comprehensive single eluent system would be most advantageous, but most likely unfeasible. Attention should be given towards development of methods for accomplishing drastic changes in the nature of the eluent in an automated system.

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