Journal of Research of the National Bureau of Standards
Volume 91, Number 2, March–April 1986

Storage and Pre-Neutron Activation Analysis Treatment for Trace Element Analysis in Urine

Alan J. Blotcky

Veterans Administration Medical Center, Omaha, NE 68105

and

Edward P. Rack

University of Nebraska-Lincoln, NE 68588

Accepted: October 29, 1985

The problems regarding storage and pre-neutron activation analysis treatment for the elements aluminum, calcium, vanadium, selenium, copper, iodine, zinc, manganese, and magnesium in a urine matrix are reviewed. The type of collection and storage procedure and pre-neutron activation analysis treatment of urine depend on the specific trace element; that is, its inherent physical and chemical properties. Specifically polyethylene in teflon containers are the most suitable for general determinations. Whether any preservative is added would depend upon the stability of the trace element and its tendency for surface adsorption. Preferably preservatives should contain no radioactivatable elements for maximum efficacy. Freeze drying or packing urine shipments under dry ice needs to be explored on an individual basis. Each pre- or post-neutron activation analysis treatment is specific and optimized for the trace element analyzed.

Key words: aluminium; calcium; iodine; magnesium; manganese; neutron activation analysis; pre-NAA treatment, urine; selenium; storage; vanadium; zinc.

Introduction

Cornelis et al. [1] describe one of the most comprehensive studies to date regarding neutron activation analysis for bulk and trace elements in urine, problems in sampling, collection, storage, sample preparation and contamination hazards during neutron irradiation. Our intent in this review is not to repeat Cornelis's study but to add to it from our experience in work involving the urine matrix [2–7].

The quantitative analysis of urine for trace elements is important for metabolic and nutritional research. Because of its ready availability and easy access, under proper conditions, it can be a vehicle for mass screening of individuals for normal and disease states. Urine is an aqueous admixture composed of dissolved and suspended waste products as well as inhaled and absorbed substances such as pollutants and/or their metabolites. The two major radioactivatable elements whose presence can interfere in the radioassay of urine are sodium and chlorine. Perhaps that is why there is a paucity of Instrumental Neutron Activation Analysis (INAA) techniques for trace element determination in the urine matrix. For the sake of brevity this discussion will be limited to Ag, Cd, Hg and the radionuclides depicted in

About the Authors: Alan J. Blotcky is with Medical Research at the Veterans Administration Medical Center while Edward P. Rack is with the Department of Chemistry at the University of Nebraska-Lincoln. The review described was supported in part by the U.S. Department of Energy, Fundamental Interaction Branch, Division of Chemical Sciences, under contract DE-FG02-84ER13231 and the University of Nebraska Research Council, NIH Biomedical Research Support Grant No. RR-07055.

[1]Figures in brackets indicate literature references.

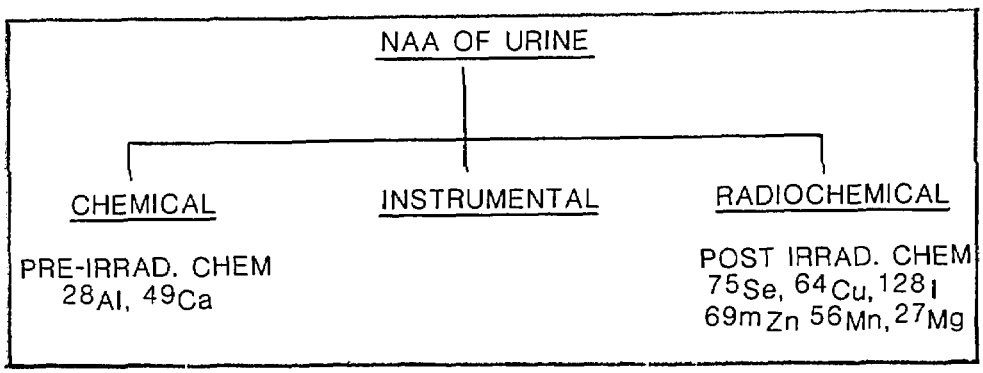

Figure 1–Trace elements determined in urine.

figure 1. One of the drawbacks of neutron activation is the limited number of reactor facilities where research and routine analysis can be performed; consequently, in many cases samples must be transported to the facility. It is of paramount importance that special precautions be taken in the collection and storage of urine samples prior to reactor irradiation. In other words, the analyst must know the history of the sample before it reaches his or her hands.

Storage of Urine Specimens for Specific Elemental Analysis

Not all storage vessels or containers are suitable for all trace elements to be investigated. It is important that the containers chosen show no absorption or adsorption of the desired trace element and that there is no loss of the trace elements during storage. West et al. [8] showed that the adsorption of silver in potable water samples to the walls of borosilicate, flint, and polyethylene was pH dependent and could be prevented for 30 days by collecting samples in sufficient sodium thiosulfate to produce a 10–15% solution.

King et al. [9] have shown that in order to prevent losses of Cd by adsorption to the walls of glass containers, water samples should be acidified with HNO_3. Since polymer surfaces do not interact with Cd aqueous solutions, sampling of Cd would be better performed employing plastic containers.

Struempler [10], found that polyethylene containers did not absorb cadmium and zinc; and acidification with dilute HNO_3 to a pH value of 2 prevented Ag, Pb, Cd, and Zn adsorption on borosilicate and silver adsorption on polyethylene surfaces. He also found that silver solutions must be kept in the dark, even under acidified conditions, to maintain stability and to minimize adsorption loss and that new polypropylene containers could not be cleaned satisfactorily for Cd and Zn. Feldmen [13] has shown that distilled water solutions containing >0.1 ng Hg/mL can be stored in glass without deterioration for as long as five months if the solution contains 5% by volume HNO_3 and 0.01% dichromate. Storage of such standards is safe in polyethylene containers for at least 10 days if the solution contains 5% HNO_3 and 0.05% dichromate.

Thiers [11] found that borosilicate glass can seriously contaminate solutions and should not be used for storage unless the analyst is confident that the contamination of the element being determined is negligible. Murphy [12] states that "Teflon FEP bottles and beakers have been used at NBS for the past several years with favorable results after thorough cleaning with nitric and hydrochloric acids to remove contaminants introduced during fabrication." High purity acids were stored for at least two weeks with no significant levels of contamination observed. It is important that any substance added to the urine for purposes of preservation does not form complexes with the desired trace element, or introduce that trace element. Some authors have suggested that the samples be lyophilized since dry, solid samples lessen the possibility of leaking or adsorption during storage. However with urine we have found that due to its high NaCl concentration there is both a volume limitation and the possibility for a potential loss due to bumping of the sample during the vacuum process. As a minimum the urine must be diluted with deionized water to allow solid freezing.

Determining what kind of stabilizing additive should be mixed with the solution can be very complex, as can be seen in the following figures showing the loss of the element with storage time for several types of containers.

As seen in figures 2 and 3 [13], the aqueous results obtained in both glass and polyethylene confirm the generally held view that aqueous solutions of mercury salts rapidly lose strength on storage because they exist as a colloidally suspended hydrolysis product.

Depicted in figures 4 and 5 [13], nitric acid at the 1% level appears to be almost as ineffective as water alone in glass and polyethylene vessels. It is more effective at the 5% level but still quite unsatisfactory.

As presented in figures 6 and 7 [13], the mixture of H_2SO_4 and potassium permanganate produces colloidal Mn oxides. These over a period of time remove the Hg

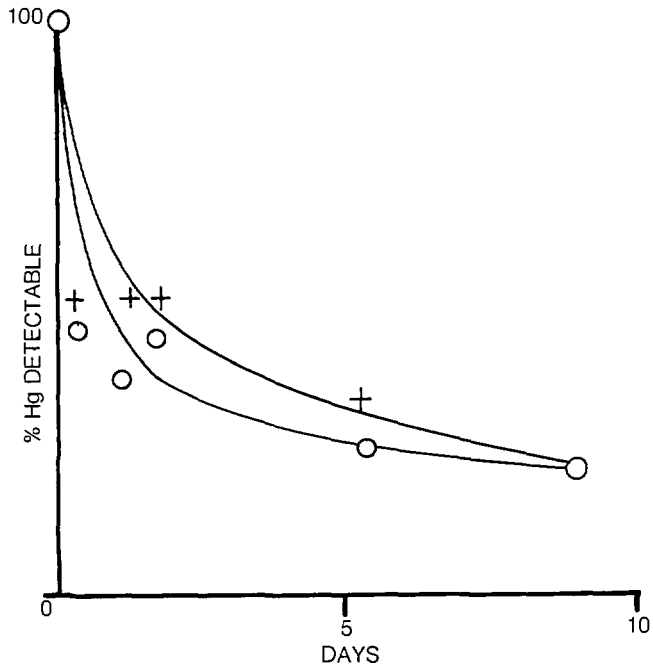

Figure 2–Stability of Hg solutions in glass (H_2O); 0, 10 ng lmL; +, lng/mL.

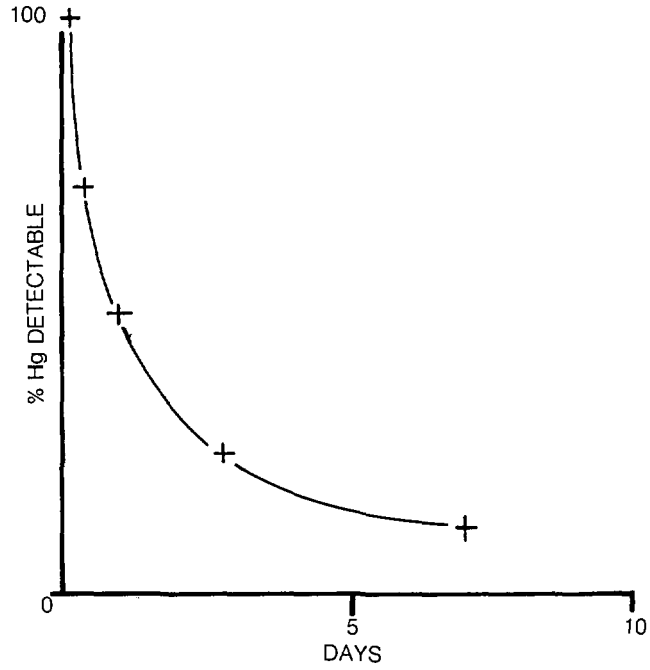

Figure 3–Stability of Hg solutions in polyethylene (H_2O) +, 0.2 ng/mL.

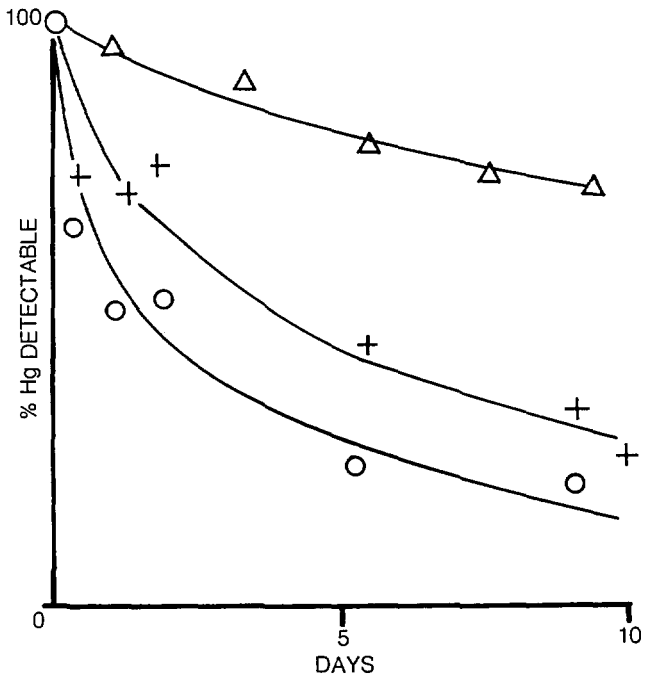

Figure 4–Stability of Hg solutions in glass (HNO_3); Δ, 10 ng/mL, 5% (v/v): +, 1 ng/mL, 1% (v/v); 0, 10 ng/mL, 1% (v/v).

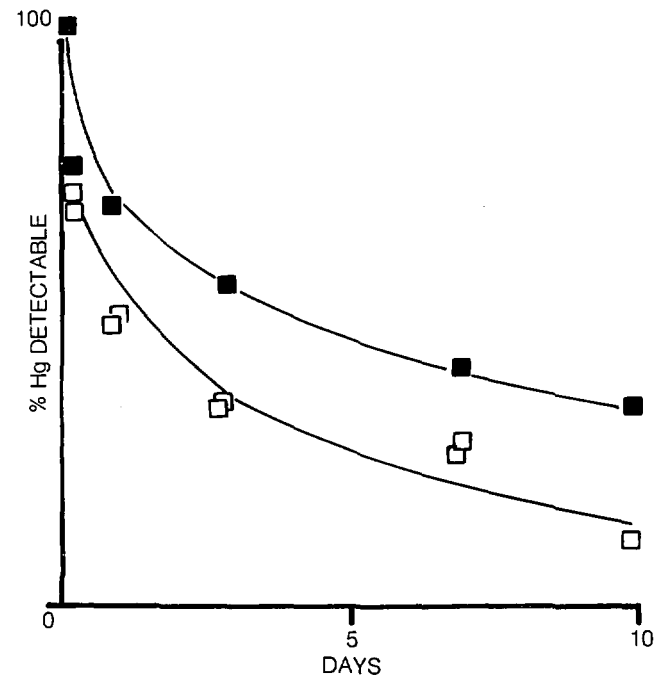

Figure 5–Stability of Hg solutions in polyethylene (HNO_3); ■ 0.2 ng/mL, 5% (v/v); □, 0.2 ng/mL, 1% (v/v).

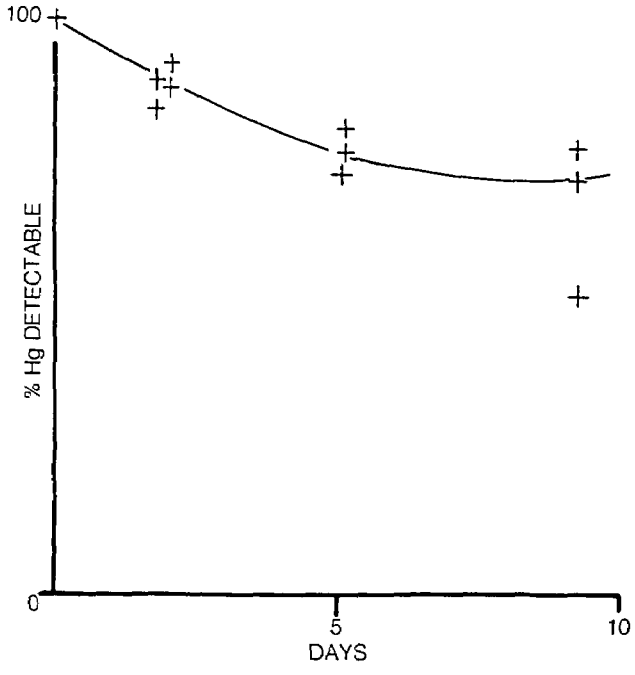

Figure 6–Stability of Hg solutions in glass (0.5% (v/v) H_2SO_4 +0.01% $KMnO_4$); +, 10 ng/mL.

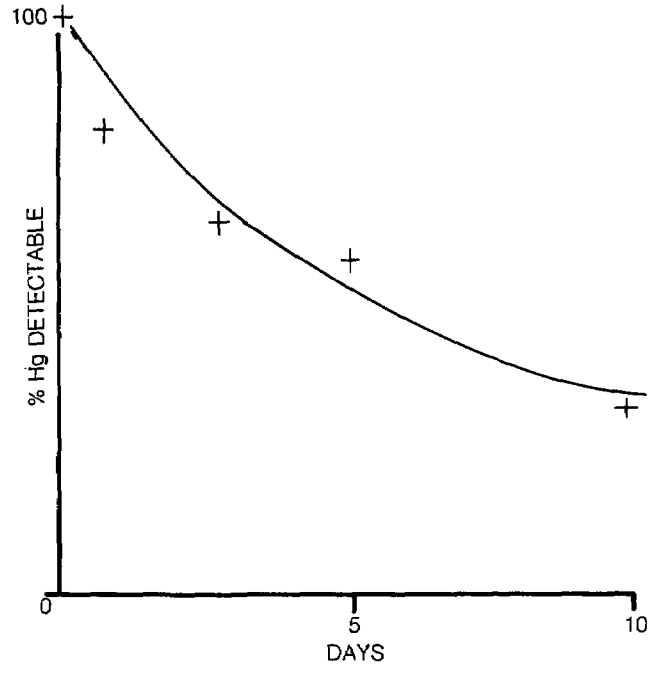

Figure 7–Stability of Hg solutions in polyethylene (0.5% (v/v) H_2SO_4+0.01% $KMnO_4$); +0.2 ng/mL.

from the solution and plate it on the side walls of the container.

In glass the combination of 1% HNO_3 and 0.01% dichromate does not prevent a rapid initial drop of Hg concentration with increasing storage time, possibly due to adherance of hydrolyzed Hg salts to the walls although little or no loss occurs after the first day. This effect is presented in figure 8 [13]. However, as can be seen in the figure, the combination of 5% HNO_3 and 0.01% dichromate is quite successful because of its ability to prevent the hydrolysis of dissolved mercury and to prevent its reduction to oxidation states lower than +2. For polyethylene containers it is necessary to increase the dichromate concentration to 0.05% as can be seen in figure 9.

The initial concentration of Cd present in water also affects the degree of loss of Cd during storage. Figure 10 shows the percentage loss of Cd as a function of time for distilled water samples at pH 10 with different Cd concentrations. The curve for 25 ppb Cd reaches a maximum value of about 35% Cd loss after 20 hours of storage in soft glass [9]. For most environmental monitoring programs the significant level of Cd is in the 1–100 ppb range.

Loss of Cd is definitely pH dependent as seen in figure 11. At pH 6.9 there is no Cd loss. Cd loss does not occur in plastic containers [9].

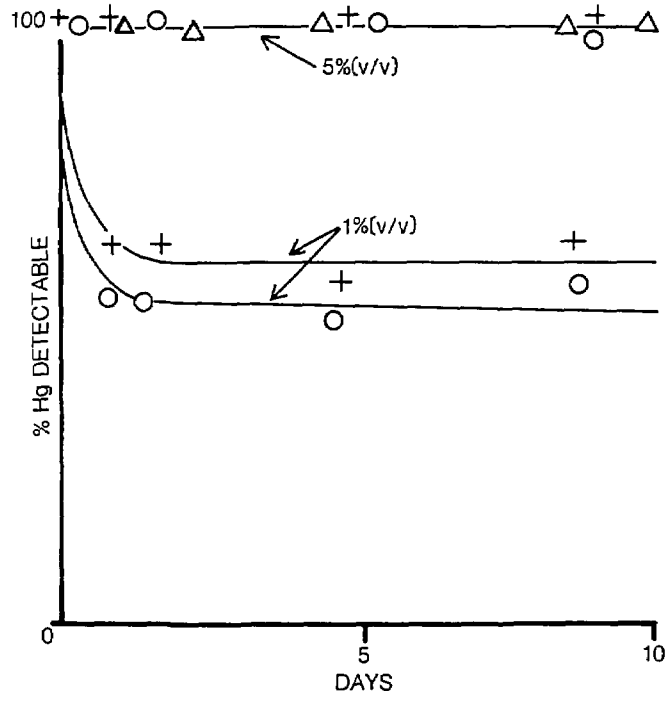

Figure 8–Stability of Hg solutions in glass (HNO_3+0.01% $K_2Cr_2O_7$); Δ, 0.1 ng/mL; +, 1 ng/mL; 0, 10 ng/mL.

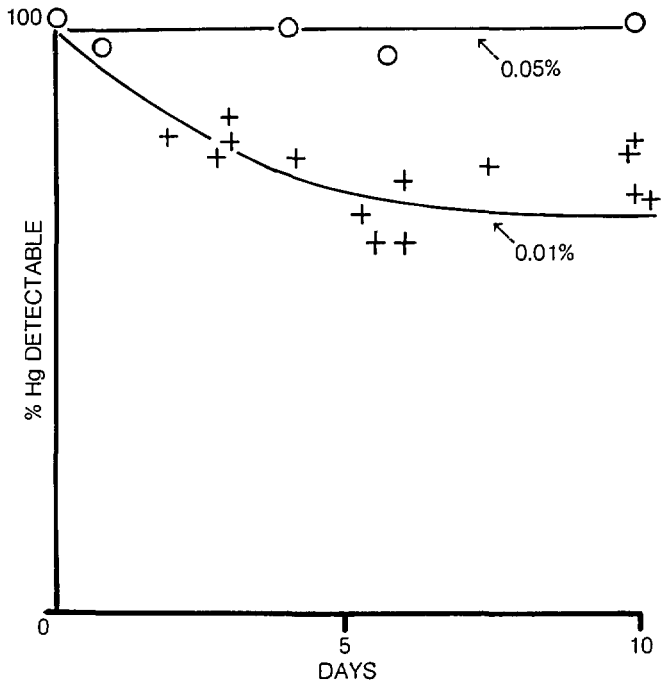

Figure 9–Stability of Hg solutions in polyethylene (5% (v/v) $HNO_3 + K_2Cr_2O_7$); +, 0, 0.2 ng/mL.

Figures 12, 13, and 14 show the loss of silver in aqueous solutions stored in different types of containers [8]. The maximum and minimum values represent the variation in multiple runs. There appears to be a narrow range of adsorption values in the case of the flint glass but adsorption begins after a shorter contact time.

Thus, we can see that the container loss must be investigated for each element analyzed and for each concentration range. Because of the nature of urine, several practical considerations must also be addressed if urine samples are to be shipped to a nuclear reactor for neutron irradiation and subsequent radioassay. Rapid freezing and dry ice shipment may offer the least potential damage to the matrix; however, for each individual elemental assay it must be determined if freezing has any deleterious effects on the analysis.

Pre-Neutron Activtion Analysis Treatment

Each trace element has its own inherent physical, chemical, and radioactivatable properties which must be considered in the pre-irradiation chemistry prior to neutron irradiation and its detection and analysis. Because of the large amount of the radioactivatable elements sodium and chlorine in urine, it is not practical or wise to employ instrumental NAA regardless of the sensitivity and selectivity of the radioassay equipment. This can graphically be seen in figure 15 which shows the spectra of raw saliva and an extracted CCl_4 phase. It can be observed that the iodine peak appears in the top spectra but the compton continuum contribution to the iodine photopeak is 77% of the total counts in the photopeak [14]. For an individual trace element several

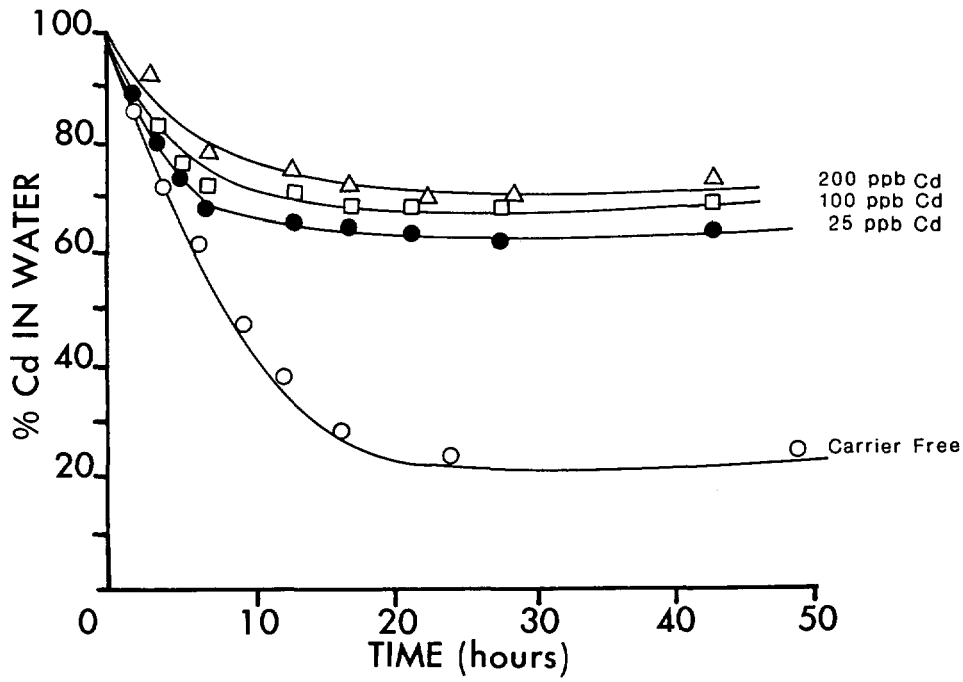

Figure 10–Percent of ^{109}Cd in water during storage with respect to the initial concentration of cadmium in solution. Soft glass.

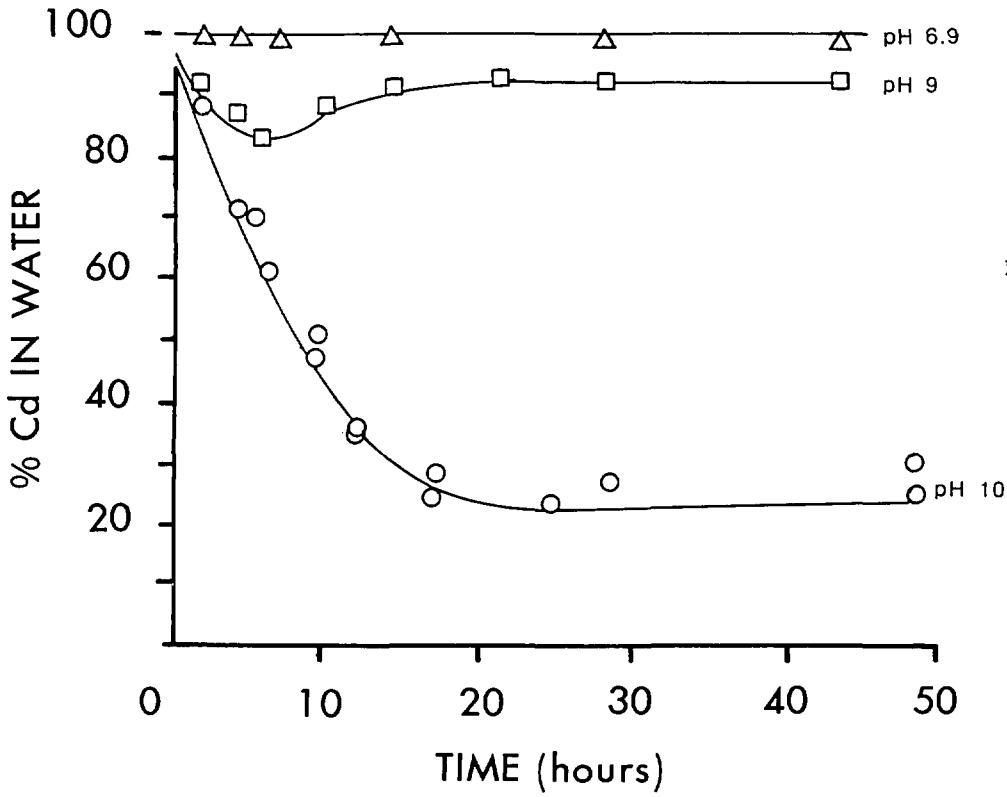

Figure 11–Percent of ^{109}Cd in water during storage asa a function of pH of solution. Soft glass.

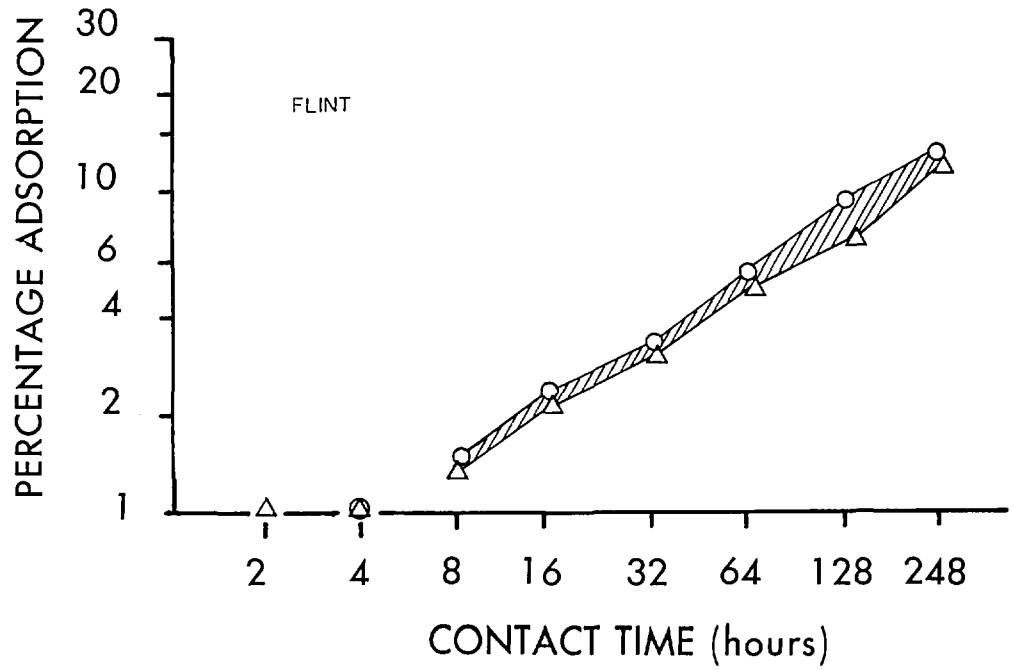

Figure 12–Adsorption range profiles for flint beakers at the 1.0 mg per liter silver concentration. Maximum (o) and minimum (Δ) plotted values are from the distilled water series.

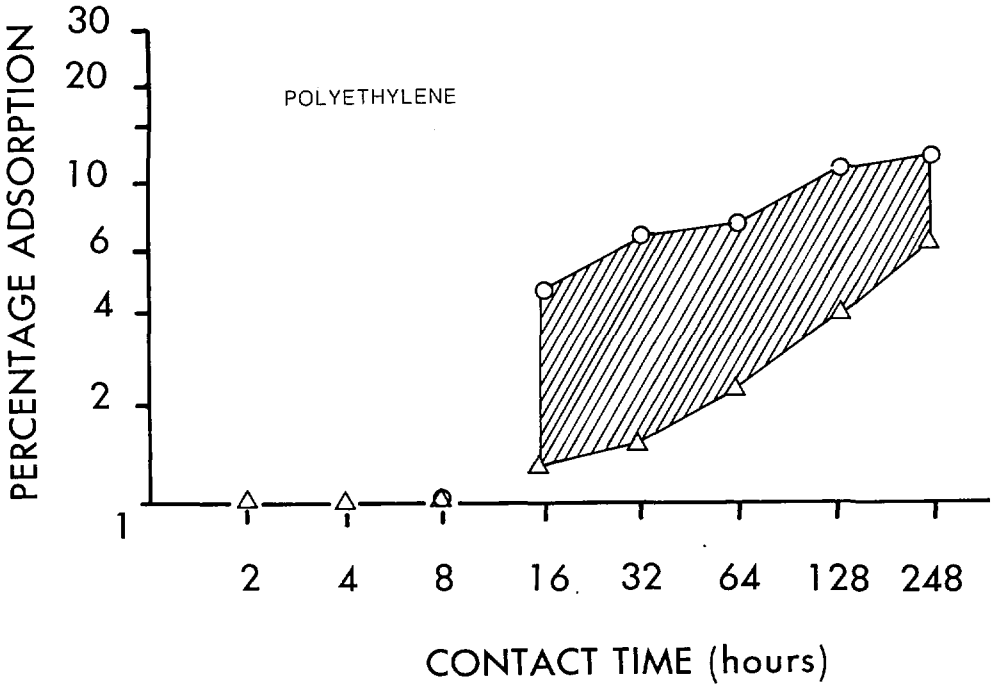

Figure 13–Adsorption range profiles for polyethylene beakers at the 1.0 mg per liter silver concentration. Maximum (o) and minimum (Δ) plotted values are from the distilled water series.

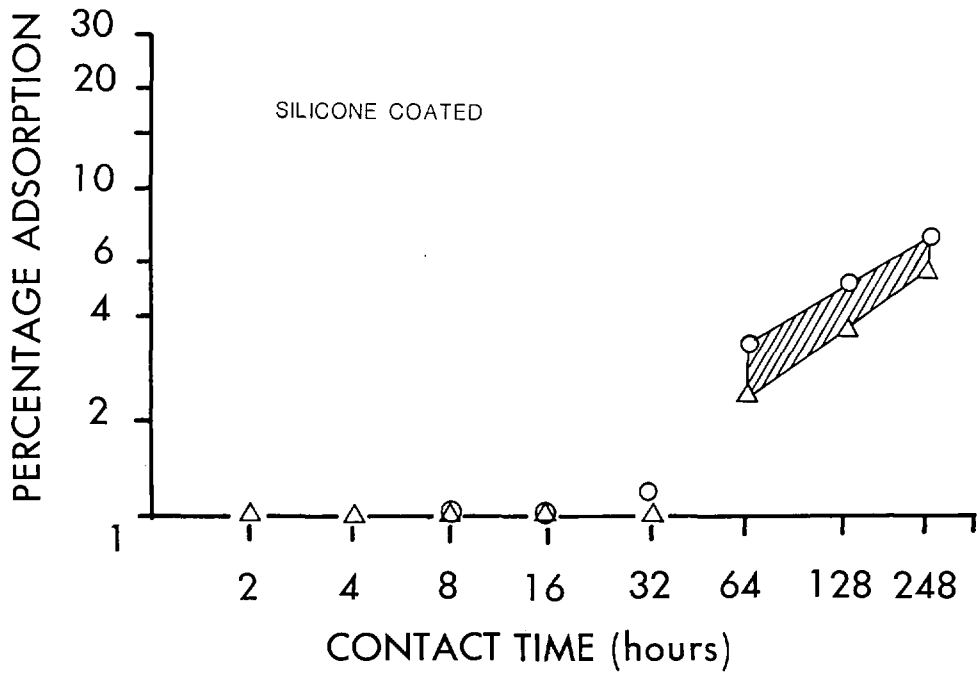

Figure 14–Adsorption range profiles for silicone coated beakers at the 1.0 ng per liter silver concentration. Maximum (o) and minimum (Δ) plotted values are from the distilled water series.

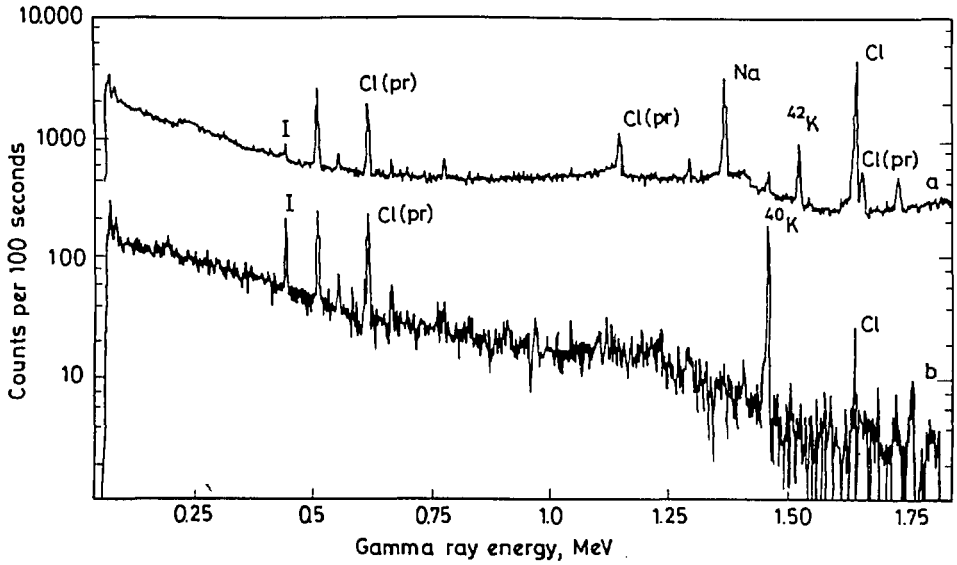

Figure 15–Typical γ-ray spectra of neutron irradiated saliva using a Ge(Li) detector—(a) raw saliva (b) extracted CCl_4 phase.

varied and different techniques can be employed for its isolation. If wet chemistry must be performed on the sample prior to neutron irradiation, it is important that the reagents employed be free of interfering activity. For example, for our aluminum determination various reagents have trace quantities of aluminum as can be seen in table 1.

Nitric acid is generally the reagent of choice in wet digestion procedures and table 2 shows that different grades of nitric acid contain varying amounts of many trace elements [12]. The reagent of choice in our laboratory for wet digestion is Baker Ultrex nitric acid.

Urine is a nonhomogenous admixture whose concentration can vary over wide limits. In some determinations it may be necessary to take into consideration the specific gravity of the urine. For example, it may be quite critical in solvent extraction procedures such as those in our determination of total iodine in urine by the Szilard-Chalmers technique, as can be seen in table 3.

Table 1. Aluminum contents of reagents.

Reagent	Mfg.	Concentration	Al	Assay
HNO_3	Baker-Ultrex	Conc.	0.02	μg/ml
HNO_3	Du Pont	Conc.	0.04	μg/ml
HNO_3	Fisher	Conc.	0.22	μg/ml
HNO_3	Mallinckrodt	Conc.	0.36	μg/ml
HF	Mallinckrodt Ar	48% WT	0.25	μg/ml
HF	Matheson Reagent	48% WT	0.69	μg/ml
H_2O	Fontenelle Springs Distilled		0.01	μg/ml
H_2O	McGaw-Distilled For Irrigation		0.03	μg/ml
RESIN	Bio-Rad Ag 50W-X8		0.25	μg/g Resin
RESIN	Dowex 50W-X8		4.7	μg/g Resin

Table 2. Impurity concentration in nitric acid.

Element	Sub-boiling Distilled (ng/g)	ACS Reagent Grade acid (ng/g)	Commercial High purity (ng/g)
Pb	0.02	0.2	0.3
Tl	—	0.2	—
Ba	.01	8	—
Te	.01	0.1	—
Sn	.01	0.1	1
In	.01	—	—
Cd	.01	0.1	0.2
Ag	0.1	0.03	0.1
Sr	.01	2	—
Se	.09	0.2	—
Zn	.04	4	8
Cu	.04	20	4
Ni	.05	20	3
Fe	.3	24	55
Cr	.05	6	130
Ca	.2	30	30
K	.2	10	11
Mg	.1	13	—
Na	1	80	—
Total Impurity	2.3 ppb	220 ppb	240 ppb

Table 3. Variation of extraction yield with specific gravity and osmolarity for urine collected at different times from one individual.

Extraction Yield (%)	Specific Gravity
63.2	1.031
63.9	1.026
67.3	1.017
70.5	1.011
81.2	1.007
84.9	1.006

Since urine contains sodium and chlorine, it is necessary to perform a separation of the desired trace element from dissolved NaCl in order to obtain a viable analysis. Table 4 is a summary of the pre- or post-neutron activation chemistry required for the trace elements listed in figure 1. While this list is not all inclusive for all trace elements that can be detected in urine, it represents some of those which have the greatest importance in the study of disease and health states. It should be noted that the procedures summarized for Ca, V, Cu, Mn, and Mg are specific for tissue and will probably need to be modified accordingly for a urine matrix.

Conclusions

It would seem that the type of collection and storage procedure and pre- or post-NAA treatment of urine depends on the specific trace element; that is, its inherent physical and chemical properties. The following generalizations can be made.

1) Polyethylene or teflon containers may be the most suitable.
2) Whether any preservative should be added would depend upon the stability of the trace element and its tendency for surface adsorption. Preferably preservatives should contain no radioactivatable elements for maximum efficacy.
3) Freeze drying or packing urine shipments under dry ice needs to be explored on an individual basis considering all factors involved.
4) Each pre- or post-NAA treatment is specific and optimized for the trace element analyzed. As a general suggestion, it is important to minimize the number of operational steps and choose reagents that do not contribute radioactivity in the activation step.

References

[1] Cornelis, R.; A. Speeck, and J. Hoste, Neutron Activation Analysis for Bulk and Trace Elements in Urine, Anal. Chimica Acta 78, 317 (1975).

[2] Blotcky, A. J.; D. Hobson, J. A. Leffler, E. P. Rack and R. R. Recker, Determination of Trace Aluminum in Urine by Neutron Activation Analysis, Anal. Chem. 48, 1084 (1976).

[3] Recker, R. R.; A. J. Blotcky, J. A. Leffler and E. P. Rack, Evidence for Aluminum Absorption from the Gastrointestinal Tract and Bone Deposition by Aluminum Carbonate Ingestion with Normal Renal Function, J. Lab. Clin. Med. 90, 810 (1977).

[4] Garmestani, K.; A. J. Blotcky, and E. P. Rack, Comparison between Neutron Activation Analysis and Graphite Furnace Atomic Absorption Spectrometry for Trace Aluminum Determination in Biological Materials, Anal. Chem. 50, 144 (1978).

[5] Blotcky, A. J.; D. M. Duven, W. M. Grauer and E. P. Rack, Determination of Iodine in Urine by Neutron Activation Analysis, Anal. Chem. 46, 838 (1974).

Table 4. Summary of pre- or post-NAA treatment for trace element analysis.

Element	Ref	Digest	Acid Sample Ratio	Temp (°C)	Resin	Elution Solution	Elution Volume	Fraction Analyzed	Pre or Post Chem.
Al	[2]	HNO_3	1:1	65	50WX8	1 M HNO_3	19 mL	Resin	Pre
V	[15]	HNO_3	1:1	65	50WX8	1 M HNO_3 0.5 M HNO_3 4 M HN_4OH	10 mL 10 mL 6 mL	Eluent	Pre
Se	[16]	H_2SO_4	1:5	50	Oxidation to red amorphous selenium			Precipitate	Post
I	[5]	No			HNO_3–H_2O_2 oxidation			CCl_4 Extraction	Post
Ca	[17]	No			Precipitate with Sat. $(NH_4)_2$ C_2O_4			Precipitate	Pre
Cu	[18]	HNO_3	1:1	65	Solvent Extraction (S.E.) HCl, dithizone, CCl_4			Organic	Post
Zn	[18]	HNO_3	1:1	65	S.E., Acetate, Sodium Thiosulfate, Dithizone, CCl_4			Organic	Post
Mn	[18]	HNO_3	1:1	65	S.E., Acetate, Chloroform, dithiocarbamate			Organic	Post
Mg	[18]	HNO_3	1:1	65	S.E., Acetate, n-butamine, TTA in chloroform			Organic	Post

[6] Firouzbakht, M. L.; S. K. Garmestani, E. P. Rack and A. J. Blotcky, Determination of Iodoamino Acids and Thyroid Hormones in a Urine Matrix by Neutron Activation Analysis, Anal. Chem. **53**, 1746 (1981).

[7] Opelanio, L. R.; E. P. Rack, A. J. Blotcky and F. W. Crow, Determination of Chlorinated Pesticides in Urine by Molecular Neutron Activation Analysis, Anal. Chem. **55**, 677 (1983).

[8] West, F. W.; P. W. West and F. W. Iddings, Adsorption of Traces of Silver on Container Surfaces, Anal. Chem. **38**, 1566 (1966).

[9] King, W. G.; J. M. Rodriguez and C. M. Wai, Losses of Trace Concentrations of Cadmium from Aqueous Solution during Storage in Glass Containers, Anal. Chem. **46**, 771 (1974).

[10] Struempler, A. W., Adsorption Characteristics of Silver, Lead, Cadmium, Zinc, and Nickel on Borosilicate Glass, Polyethylene, and Polypropylene Container Surfaces, Anal. Chem. **45**, 2251 (1973).

[11] Thiers, R. E., in "Trace Analysis" pp 637–66. J. H. Yoe, H. J. Kock, Eds. Wiley Interscience Publishers: New York (1975).

[12] Murphy, T. J. The Role of the Analytical Blank in Accurate Trace Analysis, Proc. Symposium on Accuracy in Trace Analysis: Sampling, Sample Handling and Analysis, Special Publication 422. P. D. LaFleur, Ed. National Bureau of Standards (1976).

[13] Feldman, C. Preservation of Dilute Mercury Solutions, Anal. Chem. **46**, 99 (1974).

[14] Blotcky, A. J.; D. Hobson and E. P. Rack, A Comparison of Destructive and Non-Destructive Neutron Activation Analysis Methods for the Determination of Trace Iodine in Saliva, Radiochem. Radioanal. Letters **24**, 291 (1976).

[15] Blotcky, A. J.; C. Falcone, V. A. Medina and E. P. Rack, Determination of Trace level Vanadium in Marine Biological Samples by Chemical Neutron Activation Analysis, Anal. Chem. **51**, 178 (1979).

[16] Weingarten, R.; Y. Shamai and T. Schlesinger, Determination of Selenium in Urine by Neutron Activation Analysis, J. Appl. Rad. Isot. **30**, 585 (1979).

[17] Janghorbani, M.; A. Sundaresan and V. R. Young, Accurate Measurement of Stable Isotopes $^{46}C_A$ and $^{48}C_A$ in Feces, Plasma and Urine in Relation to Human Nutrition of Calcium, Radio Chemica. Acta. **113**, 267 (1981).

[18] Hahn, K. J.; D. J. Tuma and J. L. Sullivan, Rapid and Simple Continuous Radio-Chemical Separation of Copper, Magnesium, Zinc, and Manganese in Biological Materials, Anal. Chem. **40**, 974 (1968).