THE DETERMINATION FOR CADMIUM IN BIOLOGICAL MATERIALS

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By

RONALD E. YOUNG, B.Sc.(HONS.)

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

December 1974

MASTER OF SCIENCE (1974) McMASTER UNIVERSITY
(Chemistry) Hamilton, Ontario

TITLE: The Determination for Cadmium in Biological Materials

AUTHOR: Ronald E. Young, B.Sc.(Hons.) (McMaster University)

SUPERVISOR: Professor K. Fritze

NUMBER OF PAGES: ii, 112

Acknowledgement

I would like to thank Dr. K. Fritze both for his patience and his assistance in the preparation of this thesis.

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ABSTRACT

With the increasing concern about pollution the need has arisen for accurate trace analysis of hazardous or toxic trace elements such as cadmium. While the modern analytical chemist has at his disposal a great many different techniques for analysis, surveys of inter-laboratory analyses have often produced serious discrepancies among results done by different methods and/or workers. The purpose of this thesis will be to review the different methods for trace analysis of cadmium in biological samples and to experimently compare two of these, atomic-absorption spectrometry and neutron-activation analysis.

INTRODUCTION

Because of the recent concern over pollution, the public has become aware of the dangers of certain trace elements such as cadmium in the environment. While its actual biological role may be poorly understood, there is growing evidence that cadmium, even in trace amounts, constitutes a health hazard (see Appendix-1). Hence there has arisen a need for a reliable means of analysis at the trace level of concentration. With the wide variety of different techniques and instruments available today this might appear to be no problem at all to the analytical chemist. Unfortunately this is not the case. As pointed out by Bowen (1, 2) elemental analysis on a standard biological material often reveals serious and disturbing differences when the analysis is done by different methods and/or people.

Bowen's standard biological material was made from the leaf tissue of Marrow Stem Kale and precautions were taken to ensure a minimum of elemental contamination. (3, 4, 5) Cadmium has been determined at least ten times in the standard kale power, seven of these by neutron activation analysis. The results are listed below:

Table 1 - Literature Values for Kale Analysis

METHOD	SEPARATION	AVERAGE (p.p.m.)	ACTUAL RESULTS	REFERENCE
N.A.A.	chemical	0.38	0.347, 0.386, 0.392 0.410	46
N.A.A.	solv.-ext.	0.50	0.48, 0.46, 0.51 0.54	9
N.A.A.	- - - -	0.63	average of 5 results	2
N.A.A.	an.-ex, chemical	0.63	0.67, 0.63, 0.60	7
Polar.	- - - -	0.73	overall average of two sets of six each. S.D. $\pm$ 10.3%	57
At. Abs	- - - -	0.75	average of 10 results S.D. $\pm$ 0.18	89
N.A.A.	an.-ex.	0.89	10 results S.D. $\pm$ 0.16	8
N.A.A.	- - - -	0.91	average of 6 results	2
Polar	- - - -	1.0	average of 4 results	2
N.A.A.	solv.ext.	1.06	1.05, 1.03, 1.07, 1.08	10

N.A.A. - Neutron Activation Analysis
Polar. - Polarography
At.Abs. - Atomic Absorption Spectrometry
solv.-ext. - solvent extraction
an.-ex. - anion exchange

If one makes the assumption that the kale powder is homogeneous, then the results, especially by neutron activation analysis, are rather disturbing. Nevertheless it is interesting to note that the quoted concentrations do not appear to follow a normal distribution but possibly show a "bunching" around the 1 p.p.m. level. However, other methods of analysis have also given trouble. Authors have often disagreed over colourimetric methods of analysis (11) and there are

several examples in the literature where differences have been found between colourimetric and atomic absorption analysis (13, 14, 15). For example Schroeder (12), in 1961, determined cadmium in over 100 naturally occurring samples, including foods, by a colourimetric method employing dithizone. Four years later he repeated all of his analyses because the former results had been found to be too low. (15)

Something appears fundamentally wrong or inadequate with some of the analytical methods or procedures involved. The purpose of this thesis will be to investigate some of the methods and to suggest a preferred method of analysis if one is found.

Naturally, there have been some attempts in the past to recommend or to standardize certain procedures. In 1969, the Analytical Methods Committee of "Analyst" published an article entitled: "The Determination of Small Amounts of Cadmium in Organic Matter" (16). In essence the article recommends three different procedures; a colourimetric method (dithizone), a polarographic method, and an atomic absorption method. However, it is the opinion of this author that certain criticisms can be made of this study.

First, their methods were tested on samples "doped" with cadmium. Since the cadmium in organic material will most likely be in a different form, their approach may be somewhat unrealistic.

Secondly, they make no more than a cursory interpretation of their results. For example, they analysed certain doped samples by the following methods:

Table 11 - Results of Cadmium Analysis

ORANGE SQUASH (Cadmium is added to a concentration of 2 p.p.m.)

CONCENTRATION (p.p.m.)

	Dithizone	Polarography	Atomie Absorption
No. of detn.	8	4	7
Range	1.3-2.1	1.9-2.0	1.5-2.0
Mean	1.59	1.93	1.80
S.D.	0.23	0.05	0.18

LIME JUICE (Cadmium is added to a concentration of 2 p.p.m.)

CONCENTRATION (p.p.m.)

	Dithizone	Polarography	Atomic Absorption
No. of detn.	7	5	7
Range	1.3-1.9	1.8-2.0	1.5-2.2
Mean	1.62	1.90	1.99
S.D.	0.24	0.10	0.27

LIME JUICE (Cadmium is added to a concentration of 20 p.p.m.)

	Dithizone	Polarography	Atomic Absorption
No. of detn.	7	6	23
Range	13.5-21.0	19.8-21.3	18.0-20.1
Mean	19.2	20.4	19.4
S.D.	2.5	0.6	0.5

While the number of determinations is low, it appears certain that the dithizone method does give lower and less reproducible results at least at this level. While one may argue that the difference is small it is not reassuring to have serious differences between methods especially if these are recommended procedures. As mentioned before, dithizone has been known to give troubles in the past (11, 13, 14, 15).

Finally, while not a criticism, the paper has no reference to other methods such as neutron activation analysis.

Before going any further, let us realistically examine the problem that confronts the analytical chemist. Basically, he must devise a method that others can use with a minimum of interferences and/or problems. To do this the analytical chemist must take two factors into account. These are (a) the likely concentration range in his sample and (b) the type of matrix involved.

(A) CONCENTRATION RANGES

Studies of various diets (15, 17, 18) have shown that the average intake of cadmium is between 0.1-0.3 mg/day. The concentration in food and water usually varies from the low or sub p.p.b. level to the low p.p.m. level (15).

i.e. p.p.b. ----------p.p.m.

vegetables cereals dairy poultry meat
water products seafoods

Analysis of cadmium in normal adult human tissue usually shows concentrations around 0.1-1.0 p.p.m. (wet weight) except for kidney and liver tissues which are usually much higher, i.e. 1-60 p.p.m. In blood and urine the concentration is usually in the low p.p.b. range. (see appendix-2 for literature values for the concentration of cadmium in different human tissues and fluids).

Hence the analytical method must be applicable to these ranges. Bowen (6) gives the detection limits for cadmium by the following methods as:

Table III - Detection Limits by Various Methods

Activation	10^{-9} gm.
Colourimetry	10^{-8} gm.
Mass Spectrometry	10^{-6} gm.
Polarography	1.5×10^{-6} gm.
Spectroscopy**	5×10^{-9} gm.
X-ray Fluorescence	9×10^{-8} gm.

* assuming a flux of 10^{12} neutrons cm^{-2} sec^{-1} and sample irradiated till saturation

** using a D.C. arc after chemical concentration

Activation analysis appears to be the most sensitive of the six methods. Since the McMaster University Reactor has a flux of 10^{13} neutrons cm^{-2} sec^{-1} one expects the detection limit to be down to 10^{-10} gm.

Winefordner and Elser (19) give the following detection limits for atomic flame and related methods.

Table IV - Detection Limits by Atomic Flame and Related Methods

Method	Absolute Detection Limit (gm)
Atomic Emission Flame Spectrometry	4×10^{-7}
RF Excitation Atomic Emission Spectrometry	6×10^{-9}
Atomic Absorption Flame Spectrometry	1×10^{-10}
Graphite Cell Atomic Absorption Spectrometry	6×10^{-14}
Atomic Fluorescence Flame Spectrometry	2×10^{-13}
Graphite Cell Atomic Fluorescence Spectrometry	3×10^{-14}
Metal Loop Atomic Fluorescence Spectrometry	2×10^{-14}

While the numbers are quite impressive they must be interpreted with a certain degree of care. Most of the techniques are relatively new and little practical work has been done at the above levels.

Usually the results were obtained using special equipment on cadmium solutions alone. As well, since some of the techniques require only a small sample volume, the relative detection limits are often several orders of magnitude higher. However some practical work has been done at the nanogram range (10^{-9} gm) using some of the above or related techniques (20, 21, 123).

(B) BIOLOGICAL MATRIX

While organic matter is mainly composed of carbon, hydrogen, oxygen, nitrogen, phosphorous and sulphur, it may contain appreciable quantities of other elements such as sodium, potassium, chlorine, bromine, zinc, iron, manganese, calcium and magnesium. Depending on the type of method of analysis involved it is this matrix that may interfere. For instance, most biological samples contain appreciable concentrations of sodium and chlorine. This creates special problems in non-destructive neutron activation analysis due to the high background from these elements. Or, in the case of atomic absorption, these same elements give rise to false absorption signals for a variety of reasons.

It is the responsibility of the analytical chemist to be aware of these problems before he starts to recommend any procedure. Unfortunately examination of the literature shows numerous cases of discrepancies, erroneous results and controversy. It is hoped that this present work will help to improve the situation.

CADMIUM - DESCRIPTION AND PROPERTIES

Cadmium is a silvery white but tarnishable metal belonging to group 2B in the periodic table along with zinc and mercury. Its main physical properties are listed below (22, 23) in Table V:

Table V - Physical Properties of Cadmium

Atomic Number	48
Atomic Weight	112.40
Outer Electron Configuration	$4d^{10}5s^2$
Ionization Potentials	
	1st - 8.00 e.v.
	2nd - 16.84 e.v.
	3rd - 38.0 e.v.
M.P.	^{o}C-321
B.P.	^{o}C-767
Heat of Vapouration	26.8 kcal/mole
E_o for $M^{2+} + 2\ e^- \longrightarrow M^o$	-o.402 volts

Because of the outer electron configuration, $4d^{10}\ 5s^2$, and the extremely high third ionization potential it forms no compounds in which the d shell is other than full and may be regarded as a non-transition element. As well except for some evidence for the +1 state in melts, +2 is the dominant oxidation state and the only stable valence in aqueous solution.

While cadmium will dissolve in hot dilute sulfuric and hydrochloric acid it most readily dissolves in dilute nitric acid. All of the common salts of cadmium are soluble in water with the exception of the sulfide, carbonate, oxalate, phosphate, ferricyanide, ferrocyanide, and hydroxide.

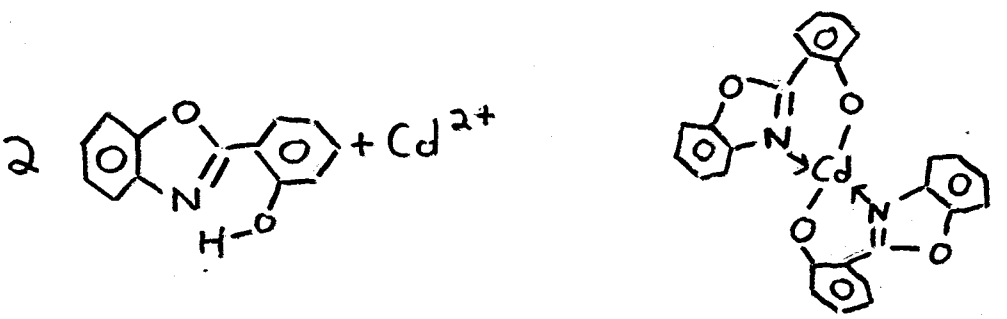
Of interest to the analytical chemist are the facts that it has a suitable E_o for polarography; it forms fairly stable anions with the halogens and this allows a rather selective anion-exchange procedure; and it readily complexes with a wide variety of complexing

agents such as dithizone, EDTA, etc.

Indicators such as xylenol orange, methyl thymol blue, and PAR (4- (2- pyridyazo) -2-napthol) may be used for endpoint detection in direct EDTA titrations (24, 25, 26).

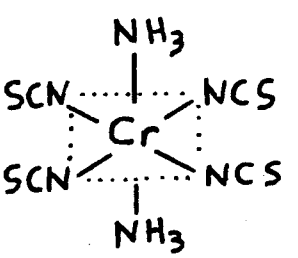
While there are a great many precipitating reagents for cadmium (24) two reagents have gradually emerged as being superior to the others. They are 2- (o-Hydroxyphenyl) -benzoxazole and ammonium reineckate.

2- (o-Hydroxyphenyl) - benzoxazole - (27, 28) The reagent is believed to react with cadmium in the following manner.

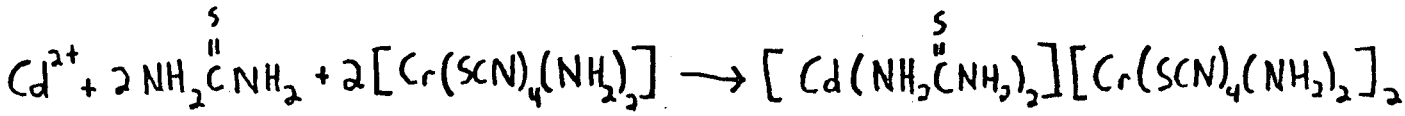


It is a fairly selective reagent and the few elements that interfere namely copper, nickel, and cobalt, may be completely eliminated by following a simple separation scheme (28). It has a gravimetric factor of 0.2109.

Ammonium Reineckate - (27, 29) The structure of the reineckate anion is believed to be:



It combines with cadmium and thiourea in the following manner:



Tin, antimony, copper, bismuth, mercury, and lead will interfere but suitable preliminary separations are available (29). It has a gravimetric factor of 0.1247.

APPARATUS AND CHEMICALS

Apparatus

Samples were irradiated in the McMaster University Nuclear Reactor either in the high flux (1.5×10^{13} neutrons/cm^2/sec) or in the pneumatic rabbit position (1×10^{12} neutrons/cm^2/sec.). Gamma spectra were taken on a Nuclear Data Multichannel Analyzer (Series 2200) using either a 45 cm^3 Ge (Li) detector or a standard 3" X 3" or a 3" X 3" well NaI (Tl) detector.

Atomic absorption work was done using a Westinghouse cadmium lamp on either a Jarrell-Ash 800 Spectrophotometer with a 5 or 10 cm slot burner; or a Heathkit EU 703 Spectrophotometer using either a Techron 5 cm. slot burner or Beckman total consumption burner. Readings from the Heathkit were taken with a digital readout.

Dry ashing of biological samples were preformed in a Blue M Muffle Furnace. pH measurements were done on a Beckman Zeromatic pH meter.

Chemicals

All cadmium solutions were prepared from high purity Cominco 69 cadmium metal shot. C.P. grade acid and bases were used throughout except in the wet ashing of samples for atomic absorption work where B.D.H. Aristar brand HNO_3 and H_2SO_4 were used along with Analar brand of NaOH.

Chelating and anion-exchange columns were made up of Chelex-100 (100-200 mesh) and Bio-Rad AG 1 X 8 (100-200 mesh) respectively.

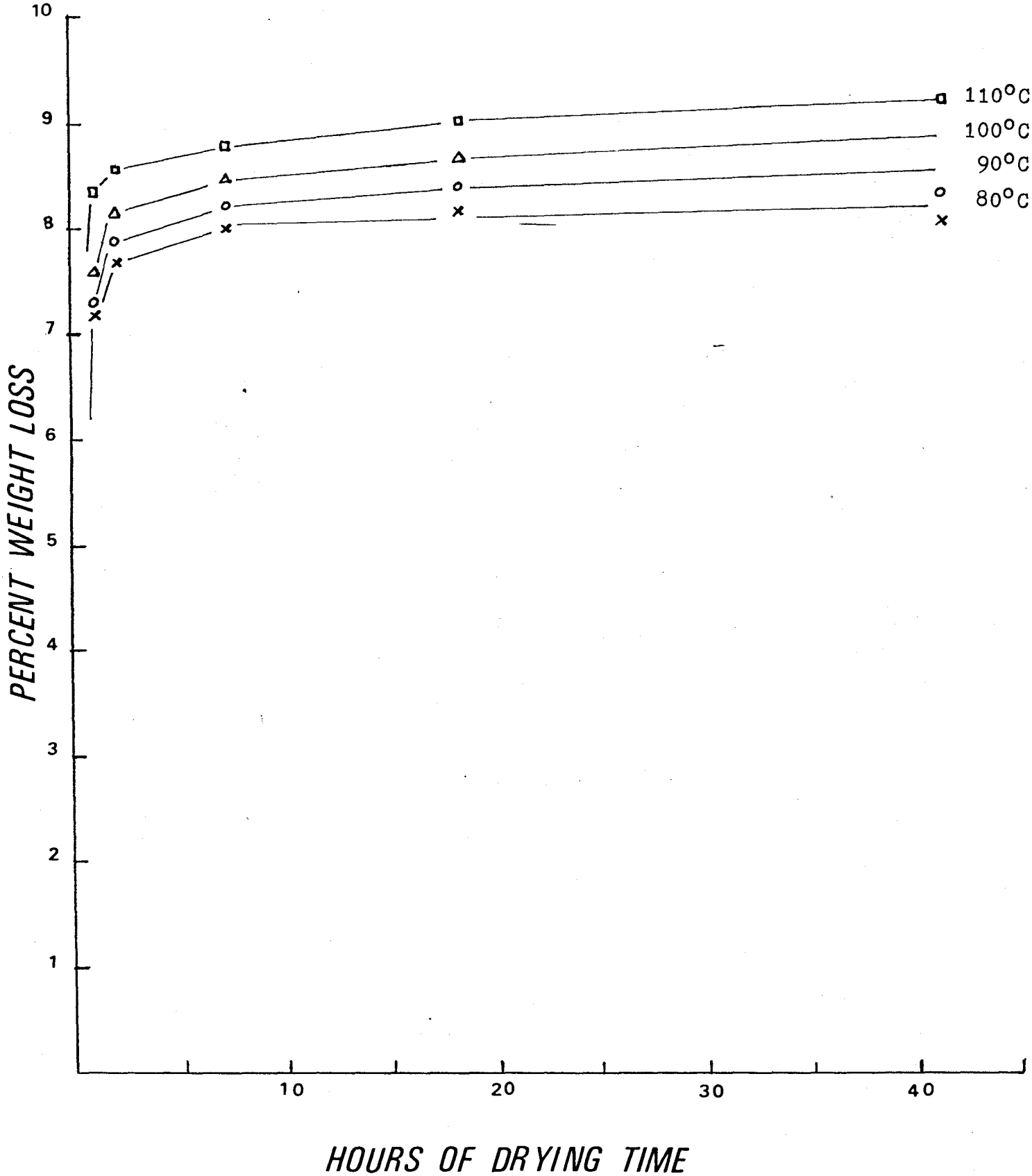
BIOLOGICAL STANDARDS

For comparisons of different analytical methods it was felt advantageous to have a standard biological material. Unfortunately Bowen's kale powder (1) was available in only limited quantities. Since cadmium tends to accumulate mainly in the kidneys (12), beef kidney was chosen as another standard material. However as pointed out by Livingston (30) there is a concentration gradient even within the kidney itself and it tends to accumulate mainly in the renal cortex. A standard was prepared from kidneys bought commercially. They were washed with deionized water, the outer layers (mainly the renal cortex) were removed with plastic utensils and samples were freezed-dried. Sample #1 was ground up using a glass mortar and pestle. Sample #2 was ground up using a blender. The samples were sieved and only the fractions that passed through a 250 micron sieve were taken. It is important to realize that the actual, biologically significant concentration is of no concern here. The essential part lies in the analytical agreement or disagreement obtained using different methods on the same matrix.

Bowen pointed out that drying his kale powder at different temperatures could lead to variations in results since it is slightly hydroscopic, containing about 5% water (6). Similarly drying curves for these standards for the beef kidney also show a variation of

weight loss with temperature (see diagram 1). For the purpose of this work all samples were dried at 90°C for 20 hours.

PERCENT WEIGHT LOSS AS A FUNCTION OF DRYING TIME AT DIFFERENT TEMPERATURES FOR BEEF KIDNEY SAMPLES



ASHING

For the analysis of cadmium in biological materials, it is usually either desirable or necessary to remove the organic matrix before analysis. This can be done in either of two ways.

(1) Wet Ashing - This is the recommended procedure (16, 31-33). Tracer studies have shown near 100% recovery with any of the mixtures of HNO_3, H_2SO_4, and $HClO_4$ (33, 34). Sulphuric acid and hydrogen peroxide have also been used but slight losses of 3-6% have been found (33, 35).

The particular choice of oxidizing agent will depend somewhat on the biological sample itself. For example, samples high in calcium may give undesirable precipitates if H_2SO_4 is used. In this work, for the beef kidney samples a 3:1 mixture of HNO_3 and H_2SO_4 was used in a preliminary step. After a clear solution was obtained the remaining organic material was removed using $HClO_4$. For the kale samples, 90% HNO_3 was finally chosen as an oxidizing agent.

Various authors (31, 33, 35) have recommended rather complicated types of glassware for wet ashing. For cadmium, it was felt that this was not necessary. In this work, a small funnel was simply placed at the mouth of a Kjeldahl flask (30 or 100 ml). This allows the escaping fumes to be partially refluxed. Tracer studies showed near 100% recovery and indicated that the method was satisfactory.

For atomic absorption work in order to keep blank values to a minimum only high-purity acids (B.D.H. Aristar brand) were used.

However, it was found later that C.P. reagent grade acids usually gave a negligible blank as well.

(2) Dry Ashing - One of the few who advocate dry ashing is Shirley (36) who claims that at 550°C using sulphuric acid, he got practically full recovery. However, most authors disagree. At this temperature, Gorsuch (34) reports losses of up to 24%, especially when using ashing aids such as HNO_3 or $Mg(NO_3)_2$. Gorsuch speculates that losses of cadmium may be due to reduction of CdO to the metal (which melts at 320°C and boils at 778°C) by the carbon present (33).

Lowering the temperatures does not solve the problem but losses are reduced. Zook (13) reported recoveries of $92\pm8\%$ at 480°C. Murthy (19) had recoveries of approximately 95% in the same temperature range. Finally at 450°C, Schroeder (12) found less than 5% difference between dry and wet ashing and Pulido (20) has reported similar findings.

Since differences did exist between authors it was decided to investigate dry ashing. This was done in the following manner:

Approximately 1 gm. of beef kidney powder was spiked with 50 ul. of a radioactive cadmium solution (containing about 1 ug Cd as the nitrate). The sample was then dried for 2 hours at 110°C and then dried overnite (16-20 hrs.) in a Blue M Muffle Furnace. Recoveries of cadmium are found in Table VI.

Table VI - Recoveries After Dry Ashing

Temp. °C	Recoveries		
450°C	--	--	-- *
500°C	100.4	99.7	100.6
550°C	98.7	99.7	99.6
600°C	99.3	100.4	99.7

*Ashing was incomplete at this temperature and recoveries were not checked.

While no attempt was made to calibrate the temperature of the furnace, the above data would indicate no loss of cadmium at moderate temperatures. Since this is contradictory to the general result, one must interpret the results with care. One might draw the conclusion that possibly the actual chemical form of the cadmium in the sample might be more important than previously thought. Previous tracer studies have omitted stating the chemical form that the cadmium is in. Therefore, discrepancies might arise from this source.

In conclusion, while wet-ashing is the preferred method, dry ashing at 500°C would probably lead to only small errors (5% or less).

NEUTRON ACTIVATION ANALYSIS

Introduction

Neutron Activation Analysis (N.A.A.) depends upon the formation of radionuclides from an element in the sample when it is bombarded by neutrons. Some of the stable isotopes of the element, when irradiated by slow or thermal neutrons, produce a radioactive species of the same atomic number but one atomic mass unit higher. Each of these radionuclides has its own characteristic radiation, half-life and mode of decay. These properties may be used for the qualitative and quantitative determination of the element of interest. The methods showing how these properties may be applied to analysis can be found in any good reference text on activation analysis.

For cadmium there are seven different isotopes produced by (n,γ) reactions (45) of which only four have been recommended for use in neutron activation analysis. These four are listed in Table VII, along with some of their properties (45).

Table VII - Useful Cadmium Isotopes for N.A.A.

Isotope	$T_{\frac{1}{2}}$	Main γ Lines (Kev)	Precursor	Natural Isotopic Abundance	Cross Section (Barns)
^{111m}Cd	49 min	245,150.8	^{110}Cd	12.39%	0.2
^{115}Cd	53.5 Hr.	527.9 492.3 (336.2*)	^{114}Cd	28.86%	1.1
^{115m}Cd	43d	934.1, 1289.9	^{114}Cd	28.86%	0.14
^{117}Cd	2.4h	273.0,1302.0	^{116}Cd	7.58%	1.4

* photopeak from ^{115m}In daughter

Another isotope ^{109}Cd ($t_{\frac{1}{2}}$=1.3 years) is often used for tracer studies or for substoichiometric analysis. (A more complete list of the isotopes and their properties is listed in Appendix 111).

As illustrated in diagram-2, radiochemical analysis is a fairly broad field. The particular isotope used and the analytical approach will depend on the concentration range and the matrix involved. However for biological samples usually the 53.3 hr. ^{115}Cd is used. There are several reasons for this. First, after irradiation the cadmium spectrum is quite complex (see diagram-3). If one employs a suitable cooling time of at least two days, the shorter lived isotopes will no longer interfere. For irradiations of less than two days interference from ^{115m}Cd is minimal as well (37). Hence the spectrum is usually just that of ^{115}Cd and its daughter ^{115m}In. (see diagram-4,5).

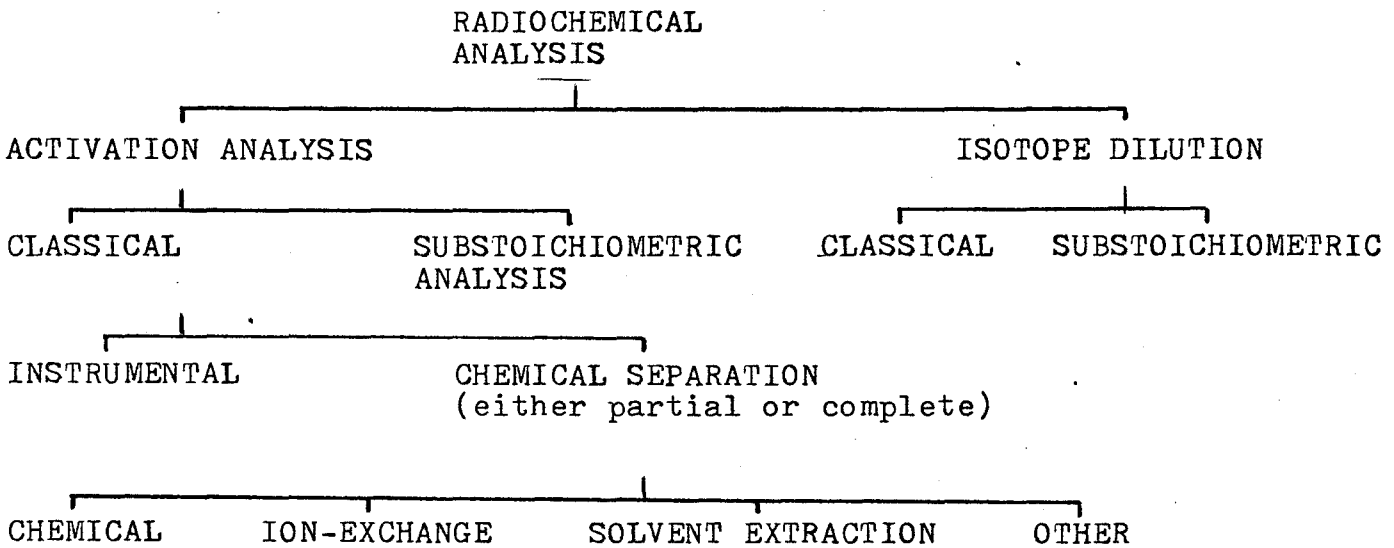
In the case of biological samples the wait of two days also allows the decay of many of the shorter lived isotopes commonly found in biological materials such as: ^{38}Cl (37 min.), ^{56}Mn (2.6 hr.), ^{24}Na (15.0 hr.), ^{64}Cu (12.7 hr.), ^{42}K (13 hr.), and ^{69m}Zn (13 hr.). This also conveniently provides a safer radioactive level to work with. However there has been one reported case in the literature where the short lived ^{111m}Cd was used. (38).

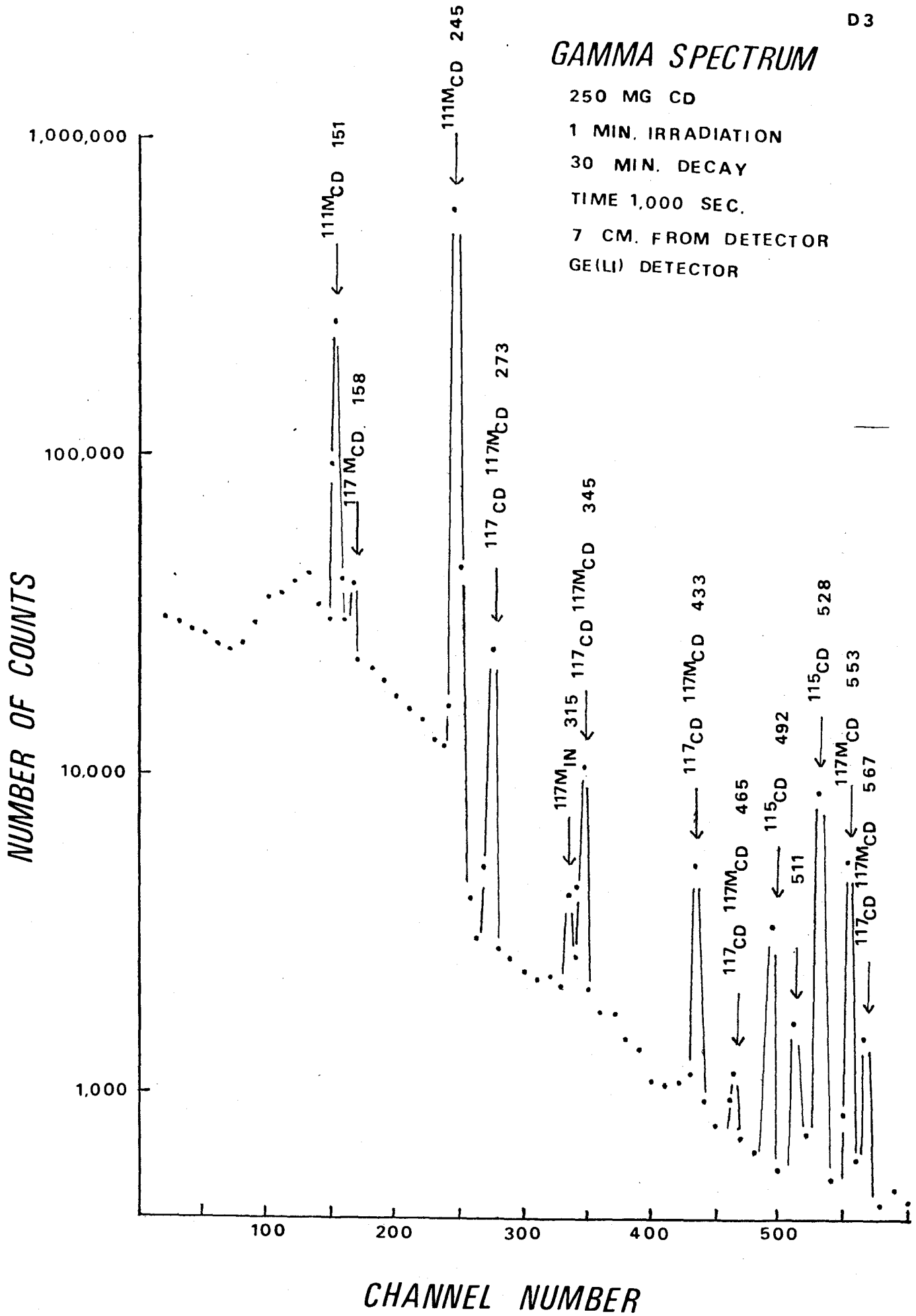
There are three factors which one should consider before proceeding further. These are:

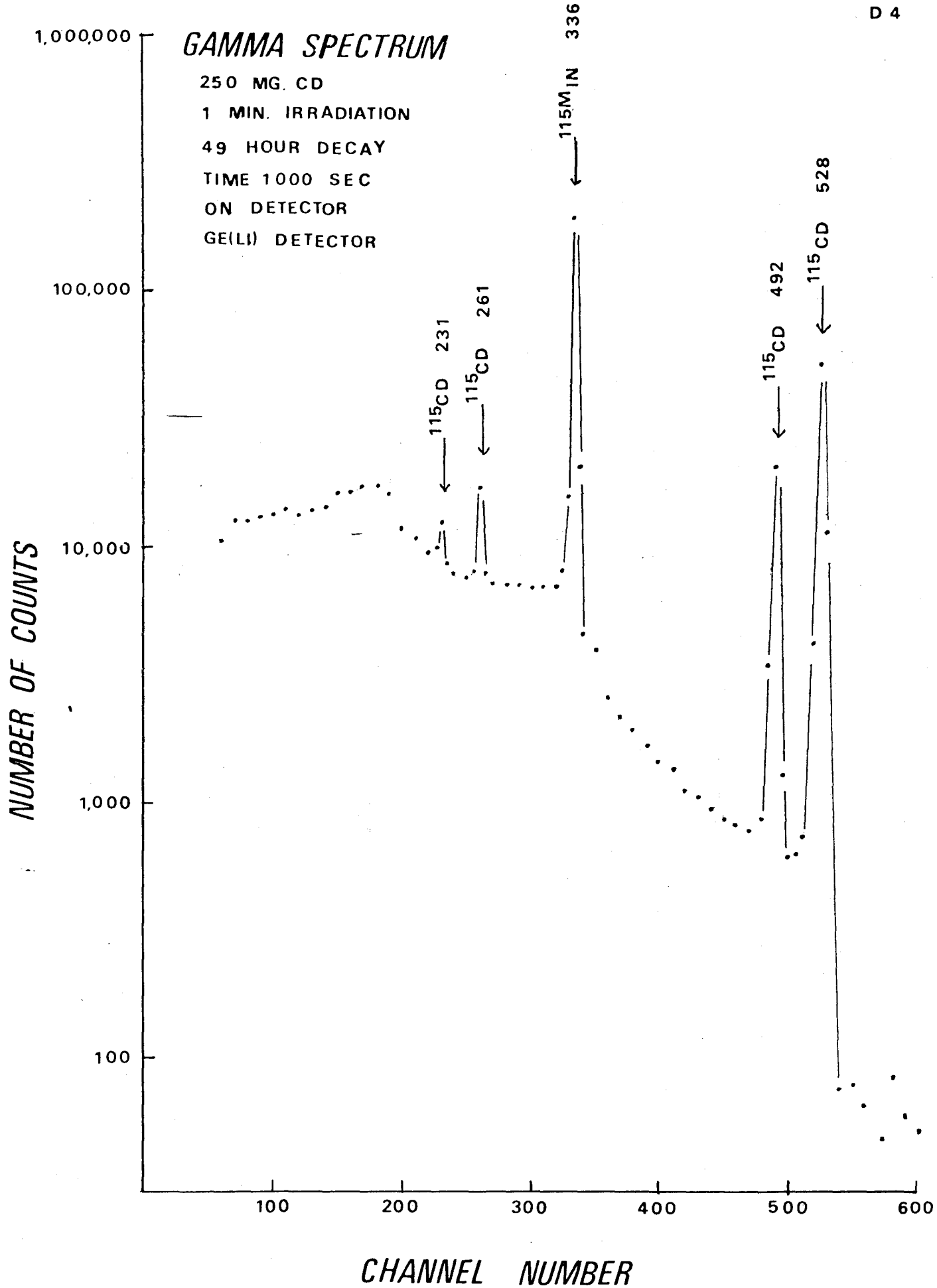
(1) $^{115}Cd/^{115m}In$ transient equilibrium

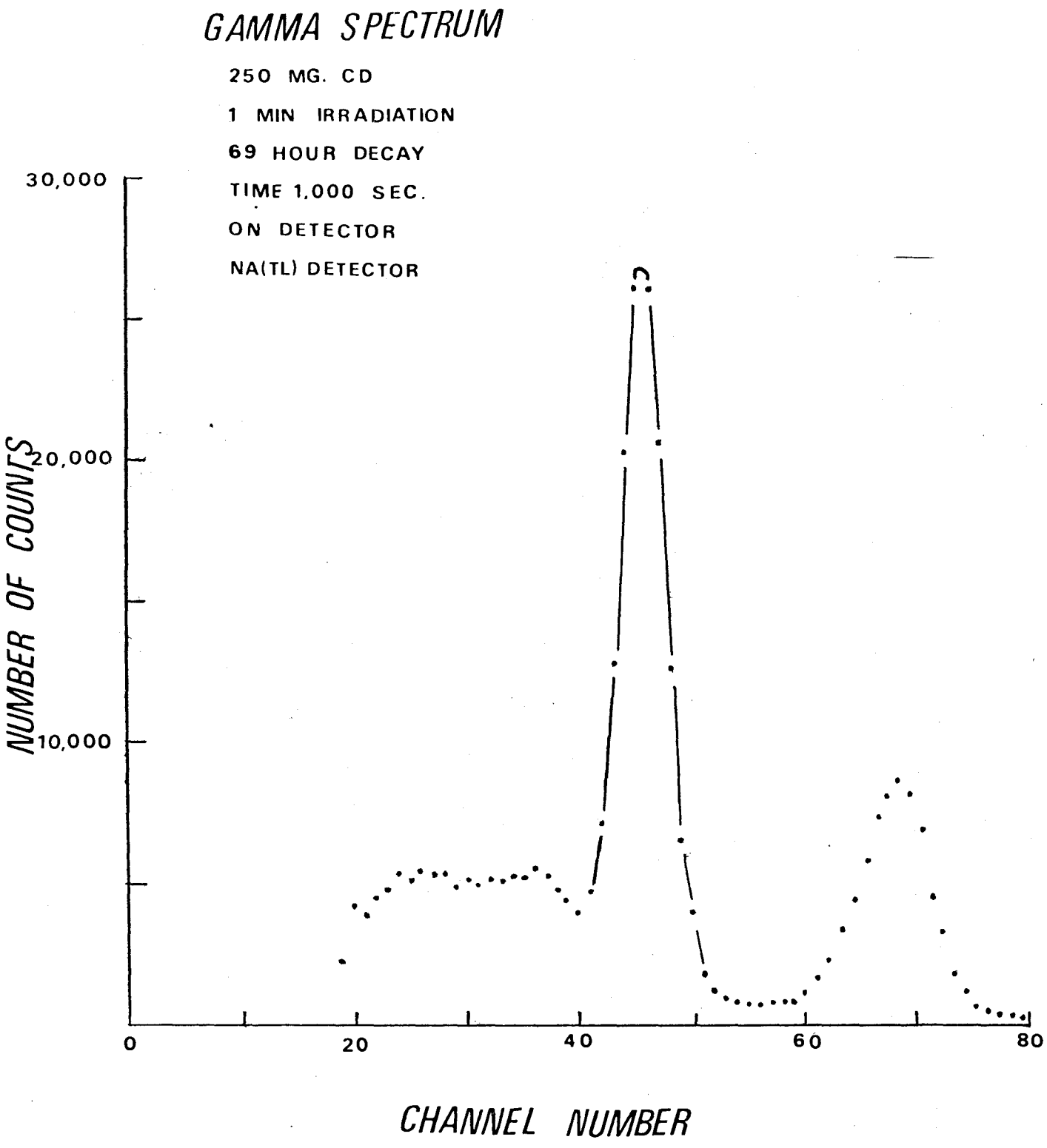
(2) Self-shielding effects

(3) Interfering nuclear reactions


RADIOCHEMICAL ANALYSIS
ACTIVATION ANALYSIS
ISOTOPE DILUTION
CLASSICAL
SUBSTOICHIOMETRIC ANALYSIS
CLASSICAL
SUBSTOICHIOMETRIC
INSTRUMENTAL
CHEMICAL SEPARATION (either partial or complete)
CHEMICAL
ION-EXCHANGE
SOLVENT EXTRACTION
OTHER


GAMMA SPECTRUM
250 MG CD
1 MIN. IRRADIATION
30 MIN. DECAY
TIME 1,000 SEC.
7 CM. FROM DETECTOR
GE(LI) DETECTOR
1,000,000
100,000
10,000
1,000
NUMBER OF COUNTS
100
200
300
400
500
CHANNEL NUMBER
111MCD 245
111MCD 151
117MCD 158
117CD 117MCD 273
117MIN 315
117CD 117MCD 345
117CD 117MCD 433
117CD 117MCD 465
115CD 492
511
115CD 528
117MCD 553
117CD 117MCD 567


GAMMA SPECTRUM
250 MG. CD
1 MIN. IRRADIATION
49 HOUR DECAY
TIME 1000 SEC
ON DETECTOR
GE(LI) DETECTOR
115MIN 336
115CD 231
115CD 261
115CD 492
115CD 528
1,000,000
100,000
10,000
1,000
100
NUMBER OF COUNTS
100
200
300
400
500
600
CHANNEL NUMBER


GAMMA SPECTRUM
250 MG. CD
1 MIN IRRADIATION
69 HOUR DECAY
TIME 1,000 SEC.
ON DETECTOR
NA(TL) DETECTOR
30,000
20,000
10,000
NUMBER OF COUNTS
0
20
40
60
80
CHANNEL NUMBER

Cadmium - Indium Equilibrium

While ^{115}Cd may be readily determined by γ-ray spectrometry via its photopeak at 527.9 kev., the daughter isotope ^{115m}In is often used instead due to its more intense photopeak at 336.2 kev. Because the mother isotope, ^{115}Cd, has a longer $T_{\frac{1}{2}}$ than the daughter isotope, ^{115m}In, the daughter isotope will grow into a so-called transient equilibrium with the mother isotope (39, 44). Therefore if one wishes to use the In-daughter photopeak there are two things which one should know. First, how long does it take to reach the transient equilibrium situation? Secondly, when is the optimum time for counting for a maximum count rate? -

Concerning transient equilibrium the literature is inconsistent. Westermark (37) suggests only several half lives of ^{115m}In (10-15 hr.?), Ljunggren et al. suggest 15-20 hr. (40), Livingston et al. overnight (16-24 hrs.?)(7), Vincent and Bilefield 24 hr. (41, 42), Chueca at least 30 hr. (8). Heurtebise 33 hrs. (43), and Das and deVries, 45 hrs. (9). Therefore it was felt necessary to plot a theoretical curve of the equilibrium which is done in the following manner.
"The number of atoms of daughter substance N_2 is connected with the activity of the mother substance by the following relationship.

$$N_2 = \frac{N_1^{\circ}\lambda_1}{\lambda_2 - \lambda_1}\left(e^{-\lambda_1 t} - e^{-\lambda_2 t}\right)$$

where:

-N^{o}_{I} is the initial activity of the mother substance

-t is the time of accumulation

- 1 and 2 are the decay constants of the mother and daughter substances.

The recorded counting intensity I_t at instant t is:

$$I_t = k_1 N_1^{\circ} \lambda_1 e^{-\lambda_1 t} + \frac{k_2 N_1^{\circ} \lambda_1 \lambda_2}{\lambda_2 - \lambda_1} \left(e^{-\lambda_1 t} - e^{-\lambda_2 t} \right)$$

where:

-k_1 and k_2 are the counting efficiencies of the mother and daughter activities" (44).

Since the first term on the right hand side is solely the contribution from ^{115}Cd and the second term is solely the contribution from ^{115m}In, the ratio of the $^{115m}In/^{115}Cd$ photopeaks can be given by the following equation:

$$\frac{^{115m}In}{^{115}Cd} = \frac{\frac{k_2 N_1^{\circ} \lambda_1 \lambda_2}{\lambda_2 - \lambda_1} \left(e^{-\lambda_1 t} - e^{-\lambda_2 t} \right)}{k_1 N_1^{\circ} \lambda_1 e^{-\lambda_1 t}}$$

This can be simplified to: $\frac{^{115m}In}{^{115}Cd} = C \left(1 - e^{(-\lambda_2 + \lambda_1)t} \right)$

C is a constant

$$= \frac{k_2}{k_1} \cdot \frac{\lambda_2}{\lambda_2 - \lambda_1}$$

The counting efficiencies k_1 and k_2 were determined by the following expression

k=(detector efficiency at a specific)$(A_1)(0.01)$

where:

-A_1 is the number of photons emitted per 100 disintergrations of the nuclide.

For Cd and In:

	$T_{\frac{1}{2}}$	$A_1(50)$	Kev	Detector Efficiency (46)
In	4.5 hr.	45.2	336.2	8.8%
Cd	53.3 hrs.	27.5	527.9	5.2%

Substituting the above values one finds C=3.04. Experimentally C was found to range from 2.95-3.36 with an average of 3.06.

To find the transient equilibrium position the function

$$f(t) = (1 - e^{-0.141t})$$

was plotted against T (see diagram-6). It showed the following values.

f(t) = 0.90 at 17 hrs.
0.99 at 33 hrs.
0.999 at 49 hrs.
0.9999 at 65 hrs.

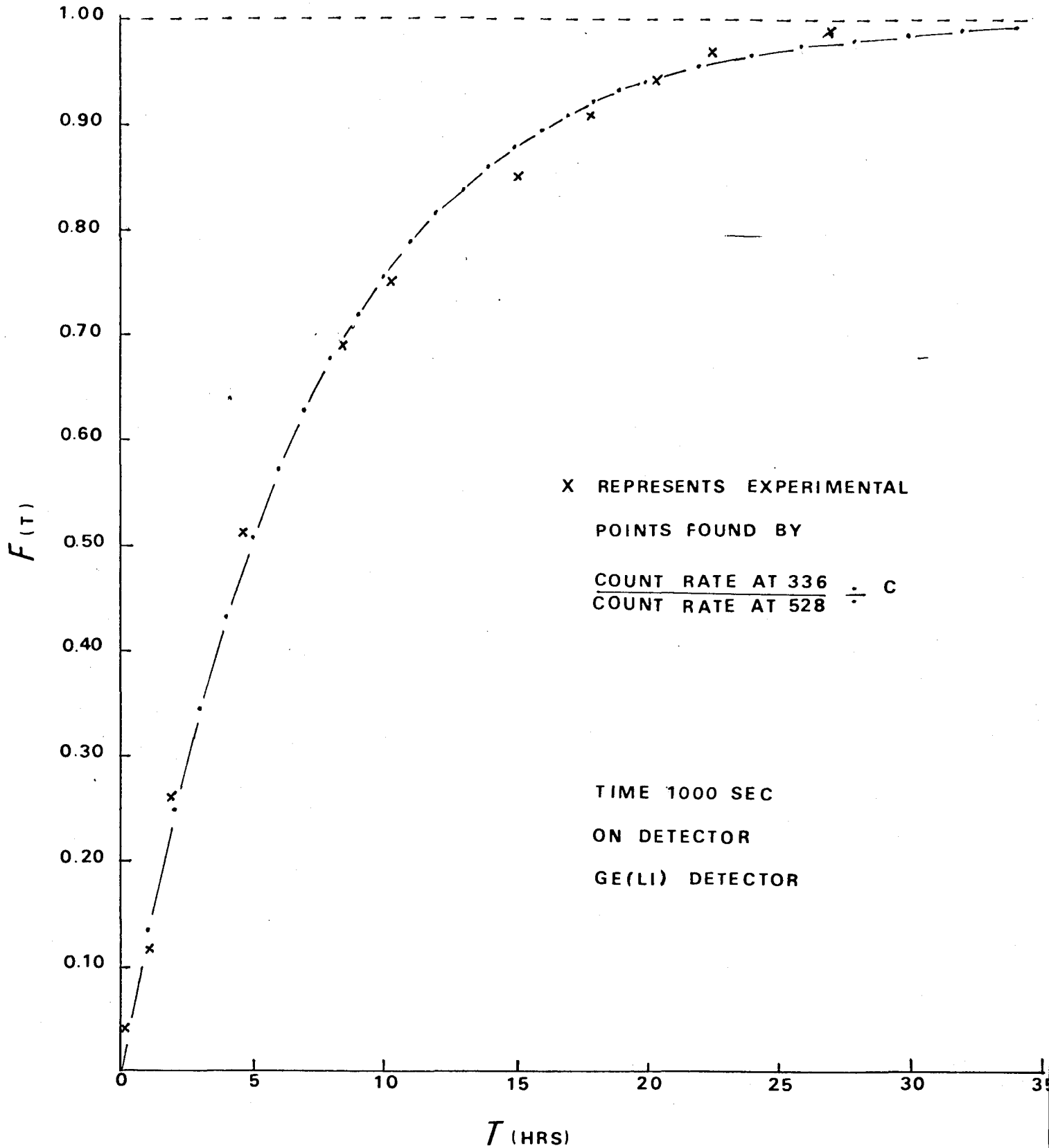
Hence Heurtebise (43) was correct that a wait of 33 hours is necessary unless one wishes to make a correction from the above diagram.

Maximum count rate may be determined from the following expression (39).

$$t_{max} = \frac{1}{\lambda_2 - \lambda_1} \ln \frac{\lambda_2}{\lambda_1}$$

$F_{(T)}$ VS T

WHERE $F(T) = (1 - e^{-0.141T})$



This could also be obtained by plotting In/Cd ratio X Cd count rate versus time (see diagram-7).

To verify the above equations the ^{115m}In daughter was separated from the ^{115}Cd on an anion-exchange column and then allowed to grow in. The results are shown in Table VIII.

Table VIII - $^{115m}In/^{115}Cd$ Equilibrium Data

Time (hr.)	$^{115m}In/^{115}Cd \div C$	^{115m}In Count rate/1000 sec*
0.2	0.04	3807
1.1	0.16	14127
2.1	0.26	23913
4.7	0.51	43916
8.2	0.69	57934
10.2	0.75	62510
14.9	0.85	66744
17.9	0.91	67364
20.4	0.94	66306
22.6	0.97	66135
27.0	0.99	64566
37.0	1.00	56908

*corrected to initial cadmium count rate of 30,000 counts/1000 sec.

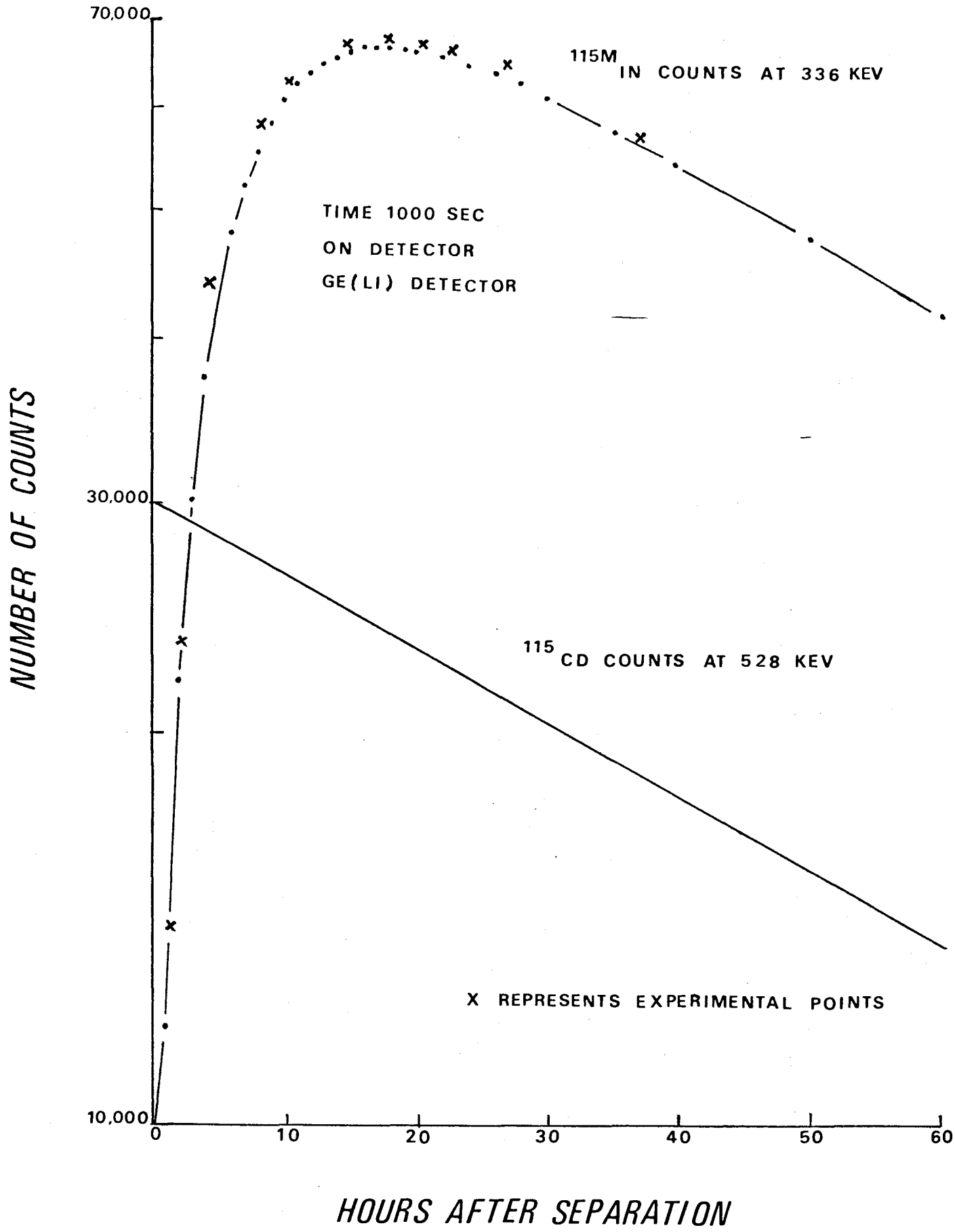
These results showed good agreement with the theoretical curves. (see diagrams-6 and-7)

For beta counting, despite the claims of Vincent and Bilefield (41, 42), decay schemes (47) show the ^{115m}In contribution to be at most only 0.15% and for most practical purposes there is no need to wait for equilibrium conditions.

Self-Shielding

The large cross section of the ^{113}Cd isotope of about 20,000 barns (48) makes self-shielding a definite possibility whenever cadmium is irradiated. However in dealing with very small quantities of cadmium

COUNT RATE vs HOURS AFTER SEPARATION



one might expect self-shielding effects to be minimal. Indeed most authors do not even bother to mention the possibility. Vincent and Bilefield (41, 42) checked self-shielding in the sub ug region and found little difference between standards at this level. Their results are given below in Table IX:

Table IX - Cadmium Self-Shielding Results of Vincent and Bilefield (41,42)

Irradiation Number

	1				2			
Weight in ug	0.926	0.672	0.107	0.0115	0.908	0.474	0.057	0.0093
Activity/ug	3594	3466	3883	3899	3835	3973	4007	4103

They concluded from the above that self-shielding was not important at this level.

However Chueca (8) in trying to explain the differences in the kale powder results re-examined the phenomenon of self-shielding. His results showed that below 1 ug there is a significant increase of about 20% in the activity per ug.

Again to resolve the controversy it was decided to investigate the matter. 50 ul. of $Cd(NO_3)_2$ solutions of varying concentrations were placed in cylindrical quartz capsules of dimensions 15mm. high and 12mm. in diameter. The solutions were evaporated to dryness. To monitor the total flux, a standard of about 1 ug was treated in an analagous manner. Each sample along with a standard was irradiated for periods of time ranging from 1-72 hours. After irradiation, the samples were transferred along with carrier to test tubes and the samples were counted after two days in a NaI(Tl) well counter. The results were all normalized so that the standard 1 ug gives 10,000 counts/1000sec.

The results are given below in Table X.

Table X - Cadmium Self-Shielding Data

Run #1		Run #2	
weight Cd (ug)	activity/ug	weight Cd (ug)	Activity/ug
60.34	9587	1425.3	8529
12.068	9331	142.73	9386
0.12068	10481	10.54	9485
0.012068	9925	0.1054	9777
		0.01054	9749

The activity/ug was then plotted versus the log of the weight in ug. See diagram -8, It is evident from the diagram that self-shielding is not important in the region of interest to the analysis done in this work. (Most standards and samples are usually in the range of 0.1-1.0 ug.)

Interfering Nuclear Reactions

Whenever one does neutron-activation analysis there is always the possibility of a contribution to the isotope of interest from some interfering nuclear reaction. For instance ^{115}Cd can be produced by three other nuclear reactions other than $^{114}Cd(n,\gamma)^{115}Cd$.

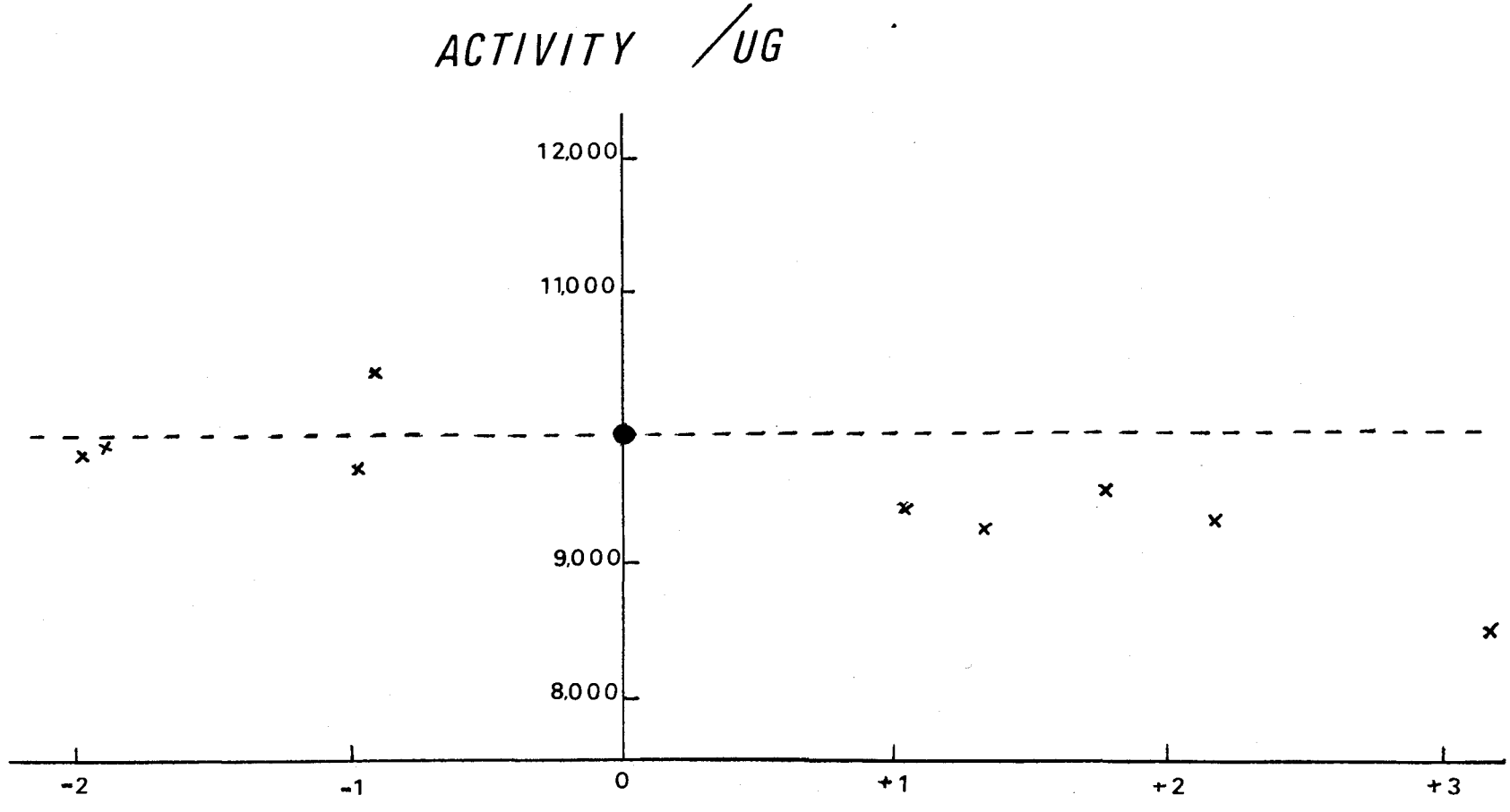
i.e. (1) ^{115}In (n, p) ^{115}Cd

(2) ^{118}Sn (n,α) ^{115}Cd

(3) ^{235}U (n,fission) ^{115}Cd

However reactions (1) and (2) have very low cross sections and in reaction (3) cadmium lies in the trough of the uranium fission yield curve (41). Combined with the low concentrations of the precursors in biological samples, as given by Bowen (67), the error in analysis from interfering nuclear reactions is negligible in nearly all cases.

CADMIUM SELF-SHIELDING



NON-DESTRUCTIVE ANALYSIS

Introduction

Various references in the literature (30, 37, 49-52) have shown that cadmium can be both detected and determined in biological samples by strictly instrumental means. This is made possible by the fact that ^{115}Cd has a longer half-life than the major elements present in the biological matrix. The general approach to analysis appears to be irradiate for about a day, wait for six days (30, 37) or longer (49, 52) and count.

Usually cadmium is only one element in a multielement survey and then the results are usually qualitative or semi-quantitative at best. Some of the work is even suspect. For example Cooper (49) claims to be able to see cadmium in normal whole blood. However using his data the cadmium content would need to be least 9 p.p.m. which is totally unrealistic (see appendix-II)

In a multi-element N.A.A. paper Yule computer-calculates the following detection limits for cadmium in six common matrices (51).

Table XI - Detection Limit of Cadmium in Various Substances by Yule

	Whole Blood	Urine	Milk	Tap Water	"Pure" Water	Polythylene Vials
Cd (in p.p.m.)	500	20	200	40	0.1	0.2

However, as will be experimentally shown later, these detection limits are probably too high. A previous paper by Yule (53), suggests that the above results may have been calculated using the 49 min. ^{111m}Cd isotope.

In 1960, T. Westermark et al. (37) stated that Cd can be determined (at least semi-quantitatively) by direct gamma scintillation counting. Basically his method involves a 6-9 day aging period followed by detection with a NaI(TI) detector and a single channel analyser. Concentration is then determined by comparison with known standards. While in general he uses samples of high Cd content, (200 p.p.m. for 0.1g. samples), he also states that he can place an upper limit on certain bladder samples (6-10 p.p.m.). While still applicable, the fact that only a single channel analyser is being used, means that certain precautions must be taken with this method (37, 54).

In the most recent paper Livingston uses a 6 day waiting period combined with a Ge(Li) detector and a multichannel analyzer (30). Previously he had published a paper on a post-irradiation separation scheme for cadmium (along with Cu, Zn, Hg) (7), but found that now with a Ge(Li) detector the separation was not necessary. Unfortunately, he fails to give a lower level of detection. This is important because like Westermark he is using samples of relatively high cadmium content. (16-305 p.p.m.)

Investigation of Non-Destructive Analysis

Several factors should be considered before non-destructive analysis is used. These include choice of isotope, irradiation and cooling times, possible interferences either from other gamma lines, comptons or background, and detection limits. Each of these is investigated in turn.

(a) Choice of Isotope - As previously mentioned the ^{115}Cd isotope is the one most used but Yule's work indicate that ^{111m}Cd might be suitable as well. This possibility was investigated in the following

manner.

About 0.1g. of beef-kidney was spiked with varying amounts of cadmium and then sealed in polythylene vials. The vials were then irradiated for 15 minutes in the pneumatic rabbit system. After the irradiation the vials were opened and the samples were quickly transferred to small beakers and the gamma spectrum was taken. However when no discernible ^{111m}Cd peaks (151 Kev and 245 Kev) were found at 500 p.p.m. the method was abandoned. Hence it would appear that ^{115}Cd should be used.

(b) Irradiation and Cooling Times - An arbitrary irradiation time of one day was used. Because of the building or saturation factor $(1 - e^{\frac{-0.693t}{T_{\frac{1}{2}}}})$ this irradiation time produces about one quarter of the possible cadmium radioactivity. While longer irradiations would increase the cadmium activity there is not expected to be any great increase in sensitivity because background from the longer lived isotopes would be higher as well.

Shortly after irradiation the gamma spectrum of biological samples is dominated by the major elements present in the sample such as sodium, potassium,chlorine and bromine. Hence trace elements such as cadmium are masked or hidden in the background. Therefore, it is necessary to let the radioisotopes giving the background decay away. Naturally the question arises, how long should one wait before analysis? To answer this, several beef kidney samples of about 0.1 - 0.2 g. were spiked with cadmium to give a final concentration of about 100 p.p.m. in the sample. These were irradiated along with standards for one day at high flux and then counted daily after irradiation using a Ge(Li) detector and a multichannel analyzer. Qualitatively it was

found that when the samples were counted before the fourth day the results were invariably low. Samples counted during the fourth and fifth day were occasionally low, but from the sixth day on they usually were satisfactory. However, after about the twelfth day, the decreasing count rate was beginning to make analysis unsatisfactory.

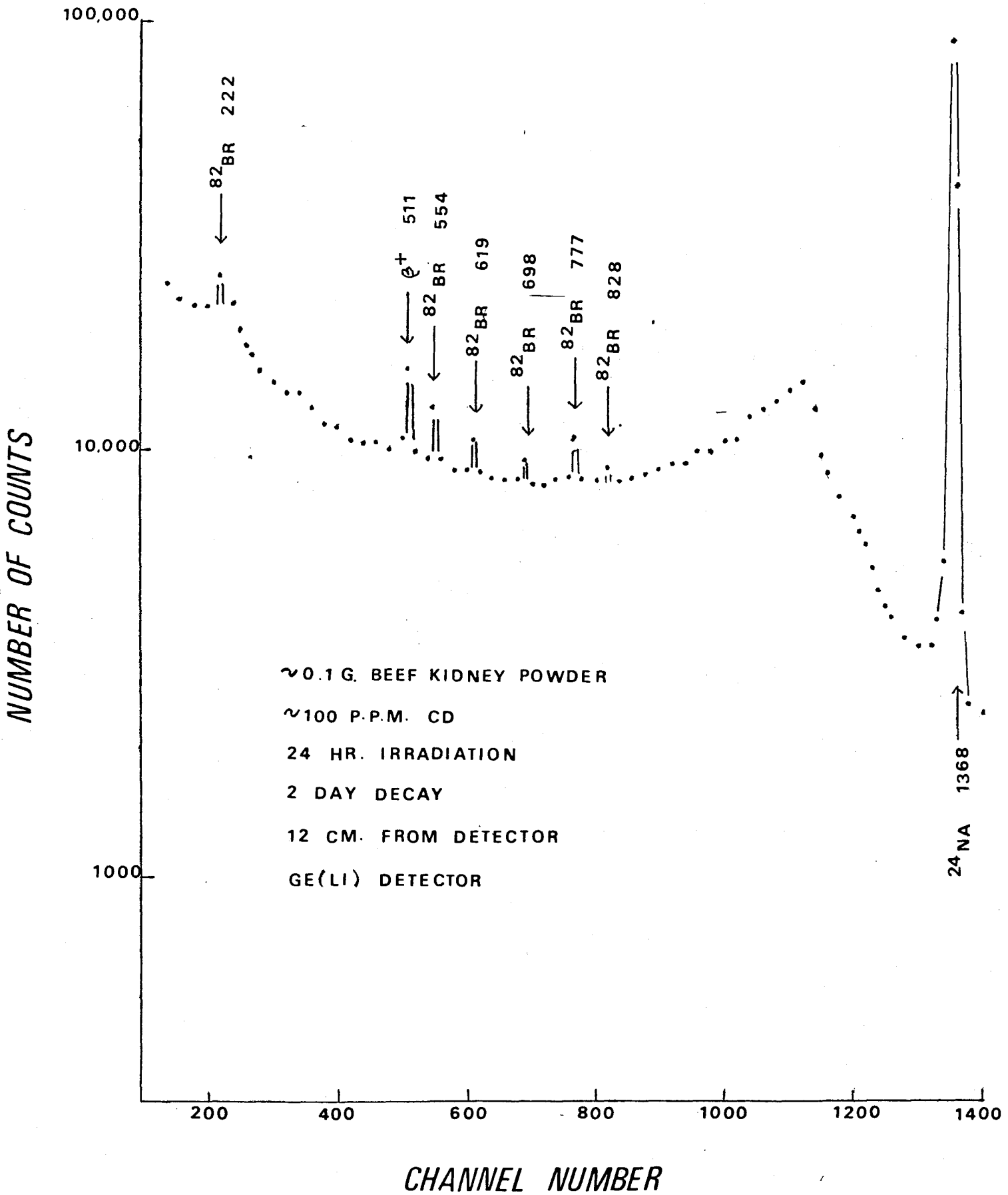
The gamma spectrum of a spiked beef kidney sample, two, four and twelve days after irradiation is shown in diagrams 9, 10 and 11 a, b.

(c) Interference from Other Gamma Lines - The use of high resolution Ge(Li) detectors and multichannel analyzers has minimized this in recent years. Examination of published gamma spectrum of various biological materials (30, 49, 52, 56) show no interference at the ^{115m}In peak at 336. Considering the usual concentration of elements in biological samples (67), and their gamma spectrums (45), there is not likely to be any interference with possibly one exception, namely the ^{59}Fe line at 334.7 Kev. But with a relative intensity of 0.4% and half-life of 45.6 days it should present little trouble. If necessary a correction could be made for it based on the high energy line of iron.

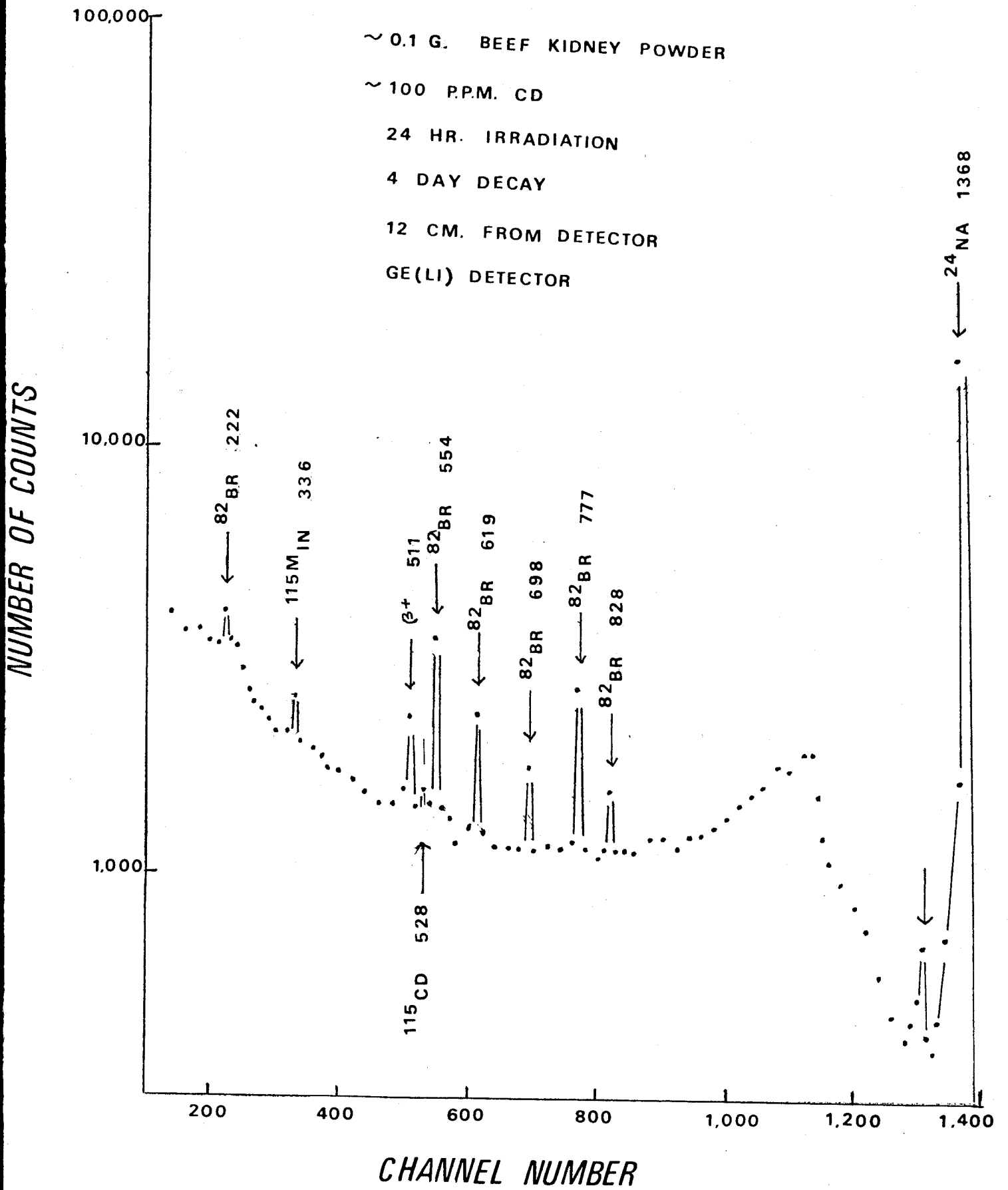
Alternately one could use the ^{115}Cd peaks at 492 and 528 kev. However, the lower count rate and the nearby strong 511 peak mainly from ^{24}Na and ^{64}Cu makes them less satisfactory.

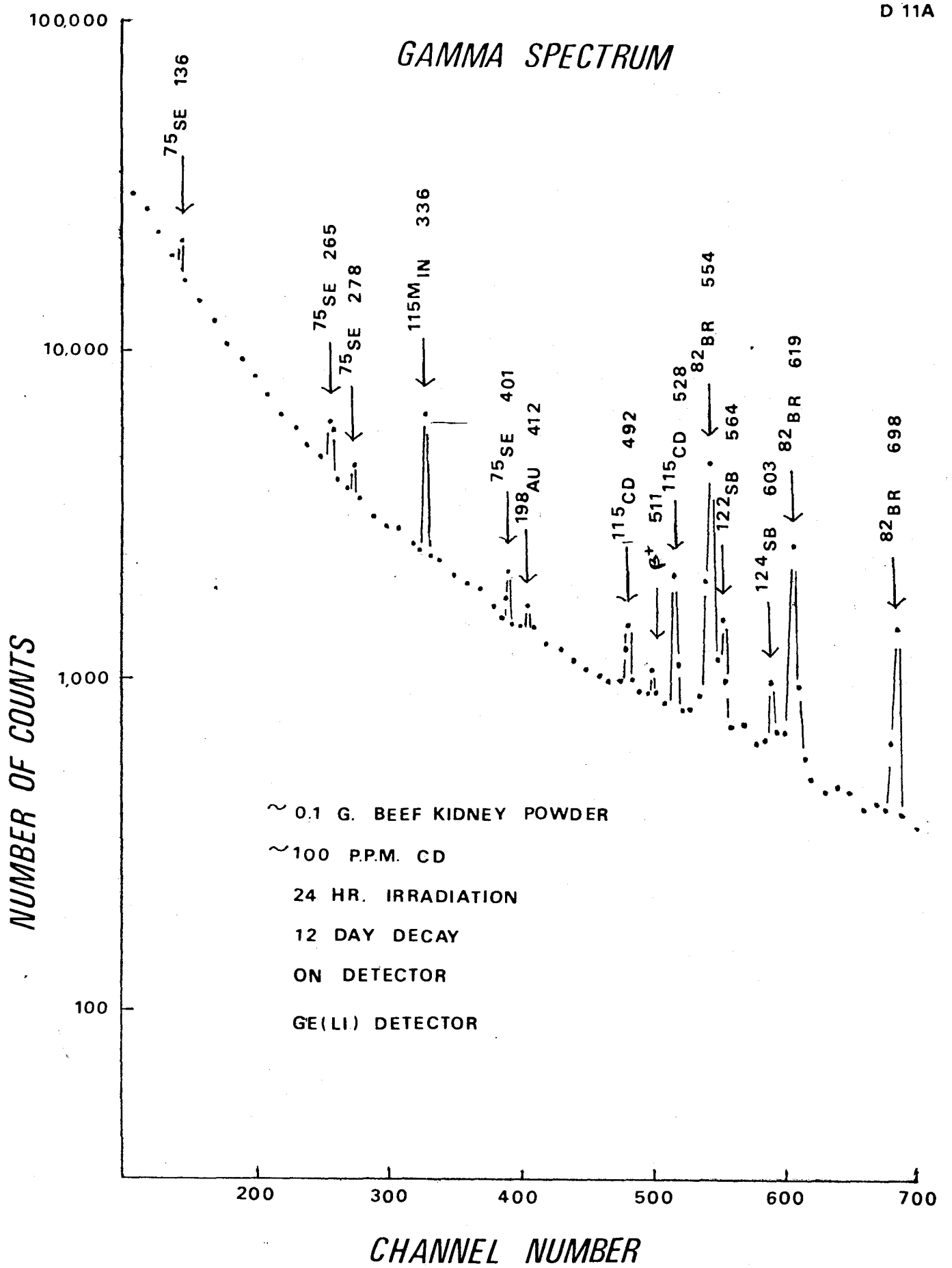
(d) Detection Limit - To find this some samples of beef kidney powder (0.1g.) were doped with various amounts of cadmium, irradiated for 24 hours at high flux along with standards. The samples and standards were allowed to decay for six days before being counted. The results are shown in Table XII.

GAMMA SPECTRUM



GAMMA SPECTRUM




GAMMA SPECTRUM
100,000
10,000
1,000
100
NUMBER OF COUNTS
200
300
400
500
600
700
CHANNEL NUMBER
75SE 136
75SE 265
75SE 278
115MIN 336
75SE 401
198AU 412
115CD 492
511
e+
115CD 528
82BR 554
122SB 564
124SB 603
82BR 619
82BR 698
~0.1 G. BEEF KIDNEY POWDER
~100 P.P.M. CD
24 HR. IRRADIATION
12 DAY DECAY
ON DETECTOR
GE(LI) DETECTOR

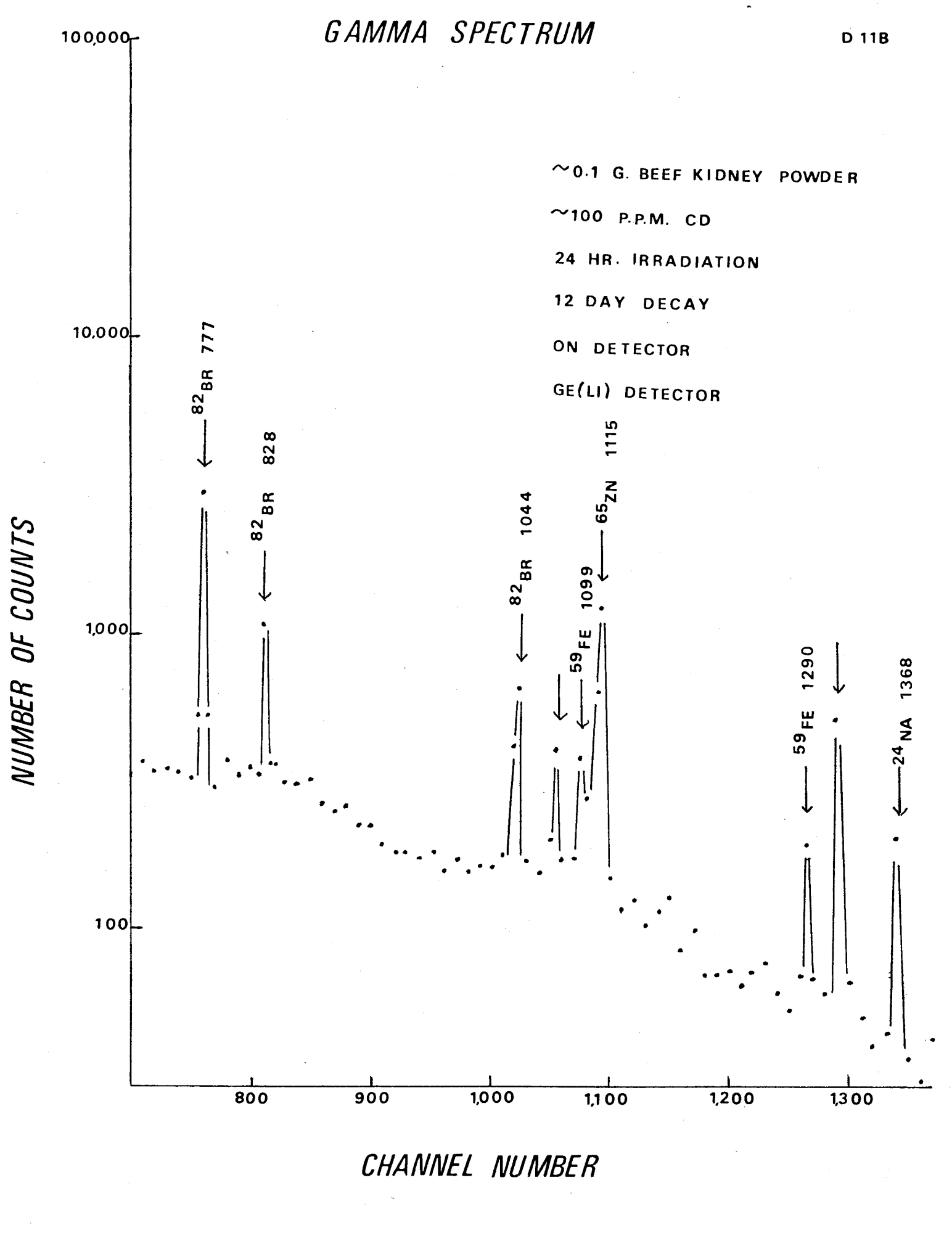
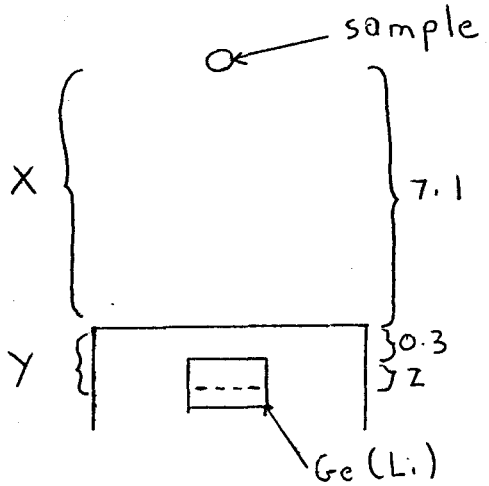

GAMMA SPECTRUM
D 11B
~0.1 G. BEEF KIDNEY POWDER
~100 P.P.M. CD
24 HR. IRRADIATION
12 DAY DECAY
ON DETECTOR
GE(LI) DETECTOR
82BR 777
82BR 828
82BR 1044
65ZN 1115
59FE 1099
59FE 1290
24NA 1368
100,000
10,000
1,000
100
800
900
1,000
1,100
1,200
1,300
NUMBER OF COUNTS
CHANNEL NUMBER

Table XII - Results of Non-Destructive Analysis

Actual Weight (ug) (p.p.m.)		Found Weight (ug)	Error
0.48	(4.9)	-----	-----*
1.03	(10.1)	1.14	+11%
2.69	(25.8)	2.11	-22%
5.50	(47.8)	5.14	-7%
8.21	(77.4)	8.03	-2%
10.51	(99.7)	10.83	+3%

*not calculated due to no discernible ^{115m}In photopeak.

A brief inspection of the above graph would suggest an experimental detection limit of about 5-10 p.p.m. Some calculations on the average background give similar results in the following manner. First it is necessary to examine the counting geometry.



Since the gamma rays will penetrate some distance inside the crystal before stopping z is some small number.

Distance to where gamma rays stop then is 7.1 + 0.3 + z.

To simplify calculations, let us make the assumption that all gamma rays stop at the same depth z.

Assume z = 1 mm)
= 3 mm)
= 5 mm)
=10 mm)
just arbitrary numbers

Then to a first approximation

Detector Efficiency $\frac{Y}{X + Y} \times 0.088$*

*detector efficiency at 336.2 kev when X=0 (46)

Background - In the above position, for a sample of beef kidney powder (0.1 gm.) irradiated for one day at 1.5 X 10^{13} neutrons/cm^3/sec. and allowed to decay for six days, the average background is about 3000-3500 counts/channel/1000 sec.

Assume the peak is in three channels and one wants minimum detection limit to be about 2 standard deviations above background.

2 S.D. of 10000 = 200 counts

Number of counts needed then is $\frac{2 \text{ S.D.}}{\text{Detector Efficiency} \times \frac{A_I}{100}} \times \frac{1}{1000}$

where again A_I is the number of photons emitted per 100 disintergrations

for X = 1	D.P.S.	2900	0.88 uC
X = 3	"	1360	0.04 uC
X = 5	"	800	0.02 uC
X =10	"	350	0.01 uC

But 1 ug irradiated for one day at high flux = 0.124 uC

Allow for 6 day decay Activity/ug = 0.02 uC

Since sample size is about 0.1 gms. then detection limits are

X = 1	40 p.p.m.
X = 3	20 p.p.m.
X = 5	10 p.p.m.
X =10	5 p.p.m.

Obviously, from these figures a detection limit of about 10 p.p.m. seems reasonable.

While the above calculations apply to a particular sample, sample size, irradiation and decay time, the detection limit of about 10 p.p.m. is probably typical within a small factor for most biological samples.

PARTIAL SEPARATIONS

The main disadvantage of non-destructive analysis is the high detection limit of about 10 p.p.m. due to the high background from the sample matrix (mainly sodium, potassium, chlorine and bromine) and the poor-counting geometry because of the high activity of the matrix. Thus even a simple form of chemical separation should improve matters considerably. Qualitatively, the only difference from a complete separation, is that for some loss in radiochemical purity one gets a saving in time and effort involved in analysis. However as the detection limit goes down the spectrum usually becomes more complex because other trace elements show up in a partial separation.

Actually very little work has been done using only partial separation as a means of analysis. T. Westermark et al (37) wet ashed their sample and did a sulfide precipitation from 1 N acid. The precipitate was filtered, washed with 1 N NaOH, dried, counted and then weighed for chemical yield. He claims that while it doesn't lead to a completely pure cadmium spectrum the 340 kev ^{115m}In peak is not contaminated and could be easily evaluated without difficulties in his samples. It should be noted that he was using a lower resolution NaI (Tl) detector at the time and claimed a detection limited of 6-10 p.p.m.

Two other partial separations were based on solvent extraction this time in multielement analysis papers. Strain et al (38) used solvent

extraction of ^{111m}Cd at pH 8 with D.E.D.C. (diethyldithiocarbamate, sodium salt) into ethyl acetate to determine several elements in arteries. Steinnes et al (10) used solvent extraction with tri-octyl-phosphine oxide (TOPO) in cyclohexane from 6N HCI to determine several elements in kale powder.

De Voe and Meinke (27) did some related work in 1959 when they studied some rapid radiochemical separations on cadmium.

Experimental

Both precipitation and solvent extraction were both briefly investigated as a means of partial separation prior to analysis. For all samples the following was done.

Samples (approximately 0.1 g. of beef kidney powder) were irradiated for 24 hours at a neutron flux of 1.5×10^{13} neutrons/cm^2/sec. The samples were allowed to cool for at least two days, cadmium carrier was added (20 mg. for precipitation, 1 mg. for solvent extraction), wet ashed using HNO_3, H_2SO_4 and $HClO_4$, separated by the appropriate method and then allowed to sit for at least two days before analysis to ensure that the transient equilibrium had been reached. The different samples were all analyzed 4-10 days after irradiation using a Ge(Li) detector and a multi-channel analyzer.

(a) Precipitation - First of all it was decided to investigate the method of Westermark (37) which uses a sulfide precipitation followed by washing with NaOH. The following was done:

After wet-ashing the radioactive sample, the resulting solution

was diluted to 50-100 ml. and adjusted with HNO_3 to be about 1 N in acid. H_2S was then bubbled through the solution for five minutes. The solution was then allowed to stand for half an hour before being suction filtered through a glass-fiber filter disc. The sample was then washed with several small portions of 1 N NaOH and water and then dried at 105°C for 1-2 hours. Another sample was treated similarly, but washed only with distilled, deionized water.

The gamma-ray spectrum of the precipitate showed a large number of non-cadmium peaks (see Table XIII). As well, it was found that there was very little difference between washing with 1 N NaOH and water. The use of I N NaOH was based on a separation scheme to eliminate sulfides of Mo, Sn, Pt, Ag, As and Sb (55).

From the gamma spectrum, the method appears suitable for analysis, but only using the higher resolution Ge(Li) detector. A NaI(Tl) detector is not suitable. For example, in the particular sample used, one would get an estimated 10-20% contribution in the ^{115m}In 336 Kev. peak from contributions from the nearly ^{203}Hg (279 kev) and ^{198}Au (412 kev) if the NaI(Tl) detector was used. As well the ^{115}Cd (492-527 kev) peak could never be used because of the strong positron annihilation peak at 511 kev.

Since the method appears suitable for analysis and because sulfide even in acid solution is not selective for cadmium, it was decided to see if two "selective" precipitating reagents for cadmium, ammonium reineckate, and 2-(o -hydroxyphenyl) -benzoxazle, would offer any improvement.

For example, the reineckate precipitate would appear to have several advantages over a sulfide precipitate.

(1) The precipitate is bulkier and hence more easily handled.

(2) Better gravimetric factor for gravimetric yield calculations.

(3) Higher degree of selectivity than a sulfide precipitate.

(4) The control of acidity is not as important.

2-(σ-hydroxyphenyl) -benzoxazole has similar advantages except for number (4). The precipitation is done in a certain pH range in basic solution. The use of a basic solution creates the disadvantage that it becomes necessary to complex certain cations to prevent them from precipitating out of solution.

The procedure for reineckate precipitation was:

After wet ashing, the sample solution was taken to dryness and the residue was dissolved in 50-100 ml of 0.1-1.0 N HCl. Sufficient freshly prepared and filtered 5% w/v thiourea solution was added to bring the thiourea concentration up to about 1% w/v in the sample solution. A saturated solution of ammonium reineckate (which is also 1% w/v in thiourea), is added in excess. This was marked by a strong pink colour in the supernatant liquid. The mixture is allowed to stand in an ice bath for about a half to one hour with frequent stirring. The cold suspension is suction-filtered through glass fiber filter discs, washed once with cold 1% w/v thiourea solution and then several times with cold 95% ethanol. Finally the precipitate is dried at 110^{o}-120^{o}C for one hour.

The procedure for 2-(hydroxyphenyl) -benzoxazole was:

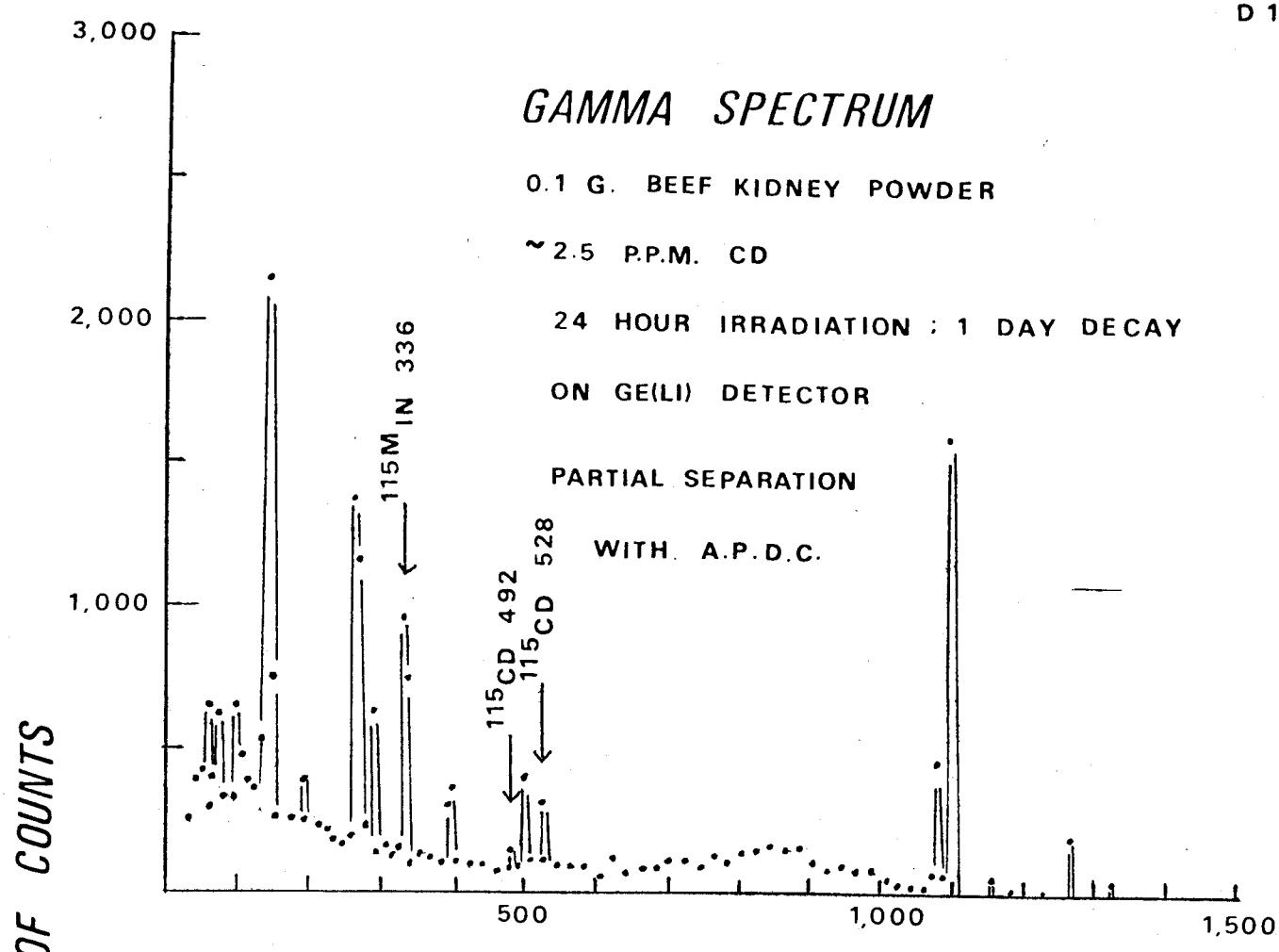
After wet ashing the solution was diluted to about 100 ml. and 2 gm. of ammonium tartrate was added. Then 10 N NaOH was added slowly with stirring until pH 11 was reached. The solution was then heated to 60°C and 10 ml. of a 1% w/v solution of 2-(o-hydroxyphenyl)-benzoxazole in 95% ethyl alcohol was added. The solution was allowed to sit for 15 minutes at 60°C and then allowed to cool to room temperature. It was then suction-filtered through glass fiber filter discs and washed with 50% ethanol several times. The precipitate was dried at 120-130°C.

The gamma spectrum of both precipitates showed a number of non-cadmium peaks as shown in Table XIII. However, both were suitable for analysis but again only with the higher resolution Ge(Li) detector. Both showed a slight improvement over the sulfide precipitate in reducing the background. The gamma spectrum of the reineckate precipitate is shown in diagram 12b.

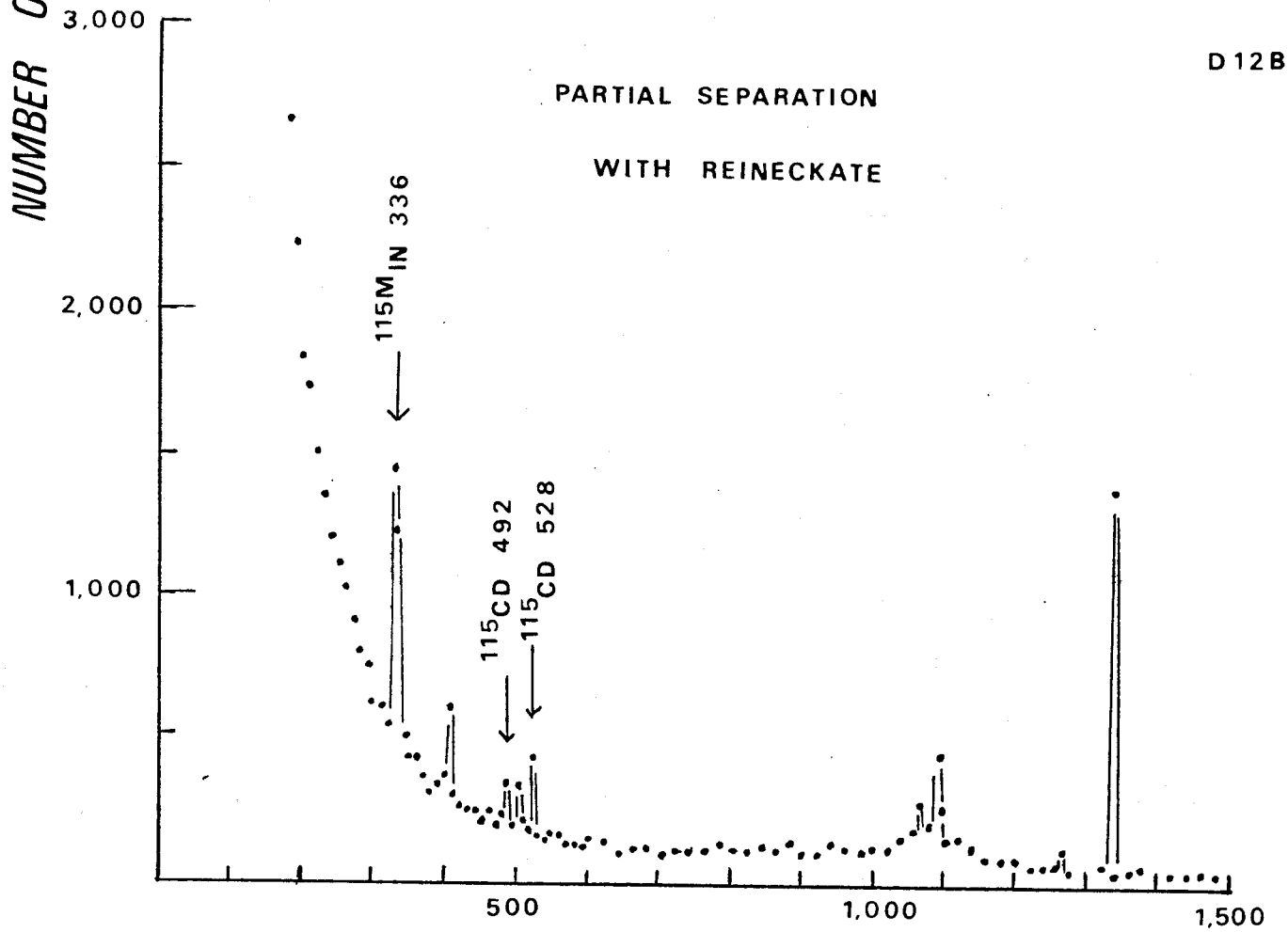
(b) Solvent Extraction - Three different reagents were investigated: ammonium pyrrolidine dithiocarbamate, (A.P.D.C.); diethyldithiocarbamate, sodium salt, (D.E.D.C.); and dithizone.

In each case after irradiation and wet-ashing the sample was diluted to 100 ml. (in the case of dithizone, two grams of sodium potassium tartrate was added) and the pH was adjusted with NaOH to 4 for A.P.D.C., 7 for D.E.D.C. and 13.5 for dithizone. The solutions were now transferred to separatory funnels.

D 12A


GAMMA SPECTRUM
0.1 G. BEEF KIDNEY POWDER
~2.5 P.P.M. CD
24 HOUR IRRADIATION : 1 DAY DECAY
ON GE(LI) DETECTOR
PARTIAL SEPARATION
WITH A.P.D.C.
115M IN 336
115CD 492
115CD 528
NUMBER OF COUNTS
3,000
2,000
1,000
500
1,000
1,500

D 12B


PARTIAL SEPARATION
WITH REINECKATE
115M IN 336
115CD 492
115CD 528
3,000
2,000
1,000
500
1,000
1,500

For A.P.D.C. and D.E.D.C., 10 ml. of a 1% w/v solution of the respective reagent was added followed by 10 ml. of methyl isolutyl ketone (M.I.B.K.) and the funnel was stoppered and shaken. Dithizone was added in CCI_4 and then the funnel was stoppered and shaken. Now in each case the aqueous phase was discarded and the organic phase was shaken twice with small portions of distilled deionized water. Finally the organic phase was transferred to a 30 ml. beaker and evaporated to dryness under an infrared lamp.

Again in all three cases the gamma spectrum showed a number of non-cadmium peaks. The A.P.D.C. spectrum is shown in Diagram 12a. Qualitatively, dithizone extraction at a high pH, gave the best separation. However, as for the precipitation method, all were suitable for analysis but with the possible exception of dithizone, only with the higher resolution Ge(Li) detector.

Use of ^{111m}Cd

Attempts to duplicate the work of Strain (38) were not successful. Using 15 min. irradiation in the rabbit position and counting one hour after irradiation no ^{111m}Cd peaks were seen with the beef kidney samples. From the background a detection limit of about 10 p.p.m. was estimated which was well above the levels that Strain (38) was determining.

Comments - Because the method of partial separation gives distinct and measurable ^{115}Cd-^{115m}In peaks it would appear to be a suitable method of analysis. While the work was done on only one sample, the general result is probably typical of most biological samples. The

Table XIII - Non-Cadmium Peaks in Partial Separations

Method (number of time preformed)

Energy (kev)	Isotope	Halflife	Sulfide(4)	Reineckate(3)	oHpB(2)	A.P.D.C.(1)	D.E.D.C.(2)	Dithizone(1)
69	(1)	-----	PA	PA	PA	PA	PA	A
	^{197}							
78	Hg	65 h	PA	PA	PA	PA	PA	A
104	(2)	-----	PS	PS	A	PA	A	A
140	(3)	-----	PA	PS	A	PA	A	A
	^{75}							
265	Se	120d	PS	PS	A	PA	A	A
280	(4)	-----	PS	PS	PA	PA	A	A
	^{198}							
412	Au	64.7h	PA	PA	PA	PA	A	A
511		-----	PA	PA	PA	PA	PA	PA
560	(5)	-----	PS	A	A	A	A	A
	^{59}							
1099	Fe	45.1d	PS	A	A	PA	PS	A
	^{65}							
1115	Zn	243d	PA	PA	PA	PA	PA	PA
	^{60}							
1173	Co	5.24y	A	A	AE	PA	PS	PA
	^{59}							
1292	Fe	45.1d	PS	A	A	PA	PS	A
	^{60}							
1332	Co	5.24y	A	A	AE	PA	PS	PA
	^{64}							
1345	Cy	12.8h	PA	PA	PA	PA	PS	PA
	^{24}							
1368	Na	15.0h	PA	PA	PA	A	A	A

PA-present in all cases; PS-present in some cases; A-Absent in all cases; AE-Absent but expected

(1) Possibly a Au x-ray
(2) Source unknown
(3) Possible combination of $^{75}_{75}$Se(136 kev; $t_{\frac{1}{2}}$-121d) and $^{99m}_{203}$Tc(141 kev; daughter of ^{99}Mo, $t_{\frac{1}{2}}$-66h)
(4) Possible combination of $^{76}_{122}$Se(280 kev; $t_{\frac{1}{2}}$-121d) and $^{}_{}$Hg(279 kev; $t_{\frac{1}{2}}$ 46.9d)
(5) Possible combination of $^{}$As(559 kev; $t_{\frac{1}{2}}$-26.4h) and $^{}$Sb(564 kev; $t_{\frac{1}{2}}$ 64.3h)

reason for this lies again in the fact that the region around the ^{115m}In 336 kev peak is clean. Partial separation makes analysis possible by reducing the high background. The background that is left after separation is mainly ^{24}Na for precipitation and ^{65}Zn for solvent extraction. With the exception of iron the presence or absence of most other trace elements has little effect on the analysis. Even the interference from iron could be minimized by choice of method (i.e. Reineckate rather than A.P.D.C.).

Since the method of analysis was suitable for samples of about 2.5 p.p.m., partial separation obviously has a lower detection limit than non-destructive analysis. For the reineckate precipitate a count rate of about 1500-20000 counts/1000 sec was obtained with an average background of about 250 counts/channel. From this one can calculate a detection limit of about 0.1 p.p.m. Using A.P.D.C. the detection limit is slightly lower, about 0.05 p.p.m. because of the lower background. This represents an improvement in the detection limit of a factor of 100-200 times over non-destructive analysis.

COMPLETE SEPARATIONS

While determination may be possible by either instrumental or partial separations most often it is desirable to do a complete separation. The main reason for doing this lies in the advantages of using a NaI(Tl) well detector. The advantages are that counting efficiency approaches 100% and errors in counting geometry are minimized. The expression "complete separation" is somewhat of a misnomer in this case because all that is required is that the spectrum be "clean" in the region of interest.

Again complete separations take advantage of some chemical or physical property of cadmium. Usually the separation scheme is one of or a combination of the following procedures.

(1) Classical chemical procedure

(2) Ion-exchange procedure

(3) Solvent extraction

It was decided to investigate each of the above types.

CHEMICAL SEPARATION

There are two approaches to this method. The first one is to start with one of the classical multi-element chemical separation schemes (24) and adopt those parts that are pertinent to cadmium analysis. The second approach is to take advantage of the selective precipitating agents for cadmium. Bowen (6) and Ljunggren et al (58) followed the second approach. Following an initial sulfide precipitation from acid solution, they dissolve the precipitate and reprecipitate the cadmium as the reineckate. Ljunggren stops here and claims detection limits on the order of a few nanograms. Bowen however takes the procedure one step further, dissolves the reineckate, and reprecipitates the cadmium as a sulfide. Bowen's method, with a few modifications, was investigated in the following manner:

A 0.1 gm. samples of beef kidney powder which had previously been irradiated for 1 day at 1.5×10^{13} neutrons/cm^2/sec. and allowed to decay for four days was dissolved along with 10 mg. of cadmium carrier in a mixture of HNO_3, H_2SO_4 and $HClO_4$ and taken to dryness. The residue was dissolved in 1 N HNO_3 and filtered through a glass fiber filter. H_2S was bubbled through for 5 minutes. The solution was then centrifuged. It was washed twice with water and centrifuged after each washing. The CdS was dissolved in 3 ml. of concentrated HCl. The solution was boiled for a few minutes to remove H_2S, and then diluted to 1 N and made 1% w/v in thiourea. A saturated solution of ammonium reineckate

(also 1% w/v in thiourea) was added dropwise with mixing until an excess was present as indicated by a strong pink colour in the supernatant. The precipitate was centrifuged and then washed twice with water. The precipitate then was dissolved in 2 ml. of concentrated HNO_3 and boiled until the resulting solution turned blue. It was diluted to 1 N (and warmed if necessary to dissolve any $Cr(NO_3)_3$ which comes from the reineckate anion) and H_2S was bubbled through for five minutes. The precipitate was centrifuged and washed twice with water.

Two days after separation and six days after irradiation the gamma spectrum was investigated. The only foreign peak in the spectrum was a 511 kev peak (possibly from ^{64}Cu). The chemical yield was also determined, using a ^{115}Cd carrier solution.

Ion Exchange

Because of its ability to form moderately strong halogenide complexes, cadmium readily lends itself to anion-exchange separations. For the separation of cadmium from the biological matrix at least three different approaches have been advanced.

The first and simplest of these is a strict anion-exchange separation scheme proposed by Chueca et al. (8) in a paper on zinc and cadmium determination in biological samples. Basically the separation scheme is to load the sample on the column with 1 N HCl, wash with 1 N HCl, and then elute the zinc with 0.01 N HCl, and finally the cadmium with 0.0005 N HCl. He claims approximately 86% chemical yield with no foreign activity in the gamma spectrum of cadmium except a little ^{65}Zn.

The second approach is a group separation of copper, zinc, cadmium, and mercury (7) by Livingston. However it has been used to determine cadmium alone (68). Basically the separation scheme loads the sample on a Dowex-2 column using 0.5 N HCl. The copper was eluted using 0.12 N HCl. The zinc was eluted with 2 N NaOH containing 2% w/v NaCl. The cadmium was then removed using 1 N HNO_3. Finally the mercury was removed using a 5% w/v solution of EDTA. After removal from the column the cadmium is precipitated as the hydroxide, redissolved in acid and reprecipitated finally as the reineckate.

Livingston claims that of the elements held on the resin by formation of insoluble oxides and hydroxides only silver, mercury (+2), thallium, and bismuth are precipitated by reinecke's salt. However, silver, mercury (+2), and thallium can be removed by a suitable silver chloride scavenge before the reineckate precipitate. Bismuth because of its poor activation characteristic does not interfere.

Using this scheme for the analysis of a kale sample, Livingston claims to get a radiochemical pure spectrum.

Again in a multielement separation scheme K. Smasahl et al (59-61) use a different technique. Cadmium is first absorbed on a Dowex-2 column using concentrated HCl, removed using H_2SO_4 and then selectively absorbed on another Dowex-2 column using either a mixture of sulphuric (0.5 M) and hydrobromic acid (0.1 N.)(59, 60), or hydrochloric acid (0.1 N) (61). While no radiochemical purity is given, chemical yields of 100% (60) and 93% (61) are claimed.

Wester (62) in still another paper essentially combines both the first and third methods. He uses Samsahl's original method of wet ashing and distillation, but then loads the anion-exchange column with less concentrated HCl and selectively elutes first iron and zinc off using HCl and H_2O_2. The cadmium is then eluted off with H_2O and H_2O_2 and then collected on a cation-exchange column in the H^+ form. He claims a chemical yield of 97%.

It was decided to investigate Chueca et al method. The method with some modifications is as follows:

After suitable irradiation and cooling periods, the sample along with 1 mg. cadmium carrier was transferred to a 250 ml. erlenmeyer flask and dissolved in a mixture of HNO_3/H_2SO_4. When the solution has cleared the final traces of organic material were removed using $HClO_4$. The solution was taken to dryness. The residue was dissolved in 2-3 ml. of 2 N HCl and then loaded onto an AG 1 X 10, 100-200 mesh column (dimensions 7.5 X 1 cm.) in the chloride form. The flask was washed three times with 2 ml. of 1 N HCl and the column with 25 ml. of 1 N HCl. The zinc was eluted with 40 ml. of 0.01 N HCl, and then the cadmium was eluted with 80 ml. of 0.0005 N HCl which was collected. The 80 ml. was evaporated down to dryness and the residue was dissolved in a few ml. of 0.01 N HCl and transferred to a test tube to be counted in a NaI(Tl) well counter.

The method was further examined with respect to the actual separation of the zinc and cadmium, the chemical yield, and the radiochemical purity.

Separation of Zinc and Cadmium - Using atomic absorption the elution curves of cadmium and zinc were studied using first just 0.01 N HCl, then just 0.0005 N HCl, and finally both as in the above procedure. As illustrated in diagram 13, a reasonably good separation between the two is obtained.

Chemical Yield - The chemical yield was determined using ^{115}Cd carrier solution and counted in a NaI(Tl) well counter. Results from three different columns are as follows:

Column A - 95%, 94%
Column B - 90%, 97% Average of 93%
Column C - 90%, 94%

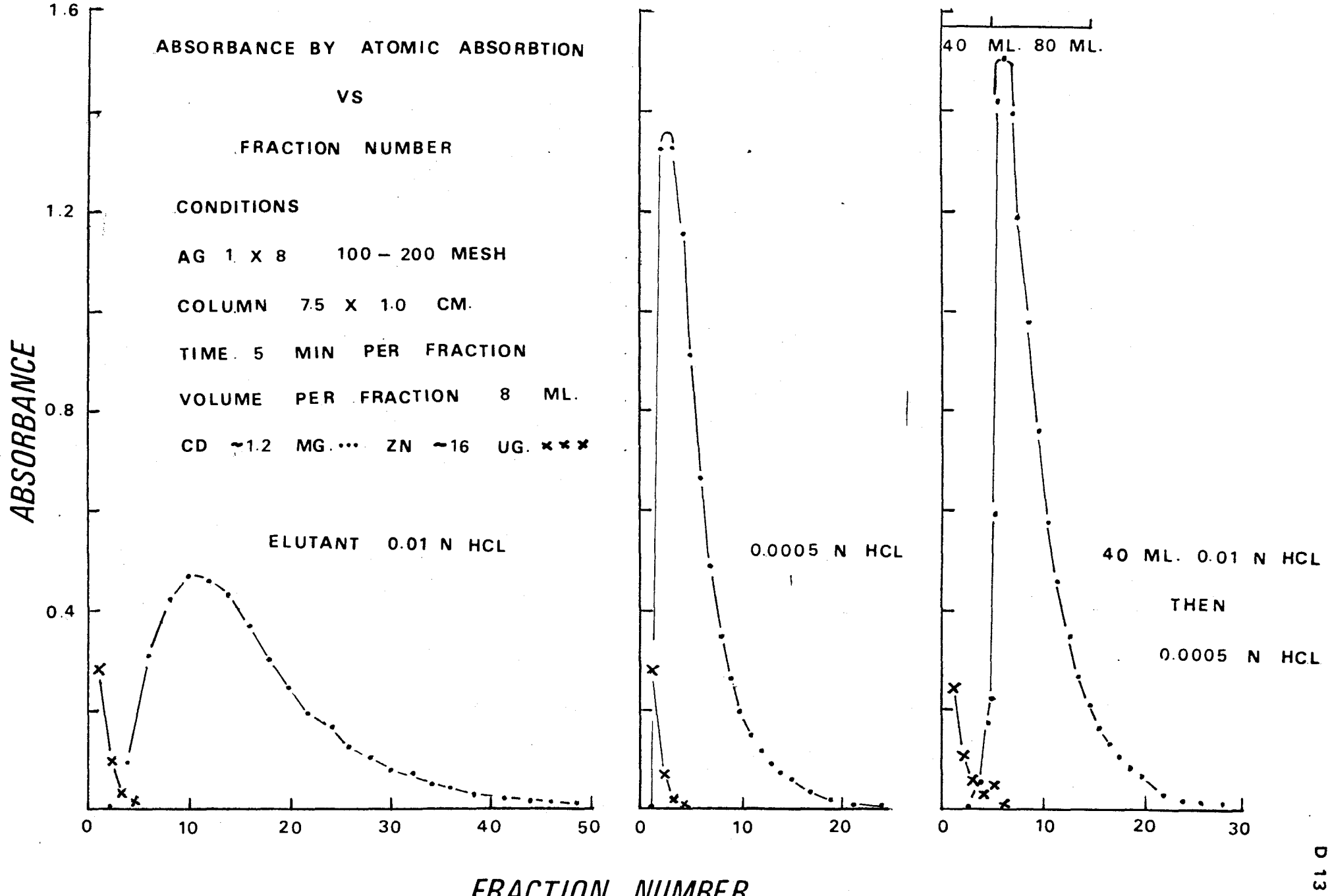
This is slightly higher than Chueca's value of $86.0 \pm 2.1\%$.

Gamma Spectrum - The only foreign peak in the spectrum of a 0.1 gm sample irradiated for 1 day and seven days after irradiation was a small zinc peak (^{65}Zn) at 1115 kev which does not interfere in the region of interest.

Solvent Extraction

Again a complete separation can be preformed using just solvent extraction. Basically the separation scheme is almost identical to some of the various dithizone methods for colourimetric analysis. Lieberman (63) and Das (9) present similar schemes both based on dithizone but differing slightly in the removal of interferences. Liebermann for example, does a preliminary extraction at pH 1-2 to remove gross interferences followed by the main extraction of Zn&Cd at pH 13-14 from a tartrate medium. Both the zinc and the cadmium are then backextracted into 1 N HCl and the zinc is

CADMIUM ELUTION CURVES



D 13

selectively removed by solvent extraction using thiocyanate and isoamyl alcohol. He claims 50-63% chemical yield with no foreign activities in the gamma spectrum. Das uses a similar procedure but uses tartrate and hydroxylamine for the first extraction of cadmium at a high pH and then backextracts into a buffered pH 2 solution. He makes no attempt at a final zinc separation.

Liebermann's method as described (63) was investigated. As claimed there were no foreign gamma lines in the spectrum. Liebermann's value for the recovery of cadmium was 50-65%.

Comment

A comparison of the three methods is shown in Table XIV.

Table XIV - Comparison of Complete Separations

Method	Chem. Yield	Gamma Impurities
Sulfide-Reineckate-Sulfide	66%	strong 511 kev
Anion-Exchange	93%	weak ^{65}Zn
Solvent Extraction	50-65%*	no interference

*Literature value

While solvent extraction with dithizone was perhaps the best method in terms of time of analysis and radiochemical purity, anion-exchange was preferred by the author for two reasons. First of all, manual handling of the sample was minimized, and secondly there was a very high and reproducible recovery of cadmium.

With a count rate of about 25,000 counts/1000 sec. for a 0.1 g. sample of beef-kidney powder containing about 2.5 p.p.m. of cadmium, and a background of about 100-150 counts per channel, one can calculate a detection limit of about 5 ppb. This is 10-20 times

less than partial separation and three orders of magnitude less than non-destructive analysis.

ALTERNATIVE METHODS OF ACTIVATION ANALYSIS

One can preform activation analysis in two other ways. The first of these involves substoichiometric analysis. The general method of substoichiometric analysis does appear to have some promise as a useful tool but unfortunately only a little work has been done on cadmium. The second approach involves the use of alternative nuclear reactions. The great loss in sensitivity by this method is partially compensated by the fact that usual background from the major elements present in the usual biological sample is nearly absent.

(A) Substoichiometric Analysis

Substoichiometric analysis is an interesting variation of both activation analysis and isotope dilution analysis. The theory and scope of the method are well described by Ruzicka and Stary (64).

Substoichiometric extraction of cadmium into dithizone in chloroform has been suggested as a method of cadmium analysis (65) using substoichiometric isotope dilution. This method has been used for the determination of cadmium in biological samples (66) apparently with good reproducibility. Due to the similiarities in separation schemes and sample size the method would appear to be an attractive alternative to the standard dithizone methods.

(B) Prompt (n,γ)

When a nucleus is bombarded with neutrons it emits prompt gamma rays which are characteristic of the element and hence can be used for analysis. Handley and DeCarlo (69) used this method to analyze cadmium in water. They claim a detection limit of about 20 ppm. Cd using a 10 ug ^{252}Cf source immersed in the water.

(C) Photon Activation

Photon activation analysis is an alternative and sometimes complimentary form of activation analysis. Cadmium is one of the elements that can be determined by this method either using the (γ,γ') reaction ($^{111}Cd(\gamma,\gamma')^{111m}Cd$) or a ($\gamma$,n) reaction ($^{106}Cd(\gamma,n)^{105}Cd$). While the analytical detection limits will depend naturally on the strength or intensity of the photon source, it appears that the method is at least several orders of magnitude less sensitive than neutron activation analysis (126, 127). While it is indeed less sensitive than N.A.A., it does have the advantage that Na, one of the major elements present in biological samples, is usually not activated and hence is no longer a serious interference (49, 127). None-the-less with a detection limit of about 10 ug (127) this means that only large samples or those high in cadmium can be analysed.

(D) Charged Particle Activation Analysis

Bankert et al. (70) used 14.7 mev protons in a microampere beam to determine several elements in water. He claims a detection limit of 1.5 ppm for cadmium.

ATOMIC ABSORPTION SPECTROMETRY

Since its introduction by Walsh (71) in the mid 1950's atomic absorption has proven itself to be a powerful and versatile means of analysis. The actual fundamentals, theory and scope of the method can be found in any of a number of good reference texts (72, 73). Because cadmium does not form involative compounds and dissociates readily in even relatively cool flames, it is ideally suited for atomic absorption. In addition the high stability of the cadmium hollow cathode lamp makes for great sensitivity as illustrated in Table XV (73).

Table XV - Atomic Absorption Data for Cadmium

Line	Sensitivity* (ppm)	Analytical Range (p.p.m.)
2288.0	0.03	0.5 - 5
3261.1	20	400 - 4000

*coal gas - air flame, 10 cm slot burner, 1% absorption

Chemical interferences in the analysis for cadmium are thought to be minimal. With the exception of the phenomenon known as molecular absorption (to be discussed later), no common cations interfere. Of the common anions, $B_4O_7^{2-}$, SiO_3^{2-}, CO_3^{2-}, HCO_3^-, $HAsO_4^{2-}$, and PO_4^{3-} have been known to decrease the absorption signal (20, 73, 74). However it has been found that these could be effectively removed by either acidification or by the addition of E.D.T.A. to the sample (74). HCl has been known to give a slight enhancement as well (72).

Most flame combinations can be used for cadmium. The most used and recommended combination is a lean air-acetylene combination. The use

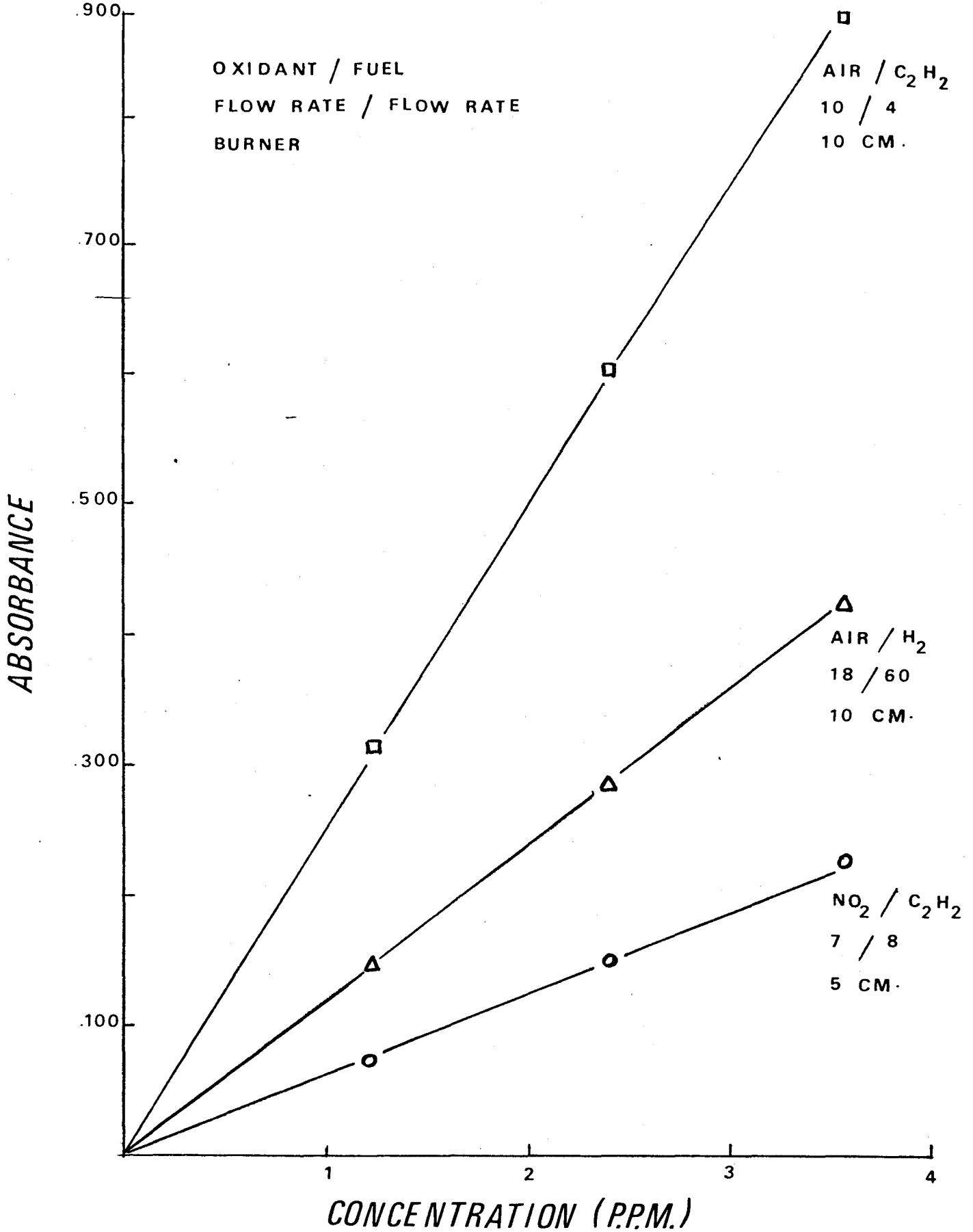
of the air-acetylene flame is due to the inherent high sensitivity of the method since the cooler air-coal gas flame is more sensitive. The air-hydrogen flame is often used as well because of the lower background absorption of the flame itself. Still the air-acetylene flame is recommended probably to reduce any interferences due to its higher temperature. However, the still hotter nitrous-oxide flame gives still lower results. For a comparison of the three flames air-hydrogen, air-acetylene, and nitrous oxide-acetylene see diagram 14.

However even with the high sensitivity of the method, the low concentration of cadmium in most biological materials means that the method often is not suitable for analysis. To overcome this problem two approaches have been taken. The first of these is instrumental modifications which include the use of longer path lengths and non-flame methods. The second of these is chemical preconcentration.

Instrumental Modifications

(1) Increasing the Path Length - Since absorption is a function of the path length as well as concentration, increasing the path length of the flame should increase the detection limit as well. Hence the use of long absorption tubes or Fuwa-Vallee tubes (75) has shown some promise. Pulido (20) has used them to determine cadmium in a variety of biological samples. He claims a detection limit of about 0.2 p.p.b. which is more than a 100-fold increase over the standard 10 cm. slot burner.

ABSORBANCE VS CONCENTRATION FOR DIFFERENT FLAMES



The main advantages of the method lie in the great increase in sensitivity and the use of a smaller sample size through the use of total consumption burners. The main disadvantage is that these long narrow tubes become contaminated easily and must be cleaned frequently. Secondly interferences from molecular absorption are quite serious and must be corrected for.

(2) Non-Flame Techniques - Because of the high sensitivity and small sample size, the use of non-flame techniques for analysis is showing great promise as an analytical tool. Work by L'vov (76) and West (77) among others has led to the design of various types of high temperature furnaces and atom reservoirs, which can be used for practical work. Related techniques such as sampling boats (78) have also shown great promise.

However certain problems with the method have not been completely worked out. Reproducibility and precision are often poor. Background absorption often creates problems in working with biological samples. Finally the high sensitivities (often 10^{-12}g. or less) are somewhat misleading. Because the sample size is often of the order of a few ul, the relative detection limit is often only slightly improved.

None the less, non-flame methods probably will become the most popular form of analysis for cadmium in a few years. A recent paper by Parker et al (123) on environmental samples, demonstrates the ease and convenience of the method. Schramel (89) has used it to determine cadmium in the kale powder.

Chemical Preconcentration

For atomic absorption, chemical preconcentration, almost invariably means solvent extraction although other methods have been used successfully.

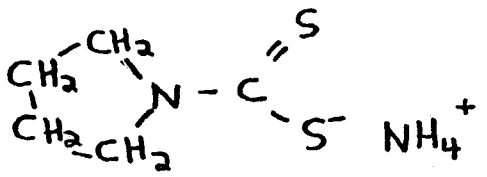
Solvent Extraction

Solvent extraction has become an established method for increasing the detection limits in atomic absorption. The increase in sensitivity is due to a possible concentration effect, and an increase in sensitivity, due to the use of an organic solvent. The relationship between physical properties of an organic solvent and enhancement of signal is still poorly understood. However, the main factor appears to be the lower viscosity which means higher flow rates and a subsequent increase in the concentration of atoms in the flame. Other factors such as boiling point and surface tension may be important as well (73, 79, 80, 81).

Today there is a wide choice of complexing agents and organic solvents to choose from. Most of these systems were originally used to determine cadmium in water. However many of them are readily adaptable to the analysis of cadmium in biological samples. Some of the more common complexing agents and organic solvents are as follows:

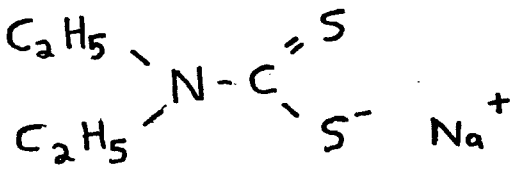
Complexing Agents

A.P.D.C. (ammonium pyrrolidine dithiocarbamate) (73, 82-88). This is widely used and perhaps the most common complexing agent today. It is

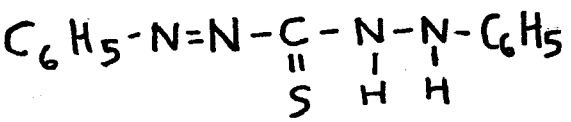


usually prepared fresh daily as a 1-5% aqueous solution and filtered before use since it has a tendency to decompose. It has been used to extract cadmium with chloroform over a pH range of 1-10 (83), and with MIBK over a pH range of 1-5 (84) and 1-6 (85). Other authors suggest it can be used over a pH range of 2-14 (72). However some difficulties have been found in the past (150) with this reagent. This possibly may be due to low quality reagents.

D.E.D.C. (diethyldithiocarbamate, sodium salt) (84, 90). Diethyltdithiocarbamate is again a widely used and common complexing agent. However the recommended pH range is much lower than A.P.D.C. (pH 6.0-7.5).



Dithizone (diphenyl thiocarbazone) (80, 91). A common reagent most often used for the colourimetric determination of cadmium. While Sachdew (91) lists a pH range of 5-9, it probably would be suitable from pH 5-14 (64). Ethyl propionate has been proposed as the organic reagent since the cadmium dithizonate is stable in it (91).



Organic Solvents

Allan (79) showed that for a wide variety of organic solvents that esters and ketones behaved best for atomic absorption work.

While Allan showed that ethyl acetate is the best solvent in terms of enhancement, he pointed out that it is unsatisfactory, because of its relatively high solubility in water. Therefore, he suggests the use of methyl isobutyl ketone (M.I.B.K.) which is the next best. M.I.B.K. has been the most common solvent used for the solvent extraction of cadmium (84-88, 90, 92). It, along with n-butyl acetate (79, 80), was used in this work.

Many other solvents have been suggested (79-81, 91) and are probably satisfactory as well.

Alternative Methods of Concentration

The use of ion exchange or chelating resins also provides a means of concentrating cadmium prior to analysis. Indeed these methods have been used to analyze cadmium in natural waters (93), and waste waters (94), and sea water (95-97). Unfortunately, there are certain disadvantages. First, they are more time consuming. Secondly, in practical work the volume required to strip the cadmium quantitatively from the column is usually greater than desired. However, the fact that some do not bind up sodium and potassium means that one might use them to minimize light scatter in certain samples.

LIGHT SCATTER AND MOLECULAR ABSORPTION

Introduction

If a flame contains appreciable quantities of solid particles that are not vapourized, then these particles can either scatter or absorb the light beam giving rise to false absorption signals (20, 73, 98-104). One of the first to realize this was Willis (98), who found in the direct analysis of urine (using the conventional 10 cm. slot burner), that when the cadmium content was low, there was a tendency to get high results. He explained this by saying solid particles of NaCl in the flame were scattering the light beam. Others (99, 102) have shown the same results with other salts. Billings (102) showed that both $MgCl_2$ and $CaCl_2$ were worse than NaCl. This effect of a false absorption signal becomes a source of serious interference when using the long path absorption tubes (20, 99).

Koirtyohann and Pickett (100, 101) have shown that this interference is mainly due to molecular absorption (sometimes called heterochromatic or background absorption) rather than light scatter.

There are several recommended methods to correct for this error. Slavin (103) and Zook (13) suggest using a nearby non-absorbing line to subtract background. However, Kahn and Manning (104) have pointed out that this leads to a systematic error due to the high slope of the NaCl absorbance curve around 2288 Å. Pulido (20) and

Koirtyohann recommend the use of hydrogen continuum lamps at the same wavelengths. Recently instrumental methods have been devised to substract this background absorption automatically (105).

Finally it should be noted that Billings (102) has pointed out that the usual method of eliminating matrix effects using the method of standard addition does not work in this case because all signals are enhanced.

Experimental

A typical enhancement of the cadmium absorption signal by NaCl and KCl was found in the following manner. A 1 p.p.m. solution of cadmium was prepared in distilled deionized water and in 1, 5, and 10% w/v solutions of either sodium chloride or potassium chloride. The absorbence signals were taken using the conventional air-C_2H_2 flame and the conventional 10 cm. slot burner. The readings were normalized to give 0.100 for the 1 p.p.m. solution in distilled deionized water. The results are shown in Table XVI:

Table XVI - Light Scattering

Absorption of 1 ug/ml soln of Cd.

	0%	1%	5%	10%
NaCl	0.100	0.104	0.118	0.128
KCl	0.100	0.105	0.109	0.111

As shown above there is an enhancement. At high concentrations of salt and/or lower concentrations of cadmium this may become a serious source of error.

EXPERIMENTAL PROCEDURE FOR ATOMIC ABSORPTION ANALYSIS

Samples (1-2 gm) were wet ashed with a mixture of HNO_3 and H_2SO_4. Final traces of organic matter was removed using $HClO_4$. Samples were diluted to about 100 ml and the pH was adjusted with either concentrated NH_4OH or 10 N NaOH. When extraction was done with A.P.D.C. 10 ml. of a 0.05 M solution of potassium hydrogen phthalate was added to provide some buffering.- The pH was adjusted to 4 for A.P.D.C. and 6.5-7.5 for D.E.D.C. Samples were transferred to separatory funnels where 10 ml. of a 1% w/v aqueous solution of the complexing agent was added. The samples were shaken for one minute. Now 10 ml of the organic phase was added and the funnels were shaken for two minutes. The organic phase was separated and used for analysis. In the course of this work it was found that droplets of water in the organic phase often lead to erroneous absorption signals. Therefore, the experimental work done on beef kidney sample #2 and the kale sample had the organic phase centrifuged to remove any water droplets.

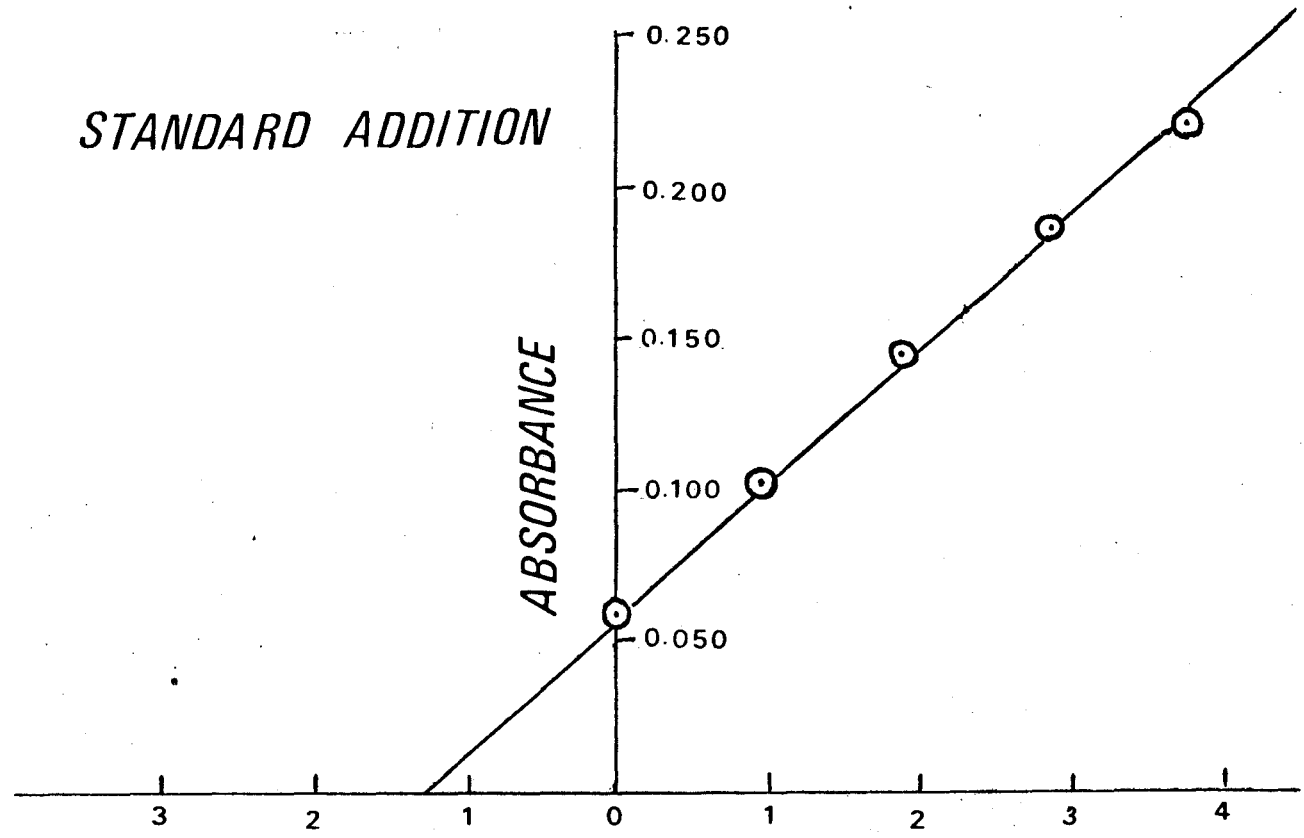
Blanks and standards were treated in an analogous manner.

A typical calibration curve is seen in Diagram 15 ab.

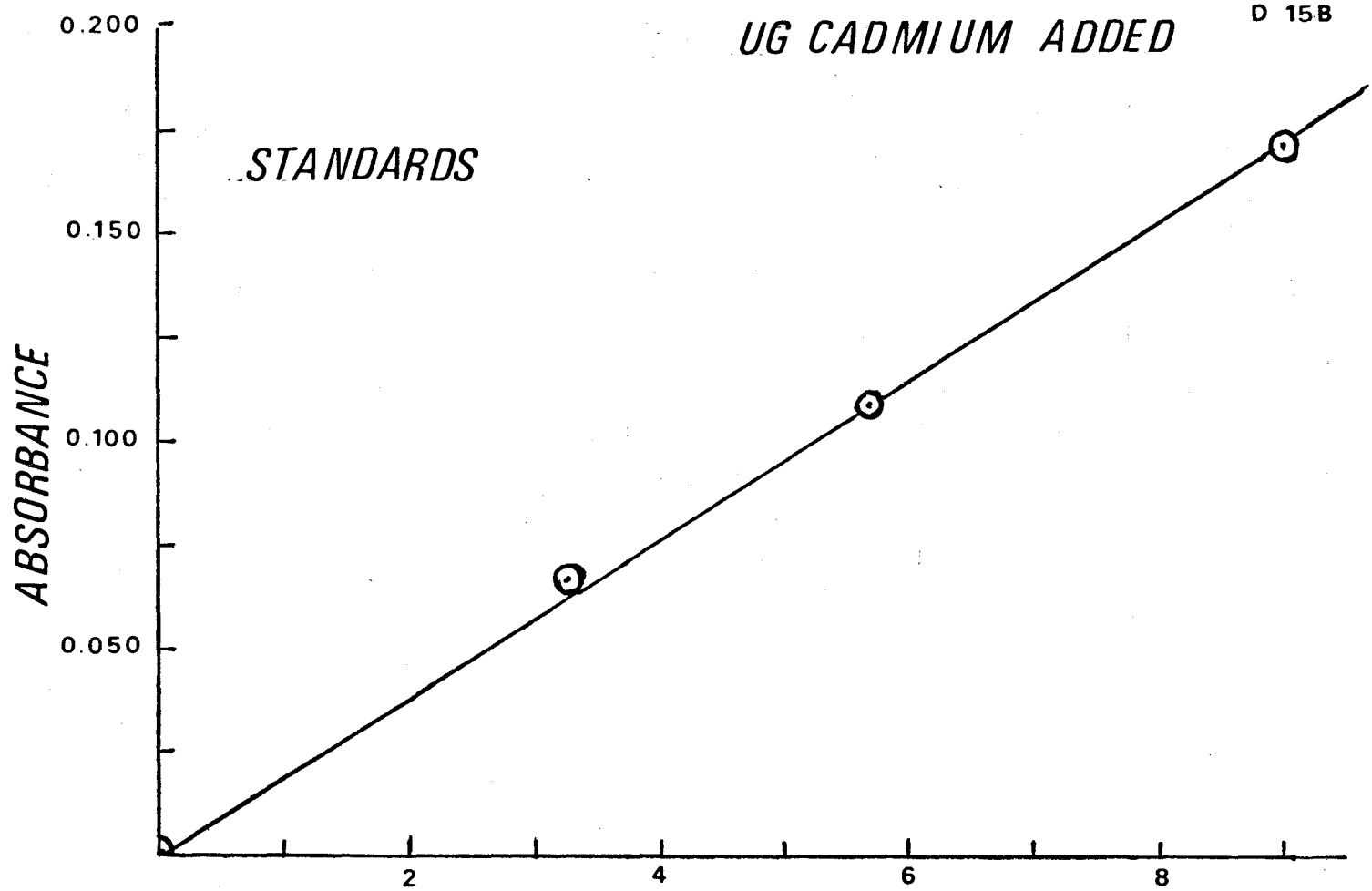
Detection Limit

The detection limit for an aqueous sample is 0.03 p.p.m. (73). Using solvent extraction one gets a 2.9 fold enhancement of signal

TYPICAL CALIBRATION CURVES



D 15B



from M.I.B.K. (81). Combined with a solvent volume of about 5 ml. this means a detection limit of 0.05 ug or a relative detection limit of 50 p.p.b. for a 1 gm. sample. This can be lowered somewhat both by the use of a smaller solvent volume and by the use of a larger sample. However, with the larger sample size, contamination from reagents may place an upper level on the detection limit.

ATOMIC FLUORESCENCE

A great deal of work has been done on the atomic fluorescence analysis of cadmium. Unfortunately most of it deals with instrumentation and detection limits and very little practical work has been done. However, some work has been done on interferences and it indicated the method is quite suitable for analysis. For instance Dagnall, West and Young (106) studied possible interferences from 100 fold excess of 41 cations and 18 anions in the analysis of 10^{-5}M cadmium solutions and found no more than a $\pm$5% interference. Goodfellow (107) found similar but slightly higher interferences using more concentrated solutions. Bratze et al (108) found significant changes in the atomic fluorescence signal with different concentrations of nitric acid and hydrochloric acid. They recommend that solutions and standards be prepared with no excess acid or at least the same concentration of acid to eliminate the error.

ATOMIC EMISSION

Gilbert (109) did a comparison of various flames using total consumption burners. The most sensitive and recommended method was with a detection limit of 0.5 p.p.m. at 326.1 mu. While the same detection limit could be reached using an oxygen-cyanogen flame and

the 228.8 m line, the 326.1 m line is preferred due to strong self-absorption in the case of the 228.8 line which leads to a parabolic working curve.

Some manufacturers have recommended the use of a 10 cm. slot burner along with an air-acetylene flame. However the detection limit is much higher in this case.

COLOURIMETRY

There are many articles in the chemical literature concerning the determination of cadmium in biological samples using some form of colourimetry usually dithizone (11, 16, 36, 110-112). These papers mainly differ in their proposed removal of possible interferences such as Cu, Ag, Ni, Co, Zn, Tl, and Bi which also coextract with cadmium over a wide pH range (36). Some of these employ the observation made by Fisher and Leopoldi (11) that cadmium can be extracted with dithizone from an approximately 5% NaOH solution.

Shirley (36) removes the interferences by prior extraction at lower pH's with dithizone and dimethylglyoxime. Sandell (11) recommends the use of di-β - naphthylthiocarbamate and dithizone in a series of extractions. However the recommended procedure (16, 112) is that of Saltzman (110). The novel feature of his method is extraction of the cadmium dithizonate from a strongly basic solution containing cyanide. The cadmium is backextracted into a weakly acidic solution and then re-extracted by dithizone-carbon tetrachloride from a strongly basic cyanide medium. Smith's method (111) is similar to Saltzman's but with a few modifications in reagents.

However, results by these multi-extraction methods often tend to be low and somewhat erratic (11). For instance in 1961 H.A. Schroeder (12) determined cadmium in over 100 naturally occurring substances. But then in 1967 he repeats all his analysis by atomic

absorption (15) because the former results were too low. Other investigators have found the same thing (13, 14), that colourimetry gives results that are lower when compared to other methods, especially atomic absorption.

EMISSION SPECTROPHOTOMETRY

Emission spectrophotometry played an important role in the study of cadmium distribution in human tissues. While in 1941 it was first reported in bovine kidney, it wasn't until 1953 that the high relative concentration of cadmium in human kidney and liver was realized (115). Over the next ten years a series of multielement surveys (115-119) on various human tissues showed the normal cadmium concentration both as a function of age and geographical origin (120) and as a factor in hypertension (68).

However, as an analytical method it lacks a great deal of sensitivity. The usual procedure is to dry ash the sample, mix the resulting ash, along with an indium standard, with graphite and run a D.C. arc using cathode excitation. The standards were prepared using a synthetic ash (120). The analytical lines used were cadmium 3216-indium 3039 with a practical working range of 50-10000 p.p.m. (117).

There have been some attempts to increase the sensitivity of the method. One of these involves the use of solvent extraction to first preconcentrate the cadmium (121). In this case different analytical lines were used being cadmium 2288-aluminum 2367.1. Nusbaum et al. (122) tried to use an instrumental modification to increase the

sensitivity. They photoelectrically measured the emission this time using the cadmium 3261.1-bismuth 2938 line but in the second order spectrum. Again for standards, a spectroscopic buffer solution was used. However, while the method may be analytical useful, their results are several orders of magnitude higher than other values given in the literature (see appendix 2).

POLAROGRAPHY

A variety of polarographic methods can be used to determine cadmium in biological samples. Basically there have been two popular approaches. The first of these involves regular polarography after a solvent extraction or preconcentration step (16) and the second approach is anodic stripping voltammetry (57, 124, 125).

While regular polarography can be used the range of analysis, 1-10 ug, is relatively high. Hence it is best suited either to large sample sizes or samples high in cadmium content.

Arodic Stripping Voltammetry is much more sensitive than regular polarography. Separation from interfering ions can be accomplished by choice of medium and potential (57, 124) or by a prior dithizone separation (125). While authors disagree about the choice of experimental conditions certain points are common in their procedures. The final volume of solution is about 10 ml. and the range of analysis is from 10^{-8} - 10^{-5}M in cadmium. This represents a detection limit of 0.01 ug. That detection limit compares well with other methods. Unfortunately electrochemical methods have never become a popular form of analysis.

INTERCOMPARISON OF RESULTS

With one exception, all the neutron activation analysis was done following the modified anion-exchange method of Chueca as previously described. The sample size for the beef kidney sample was typically 0.1-0.2 g. For the kale it was higher from 0.2-0.4 g. Quantitative results were based on comparison with standards that were co-irradiated in the same irradiation can as the sample. Chemical yields for the beef kidney samples were determined by titrating the remains of the cadmium carrier ($\sim$1 mg.) with E.D.T.A. using Xylenol Orange. The chemical yields were high and reasonably reproducible varying from 90-100%.

The chemical yields for the kale samples were determined by atomic absorption after a cooling period of one month followed by dilution to 100 ml. The chemical yields for the kale samples were quite erratic. This was believed due to the fact that when the dissolved samples were taken to dryness, a white residue remained which did not dissolve in the 2 N HCl.

Errors in the results were determined from the following formula:

$$\frac{\Delta \text{ result}}{\text{result}} = \left(\frac{\sqrt{\text{no of sample counts}}}{\text{no of sample counts}} + \frac{\sqrt{\text{no of standard counts}}}{\text{no of standard counts}} + \frac{\Delta \text{ chemical yield}}{\text{chemical yield}}\right)$$

The atomic absorption analysis was preformed using solvent extraction of the cadmium with either A.P.D.C. or D.E.D.C. as the complexing agent and M.I.B.K. or n-butyl acetate as the organic phase, as previously described. Quantitative results were based on either comparison with standards or by standard addition.

Errors in the results were calculated from the calibration curve.

BEEF KIDNEY STANDARD #1 RESULTS OF ANALYSIS

Atomic Absorption Analysis

Run	Method	Complexing Agent	pH	Solvent	Results (p.p.m.)
1	Standards	DEDC	6.0-7.5	n-Bu-Ac	4.7 ± 0.1
2	Standards	DEDC	6.0-7.5	n-Bu-Ac	4.7 ± 0.1
	"	"	"	"	4.6 ± 0.1
3	Standards	DEDC	6.0-7.5	n-Bu-Ac	5.1 ± 0.1
	"	"	"	"	5.2 ± 0.1
4	Standards	DEDC	6.0-7.5	N-Bu-Ac	4.8 ± 0.2
	"	"	"	"	30*
5	Standards	APDC	3.5-4.0	MIKB	5.0 ± 0.1
	"	"	"	"	5.1 ± 0.1
	"	"	"	"	5.1 ± 0.2

*not included in average
Mean = 4.92
Standard Deviation = 0.22

BEEF KIDNEY STANDARD #1

Neutron Activation Analysis

Run	Isotope	Separation	Results (p.p.m.)
1	^{115}Cd-^{115m}In	Reineckate precipitate + Anion Exchange	4.2 ± 0.3
2	^{115}Cd-^{115m}In	Anion Exchange	4.1 ± 0.2
	"	"	5.2 ± 0.2
	"	"	4.7 ± 0.2
3	^{115}Cd-^{115m}In	Anion Exchange	5.9 ± 0.2
	"	"	4.8 ± 0.2
	"	"	5.8 ± 0.2
4	^{115}Cd-^{115m}In	Anion Exchange	5.6 ± 0.2
	"	"	5.5 ± 0.2
	"	"	5.5 ± 0.2

Mean = 5.13
Standard Deviation = 0.65

BEEF KIDNEY STANDARD #2

Atomic Absorption Analysis

Run	Method	Complexing Agent	pH	Solvent	Results (p.p.m.)
1	Standards	DEDC	6.0-7.5	n-Bu-Ac	2.2 ± 0.1
	"	"	"	"	2.2 ± 0.1
2	Standards	DEDC	6.0-7.5	n-Bu-Ac	3.0 ± 0.2
	"	"	"	"	2.7 ± 0.2
	"	"	"	"	2.6 ± 0.2
3	Standards	DEDC	6.0-7.5	MIBK	2.2 ± 0.1
	"	"	"	"	2.2 ± 0.1
	"	"	"	"	2.2 ± 0.1
4	Standards	DEDC	6.0-7.5	MIBK	2.3 ± 0.1
	"	"	"	"	2.3 ± 0.1
	"	"	"	"	2.2 ± 0.3
5	Standard Addition	DEDC	6.0-7.5	MIBK	2.5 ± 0.1
6	Standard Addition	DEDC	6.0-7.5	MIBK	2.7 ± 0.1

Mean = 2.42
Standard Deviation = 0.26

BEEF KIDNEY STANDARD #2

Neutron Activation Analysis

Run	Isotope		Separation	Results (p.p.m.)
1	^{115}Cd-	^{115m}In	Anion Exchange	2.5 ± 0.2
	"		"	2.5 ± 0.2
	"		"	2.7 ± 0.2
2	^{115}Cd-	^{115m}In	Anion Exchange	2.2 ± 0.2
	"		"	2.4 ± 0.2
	"		"	2.4 ± 0.2
3	^{115}Cd-	^{115m}In	Anion Exchange	2.4 ± 0.1
	"		"	2.4 ± 0.1
	"		"	2.5 ± 0.1
4	^{115}Cd-	^{115m}In	Anion Exchange	2.6 ± 0.1
	"		"	2.8 ± 0.1
	"		"	2.3 ± 0.1

Mean = 2.48
Standard Deviation = 0.17

KALE RESULTS OF ANALYSIS

Neutron Activation Analysis

Run	Sample Size	Separation	Chemical Yield	Conc (p.p.m.)
1	0.3256	Anion Exchange	49.5	0.42 ± 0.03
	0.2984	"	32.0	0.49 ± 0.07
	0.3197	"	83.0	0.43 ± 0.06
	0.3494	"	21.0	0.37 ± 0.04
	0.2933	"	45.0	0.42 ± 0.04
2	0.3476	Anion Exchange	11.0	1.03 ± 0.13
	0.3233	"	63.0	0.90 ± 0.06
	0.3867	"	4.4	1.33 ± 0.07*
3	0.2439	Anion Exchange	66.9	0.96 ± 0.03
	0.3278	"	82.5	1.03 ± 0.04
	0.3113	"	32.1	2.41 ± 0.07*+
4	0.2907	Anion Exchange	54.7	0.91 ± 0.04
	0.3175	"	74.7	1.73 ± 0.06*+
	0.2775	"	85.6	0.93 ± 0.03
	0.2705	"	53.9	0.89 ± 0.05
	0.3032	"	60.8	0.91 ± 0.05
	0.3239	"	40.8	0.90 ± 0.05

*not included in interpretation A

+not included in interpretation B

Two conclusions are possible.

A) Homogenuity Conclusion-The concentration of cadmium in kale is 0.94 ± 0.05 p.p.m. This conclusion excludes Run-1 and three other outlying results which are not within three standard deviations of the other nine results.

B) Inhomogenuity or Poor Precision and Accuracy Conclusion. The concentration of cadmium in kale is 0.79 ± 0.30 p.p.m. This includes all results except for two outlying results which are not within three standard deviations of the other fifteen results.

KALE RESULTS OF ANALYSIS

Run	Sample(g)	Method	Complexing Agent	pH	Solvent	Results (ppm)
1	2.0581	Standards	A.P.D.C.	3.5-4.0	MIBK	0.61 ± 0.03
2	2.1262	Standards	A.P.D.C.	3.5-4.0	MIBK	0.81 ± 0.05
	1.9169	"	"	"	"	0.67 ± 0.05

Mean = 0.70 p.p.m.
Standard Deviation = $\pm$0.10 p.p.m.

OVERALL KALE RESULTS (For inhomogenuity or Poor Precision and Accuracy conclusion)

18 Analysis Conc. 0.78 Standard Deviation = $\pm$0.29 p.p.m.

The results show good agreement between Neutron Activation Analysis and Atomic Absorption Analysis as summarized in Table XVII.

Table XVII - Results of Analysis

Method	Sample	B.K.S. #1 (P.P.M.)	B.K.S. #2 (P.P.M.)	Kale (P.P.M.)
Atomic Absorption		4.92 ± 0.22	2.42 ± 0.26	0.70 ± 0.10
Neutron Activation Analysis		5.13 ± 0.65	2.48 ± 0.17	0.94 ± 0.05 A
				0.79 ± 0.30 B

CONCLUSION

This work has dealt with a general review of the analysis for cadmium in biological samples as well as a detailed investigation into two methods of analysis, Neutron Activation Analysis and Atomic Absorption Analysis. In the course of this work some general points have been made and some differences have been investigated and hopefully resolved. It is now important to review some of these points.

Perhaps the first point is that in biological materials cadmium appears to be ubiquitous and ranges in concentration from the low p.p.b. to the low p.p.m. range. Most instrumental methods are sensitive enough to work in this range or can be used after a suitable preconcentration step.

In most cases it is necessary to destroy the organic matrix before analysis can be preformed. This can be done either by wet or dry ashing. While it is unanimously agreed that wet ashing is satisfactory, there exists quite divided opinion over the merits of dry ashing. Tracer studies in this work have shown no losses of cadmium when present as the nitrate at temperatures up to 600^{o}C. However this is in marked contrast to the results of other authors. A possible explanation for this may lie in the chemical form of the tracer used. Unfortunately most authors neglect to give it so it is not possible to draw

any definite conclusions. However those who have used both wet and dry ashing at 500°C have found little if any difference in the results. Hence one probably can say that dry ashing the sample at 500°C would be satisfactory for most analysis.

Regarding the use of Neutron Activation Analysis for analysis of the sample, this work has cleared up a few of the difficulties. The possible use of ^{111m}Cd either for non-destructive analysis or analysis following separation was found unsatisfactory for practical samples because the detection limit is too high. Hence ^{115}Cd is the isotope of choice.

Regarding the $^{115}Cd/^{115m}In$ equilibrium, a wait of 33 hours is necessary for the equilibrium to be 99% complete. As well the maximum count rate if needed is obtained 17.6 hours after irradiation or separation.

Non-Destructive Analysis can be used to determine cadmium in biological materials. Unfortunately the detection limit is quite high, around 10 p.p.m. As well counting must be delayed at least six days after irradiation in order to let the high background (mainly ^{24}Na, ^{82}Br, ^{42}K) die away.

Partial separation, either by solvent extraction or precipitation, appears to be a satisfactory if relatively unused method of analysis. With a detection limit of about 50-100 p.p.b., it might yet find practical applications either in monitoring samples or as part of a multielement scheme. It's major disadvantage compared to complete separation is a lower count rate due to a poorer counting geometry and less efficient detector.

Complete separation remains the best method of analysis. The choice of method is up to the analyst. Solvent extraction is perhaps the quickest method but requires the greatest manual handling of the radioactive sample. Anion exchange on the other hand is perhaps the slowest method but requires the least handling of the sample. If one counts the ^{115m}In photo-peak, speed of analysis is not too important because one must wait for the transient equilibrium to be reached. The detection limit is about 5 p.p.b.

Now one must return to the original problem of what is wrong with the kale analysis in Table-I. To answer this question one turns to the results of this work on the kale analysis. However when one examines the N.A.A. results one sees an apparent difference between Run-1 and Runs-2,3,4. Before proceeding any further one must first check to see if this apparent difference may possibly be accounted for by the reproducibility available by N.A.A., by inexperience on the part of the operator, or by a gross error in procedure.

Now let us examine in detail the possibilities of errors in the N.A.A. method. The activity of the sample after irradiation, cooling and separation is given by the following equation:

$$A = \frac{N.F.O.\ o\ .S.D.}{Y} \qquad (1)$$

Where

N = number of cadmium atoms. This is equal to $\frac{\text{wt.}}{112.41}.6.02\times10^{23}$

F = fractional abundance of the isotope of interest.

O = neutron flux in neutrons $cm.^{-2}sec^{-1}$.

o = atomic cross section expressed in barnes ($10^{-24}cm.^2$)

S = represents the saturation or buildup factor which is $(1-e^{-+1}$ where $+_1$ is the irradiation time

D = represents the decay factor after irradiation and is given by e^{-+1} where $+_2$ is the time from irradiation to counting.

Y = chemical yield after separation or handling.

Using the above equation it is possible to determine the number of cadmium atoms solely from the activity of the sample if the other parameters are known. This is rarely done because of difficulties in accurately measuring the flux and uncertainties in o. Hence standards which are coirradiated in the same irradiation can are used for comparison. If one rearranges equation-1 and makes some substitutions then the weight of cadmium in the sample can be expressed as;

$$\text{wt.} = \text{wt.}\ \frac{(A)}{(A')} \cdot \frac{(O')}{(O)} \cdot \frac{(D')}{(D)} \cdot \frac{(Y)}{(Y')}$$

where ' refers to the standard.

Now it is necessary to examine each parameter in turn to see how each might contribute to an overall error or to poor precision. 1) wt.' - The cadmium standards were measured into quartz vials using a Hamilton syringe and then were taken down to dryness before irradiation. The original solutions were prepared from high purity cadmium shot using an analytical balance and volumetric equipment. The largest source of error there would lie in the accuracy and reproducibility offered by the syringe. The estimated error here is about 1-2%.

2) $\frac{(A)}{(A')}$ - There are three potential sources of error here in this term.

First there is an error in the counting statistics themselves. The count rate for the kale samples was about 4,000-10,000 counts/1000 sec. One expects an error of a few percent from counting statistics.

The second possible error lies in differences in count rate. Large differences in count rate can cause serious errors especially at high count rates due to detector dead time losses. However in this work, the count rates were comparable and low enough that this error is probably negligible.

The third possible error lies in differences in counting geometry. The samples were all counted in a NaI(Tl) well detector. Errors in geometry should be small but possibly are on the order of a few percent.

The overall error in this term may be at worst 5-10% for the kale samples.

3) $\frac{(O)}{(O')}$ - There is a small flux inhomogenuity within the irradiation can. This is only about 1-2%. (114)

4) $\frac{(D')}{(D)}$ - Since the sample and standard are not counted at the

same time there is a potential error here. However since the time difference is small compared to the halflife and because corrections are easily made for this than this error is negligible.

5) $\frac{(Y)}{(Y')}$ - The chemical yield for the sample was determined by atomic absorption. Possible error 5%. No chemical yield was determined for the standard. A possible 1% loss in handling seems reasonable.

The overall worst reproducibility from the above factors is only 10-20%. Hence the difference between Run-1 and Runs-2, 3,4 cannot be blamed on the method.

The second explanation for these poor results could be inexperience on the part of the operator. In work of this nature (i.e. a Master of Science Thesis) this always remains a possibility. However if one examines the results of the analysis on the beef kidney samples, one notices an improvement with time. While the agreement between N.A.A. and Atomic Absorption is satisfactory for Beef Kidney Sample-1, there is a great deal of improvement in agreement between the two methods for Beef Kidney Sample-2. This improvement merely reflects a growing expertise on the part of the operator. Hence one would not expect a third and final sample set to show the worst agreement.

There remains the possibility of a gross error in Run-1 as well. Since the results of Run-1 are about one half of the other results by Runs-2,3,4 an error in the preparation of standards (such as an error in dilution) could account for the

difference. While this type of error is unlikely, it still remains a possibility. Having decided that there is nothing wrong with either the N.A.A. or the operator, then one can draw two differenct conclusions from the results of this work. The first of these is A) Homogenuity Conclusion which maintains that the cadmium concentration is 0.94 ± 0.05 p.p.m. The second one of these is the B) Inhomogenuity or Poor Precision and Accuracy Conclusion which maintains that the cadmium concentration is 0.78 ± 0.29 p.p.m. Now to examine each of these interpretations in turn.

A) Homogenuity Conclusion

Since kale has been recommended as a biological standard and since this work deals with a reasonable small "bulk" sample, one can probably assume that the sample is homogeneous. Hence the results of Run-1, of the N.A.A. on the kale must contain some form of gross error. After the outlying results have been rejected, one gets 0.94 ± 0.05 for nine results. This is in excellent agreement with two other results from Table-I; 0.89 ± 0.16 for ten results and 0.91 for six results.

Now there does exist a difference between the N.A.A. and the Atomic Absorption results for the kale samples. However this difference may be misleading for the following reasons. First of all the number of analysis by atomic absorption is low (3) and possibly unreliable. More results by atomic absorption might give a better agreement. Secondly there is a possibility of a chemical interference in the kale results. This is based on the fact that the chemical yield for the N.A.A. was per-

sistently low and erratic for the kale samples. Since the atomic absorption results were done using standards for comparison, this error would pass undetected. A second method such as standard addition might give better results. Because the Atomic Absorption Analysis results are suspect they are not included in the mean.

As well low results in the literature could be due to gross errors in technique such as using solid cadmium metal as a standard (appreciable self-shielding) or counting the ^{115m}In photopeak at 336 Kev before allowing the transient equilibrium to grow in.

B) Inhomogenuity or Poor Precision and Accuracy Conclusion

There are several strong arguments supporting the inclusion of Run-1 of the N.A.A. The first argument concerns the close agreement between N.A.A. and Atomic Aborption Analysis which has been shown in Beef Kidney Samples-1 and -2. Hence one would expect the same close agreement with the kale analysis which one did obtain if one includes Run-1. In all three cases the two methods are within one standard deviation of each other. In the Homogenuity Conclusion they are not. Furthermore one can run a Student t-test on the results by the two methods to find the probability that the difference between methods is due to random error using the formula (145):

$$+ = \frac{X_a - X_b}{\sqrt{\frac{(N_a + N_b)}{(N_a N_b)} \frac{(N_a S_a^2 + N_b S_b^2)}{(N_a + N_b - 2)}}}$$

where

X = the average

N = the number of analysis

S = the standard deviation

Only in the case of the Homogenuity Conclusion, is it statistically highly significant, that the difference between results by the methods cannot be explained by random error, i.e. there is a difference between methods.

Now if one re-examines the literature and looks at the overall average concentration, one finds excellent agreement between the literature results and this conclusion as shown in Table XVIII.

Table XVIII Comparison of Overall Results

	Number of Analysis	Number of groups	Average Concentration
Literature	62	10	0.766
This Work	18	1	0.778
Overall	80	10	0.768

If this overall agreement isn't good enough, there is a similar excellent agreement between methods as shown in Table-XIX.

Table-XIX Comparison of Overall Results by Method

Method	Number of groups	Literature Number of Analysis	Average Concentration	This Work Number of Analysis	This Work Average Conc.
N.A.A.	7	36	0.76	15	0.79
Polar	2	16	0.80	--	----
At. Abs.	1	10	0.75	3	0.70

This breakdown further confirms the previous agreement and now shows agreement between not two but three different analytical methods. As well at this point it should be mentioned that Sinko et al's (57) polarographic method used both standards (0.75 for six analysis) and standard addition (0.72 for six analysis).

If one assumes conclusion B) to be correct, then one would say that the average concentration of cadmium in kale is 0.77 p.p.m. However it is difficult to explain the wide differences between individual runs, especially when one considers the high degree of precision within each run. But upon careful re-examination of the literature one is drawn to either or both of the following conclusions. The first possible conclusion is that the distribution of cadmium in the kale is not homogeneous. A degree of inhomogenuity, possibly combined with a sampling error due to the typically small size of the sample (usually 100 mg.) would explain the scatter in results. A possible explanation for the inhomogenuity may lie in the basic preparation of the kale powder. Zook (13) has shown that in the milling of grains one did get an enrichment of cadmium in certain of the products. The second possible conclusion is that the precision of trace analysis is poor at the p.p.m. level. Consequently the results of a single set of data may only be reliable within a factor of two.

The concept that there may be inhomogenuities with regard to certain of the trace metals in the kale has been mentioned several times in the literature. Bowen himself (1) says that

certain high values for Al and Ti may be due to contamination from dust. Fritze and Robertson (146) analyzed Al in kale in more detail. They advanced the hypothesis that the majority of Al in the kale was present as a separate phase (such as mineral dust) rather than in the actual tissue. Using N.A.A., Nadkarni and Ehmann (56) looked at fifteen elements in kale by non-destructive analysis and two more elements after chemical separation. For one of these elements, Zr, they concluded, because of their total lack of precision in their results for that element, that the distribution of Zr was inhomogeneous. Furthermore they also postulated that the high relative standard deviation for certain elements (seven elements La, As, Ag, Co, Cr, Sb and Hg had a relative standard deviation of greater than 15%) "may reflect a nonhomogeneous distribution of the element in kale rather than just analytical error" (56).

Careful re-examination of Bowen's work (1) supports this hypothesis. First of all there are fourteen elements with a relative standard deviation of 20% or greater as shown in Table XX.

Table XX Results of Analysis on Certain Elements in Bowen's Kale(1)

Element	Number of Labs	Number of Analysis	Methods	Results(p.p.m.)	R.S.D.
Ag	1	3	1	0.50 ± 0.10	20%
Al*	4	16	1	35.5 ± 7.3	21%
As*	3	16	1	0.127 ± 0.029	29%
Au	2	7	1	0.00222 ± 0.00055	25%
Cl	4	21	2	3330 ± 1060	31%
Cr	4	13	3	0.331 ± 0.155	47%
Ga	2	6	1	0.045 ± 0.020	44%
I	3	12	1	0.0800 ± 0.0234	29%
Mo	9	44	2	2.33 ± 0.47	20%
Na**	9	52	3	2594 ± 617	24%
Ni*	1	4	1	2.56 ± 1.56	61%
Pb	4	21	3	3.21 ± 1.61	50%
Sn	1	4	1	0.160 ± 0.037	23%
W	2	8	1	0.0605 ± 0.00123	20%

*OVERALL RESULTS INCONSISTENT - This may represent only a fraction of the total analysis and may be misleading.

**SIGNIFICANT DIFFERENCE BETWEEN TECHNIQUES - Hence overall results may be misleading.

Table XXI R.S.D. of Various Elements in Kale in Difference Concentration Ranges

Relative Standard Deviation	Concentration Ranges (p.p.m.) 0.1	0.1 - 1.0	1.0 - 10	10 - 100	100
0 10		Se		B, Br, Mn	Ca,K,Mg, N,P,Si
=10 20	Co,Cs,La, Sb,Sc		Ba, Cd, Cu,F	Rb,Sr, Zn	S, Fe
20	Au,Ga,I, W	Ag,As* Cr,Sn	Mo, Ni*, Pb	Al*	Cl,Na**

*OVERALL RESULTS INCONSISTENT - This may represent only a fraction of the total analysis and may be misleading.

**SIGNIFICANT DIFFERENCES BETWEEN TECHNIQUES - Hence overall results may be misleading.

Secondly as shown in Table XXI, twenty out of twenty-one elements, having a concentration of 10 p.p.m. or less, have a precision of greater than 10%. Indeed eleven out of the twenty-one have a precision of 20% or more. This poor precision for the majority of elements may be taken as indicative of possible non-homogenuity in the sample with regards to the trace elements.

Now if the distribution of cadmium in the kale is slightly inhomogeneous, then one would expect that increase in sample size or number of analysis would tend to level out inhomogenuities. Indeed this was found. The individual atomic absorption results with the larger sample size were closer to the mean than most.

of the individual N.A.A. results. As well the three groups which did the most number of analysis by N.A.A. are all within one standard deviation of the average. (Sinko et al, 12 results, $0.73 \pm 10\%$ (57), Schramel, 10 results 0.75 ± 0.18 (89); this work, 15 results, 0.79 ± 0.30; Chueca et al, 10 results, 0.89 ± 0.16 (8)).

Now the second interpretation that one may reach is that there is a problem in the analysis. This problem is that the ~~preeision~~ and accuracy of analysis is poor at the p.p.m. level of concentration. Consequently the results of a single set of data may only be reliable within a small factor of two or so. This conclusion is based on earlier work done by Cook, Crespi, and Minczewski (147). They sent out a common sample, an aqueous solution containing approximately 5 p.p.m. of Cu and Mn, and 15 p.p.m. of Cr and Hg, to nine different laboratories to be analyzed by four different techniques, spectrography, spectrophotometry, polarography, and N.A.A. Conditions were chosen such that interferences from the matrix would be minimal and any variation in results could largely be attributed to the technique used. The results of their survey was quite interesting.

As shown in Table XXII, the overall average of all the results was quite accurate. However as shown in Table XXII and XXIII the precision and accuracy of individual runs was quite poor. To quote the original paper. "The statistical analysis of the results (551 in all) shows, with the limitations as to generality imposed by the design of the experiment, that the expected precisions of a single determination in the routine

application of each of the techniques are: spectrogrpahy ± 40%; polarography ± 25%; activation ± 20% and spectrophotometry ± 10% at the 0.05 probability level.

Table XXII Summary of Overall Results by Cook et al (147)

Technique	Number of Determinations	Average (Normalized to 100)	Standard Deviation of Single Determination at 95% Confidence Limit
Spectrography	124	99.8	37.8
Spectophotometry	172	100.8	9.8
Polarography	116	102.0	26.1
Activation	139	102.5	20.4
All Methods	551	101.3	24.3

Table XXIII Range of Normalized Values of Cook et al (147)

Element	Spectrography	Spectrophotometry	Polarography	Activation	Overall
Cu	71.1-134.1	99.1-103.1	95.1-105.7	54.8-129.0	54.8-134.1
Cr	84.0-104.2	93.1-102.9	90.0-103.0	91.4-101.8	84.0-104.2
Mn	58.8-113.4	96.0-114.8	94.1-141.1	96.4-118.4	58.8-141.1
Hg	47.4-105.2	89.3-106.2	84.6-99.2	95.1-122.4	47.4-122.4

The accuracy follows the same pattern though the spread of results within a technique is wider. When all results are considered together the precision is ± 25% (147).

If one assumes that the precision at the 0.05 probability level is equal to ± 2 R.S.D. then one would say that the overall precision is about 10 - 15% at this concentration level for this

matrix. Now if this is the precision possible at this concentration level then one might explain the results of Table XXI in terms of precision rather than inhomogenuity. i.e. The reason that twenty out of twenty-one elements, with concentrations in the kale of 10 ppm or less, have precisions equal to or greater than 10% is because this is the inherent precision possible at this level. Levels of precision greater than 20% may be due to certain other factors such as lower concentration ranges involved or matrix interferences.

As well Cook et al go on to say the following about differences between laboratories. "This means that differences of up to 80% in spectrography, 50% in polarography, 40% in activation, and 20% in spectrophotometry between single determinations in two laboratories should be considered statistically possible, although the probability of the two extremes being obtained in one particular case is very small. "(147)

Finally there are two other points which help to support the Inhomogenuity or Problems in Analysis Theory. First, results of analysis on standard materials nearly always show an improvement with time as methods become more refined. However there has been no change in the cadmium content with time. In 1969 Bowen (2) published a summary of results of analysis on the kale. Assuming a one year delay in publication, one can probably say that the results represent most of the work up to and possibly including 1968. These results are shown in Table XXIV. There has been five other reports of the concentration of cadmium in kale, four of these since 1968. These results are also shown in Table XXIV.

What is interesting is that there has been essentially no change in the overall average with time. This is contrary to what one would expect if one assumes that the original poor agreement was due to some error in analysis.

Table XXIV Results of Kale Analysis for Cadmium at Different Times

Bowen's List (1969 or before)			Others	
Number of Analysis	Average Concentration (p.p.m.)	Year	Number of Analysis	Average Concentration (p.p.m.)
4	0.38	1967	3	0.63
5	0.63	1969	10	0.89
6	0.91	1971	4	0.50
4	1.0	1971	4	1.06
		1972	12	0.73
		1975	10	0.75
Overall Average	0.74			0.78

This lack of improvement with time may be indicative of sample inhomogenuity.

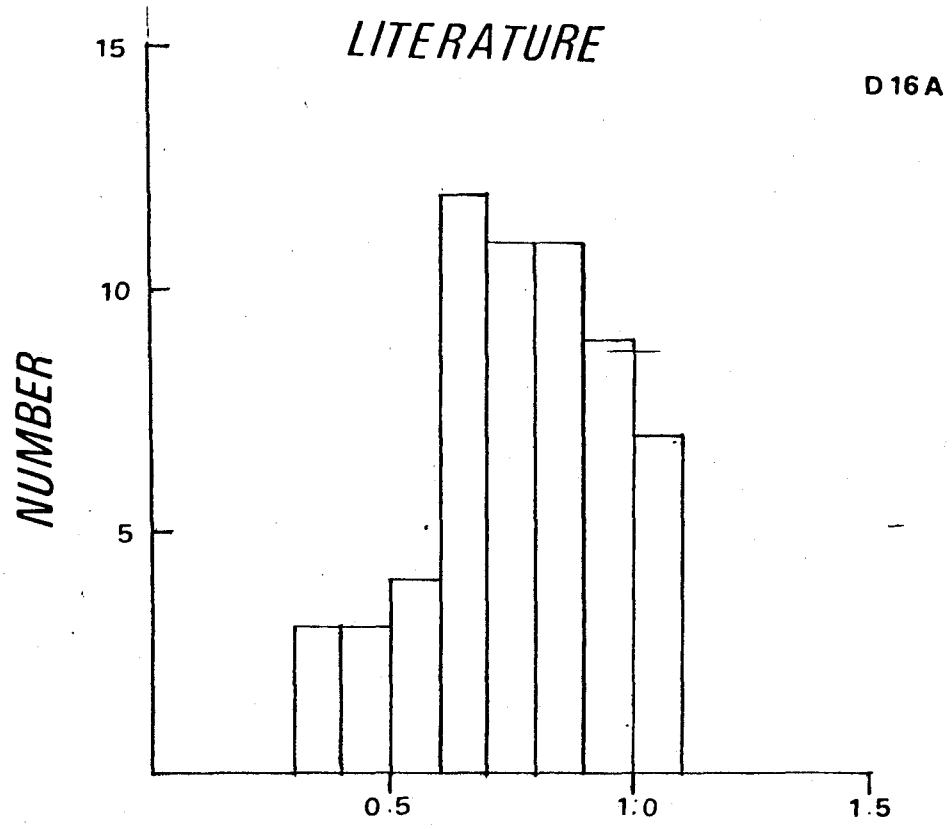
Secondly even if the sample shows a degree of inhomogenuity and/or problems in accuracy or precision, one still expects to see a "Normal" or "Gaussian" distribution about a true value if enough analysis are done. Since the overall number of analysis is low (70 in all) one has to resort to a histogram. Because not all of the individual analysis are known one has to estimate the distribution of certain results. This is done in Table XXV. Histograms of the literature results, the results of this work, and the overall results are shown in Diagram 16 a,b,c.

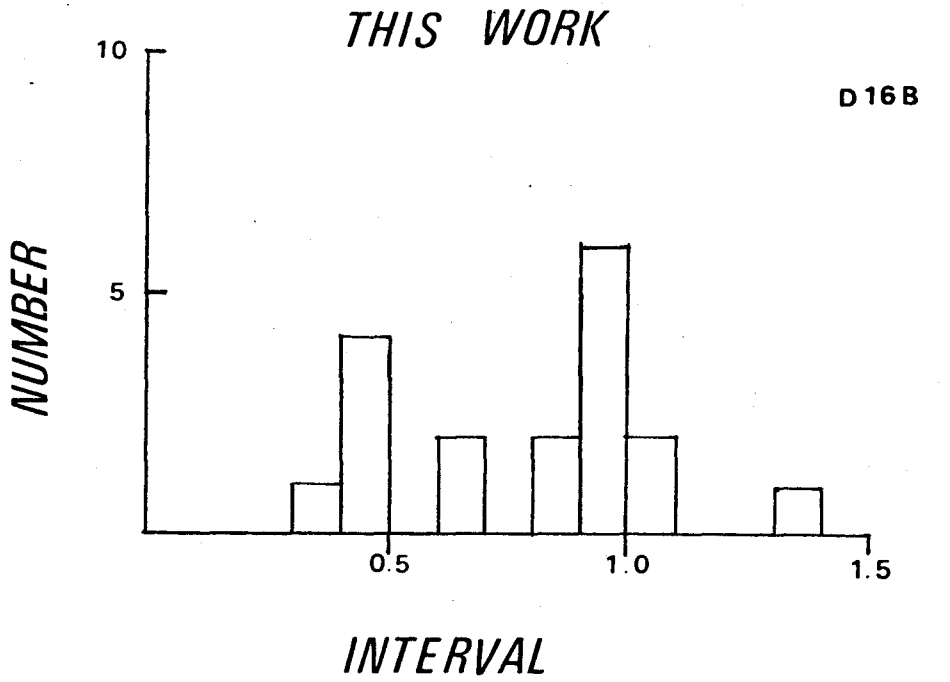
Table XXV Data for Histograms

Analysis	Average	Number in Histogram Interval														
		0	.1	.2	.3	.4	.5	.6	.7	.8	9.	1.0	11.	1.2	1.3	1.4	1.5
1	0.347				3	1											
2	0.50					2	2										
3	0.63						1	4	*								
4	0.63							3									
5	0.73							3	7	2	*						
6	0.75						1	2	4	2	1	*					
7	0.89								2	4	3	1	*				
8	0.91									3	3		*				
9	1.00										2	2	*				
10	1.06											4					
Total					3	3	4	12	11	11	9	7					
This work					1	4	0	2	0	2	6	2	0	0	1		
Overall					4	7	4	14	11	13	15	9	0	0	1		

*Estimated Distribution

HISTOGRAMS OF RESULTS

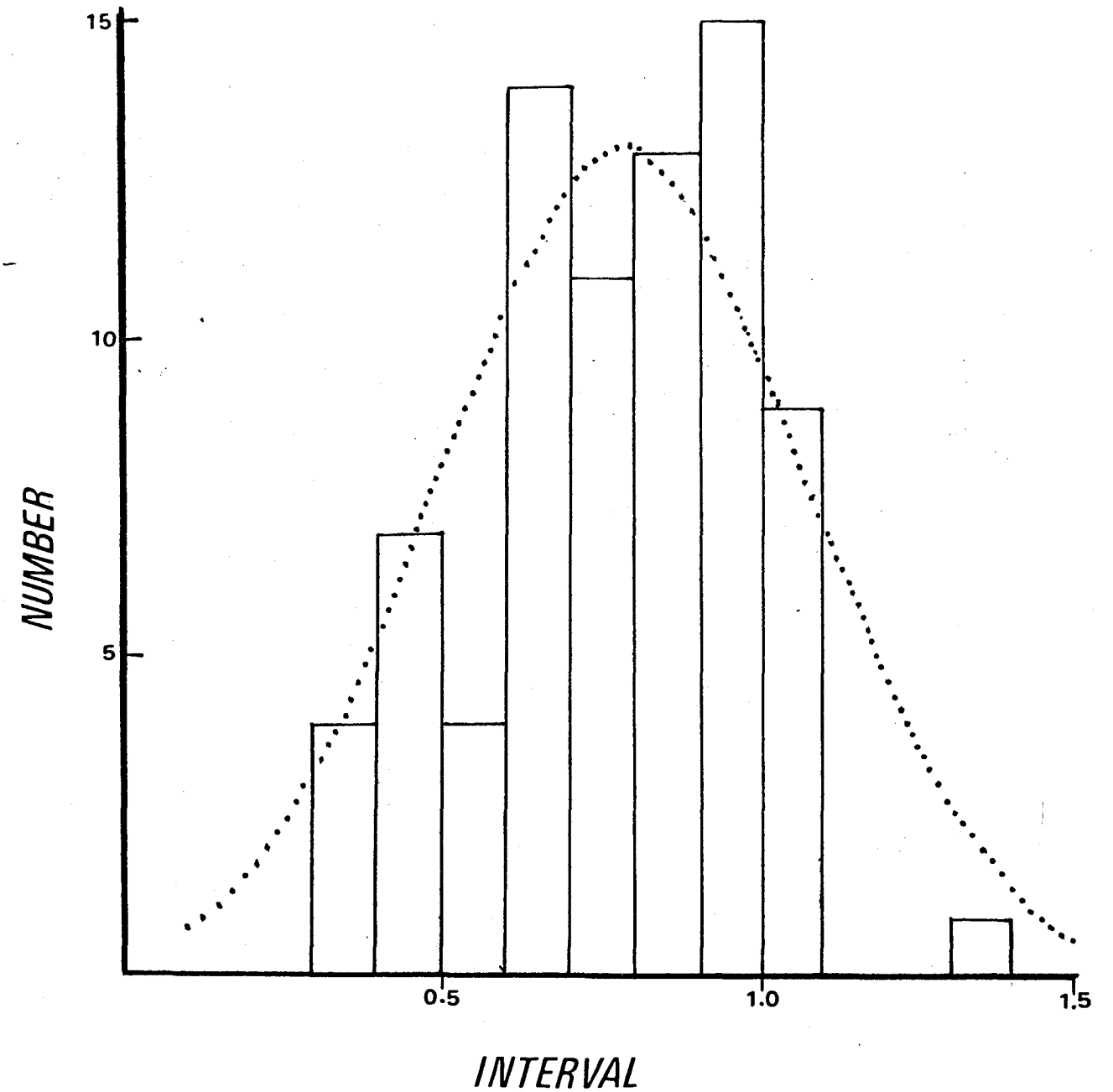




HISTOGRAM OF

OVERALL RESULTS

D 16 C



In diagram 16c, a "Gaussian" curve is also shown superimposed on the histogram. The curve was derived from the equation

$$Y = \frac{N\, e^{-(x-u)^2/2_o^2}}{o(2\ )^{\frac{1}{2}}} \qquad (149)$$

where

N is a normalization factor chosen to be thirteen and u is the average and o is the standard deviation for the Inhomogenuity Conclusion.

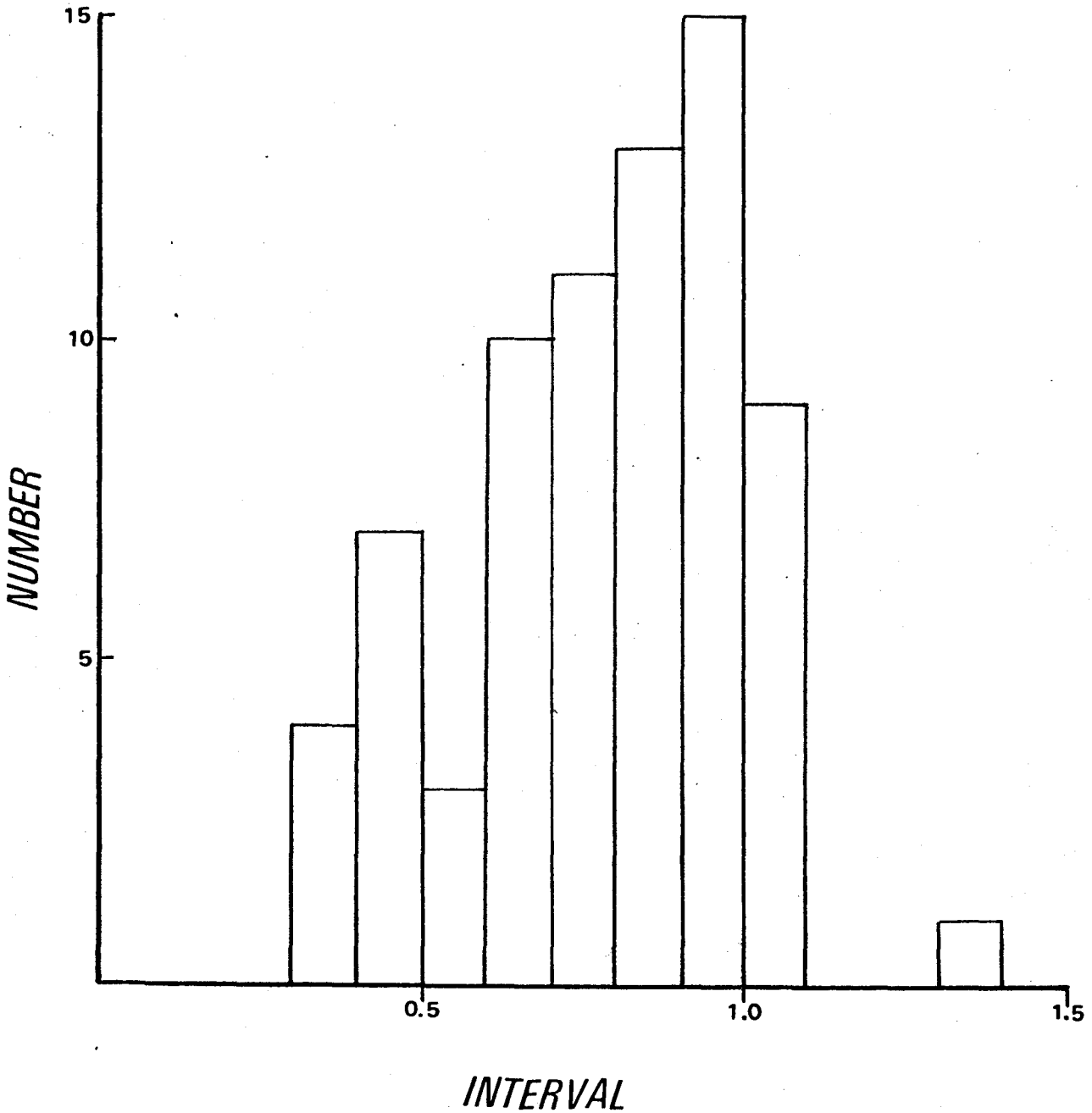
The agreement between the histogram and the normal error curve appears to be poor. However when one realizes that the error curve was derived from this work as shown in Diagram 16b, than even this amount of agreement is somewhat surprising. However one is unable to draw any definite conclusions on a "Gaussian" distribution. However the histogram does show a range of values with the true value probably lying between 0.6-1.0 p.p.m. If the Homogenuity Conclusion was correct then on would expect a bunching of results about the 0.9-1.0 p.p.m. interval. However this isn't found.

Actually another interpretation is possible. Two of the results in Table 1 may be the same. This is in reference to the two 0.63 p.p.m. results. Since both are dated before 1969, the one result 0.63 p.p.m. (average of five results) may actually be referring to the other result. If so, then one can remove that result from the average. This makes little difference in the overall average, so the previous arguments hold. However the shape of the histogram changes as shown in Diagram 16D. This

HISTOGRAM OF

CORRECTED RESULTS

D17



is interesting because it suggests that the distribution may be "skewed" and not "Gaussian". A "skewed" distribution of the cadmium in the kale would explain most of the conflict in interpretation of the results. The bunching of results around 0.9 p.p.m. would be expected since they are the most probably results. The spread of results and the overall average around 0.8 p.p.m. could also be explained. Actually one is not justified in saying that there is a "skewed" distribution based on the little information available. However it remains an interesting possibility which would explain most of the results that have been found.

In the opinion of the author the weight of evidence strongly suggests that the Inhomogenuity or Poor Precision and Accuracy Conclusion is correct, i.e. the concentration of cadmium in kale is 0.77 p.p.m. While it is not reassuring to look at a wide spread in results, comparisons with other works on standard materials at this concentration range shows that this is a familiar occurence. It would be difficult to determine whether inhomogenuity or inherent precisions and accuracy at the p.p.m. level is the major course of the poor agreement in the overall kale analysis. Indeed both may play an important role in explaining differences. For instance the poor precision obtained for the kale (R.S.D. 37%) seems to be greater than one would expect for this level of concentration. This, combined with a possible non-Gaussian Distribution indicated that poor precision

and accuracy alone cannot account for the spread in results. So a degree of inhomogenuity should be included. Actually slight inhomogenuity may reasonably be expected in any solid sample of this type where the original material is not completely homogeneous (and may consist of two or more phases). It is also possible that the typical sample size of 0.1 gram is not large enough to average out this homogenuity.

The use of a larger sample size would be expected to improve matters. However one still has the problem of the precision available by the method at this level of concentration. As well there are some practical limits and problems with larger samples. For N.A.A. larger sample size is limited by the size of the irradiation can and density of the kale powder. Contamination from reagents may become a problem with larger samples if wet ashing is used. Finally the use of larger sample sizes may be undesirable or impossible if only small quantities are available.

In conclusion this thesis has dealt with a general review of cadmium analysis in biological materials, and in particular the standard kale power; and has examined two techniques, Neutron Activation Analysis and Atomic Absorption Spectrophotometry in some detail.

The author would like to gratefully thank the National Research Council of Canada for the scholarship which permitted this work.

Appendix I

BIOLOGICAL ROLE OF CADMIUM

The biological interest in cadmium is largely confined to its possible interaction with zinc and other essential metals, its toxic properties, and possible relationship to hypertension and heart disease.

Cadmium is absent in the tissues of the new-born but tends to accumulate with age till about age fifty after which it declines slightly (12). It is concentrated mainly in the kidney and liver. A metalloprotein known as metallothionein has been isolated from kidneys. It has a molecular weight of about 6,600 and contains six metal atoms per molecule. The metal atoms are either zinc or cadmium. Since metallothionein has no known biological role, it has been suggested that it is merely a sequestering agent for cadmium with the cadmium competing favourably with the zinc for binding thiol sites in the molecule. As well cadmium has been known to inhibit certain enzymes (113, 136, 137).

The toxic properties of cadmium are well known. Indeed one author says: "Cadmium has probably more lethal possibilities than any of the other metals" (138). He is referring to the fact that the metal is seldom used in its pure form but usually is alloyed with other harmless metals to give a possible lethal combination. There are numerous cases where the use of cadmium-

plated vessels in the preparation of food or drink has led to poisoning. The symptoms appear as a "violent, acute gastristis which occurs immediately after ingestion of a liquid or a readily dissolved solid of acid nature containing a soluble cadmium compound. It apparently attacks everyone who partakes of the contaminated food" (139).

Perhaps a more serious menance is the absorption of cadmium from the air through the respiratory tract. Since the fumes of cadmium, or cadmium oxide are relatively colourless and odorless, there have been several reported cases where workers have died from overexposure to cadmium fumes (131,132,138). The persons "exposed to cadmium oxide fumes usually complain of a dryness in the throat or a sensation similar to a sore throat. This is accompanied or quickly followed by cough and later by headache and dizziness. The symptons often resemble influenza" (138).

The preceding cases of poisoning have been caused by exposure to relatively large amounts of the metal. However, there is growing and frightening evidence that cadmium in lower concentrations may be dangerous as well. The work of Schroeder (140,141) and Perry (142) has shown that cadmium does induce hypertension in rats and correlation has been found between hypertension in human subjects and cadmium concentration in the urine (143) and kidney tissue (68). Furthermore Carroll (144) showed a marked correlation between death rates from hypertension and arteriosclerotic heart disease and the cadmium content in the air of certain American cities. He shows as well that there is no correlation with air pollution in general. Zinc, a closely related element, is the

only other pollutant which correlates significantly with the above diseases.

Longer and more severe exposure to cadmium can also be fatal. In Japan, in the last twenty years, there have been many cases of the "Ouchi-Ouchi" disease directly related to cadmium. "Characteristically the disease takes a long course of increasing painfulness which, beginning with simple symptoms such as 'lumbago' or 'joint pains' ends with total and agonized immobility as a result of skeletal collapse. Cadmium leads to bone porosity and to the total inhibition of bone repair mechanisms so that, stage by stage as the disease progresses, the load bearing bones of the skeleton suffer deformation, fracture and collapse" (113).

In summary, cadmium has no known biological function and inhibits certain enzymes. In large quanitities it is toxic. In smaller quantities it causes hypertension. Prolonged exposure to it leads to pronounced skeletal damage.

Appendix II

CADMIUM IN HUMAN TISSUES AND FLUIDS

The following three tables are a partial collection of literature values and are by no means complete.

Table XXVI

BLOOD

Concentration	Range	No. of Determinations	Method	Reference
17.7 p.p.b.*	5-141.6 p.p.b.	110*	Atomic Absorption	128
8.5 p.p.b.	3.4-53.3 p.p.b.	153	Emis. Spec.	121
2.09p.p.m.**	±0.05 p.p.m.(SE)	47	Emis. Spec.	129
3.3.p.p.b.	±2.4 p.p.b.	--	Atomic Absorption	84
2.7p.p.b.	0 - 9 p.p.b.	17	Atomic Absorption	87

Table XXVII

URINE

Concentration	Range	No.of Determinations	Method	Reference
1.59 p.p.b.	0,5-10.8p.p.b.	154	Emis. Spec.	121
9 p.p.b.	3-13 p.p.b.	10	Atomic Absorption	20
-----	2-20 p.p.b.	--	Atomic Absorption	84
12.7 p.p.b.	7-22 p.p.b.	22	Emis. Spec.	130
10.0 p.p.b.	1.8-22.6	28	Col.	131

*243 samples only 110 above 5 p.p.b. which were used
Most likely average should be around 10 p.p.b.

**Author later suggests his values may be high (80). Other values for different tissues are several orders of magnitude higher than other values (85).

Table XXVIII

HUMAN TISSUES

Tissue	p.p.m. (wet weight)	Method of Analysis	Reference
Tissues	0.1-0.6	Dithizone	132
	0.2-0.8	Atomic Absorption	84
	8-17*	Emis. Spec.	122
Artery	0.2	N.A.A.	134
	0.4	Dithizone	132
Brain	0.12	Dithizone	132
Bone Marrow	0.3	Dithizone	132
Gall Bladder	0.6	Dithizone	132
Heart	0.012	N.A.A.	135
	0.2	Dithizone	132
	11*	Emis. Spec.	122
Kidney	9-18	Dithizone	132
	12	Atomic Absorption	133
	20-60	Atomic Absorption	84
	49*	Emis. Spec.	122
Liver	2	Atomic Absorption	133
	2-3	Dithizone	132
	13	Emis. Spec.	122
Lungs	0.26	N.A.A.	134
	0.7	Dithizone	132
	9	Emis. Spec.	122
Skin	0.2	Dithizone	132
Stomach	0.3	Dithizone	132
Tongue	0.13	N.A.A.	134
	0.2	Dithizone	132
Vein	0.15	N.A.A.	134

*Author later suggests his values may be high (80). Other values for different tissues are several orders of magnitude higher than other values. (85)

Appendix III

CADMIUM ISOTOPES BY (n,γ)

Isotope	Halflife	Precursor	Natural Abundance %	Cross Section (barnes)	Energy (kev)	Relative Intensity	Associated Intensity
^{107}Cd	6.49h	^{106}Cd	1.22	1.00	13.0	100.0	4.800
					325.0	0.7	0.034
					423.0	0.6	0.028
					796.0	1.7	0.081
					829.0	3.9	0.187
					898.0	0.3	0.016
^{111m}Cd	48.6h	^{110}Cd	12.39	0.10	150.8	31.9	30.400
					245.4	100.0	95.200
^{113m}Cd	13.6y	^{112}Cd	24.07	0.03	263.7	100.0	0.100
^{115}Cd	53.5h	^{114}Cd	28.86	1.10	231.4	2.5	0.700
					260.9	6.7	1.850
					492.3	29.5	8.100
					527.9	100.0	27.500
^{115m}Cd	43.0d	^{114}Cd	28.86	0.14	106.0	0.5	0.010
					157.0	0.6	1.020
					484.9	18.0	0.450
					492.0	0.6	0.020
					934.1	100.0	2.500
					1133.0	4.2	0.110
					1289.9	41.0	1.030
					1450.0	0.9	0.020
^{117}Cd	144m	^{116}Cd	7.58	1.40	72.0	3.0	0.650
					88.0	18.0	3.410
					99.0	2.0	0.430
					161.0	1.0	0.220
					221.0	4.0	0.830
					273.3	100.0	21.700
					291.0	1.0	0.220
					345.0	62.0	13.500
					368.0	9.0	2.000
					433.0	37.0	8.000
					465.0	4.0	0.900
					567.0	11.0	2.400
					635.0	3.0	0.650
					745.0	4.0	0.870
					834.0	6.0	1.300
^{117}Cd	144m	^{116}Cd	7.58	1.40	860.0	9.0	2.000
					881.0	13.0	2.800
					932.1	2.0	0.430
					948.0	4.0	0.870
					1028.0	10.0	2.200

Isotope	Halflife	Precursor	Natural Abundance %	Cross Section (barnes)	Energy (kev)	Relative Intensity	Associated Intensity
					1053.0	10.0	2.200
					1068.0	16.0	3.500
					1140.0	5.0	1.100
					1235.0	9.0	2.000
					1249.0	6.0	1.300
					1259.0	6.0	1.300
					1302.0	80.0	17.400
					1315.0	2.0	0.430
					1336.0	14.0	3.000
					1373.0	2.0	0.430
					1406.0	4.0	0.870
					1430.0	17.0	3.700
					1448.0	4.0	0.870
					1560.0	14.0	3.000
					1576.1	55.0	11.900
					1718.0	8.0	1.700
					1907.0	2.0	0.430
					1997.4	49.0	10.600
					2095.0	9.0	2.000
					2178.0	2.0	0.430
					2324.0	16.0	3.500
117m		116			2412.0	3.0	0.650
Cd	3.4h	Cd	7.58	0.70	89.0	---	--
					161.0	---	--
					221.0	---	--
					273.0	100.0	--
					291.0	---	--
					345.0	22.2	--
					368.0	29.4	--
					433.0	22.2	--
					465.0	---	--
					567.0	33.3	--
					635.0	9.8	--
					702.0	14.7	--
					715.0	22.2	--
					745.0	14.7	--
					860.0	14.7	--
					881.0	55.6	--
					932.0	9.8	--
					1028.0	19.6	--
117m		116					
Cd	3.4h	Cd	7.58	0.70	1068.0	50.0	--
					1117.0	22.2	--
					1235.0	44.4	--
					1249.0	16.7	--
					1259.0	9.8	--
					1336.0	44.4	--
					1406.0	44.4	--
					1430.0	55.6	--
					1560.0	33.3	--
					1682.0	14.7	--
					1723.0	44.4	--
					1997.0	83.3	--
			97		2095.0	9.8	--
					2319.0	16.7	--

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