PRNC012

RESONANCE IN RADIATION EF#ECTS

?Technical Report No.1

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11. CHARACTERIZATION OF A MONOCHROMATIC HIGH INTENSITY VARIABLE WAVELENGTH X-RAY SOURCE IN THE 5-20 KEV REGION

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Progress Report #1

Work perforsed at Puerto Rico Nuclear Ceater

Mayaguez, P.R., under U.S. Atomic Energy.

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January 1963

---Page Break---

SABLE GP CONTENTS

Preface

Section I. Rasonance tadtatton Bffects of Low Eneray ono chromatic X-Rays on Catalase

Section II, Characterization of « Monochromatic High Int

ty

Varlable Wavelength X-Ray Source in the 3.20 Kev

Region

A. Summary.

3. Ineroduction

©. General Properties of X-ray Production and Diffraction .

---Page Break---

Intensity and Baersy

B. Characteristics of X-ray Tube.

Anelysis for Marmonies in X-ray Bean.

A, Absorption Method

2, Double Distraction ..

3. Application

G, Ortentation of X-ray Tube, Protractor, and Soller

SLite for Optimum Intensity and Resolution «

1, Tube Position...

2. Small Protractor Posts:

a

1¢ of Soller siite

4H. Literature Referencs

Lest of rigur

List of Tables

---Page Break---

pRarAce

A 5. oowwsas

Reasonably definitive ans guss Wve sesuite from @ ae azprouc to the question; ?What as che effect of ionizing radiation on cate, are now beginning to energe. This nev approach involves study of tfects produced by radiation from adjustable monochromatic soures In general x-radiation effects have been observed to vary slovly as function of radiation energy. However, Little vork has been done in the region of x-ray energies below twenty ktlovolts, a region of considerable saportance since {t contains the K-edge absorption energies of the constituent atous of most Living systems. X-radiation of such energies is produced from ineident radiation of such higher energies (as cobait-60 gsema, 250 Kw x-rays) by degradation through Compton

Le was felt taportant, therefore, to atudy x-radiation effects in

the 5-20 Kv energy range upon biological systeas, vhich are comp

primarily of Light elenente with but traces of sediun atoaic veight

elements, As a first system for study, the ensyne catalase, contein~

Sng four atous of iron in ite porphyrin ring structure, was chosen and

?the question aske

Does radiation absorbed by the iron atom produce

nore danage (inactivation) per electron volt absorbed than radiation

absorbs

only by the Lighe elements (carbon, hydrogen, oxygen, ete.),

which make up the bulk of Ehe catalase solecule? Experisencs vere

deeigned to show or disprove che presence of a true action epectrun of radiation danage in the kilovolt region. The presence of such « spectru indicates unique effects of such radiation, entirely divorced

from the general "indirect" effects of radiation (vhich say be simulated

chenteally).

---Page Break---

Wap at che University

usder the dix

De. Henry Gosberg, has deen 200%:

18 probles for soue <i.

?Two separate studies have been carcied out by this group on the eazyae

al

(one was done by Or. Ardath H. Emons and another by Hr. Peter

Pa

Kevoudakis. Both denonstrate that 100 electron volts absorbed at

photon energies just beyond the K.

sorption edge of tron produce

to react vith hydrogen peroxide,

on the other hand, tests made by Dr. William Clendenning in the

sane group on the free radical yield in L-brom-butane, as

erained

by reaction with DPPH, shoved ao unique response as @ function of

energy. Likewise, studies aade by Dr. Marvin Atkine on damage through

frradiation at the "L?

nége of certain mercury organo-aetallic coapounds yield

negative results.

ly, Dr. Gouberg moved to Puerto Rico vhere a new grou

attacking the sane problem but with different equipment and under

4tfferent environmental conditions, was

eablished, The Ann Arbor

Broup is continuing its study under the Leadershtp of Dr. Hoyt Whipple.

Im Puerto Rico, Dr. Robert Luse has made a completely independent rerun

of the cai

fe expecinente and has confirmed the extatence of « unique

ly high damage rate for photon energies in the vicinity of the K-

rption edge in catalas

Tm the course of this work, Dr. Vazquez Martiner, alas of the

pave

staff, has developed extremely effective techniques for obtaining

?substantial yields of sonochcomatie x-rays for irradtation gucpos

from

andaré Cenerai lectric Co. XRD-type apectroae.

---Page Break---

ota

Ancidental, but significant problecs save Leen solved oF are bei

worked on in the cour

of thie veusarce, These include 4 teve ai

celoriseter for lov eneray x-rays, being developed in Ann Arbor, and

igh

asitivity chemical dosimetry, being developed in Puerto Rico.

Work 18 now in progres

HE PRNC on the x-ray action spectrum of

carboxypeptidase (a rine metallo-ensyme) and on £. coli

---Page Break---

RESONANCE RADIATION LZPEC"S OF LOW ENERGY MONOCHROMATIC X-RAYS ON CATALASE

Robert A, Luse

A. summany

Expertvents using monochromatic x-radiation ia the energy range

6.4 - 8.3 Kev have shown increased inactivation of the metalloenzyae

caval

at or near the Keabsorption edge of tron (7.11 Kev). This

Ae taken to confirm the resonance radiation hypothesis of Gosberg and

previous experizental work of Emons and Paraskevoudakts

radiation intensities of 2 x 1044 photons per hour have been

measured in che casple holder with Fricke ferrous amoniun sulfate

dosimeter. A sore sensitive method for detection of the ferric toa

produc

has been developed, using the ferric-hiccyanate complex.

erRODUCTION

Previous work by Emmons (3) and by Parasxevoudakis (8) has iadi~ cated that there is an enhanced inactivetion of che macalloenzyne catalase by monochroustic x-rays at vavelengths near the K: sorption j0 Of Aron. Indeed, @ plot of enzyme inactivation 1 function of the x-radiation wavelength (or photon energy) follows closely the normalized sass absorption spectrua for tron (see 2438. I-L and 1-2). Since no such resonance radiation effects have been reported by other workers, it was desired co confirm the vork of Emons and of Paraske: voudakis using other equipasnt and personnel. Toe present report

As concerned with such confirmatory axperineace,

---Page Break---

EXPERIMENTAL PROCEDURES

1, Developuent of presen: icradiacion system,

To obtain x-radiation of precise energy, the irradiation

system described in thie report by Vézques Martines vas utilized.

Were 4 portion of the x-ray energy produced in the x-ray tube vas

selected by colLiaation and cryetel diffraction, a0 that « bean of

?monochromatic x-rays having photon energies within the 4-50 Kev range

sod with « high purity of energy (+ 50 ev) was available.

?The characteriaticn of this (radiation Seam defined the re-

uisenents for the holder tn which the enzyme solutions were placed,

vis: irradiation chamber dimensions 9

- wide, and 4 am, deep; vercical solution height not to exceed 6 mat

Trrad{ation of solutions of catalase vas carried out in the sazple holder sketched in Fig. 1-3.? This holder te constructed of

Vieasurenent of che bean area and position was done by placing « 4 x 4 cm sheet of X-ray file (in Light proof envelope) directly in front of the sanple holder. After a short irradiation period, the position of the film relative to the holder was marked by piercing envelope, film, and holder with a sharp scribe, After photographic development of ?the fila, it vas replaced on the holder, and the holder adjusted co coincide with the darkened area of the file. Later, very accurate characterization of the distribution of energy ?croas the X-ray beam was done by Vasquez (15).

curing the course of early work, there were utilized three other

?staple holders having the characteriatica tabulated belov. Experi-

ence gained in their use alloved design of the present holder.

colt Construction Capacity Solution thicknen

?A round polyethylene bottle top 1,5 ab oo

2 facketed lucite block 10 16

© Wekee block vith small com

Pertoonts ("Michigan cell") 0,2 2

Cells A and 2 had Mylar windows, coll C was covered wich acoteh cape

until it was found that contact with such

of catalase solution

---Page Break---

Percent of Catalase

Activity Lost

?Activity Lost

Percent of Catalase

Note:

(24) 0/9 pazyyeur0y

j | |,

Catalase Solution Lots of Acts

xeray Energy. data of Eamons, Ref. 1,2,

absorption curve te given by the brocken curve.

ity as a Function of

Norsalized iron-aase

?r a ?»

? Fe ih s

t - i

mo = ?3

Pig. 1-2. Refer co caption above. paca

of Paraskevoudaki.

for Pig. 1-2, 1.2 x 1019 x-rays /ea?,

was 9.5 x 1010 xerays/ead-ne. for Pig.

fo. sd from 1.5 x 1010 ae

xerays/ea var at 10 kev.

For Fig. I-L, each sample absorbed a dose of 1.8 x 1012

The incident dose rate

TL, Por Fig. 12 th

3 kev to 7.0 x 10H

---Page Break---

Fig. 13,

* Distance becveen chanber and

sample compartnente = 2.5

Sketch of sample holder

---Page Break---

sectaccylate plastic (echt) with 4.001 inca chick Ou Pont Myiae

polyester file

Keeaches 29 ice face; Loose cacertale are velazivel

eraneparent to Low

necky x-r4y6 and ace non-reactive chemically with

che biological sasple. Comparteant thickness vas determined by balan-

cing che factors of solution absorption and sample size: a2 am

sample thickness vas seasured to absorb approximately 95% of incident

7 Kev photons, yet this thickness vould permit only a 100 pl sample,

too small for accurate manipulation and analysis. A depth of 4 am.

permitted a 220 pl. ang

oe

ditions of complete absorption was avoided by mechanical atirring wich

sking of solution under such con-

20.5 mm, glase rod tn

ted into the solution and rotated at 88 rpm.

?To winimize evaportation and inactivation of the catalas solution,

samples vere covered and maintained at 5°C. by passing vater froa a

?constant tenperature refrigerated bath through the holder block.

2, Developaent of ferrous-ferric micro-dosinetry system.

The prime requirement specified for the dosineter systen vas

the ability of direct eubseitution for the sample, so that values of

radiation intensity measured with the donieeter correspond directly

with thos

absorbed by the biological sample. Other considerations

were simplicity of use and reliability im the low do

ranges involved

in thts vork,

The Fricke ferrous-ferric dosimeter {8 the most commonly used

land best characterized ?secondary standard? avatlabl

TRis dosinerer

relles on che oxidation by sonizing radiation of ferrous ion to ferrte

on, and determination of the concentration of fersie ion formed by

T

a sompared wich primary standarde suck as cols.

sassry of hat (7)

---Page Break---

ite Light absorption ae 50% at (ef. Se:

sd Alten, 12).Appiie.

of chia dosimeter 4s prinacily iva ie wothods for ferric soa

analysis. Recent work by Scharf and Lee (11) has shown that a sore

sensitive

say for ferric tons 49 messurenent of the absorbance at

226 op; here the aolar absorptivity? of ferric ton is 4565 Liter sole?!

cmt compared to the value 2196 at 204 ap waveleny

?That che ab-

sorption spectrum for the ferric amoniua sulfate solution used ia this work coincides with that of the ferric sulfate solution used by Seharé

?and Lee vas confirmed by Laboratory searurenent.

[A considerably sore sensitive assay of ferric ion concentration

ts weasureneat of che absorbance of the red-orange ferric-thtocyanate

couplex; the wolar absorptivity of this complex is 10,000 - 14,000

Meer aote? eat

aC 480 my (see Pig. 1-4). Pribicevic, Gal, and

Draganic (10) have characterized ehis complex formation and proposed

tee use

dosinetry system in the 300 - 100 x6

dose range. Not all

thetr resulte could be confirmed, 40 that further characterization and modification of the system were undertaken. Differences vere found ia the location of the absorption maximum (480 mp, not 470 mp) and in ede potassiua thtocyanate concentration yielding greatest couplex-absorbance

(2.0.8, not 0.65 8)5 the

changes were incorporated into the procedure developed. In addition, to avoid dilution of the irradiaced solution,

optical measureaents vere taken vith the

cman DU spectrophotoneter in cuvettes of 3 am x 10 an x 25 om chasber disensions; as litle as

0.22 at of solution my be assayed in such cuvettes.

2 the term molar absorptivity {e equivalent to cae older extinction coeffielent and molar absorption coefficient. follows the prefecrad ucage of the editors of A.

---Page Break---

De 480 ou

(wien ehfocyenatey

Absorbance

! A= 224 aw

X= 304 eu

2 + © @ 0

Concentration of ferric son at (soles/t) x 37>

Fig. 1-4 Compariuon of seneisivity of var

, Getorsining ferric ton in cor:

---Page Break---

The procedure for sesuy o- ious cosinecar was as

follow

To the four aiccscwwersee

se aces portions (approxieatar

Ay 0,23 al in voluse) of 5 x 103 mM ferrous amosiue sulface in 0.8 8

sulfuric acid, treated vis: a) not irradiated, stored in refrigerator,

b) irradiated for the specified time ac 5*C. with aechanical stirring,

?) and 4) controls 1 and 2, kept in bolder compartnent

B and ¢ during

irradiation. Measurements of absorbance at 224 and 306 mp vere mad

using che stored control

blank, One of the controls was then set, ide and 220 pl. of the ferrous solution containing ferric ammonium aulface to the extent of 1 x 10-3 M ferric fon was placed in thie cuvette. Fifty mg. portions of potassium thiocyanate were then added

th cell and dissolved by shaking. Absorbance at 480 mp vas

measured after 10 min, The value of the solar at

vrptivity vas calcu

ated from the standard ferric solution and used to eatinate the ferric

Son concentration in the irradiated and control sanpl

3. Assay for catalase activity.

caval

concentrations were determined using eacentially the standard assay developed by Beers and Sizer (2), in which the disappearance of hydrogen peroxide {0 folloved spectrophotonetrically at

212 ap. catalase activity war expres

in unite of enzyae (v) per

Teese authors used 240 mp vavelength for seasurenent duc show that ?any wavelength in the 200 - 300 mp region 1s appropriate, The 212 mi wavelength was used for two reasons: to repeat work of Eanons and Paraskevoudakis, and co utilize che Larger (oy cearly five tines) molar absorptivity of hydrogen peroxide at the lover wavelength.

---Page Break---

me. of protein, where one wait 26 agua <0 will catalyze the decomposicioa o! 2 = coaote of hydrogen pevoxice pee minute under apecified conditions.

Assay reagents vere as follows: Enzyne solution - An approximately 4,5 mg % solution of catalase (Worthington Biocheaical Co, lyophilized material, lot no. CTL $535) was prepared in 0.067 H pocassiun phosphate buffer, pH 6.80 (made from 0.067 M solutions of aiiz?04.H,0 and NazPO4.12H,0), Such solutions were stored at S*c. in

red "low actinis

volumetric flasks, Calculation of enzyme concen

tration by spectrophotonetric measurenent was done using the molar

sosorpeivity value 340 oat aac at 405 ap for horse Liver catatase.?

Subserat

ution - A 0.03% (8.8 x 10 -34) solution of hydrogen per~

?oxide vas prepared from Fisher reagent grade 30% material by 1:1000

Atlution with 0,057 4 phosphate buffer, pli 6.80, This eolution was prepared fresh daily before use, as dilute peroxide solutions are not

stable at room tenperature, Water used for enzyme and substzat

lutions vas distilled in glass from previously demineralized water.

Buffers and vater vere scored in polyethylene bottles to minimize

trace metal contamination.

T

?Such terminology follows recomendations aide by che Comission

fon Enzymes of the International Union of Blocheatstry (see

?Thompson, 13).

Such 4 calculation indica

that 4 4.80 mg. % solution, nominally

2.1 x 10-7 M (solecular weight of 225,000) was accuatly 1.0 x 10-7

©, ?This means chat the lyophilized astertal has to approximately half its cotal wesahe.

gated moisture

---Page Break---

?The as

ny procedure vas 4s follows: The sampie of catwveoe so

ution (normally 0.1 - 0.2 al. in voluse} vas placed ta & sgecseazhocse

seoter cuvette (1.0 em. path iengtis, 4 al. capacity, fused sutica «

leraviolet transmission). At zero rei

ion tise, 2 al. of substrate vas rapidly pipetted into the cuvette, and the change in percent tras sitcance at 212 mi of the solution measured over the first 90 sec. of sction tine, This change in transaiteance vas measured using a Servo/riter atrip chart recorder attached to the Beckasn DU spectroPhotoneter with a Becknan energy recording attacheent (ERA). The Lintts of pen travel on che chart were defined prior co this seasurenent by ?adjusting che pen to OZ transmittance with the spectrophotonater dark current adjust with no Light striking the photocell, and chen to 100% erenamittance with the ERA "100% adjust knob with Light passing through 4 solution of buffer only. No attempt wes made co add che hyerogen peroxide to the enzyme and to start the recorder simultaneously;

the

substrate was added at cero time (as Judged from a sveep second hand

thmer), the spectrophotometer shutter was opened, and the recorder was

started, in a sequence requiring about seven

?The ninety second

interval vas accurately seasured, at vhich point che shutter vas clo

cusing « rapid deflection of the chart pen to 2410. Points on the

chart corresponding £0 60 sec. vere deteruined by carefully seasuring

back from ents

flection point with a ruler, Since chart speed was

18.3 em. min." che 60-90 «4

+ interval was equivalent co 9.15 ca.

In sone weasurenents, increased sensitivity in the chart recording was

achieved by setting the 100% T margin with 4 solution of

ured

bance approxisately 0.9. By chie method, absorbances in the

0.9 - 2 region were sore accurately measured, and che slope of the

curve ET vs. cine inereased by a factor of approxiaacely sight

---Page Break---

?The values Of 12 cai from Che caacc etsip seco:

converted to absorbance values by te ceraciocship

absorbance log (100/percent cransuiccasce oan.

and thave absorbance values used in the equation

fenzyne unite per mg. =A per atnure x 1000

Aes eeirbat pee at.

Of reaction mixture (

2»

where 146 io the molar absorptivity of hydrogen peroxide at

212 mp (seasured An thts Laboratory).

7

at the sane tise chat che molar absorptivity at 240 5) ves measured

and touad to agroe vith published values

---Page Break---

The intensity of x

radiation in the 6.5 - 7:5 kev photon energy

wed by che ferrous dosineter, 16 shovn in Pig, 1-5, based

?The folloving equations were

in calculating this daca:

?Amount of ferric ion (QEDIBes temple seerectd tory

{ }- blank at) of neasurencet.

in sample, as noles/at,

x 103

sels soprptivty of fersie tony

tN of measurement

or, c= A/1000? (ogee. 3)

Molar absorptivity of ferric ton at 224 and 306 mp {a 2196 and

4965 Liter mole"l carl respectively at 25°C, The molar absorptivity of

the thiocyanate complex at 480 ay was estinated from ferric atandarde,

eribed previously.

6.02 x 1023

aap be Ye

by the sample, in erge al?! he cone

, Cc rate of radiation absorption

ny

where © = concentration of ferric ton, aoles/al.

£ = factor for conversion of electron volts to erga,

1.6 x 10°! exgyev

Yo the ferrous ion yield (G value), equal to 0.135 tone

?oxidized per electron volt, in the 8 - 10 Kev range

(Cottin and La Porte, 4)

tm period of irradiation, in hours

of, 1 (as ergs att hele S + 7.16 x aol? (eqes. 42)

1, forthermore, as photons al?) art « c 4

© Wper pions,

L

TEx Coste, 503

---Page Break---

sorbed in sample

heel x 10"

ean tncensity

?ae (photons -

7 a

Photon energy, as Kev

Pig. T-5. Monochroaatic x-ray bees intensity

?as function of photon eneray

---Page Break---

?CUD 201 paw sagas 50 v2¥p Boyen sanz¥reder 303 pey221209 9100 sonyeA AIFATIALONGE

WTO vw

INEKEANSVEK °T aTEVE

---Page Break---

(e¢ea. 535

1, as photons

2 sosgie

= = (1, as photons. atl nel, x (saaple votune in al.)

(egtn. 6)

Total dose delivered co sempl

arose (egea. 7)

The most iaportant features of these data are a) chat the beam

Ancensity is approximately 2x 1011 photons per hour in the 6.4 = 7.2

Kev photon energy region, b) that the intensity is increasing with increasing photon energy (as found by Paraskevoudakis), and c) that

ehere 4 no re

wance radiation effect in the ferrous dosineter at

sorption edge of tron, This 1

finding 18 as expected, since

the radiation effect measured 4e an oxidation, the ¢ value for which 1

?energy independent over at Least one hundred orders of magnitude.

2, Resonance radiation effects in catali

?The extent of catala

inactivation by aonochronatic x-radiation

4a shown graphically in vig. 1-6. This curve ie based only on eos

4, from experisente in which inactivation of control catalase solutions by scatter radiation vas miniazed by additional shtelding

(rable 2), That chere is enhancement of catalase inactivation by

radiation of energies at or near the Ky and K-adsorption edges of iron 4s obvious, and confirms the findings of Emons and Paraskevoulakis.

1m general, che shape of the curve approxinates that of the tron-aass

absorption curve,

Vertical arms from these points indicate the excent of difference

tn duplicace assays on irradiated sample, Horizontal aras indicate

the variation ia ptoron energies withia the x-ray Seas (+ $0 electron

volts),

---Page Break---

Percent catalase inactivation

65 70 75 80 as

Photon energy, Kev

Pig. 1-6. Resonance radiation effect of aonochsomatic

arrays on catalase

Tocal dose absorbed by sample = 1.4 x 1029 ptocong

(except for starred point, where dose = 0.2% 10"

---Page Break---

06 36'e8 cor'se | cee oy

99 co'' 96 ras ore o'sy

sa 698 68 uw | see vos | zx/over

09'r9 98 s6'06 wu] we eis | esverer

as'e9 1988 18°96 ui | cs sons | esvover!

06 96 6 a | ove | asces | zosertt

WY PRAT TOY TH IIOD TAY THING | TATE UY [AAT | THIET |G

posed | yng | art] fesey

sua o

NOLLVEGYE-X DLLYMOWHOONOR AB NOLIVALIOWNI 2S¥I¥LND. ?2 318¥E

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CONCLUSIONS AND D:ScuSSION

Te te incerescing co compa:

preseat cose rates and corel coses

With those of Bmons and Paraskevoudakis, A summary of chese data is

siven in Table 3. Emons reports dose rates watch a1

0.1 co 0,025

those found in his work,

1d total doses which are 0.02-0.03 pre

TABLE 3. SUMMARY OP DOSE RATES AXD TOTAL DOSES ASSORDED

IN CATALASE SAMPLES

Dote racey | total dose, | sample voluae

oveetigntor | ae'tv'neet | Se'photone? | ?PS 3

17x 100 13.3 x tole | 0.18

Emons ~ fret order | 4.5 x 10? ito 5 x told | 0.18 oF 1.957

Pehrnteneet | carat 2.2 eat | aa

Luse - first order 2x 10ll 1s x 10d | +23

Vazquez - first order | 1.7 x 109 | - | -

C0 hon are cot i |

values. That the ext

Ne Anactivation obtained ie similar

(20-40%) for both arudies using first order radiation caste soae doube

on the former dosimetry. mons found that the intensity of xeradiation

(or energy f1ux) as measured by the SP-1

tector vas about ten percent

chat measured with the Fricke dosiseter. Comparison of the dose rai

values measured by Luge with the Fricke

4 sintlar electronic

s£fictency is approxiaately one percent, that ie, the counter registers

fone out of every 100 incident counts. A possible reason for the ten~

fold difference is that che surface area of Bmons! dosineter vas not

matched to the x-ray deam ai

1» #0 that the apparenc eneray flux per

en? vas in error, Por example, it seens chat amons sted 4 sanple holder

im hin dosinetry studies having a surface area equal co 2.26 cad, viere

---Page Break---

the bees area aay have been only adout 0.26 ca? (Zoesidie

defined by the collimating slits uses, ace 0.6 x 0.3 oa.) Im che peacens

work, chia discrepancy is elisinaced since the saaple surface exposes co

the xray beam is exactly the same as che beam ar

tn steplitied form,

the scheme below illustrates thie situation,

source enite 100 by; SPC-1 detector

i regtatere 1 photon

tn beam area a

1¢ 1, dosimeter absorbs 100 hv/A = 100/a; counter efficiency

= 1/100

im case 2, dosineter absorbs 100 hv/A' = 10 hv/a; apparent counter

efftetency = 1/10

?An apparent quantum yield for catalase inactivation at the Ke

4 absorption edge of iron may be calculated from present experimental

reales, vie:

Quantum yield = molecules inactivated / photons absorbed, where

molecules of catalase inactivated =

x 1077 aolee/1)x(0.25/1000 tters)x(/3 inactivarion)x(6.02 x 1023)

photons of 7.11 kev energy absorbed =

@ x 10"! e/ar) x 72 bee.

Hence the

parent quantum yie!

(x 10'2) 7 (1.4 x 1019), oF 0.36 molecules/photon.!

Yee quantua yield calculated {4 200-fold less than the 70 solecules Soactivated per photon caported by Emons using dry catalase. Quantum yields considerably greacer than unity have not been reported Zor encyaes in the many studies of con-resonance radiation effects. A pact of this

\ Atacrepancy aay 08 due co arzor in estimation of doses uvaorved in sample

---Page Break---

Te aust be reelized chat chia apparent quantua yie.s Ls cor che asus

che crue quantua yield, since the above La not corcecced for ao

sorption by the solvent. aa e

mate of the relative absorption of 7
Kav photons by solvent and solute ina 1 x 10" M catalase solution may

be made as follows (cf. date of Banon

Total Absorptio age Absorption,

Material Coefficient, nt/s. concentration, g/l. ctl

nas 0.022 0.26

0.087 + 13.60 13.7 1000 13,700

8902)

se absorbs 1/57,000 the energy absorbed by che solution.

?An exact measure of the quantum yield can be obtatned only from

experiments using éry enzyae preparations. Hovever, rough

einace

?my possibly de aade by extrapolation from 4 aeries of enzyme concentrations.

Calculation also aay be aude of the G value for catalase bused on

nt resulta, Since 0.36 molecules are inactivates ger photon of 7100 ev

energy absorbed by the solution the G value equals 0.005 aolecuies/100 ev.

Tate Le approxinately half the value of 0.009 previously reported for x-

radiation of much bigher energies where the resonant effect is not present.

---Page Break---

¥, ?ROPOSALS FOR PUTURE RESEARCH

?As present results confirs the presence of resonant effect in che

xeray inactivation of catalase, it {a proposed to enter che

cond phase

of the research--examination of second matalloenzyne.! carboxy peptidase A has deen chosen for study for several reasons, vis:

4) Ate radiosensitivity should allo much shorter {rradLation periods so that use of the present low-intensity x-ray source ta feastdie;

) Sea physical chemical characterization haa Seen well developed by Vallee and covorkers (3, 14), 10 chat {t provides a rather ?voll defined ensyne for study:

c) 4& Se presently unique in that the single sinc atom present per molecule vhich is necessary for peptidase and esterase activicy may be replaced with sercury, cadmiua, lead, cobalt, ?ickel, or eanganese to give a series of nev metallocarboxypeptidases displaying specific activities tovarde a srten of

new substrate

charactertatte of the particular setal which Le incorporated.

?Tate ability of substitution of the metal Aton ansociaced with che active site of caxboxypeptidase permits the folloving experiment: Irradiation vith monochromatic x-rays of carboxypeptidases concaining different metals, e.g, zine and n{ckel, at the K-absorption ?edgen of che reopective aatale, Enhanced inactivation of the sine~

?enryne would be expected at 9.66 Kev (xine Kgs), inactivation of the

Irradiation of catalase at bigher doses vould bese uait until 4 sore intense x-ray source 2 available.

---Page Break---

=m

nickel enzyme at 8.33 Kev (nickel Ky); mo inactivation should be produced in the sine enzyse by photons of

23 Kev, nor in che nickel
enzyne by photons of 9.66 Kev energy.
Phase three of the experimental program will be « study of Living

syatens vhere the resonance effect may be observed. Were the singh

celled bacterivn Recherichia colt provides « well-characterized teat
orgeniem, In addition, {¢ contains the sine metalloensyae alkaline

Phosphatase, which is obtainable in pure form (9). It 4a

repost
compare the resonance radiation effect in thie enayue (inactivation ve.
Photon energy) vith the resonance radiation effect in the bacterium

(percent survival ve, photon energy). Should the enzyae be sal for bacterial reproduction, the two affects should be statiec.?

?A whole new range of experinents can be initiated vhen vacua x-ray

?equipment becomes available. With photons of 2.1 and 2.5 Kev energies,

?the resonance radiation effects may be studied in sulfur and phosphorus

?tous renpectively. At this point, the {eportance of disulfide bonds

to three-disensional enzyme structure and the role of inorganic phosphate

stous in the active aite of certain ansynes (

alialine phosphat

may be studied,

Te te hoped ehat auch resonance radiation studies vill introduce

4 new dimension of specificity in the field of radiobiolog. Low enecay

sonochromatic x-radiation offers @ tool as ?clean? 4s monochromatic vltravioler Light, but in an energy range one to chree thousand: fold

that Of UW. With auch # Cool, 4 host of nev radfobiological studies ta

the Laportant field of enzyme structure and function becoses possible,

Te 9 interesting co speculate on enzyne-nucleic acid interrelations

at thie point,

---Page Break---

6. apperorx

1. Notes on catalace

ay calculation

?The method of calculating catalase activity given in this re

port differs from that used by Emmons (and precumably, Paraskevoudakis),

wo oats use of the folloving relationshs
te (90-2 2 cn

sda Gee Ge. 9

snd fy = 1.2 x 10°2 (concenteatton:8 even. 2)

Ky vas determined tn each

say by plotting che percent transnittance values measured by the recording system as log (100 - 7) and determiaing the 4

va

e of his Line over the first 60 sec. of enzyme reaction,

- (anny

gy vee at a np stow on te) = 13g ee

(eats. 3)

?That values so calculated are negative seene unrecognized by fmmons.

An es

ieieal Linear relationship was found between log x; and tog

?enzyme concentration in the 0.3 - 2x 107 M range. The equation best

eseting this Line (eqen. 2) wes us

subsequently to eatinate enzyne

concentration in controle and irradiated seaples.

?Tois method was rejected because i¢ i+ not an accurate expression

of the fundavental kinetics of the enzyse reaction. The reaction of

catalase with hydrogen peroxide 1s described by first order kinetics

during the initial part of the reaction (before product concentration

Feaches @ point where che enzyse {8 poisoned, The equations describing such fiat order ceactions are

Ky = L loge (S,/5,) where Sy and 51 = substrate concentrations

= at time 0 Sné tine 1

Keqea, 4)

---Page Break---

=

and Ky = k (enzyae concentration) Kegea. 3)

Beers and Sizer (2) have shown chat 0.01 = 0.02 hydro

peroxice

subscrate follove Be

18 Lav for wavelengths of 210 - 300 mp, i.e.

Ay 4a proportional co $, nence

Ky et loge (o/A). (eata. 6)

Substitution of percent tranmittance for absorbance results ta an

?equation of che form

= Joke lok (10/100) . loge (og 100 = Log ta)

4° S08 Glog Cyto "eM Gsptoo eS

(eats. 7)

(the term (Log 100 - log 7) ts not equal to log (100-1). Bg. if

T= 22%, log 100 - log T= 0.688; Log (100 - 1) = 0.892).

Comparison of the shapes of ehe curv

obtained by plotting equa

tfons I and 9 against enzyae concentration may be nade using #impli-

fed dace. See Table 4 and Pige, 1-7 and 1.

That Emons obtained «

straight Line relation between log Ky and log & {8 fortuttous and some

indication of the inuensitivity of « log function of T. Such insensi-

tivity ta che

jure of enzyme concentration may be responsible for

the wide variations a enzyme inactivation reported,

---Page Break---

oar

TASLE 4. ILLUSTRATION OF DIFFERENT xETH00S OF

HANDLING KINETIC DATA PROM CATALASE REACTION

Lat hy 20000, Ay * 03, 0.6, 0uby 0.2 for various concentrations of com a.

ktaeteceey ies)

Le

ae

tot ggg ca6% | toa

eee tn

= 1827 333

ao | as

coef as

- |

---Page Break---

loge (Ao/A1) oF Toke

Rowe (ho/A1) tog, (200-71)

* (oo-t)

2 + 6 ®

Eazyae concentration

(arbitrary units)

Figs 1-7. Comparison of absorbance and percent

txanaaission plote vs, ensype concentration

---Page Break---

Apparent rate constant, Kj (negative values

ae

10}

08

° 10

og, of enzyme concentration (arbitrary units)

Pig. 1-8. Apparent velocity constant

(aimplified daca)

20

eration

---Page Break---

= 30+

2, Survey of radiation levels about x-Lrradtation equipaent

A survey of the radiation levels extetiag about the x-ray 476c

prior to ite routine use for Srradiations and Laces after

additional shielding was fouad necessary ia the vicinity of the sample

older. A Wuclear-Chicage model 2612 survey seter equipped with chin-

window probe for beta-counting vas used, and results found

ta Table 5,

re tated

?TABLE 5. RADIATION LEVELS ABOUT X-IRRADIATION EQUIPMENT

watitred

cation counts minh _pe-l

Gosionater table, right end, 0-24" above table 100-1500.

Gontometer table, left end, 0-24" above table 100-150 0.30.5

Gontoaster table, front, 0-24" above

te 100-150,

Contoneter table, in Line with

cond collimator

slit, 0-26" above table ?5000-20000

?At saaple holder, with single shield (3/16" Lead

sheet) at diffraction exystal 60,000 20

At sample holder, wich additional shteld at crystal

and over second collimator, horizontal 350 La

Ditto, vertical seasurenent . 1500 5

Ditto, with additional shielding over exyetal

and Firat collieator 1200 ?

To minimize the effects of scatter radiation striking ce controls

and irradiated sample from above, lead ehtelds 1/16" thick were placed

?Atop the compartaant in the sample holde:

---Page Break---

3. Preliminary experiments on che culture of 2. coli!

Te ts desired in the future to teat the effect of resocacce

Hation in in vivo systess. Im preparation for this work, the con

4ittons for culture of the unicellular bacteriua Zscherichta colt were

A culture of £. colt obtained from the U.?.8, Biology

Departeent vas inoculated in a sterile medivm containing 1000 al. water,

7.0 g. K2HPO4, 3.0

7FO4, 0.5 @ sodium eftrate .2120, 0.1 g.

MgS04.7820, 1.0 &. (Wity)7804, and 2.0 g. glucose, After 15 days groveh

aC 35°C ehe culture was stored at 5*C to inhibit further grovth. For

Purposes of counting the celle/al. of thts suspension, « 0.1 al, aliquot

vas diluted 1:100,

1000, 1:10,000, 1:100,000 and 1:10,000,000. A 0.1 fal. aliquot of each of chese dilutions was inoculated vith che tip of 4 pipet on peptone-beef extract ager (Salttnore Bacteriological Laboratories sutrient no, 01-125) in Petri dishes. Samples of 0.1 al. of

sterilized distilled vater were u

control. Counts

of visually-
observable colonies after 2-day ineubation at 35°C indicated 1:100,000 dilution would give most accurate plate counts within the standard

region of seasurement (30300 colonies per plate)

Repetition of eis
?snitial experiment using the pour plate modification yielded che data

tm Table 6, the precision betveen replicate plates 1a high, 40 that

ets technique should permit « rather sensitive measure of resonant

eneray effects

Carried our by wisw X. Vargas of U.P.R, Chemistry Departaune.

---Page Break---

=: |

TABLE 6. PLATE COUNT REPLICATION IN £, COLT CULTURING \

Semple Dilution Colonies/plate

a ast08 ws

az 1:105 us

a 1105 vs

ra ano? 168

BL 1208 a

22 anto® 2 |

a Astoé 2

? 1108 2

a ?control °

2 control 1

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8%)

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l. LETERATURE azrERENcES

Anon. Spee trometry Nomenclature, Anal. Chenistsy 34, 1852 (3961)

roe,

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for Measuring the Breakdown of iydrogen Peroxide by cater
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cane Of Zacherichta cold: A Zine Necaltooazyse", 3iocheats

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yelow 1060

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ew York.

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=a.

SHABACTERIZATION OF A YONOCHROMATIC HIGH INTENSITY VARIABLE WAVELENGTH X-RAY SOURCE IN THE 5-20 GV GION

Plorencio vizquez

AL summuay

The x-ray eatenion system utilized for the present cesonnce radiation studies has been characterized quantitatively a# to intensity and photon energy distribution, and second harmon{e contamination,

?The monochromatic x-ray beam resulting from crystal diffraction and colliaation vas analyzed horizontally serosa ite front for a) taeenaity distribution, utilizing 4 special moving slit devices and) photon eneray discribucion, using double diffraction by a second analyzer crystal.

Eecieation of che extent of

scond harmonic energies vas made from

2) absorption measurenants relying on the different sass absorption

coefficients at the first and second harmonic wavelengths and ») double

asgfraction we

jureaents in which photons with second harmonic energies

wore analyzed separately. Correction for percentage of reflection by

second harsonic energie also ws

decernined by the double <iftracto-

?caver sechod, Contasioation by higher harmonics vas shown to be consider=

able at higher operating voltages; monochromatic beans can be obtained

?only by proper selection of tube potential.

?The effects on che beam of positioning the various components of

che xcray system (Cube, diffraction crystal, tvo soller slits) were

deversined and

we system as selected hich provides niga uniformity of photon energy distribution,

Asa result, @ diffraction system vas developed which peraite irradsation wich photons of usiforn energy distribution (+ 50 a.v, in 6000 - 9000). Such selectivity of photon energies 18 not possible wich Fluorescent exisston systens.

---Page Break---

8. aNTaqDUCTION '

?To study the effect of K-ray irradiation on satcer, 1¢ is ispore-

oly the effects of the X-ray energy, che total doe transformations involved and, on that basis, a clear definition of the wechanien for production of change by irradiation.

In particular, we have concentrated on the study of effects produced as a function of X-ray energy. Resonant effects aay occur, and At 46 necessary to utilize an Xeray source in vhich photon energy can | bbe changed slovly and by appropriate steps.

We must also be concerned vith the setting of the irradiating beam ?at the proper energy and vith obtaining the auxious possible iatensity. once these prerequisites are present, ve should be able to deliver che | required dose in a reasonable tine. |

?Two other important conditions must be met here: {

2) Setting che dees as vide posible in order to avoid che probleas arising from very saul irradiated samples, and

») obtaining « beam of uniform energy and intensity 40 4s to

deliver to all particles in the sample, no matter their

potieion, « homogeneous external radiation field.

TE 4a dstfioult Co nase all the shove requirements simultaneously

Decause sone of then are contradictory, Yor exasple, the Sigher che

eneray resolution of the beam, the lover che intensity. Also, the

coatinuous range of energies {a only possible vich lover iacensicien

han eho.

jatlable when discrete en{asion X-ray energien are utilized,

?This problen has given rise to cwo different monochromatic irradiation

rechatques:

---Page Break---

0.

2) The us

of high intensities at discrete eaeray pointe.

?This method vas used by M,C. Atking and ¥, R. Clandiaaing..

9) The use of lover intensities, but vith almost contimous

soeray adjustaent. Tata {a the technique studied 1a che

present report.

ALL the work reported was done with a General Zlectric X-ray

dtefraction uate XR0-5 shomn in Pig. 1-1 located in the Laboratories

of the Departuant of Physics of the University of Puerto Rico at

Mayaguez. Neverthele

our results may be applied co aay ocher

statlar sachine,

---Page Break---

S-OK 2700 vosa9NAT FFG Ans-K opsv09Tm YesAVAD Jo géesBorONE ?T-IT ?ATA

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eu? el

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C. GENERAL PROFEATIES O7 X-RAY PRODUCTION AND 52//SACTION

Bach factor antering into che technique of rrsdiacion, and ce

relative spo:

1ce 9f each factor must be known, Brief deseriptions

of the ost inceresting problems involved in X-ray production and diffraction are included here,

1, xeray production

nen, in a X-ray cube, a voltage Vo 1s applied between the

target and the cathode, all electrons coming from the cathode dmpinge

fon the target in such a way chat photons are produced, with a distri-

bution of intensities

ad energies.

TE we plot the intensses

?sgainse the energies as shovm tn

Fig. 11-2, we get, in general, a continuous distribution of intensity starting ar point "A" wich peaks at definite energies appearing aftervarde,

inrensity

606.

fo tek ? wavenenaTn at

wwe enemy ev,

Pig, 1-2. xeeay em

---Page Break---

there are two ruasons for these two superinposed aistributions.

linen an elecevon wich energy

" supinges on carget ic aay lose ice

energy by seeps AE with the corresponding appearance of phocons with

energies according to the equation

or)

Thais {e che so-called Bremsstrablung effect. Since the maximum

energy of the electron ts (V.e) the maximum possible anergy of photons

ks given by:

My = B= Voce

fand is shown in point A, defined by the applied voltage Vo.

?The disceibution extends from cha Liatt hv = hv co hv =o and 8

Andependent of tae target material.

There is also the possibility chat che impinging electron aay eject

another electron from any orbit of the target atoms, As soon as the

vacancy 1s produced, an exterior orbital electron falls into it and

4 photon is produced with an energy given by the difference of the

energies of the tvo corresponding orbits, This last effect is known as the fluorescence effect and 18 the ri

a for the high intensity peaks

shown ie Pig. 11-2. For this case; we will get a particular peak if the |

impinging electron haz enough anargy to eject the electron of « definite }

orbit, The peak distribution depends

he electron orbite of cerget

Betog incerested in radiations with 4 contisuous change of

?energies around a definite value and vith constant int

ity, ve use

radiation from the continuous part of the X-ray spectra.

Let us summarize some properties of this part of che epectes,

L.+ The intensity in a narrow band of energy d(3¥) t4 pro 4 portional co the tube pover (V.4.)

---Page Break---

sae

?The total intensicy and the intensity in a sarcow band are

proportional co Z (atone auaber of he target)

?The Limte wavelength of the spectra at point

a

(ig. 11-2) 8

hace Ay to given in Angstrom unite ven ¥ Ge express votts.

ror a particular energy (h¥), according to the voltage applied,

the cube can,

At parmonice of the first energy, giv

wy

?hy, 3h¥, and 50 on.

5.+ As che maxiqun voltage applied to the tube is increased,

the spectra shife to che left as can be seen in Fig. 11-2,

?The peaks in ehis figure do not correspond to any specific

atom but are "repr

oe

Te can be seen that the useful

sones of irradiation are thote Labelled x, y, 2. Por thes

the intensity distributions are lost unform, vith maxteun

snvenasess

near che spectrua Lintt, as should be the case,

2. xeray diffraction

An Xray beas incident on a crystal is diffracted according to

Bragg's Law:

aA = 26 1

here n is an integer, A is the wavelength of diffracted x-rayt

interplanar spaciag berveen successive atomte planes tn che

© is the angle betve.

che atoate plane and both the incident and che

reflected beane,

?The deen obtained after diffraction {8 sonochromatic with che

following Linteations:

---Page Break---

-a-

Liv Ab che Aneideat angle 1s defined by slice with « definice

width che aiéfcacced beam also has 4 Dand of photon energies tv + Q ay

according to the incident angles @ +e,

2.+ The crystal produces dispersion which also causes energy

aitfraction,

3.+ If there are photons in the incident bean with energies ZV,

hv =-+ abv, they are also diffracted,

So Tn distracts

the intensity of the {ncident bean is strongly
reduced, The percentage diffracted 4s quite dependent on the type of
eryatal. Also, depending to the crystal, the percentage diffracted is

quite different for fundamental, eecond, third

harmonies, in such a
way chat sone crystals strongly reduce diffracted haronice,
5.+ The sragg equation Limits to 2d the maxima vavelength chat

can be measured wich « given erysta

since ein 0 cannot excead vaity.

5. A relation between the width of the beam and the Leagth of

the crystal is easily observed $a Pig. 11~

V.- the analyzer crystal, by its atomic structure, determines che

---Page Break---

the efftetency of che cxystal is nor very igh, due i can vary aut stantially from one crystal type t another. Selection of the exyscal a therefore an soportant decision,

?vo theories have been developed to explain analyzer crystal reflecting power. The first, developed by Darvin and quoted in Janes (8) ssounes that the crystal consists of perfect parallel planes of atoas

with equal spacing between planes

2 t

tp) Lt teos @

Tein? @

where &, is che reflecting pover of the "perfect" crystal

A 4s che wavelength diffracted

NX {s che susber of atoas per unit volume

1 FI is che atone scattering factor for the diftracted wave direction

20. | Pinas @ naxinun value of 2 for angle 20 = 0, and decreases

as 28 sncreass

a2, te che classical ?electron radiue??

2 rt adi

---Page Break---

comrsta, Leer

chu

?worm

Vaud

21g. 11-3, Cryatal Length

---Page Break---

structure in che crystal; the crystal consists of sual "crystallites",

ach SLightly skewed from the average direction for che group 4s a whole:

2242

Se" et Ty!

Where 4 15 che reflecting pover of the ?aosaic" crystal

4 is the Linear absorption coefficient of the crystal for x rays

engee,

---Page Break---

=e

Ns the under of unte etructural groups (cxy

ae 90

vate votuse

Ysa che scatcering factor for the ateustuctl group and ay be

wristen Tate oo M,

ace (4a the scateering factor of the "(" ato, and approaches
2 for small angles provided A is much shorter than the absorption vavelengths of the atom, The tera eM corrects for

the temperature of the crystal. Por our vork, thts factor may
bbe neglected,
Note that Rp contains the mumber of atone (or electrons) per unit voluae

sand che scattering factor £ , which 4s aleo proportional to 2, doveve

fm contains borh factors co the 2nd. pover. Thus, Re will always be much

Larger than Rp, In nature, most crystels are much closer co ?aosaic!!

tn structure,

?Adopting 2, calculations were made for eryata!

of Lithtus Pluori¢e,

Alusinus, Sodiun Chloride and Quarts, The corr

sponding values of Ra

LiF (200) = 1543 x 10-6

AL QM = 670 x 1078

Nac (200) - 360 x 10°6

Quarts (1011) 490 x 10°6 (experimental)

These indicace that, for a vavelength of 1,739 AY (K absorption edge of

1100), 4 Léthivm Fluoride exyetal 1s superior. In this case, p, the

Linear absorp

8 coefficient is

mnall, and the maber of acons per unit

volume Se bigs, This is sufficteat to compensate of the snaller muaber

of eiecteons per atom,

Novever, the diftracted energy {6 still oaly «

saall part of che incident energy.

---Page Break---

|. ANTENSITY AND ENERGY DISTRIBUTION

Most of the studies in chie

Jecion were made ith tuo special ce

vices built tn our

1. The first provides for detailed measurement

of intensity distetbution acros

the diffracted x-ray bean.

?The second device is used co analyze the photon energy content at alt points across the bet

Both instruments are described below.

1, Intensity distribution

[A photograph of the device used 4s shown in ig. IT-4 with the corresponding sketch in Fig. 11-5.

?The device replaces the normal support of the X-ray detector aesger counter vich @ evo section mechanism, The lover section is fixed to the goniometer protractor and the upper section carries the geiger counter in such a way a6 to provide a sliding motion normal to the X-ray bean, A lead screw moves the counter to the desired position, The window of the counter {2 covered with a shield containing « well-defined slit. Photons from the beam enter the counter only through ehis slit, ay slid= ing the window slit across the beam, and counting at different points, che

intensity distribution can be establish

TE the wideh of the window slit is auch le

chan che width of

the individual medium resolution (MR) soller slits ve can also gat che

intensity Aistrtbution aeons the individual aoller slits, as shovm in

Pig. 11-6, Ay, Here, each eaxtoua corresponds to che center of a soller

slit, If che width of che window lie {8 atmtlar co chat of the MR soller

slits, ve obcain a sean intensity distribution across the beam Like that

draw ta ig. 116, By. Figs 11H

+ Ch, chowe, at che bottom, # crossection

of the goller slis

yably and, at the cop, a file negative caken at the

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3708 ROULMTOSA HOM

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Fig. 11-6, Intensity diatetbution

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sewple posicion, 1? can be seen how ene shadows in che film cot:

wich the NG sotle

Fig.

+ Ags By a0d Cp, show similar diagrams for the case of coe

high resolution soller slit system. Here, 19 saxina occur, corresponding

to 19 seller elite, The MR soller containe only 9 corresponding elite,

?me incensity of the vhole beam can be obtained by int

pacing under

the curves, If the slit has a widch w, the distance between two co

secutive counting pointe 4s b, and the seasur

intensities are given by

Sty S2y -*> Say then the tocal intensity ia:

esegb ES

sade te

Ye should be noted thar ve obtained the same results, within @ 0.3%

Asefeconce with the narrower elite

with the vider slita, when correct=

tons for tne dead tine of the counter vere made, Using reading periods

of 10 seconds duration, the intensity of the whole bean vas obtained with

good statistical accuracy.

[A similar procedure was used in comparing intensities for the

azeterone cases.

2. Double diffraction - Photon energy analysis.

In order co determine energy distribution of the

an, a special

crystal analyzer device vas us

It {e shown in Fig. 11-7 by # photo-

eaph, and an Pig. 11-8 by a sketch.

?The device consis " vageh can tide ia

direerion

2 (avesal) across the beam, A rotating ezyscal holder,

hich is an aecessucy cormally used in spectrometer techniques, 48 mounted

fon top of the place, After Fixing the bi

+ the erystal is soved Late

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bean by means of & Lead seray, provising

exact axial posicion

nocion in direction ?a-a", Precise setcing of the corresponding acgie desized ts obtained from the rotary table of the crystal older. A support brackee holds 4 shield and s1i¢ in such a wey that photons nich arrive at the second crystal have coae from the firet exystal without intervening scatter.

A councer se mounced on the plate, in such a way as to permit rotation around che exystal axis, It is turned by means of « circular

steel strip and a screw, a6 shove in Fig. 11-8, Another slit and shield

are attached to the counter so a9 fo block scattered radiation from the

firse crystal

|

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MEDIUM RESOLUTION SOLLER SUT

{isinm BETWEEN Mares)

ig RESOLUTION SoLLER SUIT

?hemm SoT WEEN poaves]

SUIT ATTACHED To_swiELO

MELO ATTACHED To LATE ?A?

Double diffraction device

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4. CHAWACTERISHICS OF x-nAY 232

Much of ene cesearch 1a this study has been done by irradiating at

energies close to the K adsorption edge of @ particulac atoate element,

Once the x

xy eneray around which ve are to {eradiate is decided, che

ext probles {s selection of che appropriate X-ray tube anode. Some

of the ericeria for che

lection are comented on briefly below.

1, Selection of anode material

?The Migher che atosic aunber of the anode the higher the 4 ation intensity, ac any fixed values of anode voltage asd curcent. vows

ever, since we wien co adjust the eneray of the radiation seaching che material under scucy, che output should change stovly as 4 function of

energy. 7

anode seterial should be free of strong sharp eatesion

Lines in the energy region under study. (See Pig. 11-2)

2. operating voltage

A precise determination of che anode voltage to be used can sade only after the permissible second harmonic contamination has been

esrablished. As a scarting point, we can apply a voltage gividg twice

?he minima photon energy desired, since up to this value, no second

harsonie is generated for che energies under study, ?this 960

mie

Aiscussed in derati io # later section,

3. Operating current

Once che voltage and the anode material have been set

ncted, the

?stximun operatiog current is determined on che basis of either the

?aximun allowable anode-pover dissipation or the maxiaun filanent

current persitted, These vary with the tube type. Our present equipest allovs 4p to $0 ma, anode current. We plan to install aew 100 aa

equipment soon,

---Page Break---

4, oucput patceen and collimation

The next scep is che measurement of the output pattern, wares

in turn determines the arrangenent of collimators in the diffraction

unit, Ta the usual digtraction study, small areas and narrow slic

openings are used to obtain igh epatial resolution, in emission

studies, nacrow slit systems are used £0 resolve eaission Lines. How

ever, ovr objective 1¢ maximum flux comensura?

with good enecgy ce

resolution. wa have used sediua resolution sotler slits before the

crystal and a high cesolution

st after the crystal, The slits are

fe in the vertical position, Ths arrangement yields cae saxinun

?useful output. The sonochromaticity of che output was

cked using

double diivaction az described above.

5. Anode shape and orientation

?The importance of the atoaie munber of the anode material ws

mentioned earlier. We will nov consider the fora of the anode surfac

The 6:

sal Electric Ch-7 tube has a target vbich {s 0.8 x 15 am, and

windows which are disposed a8 thon in Fig. 11-9, The surface of the

target is perpendicular to the longitudinal axis of che cube, 7ig. 11-9

also shovs che eaission pattern; caken with two files at

ores

position of the first goller slic, We found chat wich wistow 3 che

dollar was act well filled, but with windows A and ¢, che results were

much {aproved,

?The General Electric AEC S0-T tube hes a target vaieh is 5x5 am.

in projection and foras an angle of 70 with the cube axis, This angle

16 important fom che viewpoint of getting higaer intensities, a question

which Vill be considered in the next section, The effect of # large

projected aces across ene width of the soller slit is to proaote

vatfors sacensai

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CA-7 TUBE oh

(2, TUNE WNDOWS! dy

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sea SECTION tesJ

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AEG-SOT TUBE Cr 1

VU ????

Std

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?taRser

MW PaazECTION

2), Target emission patterns

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eo

| ANALYSIS FOR HARMONICS IN XvAAY 3zAH,

As sas seated earlier, the higher che voltage applied to the cuve, che higher che incensity obtained. However, chere exists a potential Vg sbove vaich the beam contains higher harmonics, which are not want

ed.

faust, cherefore, determine the maxioum volcage that can be applied, caicing into account the percentage of second harmonte which can be coleraced

Jas Were used to find the percentage of second haraonte

present. Toe fizet one ie bases vu the different mass absorption co-

efticiencs of an absorber for two 4

ffeccue waveleagehs. The second ie

based on double if fraction.

Le Absorpeion method

Let us suppose that the diffraction unit ta arranged for irradi~

ation as shows a Fig, 11-10.

?the probles 4s co find at a point A, where the sample is to be

placed, the percentage of the second harmonic as 4 function of volcag

Sioce che position of che counter 18 close co potne A, the tntansicy

of the seus Ls che sane as EAC received by the counter.

hence 1 T, is the intensity a# measured by the covacer, thea

@ Gry + ws

where of and @ are the efficienet

of the counter for energies ¥ and

2 oocaknes fom

we put sa piace "B", an absorber with a Large ¢ifference in

the absozption coefficient for the energies ¥ and 2¥, and in which

energy ¥ 16 pace strongly absorbed, then the intensity ax given by tha

= EDM cay ED ay

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= 56

te vateh:

. and (8

D Dy ed 8),

?energies v and 2¥

sre the mase absorption coeéfictente for

>) p i4 absorber density

© 4 4s sbeorber entekaces

rom equations (1) and (2) we get

&, s

apy Aaa

tyes bo. F

. ?

a

- BM

Thy

With d fixed, values of che factors A and B may be calcula:

1d of tables of monochromatic absorption coefficients or by ueasure-

seent, using the X-ray unit,

If divece measurements a:

?made for a given frequency, ¥ , the X-ray

unit mst be operated initially at a voltage lover than Vo, at vhich the

second haronie will appear,

The intensities T and To, with and without the absorber

od to cheat,

T/ig-3 Pe Pe

Zo obtata the second t4ctor, the apectromtter angle 18 set for

frequency 2 and a voltage above Vp but Less than 2p t4 uses

Once ve have the evo £4ctore seeded and the ot, A efficiencies of

fhe counter, determinations are made of 12/11 at ditterent veltages oo

4s to obtain the percentay

of second harmonic present,

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Pig. 11-10, Component orientation for second harnonie studies

(abeorption method)

Ss

cbectate Te PROTORS SEN EER +

Dnetm ee wm? ae

x New

poomon ron Fp ane

Pig. Lieli, Componant orientation for second aarmonic scudi

(double diffraction setso3)

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=

2, Double diffraction

Por this uethod, the proper arrangement of the auin diffraction

oie and che added double diffraction unit are

shown in Pig. II-L1.

Let us suppose Line A-A to correspond to the front surface of

?che sample, and let us select with « slit in point 4 4 fraction of the

total beam which we are to anelyze, Also, let us suypote the mixed beam

to be composed of intensities I, and 1py, Placing « second cryatal with

appropriate angle @p(v) to diftract energy ¥, and seasuring 1,(v) inten:

?stey at the counter, we have

@ rH -?e

er(4e0 (¥)

where

Nez) 4 che cryatal percentage of reflection and

Aco) 1s tha counter efficiency

In the

we way, with the crystal in position G2 (24) ve get

= nen

@ 1an+ Bea,

Hence the pere

¢ of the second harwonte present {9 given by

©) LY = HAMA Ao

TO) HO meq

Por precision measurenents, {t is necessary to cake into account

4teferent absorption by air of photons with energies Y and 2Y between

Point A and counter. However, by computation the above vas found to

of the short distance between the crystal end

the counter,

Another inportant correction mst be made for the second haraonte

photons measured in 1 (¥). The correct equation shovid be

TDD gM + TrA'er2MA'eo (20

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ce YM erin ede ps oN cen ?#

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fig ? SAM wR nti en fon yh: wt

ripe Rd oe pi eg ag

cto .

Fe wm Seb as oe oe deg ee Ste

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sa sore accurate in chat 1¢ accouate for the value of 14.22 KV2, vas

Ae the starcing point for second haruoate production, The curves were caken with the crystal set co diffract 7.111 Kev enersy ptotons, Both

curves change slope at about 21 KVP and 28.5 KVP, vbich indicates the

\co of che chicd and fourth harmonies at these operating

appears

voltages.

Both curves are similar at the lower voltages, ence both

swethods crosa-check each other. For the higher voltages it is neces

to use gta. 4 instead of Bata, 1.

In the present problem vhere ve were interested in avoiding all

second harmonies, the voltage 14 KVP vas chosen for all irradiations.

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G. ORLENTATION OF X-RAY TUBE, PROTRACTOR, AND SOLLER SLITS POR OPTENM INTENSITY AND RESOLUTION

In the following sections the relative positions of che spectro mater elements are studied with the general objective of gettiag the

highest possible uniform distribution of intensity. Particular at=

tention is given to obtaining maximin energy resolution.

Analysis of che general procedure i# b+

1 made by following the

?steps used in the particular case of irradiating at the XK absorption

of tron.

1, Tube position

The X-ray tubs must be positioned correctly co obtain « eye

metrical intensity dtatetbution,

To obta:

symmetrical dtatribution along the hetght of the

soller slit, 4¢ 9 necessary to place the tube vith carget surface

parallel to the rotation axis of the crystal. The centerling of che soller mist be at the sane level as the center of the carget. This

adjustment As made vith sereve 4, My P, @ and with the levelling acreve

AL By 0, a8 shown fn Pig. 11-13.

?The cube position my be changed, in addition, by rocati

4+ few degrees around an axia perpendicular to the plane of the figure.

Thies last possibility {9 Limtee

by the tube holder walls and by the

insulation of the high cension cable, The advantage gained ia pute

ting the cube in such # skewed position 42 a slightly Larger angle

etween the carget surface and beam center Line, thus increasing the

?flux tnto the sotler colliaator. When the target plane of the cube ta

Perpendicular to che tube axta, ehis device aay be useful, There are

other cechaiques

16 adjustments for this type of tube, described ta

Later sections, voich also may be used co tneres

eseful ourpuc.

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THE TABLE

Nook FixeD AT TO*

FROM TUBE AXIS

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ROTATING AROUND ©

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Fines To Tae TABLE

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ig. 11-13, Tube and diffraction wale vith suall procractor

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me

In che case of cube with che carget plane ac an angle of soous =<,

che adescionel angle obtained ia the stew position is insigatétcacc.

The C. £ Aeeay cube CA-7 haw a Flat anode. This tube is ordiparity weed for crystal structure diffraction studies. The tube with he anode ac 70° is che Machlett AEG 50-T, which a ordinarily used to excite characteristic radiation in sampler to be analyzed. Usually, chio cube is placed 6o chat the radiation passes dovmuard into « box containing the specizen to be analyzed. Yor our vork, the tube ia rotated 90° along the cube axis to obtain aide d{echarge, a# shown in Pig. 11-13.

Anccher adjuscment available {n tube position 4s placement along the cube axis. The tube is moved forvard in small ?steps until the output pactern reaching the crystal posttion ts symmetrical around the crystal axis, This dacemmination may be made by survey with the getger counter, covered by a aask with a chin slic, Saali proteactor posteton

When using a CA? tube ia the ARD-5 diffraction uate, it is

possible to change the angle between the surface of the target and the Akrection of che beam by rotating the base plate around point A, a# shove ta Pig. 1-14,

?The omission intensity pattern of the CA-7 cube {a siaitar to

that shown in Fig. 11-14, If we need a bean angle of "" degrees, to

FALL ene wideh of che soller slit, 1¢ is better co obtain the be

the higher angie so chat higher intensity 12, 1'2, and deccer distes-Ducion are odcained, Yor our problem the Aigher angle of the snail

protractor was always used,

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as

tse of aciler slice

?The 100-5 Ls equipped with soller slic collimators ta waaca che plates are stacked one above the other, with each plate parallel to the horizontal plane. As supplied by the manufacturer, che soller site are designed to mininize vertical dispersion of the bess, Resolution

?

ermined by separate vertical slit systems, which allow for beans with angular epread of 3°, 1%, 0.4%, ete. The small angle slit eysten transnite 4 such sealer number of photons, but spatial resolution is high, This eysten ie used with characteristic emission radiation from

4 Che? tube co study, for example, powder diffraction or crystal lattice

spacing. .

Por our problem, che need is to direct a relatively parallel bess of white radiation at an analyzer crystal, and then to accept a relarively monochromatic beam from that crystal co be directed against a target, This latcer beam aay be fairly large, about 0.5.em square, but tt should be a8 sonochromatic possible, commensurate with intensity.

It was decided co try soller slit systems co provide che initial parallel dean and also to collinace the sonochronatic bean. The soller alde system provides in effect saveral parallel narrow slits. Mediu ?and high resolution (WR and HR) soller slits were available with diweensions as shown in Fig. TT-15.

?As indicated previously, provision is made by che manufacturer only for chair use with plates horizontal. In our work, chay were used

?with the places vertical, Por test purposes they were fixed to the

support, woich hae positive alignment grooves, wich masking cape wrapped

around the oiler sie cage and the support. The positions occupied by

the soller ciies and cha arrangement used for seasucing che energy

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nouns ¥-¥

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are

sesolucion ace ssowa ia Fa. 11-15,

che arrangesente tested, the output from each inci

vidual slic of che soller slic system dn the ausber 2 position vas measured, obtaining intensity as 4 function of photot

sults for cosbination of the High Resolution alit system in position

2B, near the target, and the Mediun Resolution slit system in position

LA, near che cube vindow, are shove in Fig, 11-16.

Pive igterent soller slit system arrangesente were vested in

chie way, with che results

shows te Pige. 11-17 and 11-18, The

horizontal straight Lines on che

ey scale indicate spread betveen

al

hk satensity points.

ou these scudies, 4¢ wae apparent that for the diferent

combinations, che cotal cransuitted intensity varied ao sore thay «bout.

2 per cent. Movever, there vere noticeable differences in the photon

energy éiscribution wichin the beam, vith the coabination used ia

eat wouls produce the saallest energy epread.

Mecuntng to Fig. IT-15, we find chat for the HR soller slit

ayatea in che 2B position, the dispersion angle is:

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can ps

= 00566 p rad. = ,00546 x 182 y 3

LE we ansume chac che system 10 adjusts

fo accept 7.11 Kays photons

for the esccoss (gesting) position, the anttetpated vavelenath (and

verag) spcens any bo cantly eetinated.

Ay = 24 con 9+ 0) + 28 con (51.2.4 0) © 1.763 Mor 7.111 Lav,

2 4 Ad cus (6 +A) = 2d cow (51.20 + 0.3) = 1.743 x 262251

az (@ +A) ¢ 3) = 1.7463 ae

21.752 at 0 7.157 Kev

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ticipated spread ia thus about +46 e.v. The experienc al venules fn Fig. I1-16 show ao more than 90

ve spread to bali

saaxtuum for any one slit.

Finally, for the case in Fig. 11-16, the energy distribution for

the whole beam vas obtained by graphical ints

ration. The beam intensity

44 function of photon energy ie repren

ed im Pig. 1I-19a, This te

the result for one slit. In Fig. II-19b, the beam intensity as a function of it6 position tn che beam cross section 18 shown, Combining the {nformtion in these curves for each seasurenent position in the dean crose section, we obtain Fig. 11-20, the energy distribution of

the photons in the vhole beaa, The energy resolution ts quite good.

?The spread to the half intensity point is only +35 ev. within + 50 fv, 81.6 per cent of all che energy {4 found, Thus, meaningful trradiation

at {ntervals of 100 e.v. can be carried out.

?The interval

jeveen the K 1 ealssion lines for elenents ?round tron is about 500 e.v, This 18 important in comparing the use

of the ?sonochromatic" radiation available from « crystal diffraction ee entasion. The closest

unit with ehar available by use of fluores

fenission Line to the K absorption edge of {ron at 7.111 Kev fe the

cobalt ey Line a? 6.930 Xev, If the effect deing sought has a etre

Gependence on energy, and is associated with the absorption edge, it

could easily be issed if only fluorescence radiation were used.

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Pig. 11-16. tuergy dteteibution tn different potats across the bean

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ion and intensity distribution across the beam

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Tacgec angsiar intensity distribution ....

Soller site positions «2...

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Sesolution and intensity distribution across cae

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Yiel9, Tosa ancensicy distribution aero

ansfay distribution at point A= 2

11-20, 2nargy Gistribution of photons ia the vhole Deum

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