

PRNC062

PRNG 62

PUERTO RICO NUCLEAR CENTER

Progress Report

RADIATION CHEMISTRY AND PHOTOCHEMISTRY
OF AQUEOUS SOLUTIONS OF OXYANIONS

FINANCED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT
NO. AT (40-1)-1899 FOR U. S. ATOMIC ENERGY COMMISSION

---Page Break---

PUERTO RICO NUCLEAR CENTER

Progress Report of the Project

RADIATION CHEMISTRY AND PHOTOCHEMISTRY OF AQUEOUS SOLUTIONS OF OXYANIONS

by

Malcolm Dantels

April, 1965

---Page Break---

RADIATION CHEMISTRY AND PHOTOCHEMISTRY OF AQUEOUS SOLUTIONS OF OXALATES

Frogace Report

April, 1965

The work supported by A.E.C, Division of Biology and Medicine falls

Into three sections.

A) Photolysis of H₂O₂ - previously reported work has been considerably extended and quantitatively analysed.

B) Radiolysis of M₂SO₄ solutions. This work has been commenced
in this year.

C) Luminescence studies on D.N.A. constituents. Though not included in the proposal, this work has been carried out on equipment purchased from A.E.C. funds, Preliminary reports are appended. This work was presented, at Fourth International Congress of Photobiology, and the Ninth Annual Meeting of Biophysical Society.

A. M₂SO₄ Radiolysis

The previously reported non-linear rates of photolysis found in the

range pil 26 have not been analysed quantitatively. Careful experiments at Long irradiation times have shown that the rate of nitrite formation eventually decreases to a steady value. As is shown in fig. 3 where a limiting rate $R_y = 0.60 \text{ pM/min.}$ is indicated: Using the fact it is then found that there is a linear relationship between the reciprocal of the rate of photolysis and the duration of photolysis (fig. 2). Extrapolation to zero time then allows determination of the true initial rate.

---Page Break---

?=

In this way all previous non-linear data obtained in studying the

effect of a) nitrate concentration; b) nitrite; c) arsenite scavenging

can be decomposed into two linear terms; 1) an initial rate (usually high)

which depends on the concentration of nitrate, or scavengers; 2) a final

low rate which is independent of these factors. The analysis of data meet

4 steps model on which all subsequent discussion is based. It is envisaged

that the initial rate is due to the escape of reactive species from the "solvent

cage" followed by their random diffusion and reaction with homogeneously

Attributed scavengers and inhibitors. The non-scavengable final rate is interpreted as the probability of decomposition or recombination within the solvent cage?

Consequently the overall course of photolysis in neutral and acid solutions is represented by:

a) $\ln(I_0/I) = k_f t$, where $f(t)$ = time = dependent

See eq. 1

b) ϕ_{P} quantum yield

Equation 1 may be written in the form:

$\ln(I_0/I) = k_f t$

Equation 1

Effect of Nitrate

When this analysis was carried out on the previously reported,

experiment

results for the effect of nitrate concentration on rate of photolysis, it was found that the initial rate is only slightly dependent on nitrate concentration (Fig. 3) and that the major effect is on the magnitude of the constant describing

?the non-linearity (fig. 4). Clearly, nitrate has a strong inhibiting effect on the secondary reactions during photolysis.

?Although the experimental results may be written:

$$P(t) = P_0 + 45, [O_3]$$

---Page Break---

Se

Stochastic considerations suggest that the relationship may have a different form which the magnitude of the variation is inadequate to demonstrate.

?The effect on secondary reactions may be described by the equation:

se a

Effect of Nitrate Concentration: Inhibition

?The slope of the concentration-time curve suggested that nitrate,

@ product of photolysis, was

inhibitor, To test this the

previously rer:

Photeiynos of af

wlth nitrite added prior to

trradiation have been extended amt quantitatively analyzed. Some experinenta

results are chova in fig. 5

?ant the overall picture is summarised in fig. 6. It ean be seen that the

initial rate 1s rapidly decreased by call concentrations of nitrate without

appreciably changing the final steady rate (or the rate constant), and follows

unt

\$a»

Acct cen ue etn fenton tiie th rah

roves ef tags eo cor tanta of the tn ears te

ceouatono tin

suse seu

Fog oe evisy report wrt #8 an fd tn i

snstry pote sue wage aon to Op a

ant sma pun oa) ty be ote ra

ct tmz) vita arsenite concentration ino a form tye af competition

---Page Break---

Kinetics, fig. 7,

a =

oe fs ta)

Adastion of Hydrogen Peroxsce

Awaentte being knowa to be a ccod soavenger for OM radieal, st bas

been decided to try othe inom scavenvere for OH. Of these, tvirogen peroxide

4s of interest in that its behavior might cive some Might on the non-fomation

of H.O, im nitrate photolyste.

Addition of H₂O, leads to en apparent increase in the rate of

Photolynie, but tvo feature are note worthy.

First, when the non-linear concentration curves are analysed st 1

found that the initial rate is only slightly higher than in the absence of

Up, » and the major effect is on the rate constant which decreases by a factor

of 10. Second, in contrast to arsenite, for which scavenging is complete at

Limit, H_p , begins to have an effect at this concentration and $4e$ really only

effective at concentrations 10-20 μ M.

It is inferred that $4,0$, does not effectively compete in the primary

radical reactions, and thus its main role is competing with 70 in the

secondary reactions.

Effect of Ethanol

The addition of ethanol leads to an increase in initial rate at

low concentrations. Acetaldehyde is formed at the same rate as nitrite

together with very low yields of H_p , «

Initial rates as a function of ethanol concentration are shown in 4a

fig. 8. (the curve is calculated from the relation:

---Page Break---

a eH, its

Geom ?on * Poo

Xe ts interred chat ethanol, in conte 20 HLP, , te about as

etteotive & scavenger as aztontte aut Gi . However tare ween to be seus

quantitative differences re?!

tea tn the Limiting quontum yields.

---Page Break---

|

i

|

|

|

oF oe oz 1

To

NI Saw

vstyayd fo veymp fo uoyou\$ © so aishtaoyd fo apes

quareddy

?Ton,

oz

ay

---Page Break---

??

Oe

---Page Break---

sysXjezes

fo 2he4 yes vo valouuaoues? Kent ? fo ?ese ?6-9/4

---Page Break---

Sp npn

os oF ot W [ONJoz ot

9

fo

?9 Wd 40, veyayeeoves

Bnyw wo ywesucr aye prywiedke? fo suepuedeg ?O14

---Page Break---

oor o oot os

? - OI Spon]

OE

9 yd yo sphyzoyd

we voyripest oy sold poppe gyi4yn fo g7effg 19 Old

---Page Break---

ayale yor siskjeyoyd fo sazos

wonpesyusoue>

fo vei, +9 ?91g

---Page Break---

o

he

OF

ywery fo pelle ?L-org

-o|°?

a

---Page Break---

eo WY {[HO33] zo ro

worpoyesruey jouoys7

wm sskppoyg fo yey peywy fo vorpersny

?891d

oT

oZ

of

---Page Break---

B. Game Raltotysis

Work caries oF

A study of chs > gana: vactsu' > induce* reactions occurring tn

aqueous sodium nitrats colwiicos a camerdy Bets cursed cat. To date

?the investigation has

Mattes vo tor dvvemstnaticn of G valueu of 10,

and H03 fn neutral cerated celutione waove nitrate censoatrations mänge

ey 2,

?free 1074ç to At. A done rate of evans 2 x 20? ev. Liter?. min? has been
caployed for al of the vork 9 date. Yeluee for G(H0%) lave ulso been

determined for oxygen-caturated solutions. A typical yield-dose eurve for

WO], formtion is show in Fig, 1 The shape of the curve at Lov dot

not been explained, however, it has been observed that the adastion of

5 mtoro moles yer liter of Nel, results in a linear rate of formation of

nitrite dn the sane dose range. The straight line whose slope gives watt

4s considered to be the initial rate of formtion of MOD is tMustrated

by the broken Line.

Fig. 2 shows the initial $G(\cdot, 0)$ values obtained by irradiating various concentrations of neutral aerated sodium nitrate solutions. A similar Plot of $G(007)$ in neutral aerated oxygenated solutions is given in Fig. 3+

Although the work is still in its initial stages, some interesting

points can be mentioned. The yield drops very rapidly with increasing

---Page Break---

ba

nitrate concentration to the molecular yield value of 0.7 where G remains essentially constant until relatively high nitrate concentrations are reached. The decrease in $G(\cdot, 0)$ in this region is probably due to the decrease in the water electron fraction. The effect of oxygen is to lower the nitrite yield. This can be attributed to the competition between oxygen and nitrate for the solvated electron.

Values for k_1 and k_2 have been determined by other workers and are:

2

20

$= 1 \text{ s}^{-1} \times 2 \text{ a? see?}$

1.9×10

20 el geet

kein x wl ag

ring ratio yy, % 2

Mike predicts that at atrrate concentrations ehove O.IM O(W) should be

tially independent of O_2 concentration, ?The fact that oxygen lovers

?the nitrite yield even at 5M Mal, indtcates that this explanation is not

?the complete one.

The first plateau in the Yoorsted" curve in Fig. 5 and the deflection

of the "oxygenated" curve occur at about the same G(%) indicating that this

O represents a total electron scavenger by 2O_2 . the increase in 60% at

higher nitrate concentrations can be considered in part to be due to a direct

action effect.

Work 1s nov i progress to devemsine (1) and G(H,0,) for douerated

solutions and to investigite the effects of intensity, pif and radical scavengers.

---Page Break---

I

(2-01

ys

%) 3

a soa

oN]

z

L

)

(nw?

---Page Break---

a af

oa

%) 38

EFT

AG

i

---Page Break---

(e171 /ern) [foNnen]

01 yt OI

5 , or

Con) 9

---Page Break---

Se FT III

x (Gavi fern) Eoney]

---Page Break---

©, Luminescence Studies on

8) Gytostne

?he luminescence behavior of the purines which coeur naturslly tn

Constituent

Pallas 48 veld recognized (Duggan et, a. 1957) and nas deen the subject of considerable research (Walass 1963, Drobntk 1964). The phosphorescence of

Pullshs (Bertchn end loesberg 2963) nas been attributed solely to the adentne so esanine restover, Eyrimtaines and their derivatives (with the exception of thymine) have been reported not to fluorescence et x11 (Udenfriend 1962).

However, folLoving cur easier work on the photochentetry of cytoete (Daniels and Grimtson 1964) ve nov vish to report the fluorescence of aqueous. felutions of cytosine at room teaperature,

Cytosine (Calbicchem A Grade) in triple distilled water gave fluorescence ?spectra such as that shown in Figure 1. the ?Spectra (emission and excitation) wre otatoed with an Aninso Syactrophotofucriaeter ecpled to a Duet type eM Cectioscope and recorded cn an Hlecteoutnetrunent le recorder. Apart from the first ard second order Rayleigh and Rasan seattoring a broad entssten with maximum at 380m 49 seen; exettation moctous ?at 235mu (wavelength une corrected).

Te il Aezendence of the intensity of the exteston ab 56Cqu has toon tersintt for 50° yentn (ee vray tn aon ogo

TOI env, according to need), using the fluorophotometer. Results are shown
16. 2 (or comparison of instrument sensitivities, we get for 20y/ad quinine
in OL RHO, at Mom, @ relative fluorescence intensity of 75).

---Page Break---

Luminescence Studies on

4) Gytostne

?The luminescence behavior of the purines which occur naturally in

JA. Constituent

DNA is well recognized (Duggan et. al. 1957) and has been the subject of

considerable research (Valaas 1963, Drobak 1964). The phosphorescence of

DNA. (Bersohn and Isenberg 1963) has been attributed solely to the adenine

and guanine residues, pyrimidines and thiole derivatives (with the exception

of thymine) have been reported not to fluoresce et alii (Udenfriend 1962).

However, following our earlier work on the photochemistry of cytosine

(Dantels and Grimston 1964) we now wish to report the fluorescence of aqueous solutions of cytosine at room temperature,

Cytosine (Calbiochem A Grade) in triple distilled water gave fluorescence spectra such as that shown in Figure 1. The spectra (emission and excitation) were obtained with an Aminco Spectrophotofluorimeter equipped with a hydrogen lamp and a monochromator and recorded on an Electro-Technic Xe¹³⁵ recorder. A space from the first and second order Rayleigh end scatterings « broad emission with maximum at 360m is seen; excitation maximum is at 295m (wavelength uncorrected),

The dependence of the intensity of the emission at 360m has been

measured for 5 x 10⁻⁵ M cytosine (and varies by a factor of 10

with NaOH only, according to need), using the microphotometer. Results are shown

in Fig. 2 (For comparison of instrument sensitivities, we get for 10⁻⁵/m² quinine 40.1 1H, at 360m, « relative fluorescence intensity of 7),

---Page Break---

It can be seen that

he

wreecence intensity shows a parked

dependence on pH can clearly be correlated with ionization of the cytosine

(Fig. 4.5) (Changatt and Davidson 1950). This pH variation confirms

our belief that the fluorescence is truly that of the cytosine and not
due to some adventitious impurity.

Further, we have investigated the concentration dependence of the

emission at various pHs, When corrected for

vial Light absorption, a1

curves show strong concentration quenching. Typical curves at λ_1 and λ_2 are shown in Figs. 3 and,

Analysis of this data shows that the quenching does not follow a simple

Stern-Volmer relation:

one in which cytosine

however the curves for #3

in the non-ionic form can be analyzed into two components and the first is quenched

by a second-order reaction $k_2 = 2.35 \times 10^8 \text{ s}^{-1} \text{ M}^{-1}$ and $k_1 = 2.06 \times 10^8 \text{ s}^{-1} \text{ M}^{-1}$

where $k_1 = k_2$ where $k_2 = 9.0 \times 10^8 \text{ s}^{-1} \text{ M}^{-1}$

(Fig. 5) and the second is quenched by $k_1 = 8.0 \times 10^8 \text{ s}^{-1} \text{ M}^{-1}$

*

at pIB.E (Fig.

Me nature of the fluor

Jcing species and the mechaniens involved in

quenching are the subject of continuing ver.

his work is part of a program euported tn part by National Institutes
of Health Grant AYOSi20 and oy A.E.C. Div. of Biology and Medicine,

?We thank Mrs. wilde Reedn de Sandoval for technical assistance.

References

Berechn R. and Taenvers I, = Bivenen. Biophys. Res. Commun 25,205 (1963)

Bere A Mind Davidten Juli, > The nucleic Acids Vol. 5 Academic Press, -¥. (1960)

saarete i and Grinison A. - Dicenen. Biophys. Res, Commun, 1964 (in press)

Drobaik J, > personal communication 1954

creme s., Rowan Kei.) Brodie 32 and Usenfriend, S. - Arch, Biochem, Biophys, 68.1 (1957)

Walass, E. ~ Acta Chem. Scand, 17.462 (1963)

---Page Break---

Captions for Figures 1-6

Fig, 1 ~ Fluorescence emission spectrum of cytosine

Fig, 2- pH dependence of the fluorescence emission at 38°C

Fig. 3 ~ Corrected relative intensity of end

cytosine concentration at pH 6.8

on as function of

Fig. 4 - Corrected relative intensity of emission as function of cytosine concentration at pH 1.8

Fig. 5 - Second-order contribution to fluorescence quenching at pH 6.8

Fig. 6 - Functional dependence of fluorescence quenching at pH 6.8

---Page Break---

100

2 2 2

$^{\circ} + a$

AJISNBLNI SAILV13a

400 500 600

WAVELENGTH MILLIMICRONS

300

---Page Break---

,

[

i

1

1

i

,

,

1

pK.

z

3

=

a

a

5

a

o

<

ABSORPTION

8 g 8

3° ° °

ALISNILNI JINIISIVONTA JAILV IIE

0.08

2

---Page Break---

AAISNZLNI JONZDS3NONIs BAILV132N

CYTOSINE mM

---Page Break---

AAISN3LNI 3IN3ISINONTS FALLVISY

[cytosine] mm

---Page Break---

(wu [2a9])

ov

---Page Break---

wu [aNISOLAD]

oz o1 °

---Page Break---

-10-

ints obi Ustnercerer aiesertse

we nave previously resorted({}) the shotedeanination by 2537°A radiation

of eytentne sn equucus solution and have dram atesticn to ite possible elgnttt-

cance in photonsotegy.

Investigation of Kinetics of this desnination nas Led to the folleving

results which, taken in conjunction with tae previously describea(®) Luntnescence

behavior, allow clear conclusions to be drawn concerning the nature and behavior of the excited state of cytosine in aqueous solution.

The denaturation is linear with absorbed intensity up to considerable percentage change, (Fig. 1) and hence represents a major, primary reaction. As can be seen from Fig. 1, the reaction is strongly dependent on concentration of cytosine, and quantum yields over a twentyfold range of concentration are shown in Fig. 2. Graphical analysis shows the reciprocal of the quantum yield to be a linear function of the reciprocal of the cytosine concentration (Fig. 3) and gives the result,

$$= 6.2220 \% (2)$$

1 + 2

Fim) % ϕ

This relationship is very important, because the form of it indicates a simple

?competition between deactivation of the chemically active excited state and reaction of it with a further molecule of cytosine. Furthermore, if it is supposed that deactivation is accompanied by emission of luminescence, then we can quantitatively account for both the Luminescence and photochemical phenomena by the

following mechanism.

ee

presented at the Fourth International Photobiological Congress, Oxford, England, July, 1951.

---Page Break---

We propose that an excited state

© hy cw

which can luminesce in reverting to ground state by a first order process,

sav

of cytosine

can react with another cytosine molecule to give a transient excited dimer

%

are at eq

see OF

and that this can be deactivated by further collision with cytosine to give

stable products, among them among

sy

of + Ly Products (mis)

Treatment of this mechanism by stationary state Kinetics, assuming 153P kp

gives the following expression for the relative fluorescence intensity

Let a & $|$ og P ?

For ee ee

(F = relative luminescence intensity, K # constant).

?aod for the quantun yield of amonia.

= lb ak Mo

a

4 (ay ? * ie wo

These equations are to be compared vith experimental equation (1) of the pre-

ceeding Communication and equation (1) of this cae, The internal consistency

of the mechanien, And heace the relationship between results obtained by two

entirely different experimental sechuiques, ie show by the evaluation of the

ratio ky from the exerintal slores ky and hg. We find ay = 3.5 2103,

a ar

im ogreement with the assumption of the mechanisn.

---Page Break---

oe

Hence we conclude that the excited state which luminesces is the same one which is undergoing Leuze to the deamination reaction.

Further conclusions

Analysis of the experimental constants leads to the

conclusion that the rate constant k_2 is from the deamination

Kinetics $k_2 = 6$.

10^6 , the known possible value which I can have

of the deamination rate at 0.7 M urea (calculated by method of

oe

G. v. seomate[®]), using » atervaicn sosttterent of 20° en? seo"). mis

gives an upper limit of $6.2 \times 10^{\circ}$ eset gor

?An alternative approach to discuss the quenching

constant in terms of $Y = 1.7 \times 10^{\circ}$ where Q_f is the intrinsic lifetime of

the emission. If emission is from a singlet state, for which τ_1 is commonly

1078 ps, then $\tau_1 = 2.7 \times 10^8$ ps, an impossibly high value. On the other

hand, \$f\$ emission is treated as a triplet level with $T_1 = 10^{-8}$ sec, then $k_1 = 1.7 \times 10^{10}$, somewhat less than the air-luoretion-controlled rate. Accordingly we conclude that the excited state is probably a triplet level.

Furthermore, we find via sorption of oxygen in aqueous solution

Leads to a relatively long-lived excited state, probably triplet, emitting weak

luminescence at 580 m μ , as unimolecular self-quenching reactions leading eventually to deamination.

A complete presentation of this work is in preparation.

Puerto Rico was learned from Yauco and Daniels

Department of Chemistry

University of Puerto Rico

Rio Piedra, Puerto Rico

(1) Hy Dandekar and A. Griscia, *Biochem. Biophys. Res. Commun.*

16, 428 (1954)

(2) Be RH 6, 2 (964)

(5) 9. Ye sommes, a Pays. Shen. (Peeexturt) 8, 284 (1956)

---Page Break---

200°

---Page Break---

W[aniso1ad]

01 40!

5

o

3

=

oo

01 x GHN) &

---Page Break---

[21180143]

?orx?

of

o-ol o-3

ov oe

Q

---Page Break---

Similar studies have been carried out on thymine. In aqueous solution at room temperature a clean extension $X = 38\text{cm}$ has been found, confirming the report of Udenfriend, the pH variation of this extension parallels the BK of thymine, increasing strongly in alkaline pH.

In view of the evidence for the cytosine emission being a phosphorescence from a long-lived state, and the similarity between the behavior of uracil and thymine, the possibility that the thymine extension is phosphorescence must also be investigated. Apparatus is being set up for lifetime

studies,

It should be noted that a very weak emission has been observed from uracil solutions at room temperature. The conditions for observing this

extension, and its characteristics, are being determined.

---Page Break---