

RADIOLYSIS OF  $^{131}\text{I}$ -O-IODOHIPPURATE: A KINETIC STUDY

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## SUMMARY

Although radiolytic decomposition of several radiopharmaceuticals has exhibited apparent first-order kinetics, the dependence of decomposition on drug concentration precludes a first-order rate process. A kinetic study of the decomposition of  $^{131}\text{I}$ -o-iodohippurate in aqueous solution was carried out to accurately assess the dependence of radiopharmaceutical decomposition on absorbed radiation dose, initial radiopharmaceutical concentration and, in addition, on the concentration of added free radical inhibitors. The fraction of  $^{131}\text{I}$ -o-iodohippurate remaining,  $(A/A_0)$ , was found to decrease exponentially with increased absorbed radiation dose. The radiolytic decomposition rate, however, exhibited a Langmuirian relationship with respect to the initial concentration of the radiopharmaceutical present ( $A_0$ ) and the initial concentration of free-radical inhibitor present ( $I_0$ ).

The equation,  $-\ln \frac{A}{A_0} = \frac{1}{a + b A_0 + e I_0} \dot{D} t$  was derived to characterize these dependencies and demonstrated good correlation with experimental results. This equation may be of value in assessing the radiolytic stability of other radiopharmaceuticals.

Key Words:  $^{131}\text{I}$ -o-iodohippurate, Radiolytic Decomposition, Kinetic Study

## INTRODUCTION

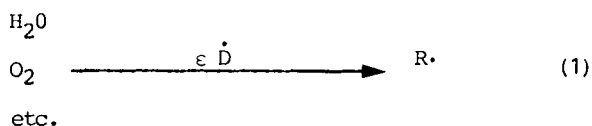
Irradiation of dilute solutions of organic and inorganic compounds due to

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either autoradiolysis or external irradiation has been shown to cause decomposition, primarily by attack on a specific solute by reactive radicals formed in the system by the absorbed radiation.<sup>1-3</sup> The rate of decomposition has been observed to increase with increasing radiation dose but to decrease with increasing initial solute concentration.<sup>4,5</sup> Also, so called free-radical scavengers such as benzyl alcohol and cysteamine have been shown to decrease the extent of solute decomposition.<sup>5</sup> At a given initial solute concentration and scavenger concentration, radiolytic decomposition appears to be a first-order process. However, dependencies on solute concentration and inhibitor concentration indicate otherwise. The kinetic treatment was developed to explain the processes involved and to characterize the effects of absorbed radiation dose, solute concentration and added free-radical inhibitor on solute decomposition, with specific application to the decomposition of radiopharmaceuticals. The derivation was patterned after that of Alder and Eyring<sup>6</sup> but differs in that it is in terms of total effective free radical species ( $R\cdot$ ), rather than in terms of specific free radical species such as  $OH\cdot$ ,  $H\cdot$  and  $HO_2\cdot$  as the individual contributions of the latter cannot be factored by present technology. The derivation also accounts for the presence of added inhibitors as well as auto-inhibition by the solute itself.

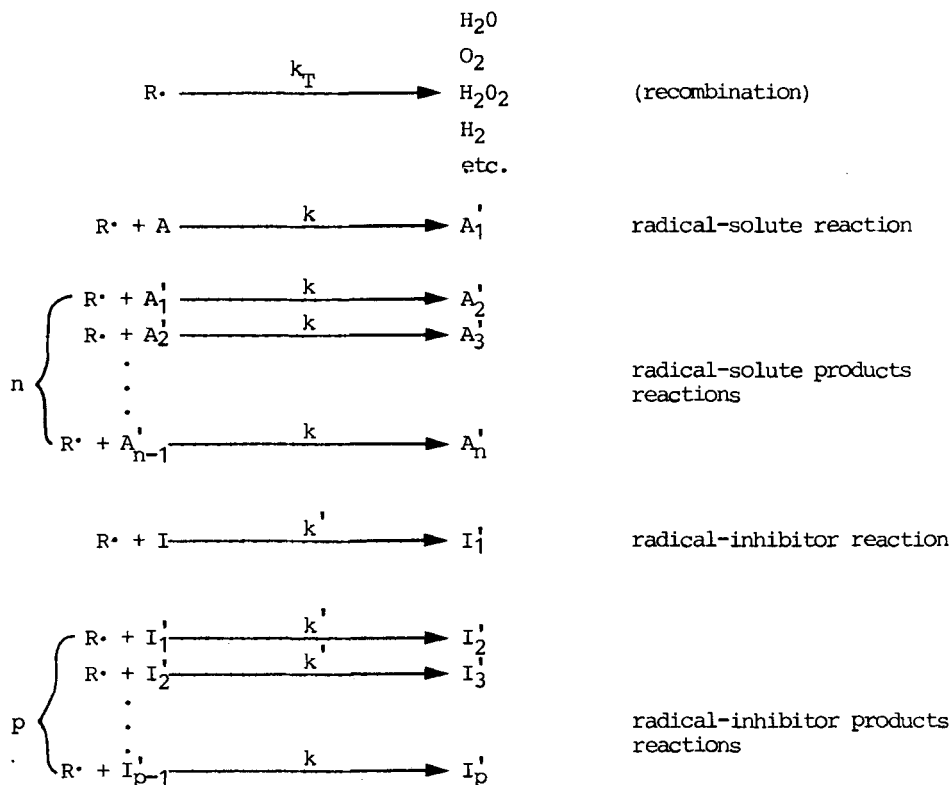
The reactive species or free-radicals most responsible for aqueous radiolytic decomposition are formed by the interaction of radiations with water and dissolved gaseous species:



In this statement,  $R\cdot$  is meant to be the effective free radical concentration,  $\dot{D}$  is the radiation dose rate per unit volume and  $\epsilon$  is the free-radical production efficiency of the absorbed radiation.

In any real system, the rates of radical formation and elimination will rapidly come into correspondence, that is, a steady state in effective radical concentra-

tion will obtain. If the radical elimination is by recombination, by reaction with the principal solute and its decomposition products and, when it exists, by reaction with added inhibitor and its decomposition products, then the following series of equations can be written to describe radical termination steps:



This series of equations assumes that the solute remains essentially unaffected as a target for free radicals even if it has undergone some radical "hits" and has thereby been modified chemically. In other words, the probability of radical collision with solute at the beginning of the process ( $t = 0$ ) will be about the same as the probability of a radical colliding with both solute and solute products, taken together, at some point in time well into the reaction. Under this circumstance, the collision frequency between radicals and intact solute would

be essentially invariant with time. The reaction efficiency is also relatively unchanged, that is, the percentage of collisions causing chemical alteration would not be expected to vary as the reaction proceeds. Therefore, the product of numbers of collisions times the percentage of successful collisions, which yields the reaction rate (free radical consumption rate by the intact organic species), will be constant and the reaction velocity with respect to the reactant will be simply proportional to the amount of reactant remaining. The rate parameter will therefore appear as a first order rate constant. Since an apparent first-order process has been observed for the radiolytic decomposition of solutes, the assumption that the reaction rates between solute and radical and solute products and radical are essentially the same appears to be valid.

Using the equations listed above, the change in solute concentration with respect to time is given by:

$$\frac{dA}{dt} = -k R \cdot A \quad (2)$$

while the change in free radical concentration is expressed as

$$\frac{dR \cdot}{dt} = \epsilon \dot{D} - \left[ k_T R \cdot + k R \cdot \left[ A + \sum_{i=1}^n (A_i') \right] + k' R \cdot \left[ I + \sum_{i=1}^p (I_i') \right] \right] \quad (3)$$

Following the derivation of Alder & Eyring<sup>6</sup>, the theoretical equation expressing the fraction of solute remaining as a function of absorbed dose rate, time, initial solute concentration and initial concentration of added inhibitor resulting from the simultaneous solution of eq. 2 and 3 becomes,

$$-\ln \frac{A}{A_0} = \frac{1}{a + b A_0 + e I_0} \dot{D} t \quad (4)$$

where,  $a = k_T/k\epsilon$ ,  $b = 1/\epsilon$  and  $e = k'/k\epsilon$

The following important facts can be derived from equation 4:

1. For any given initial solute concentration, a direct relationship will be observed between the log (fraction of residual solute) and the total absorbed dose,  $\dot{D} t$ .

2. If the absorbed dose rate,  $D$ , is essentially constant, a first order relationship will be demonstrated between the fraction of solute remaining and time.
3. As the absorbed dose rate is increased, the rate of solute decomposition is increased in exact proportion.
4. The radiolytic decomposition rate should exhibit a Langmuirian relationship with respect to the reciprocal initial solute concentration.
5. The radiolytic decomposition rate should exhibit a Langmuirian relationship with respect to the reciprocal initial inhibitor concentration.

#### MATERIALS AND METHODS

Three separate experiments were run to determine the validity of equation 4 with regard to absorbed radiation dose rate, initial solute concentration and initial inhibitor concentration.

##### Effect of Absorbed Radiation Dose Rate:

Aqueous solutions of I-131-o-iodohippurate were obtained from a commercial source\* having a specific concentration of 1 mCi/ml and a molar concentration of  $4.4 \times 10^{-3}\text{M}$  (1.31 mg/ml). Radionuclidic purity was determined by multichannel analysis utilizing a lithium drifted germanium detector\*\* in conjunction with a 512 channel multichannel analyzer.\*\*\* Gamma spectral analysis yielded no apparent radionuclidic impurities for any of the I-131-o-iodohippurate solutions used.

Radiochemical purity of the I-131-o-iodohippurate solutions was assessed by two ascending chromatography techniques: thin layer chromatography using Seprachrom ITLC-SG\*\*\*\* strips as the support and chloroform: acetic acid (9:1) as the mobile phase (system A) and paper chromatography using Whatman #1 paper as the support

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\*\*Ortec Model 8000-45, Oak Ridge, TN

\*\*\*Nucleonic Data Inc., Schaumburg, IL

\*\*\*\*Gelman Instrument Co., Ann Arbor, MI

and n-butanol : acetic acid : water (4:1:1) as the mobile phase (system B).

Five ml of I-131-o-iodohippurate solution were spotted on 3 x 15 cm. paper and ITLC-SG strips. The strips were developed in the respective solvent systems and analyzed by radiochromatogram strip-scanning. The areas under each peak on the chromatogram were quantitated using a digital electronic peak integrator.

I-131-o-iodohippurate has been shown to exhibit an Rf value of 1.0 in chromatography system A<sup>7</sup> and an Rf value of 0.92 in the paper chromatography system.<sup>8</sup> The major radiochemical impurity, iodide ion, was shown to have an Rf value of 0.1 in system A and 0.23 in system B. Analysis of three separate lots of I-131-o-iodohippurate showed no detectable levels of radiochemical impurity in any solution at the time of receipt.

Sample solutions were prepared containing 50  $\mu$ Ci of radioactivity and 1 mg of o-iodohippurate per ml. Stable o-iodohippurate\* was added as carrier to aliquots of the radioactive solution in order to dilute the radioactivity to a level which could be neglected as a major source of product decomposition. The level of radioactivity was still sufficient for analytical purposes. The aliquots were then brought to volume with distilled water such that each ml of sample contained 50  $\mu$ Ci of radioactivity and 1.0 mg of o-iodohippurate. One ml volumes of sample were placed in 5 ml multi-dose vials, stoppered and crimped closed.

The samples were placed annularly at several distances from a 7,200 Curie Cobalt-60 radiation source\*\* and irradiated for 1 hour.

Immediately following irradiation, each sample was removed from the irradiation room and the solutions analyzed. All chromatogram strips were pre-spotted with approximately 20 ml of 0.5 N sodium thiosulfate to prevent the loss of inorganic iodine which may be present in elemental form. The strips were developed under nitrogen and protected from light. The developed chromatograms were analyzed by strip-scanning techniques and quantitated using a digital peak integrator. The experiment was repeated for samples irradiated

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for 2, 3 and 4 hours and the entire experiment was carried out three times to reduce statistical variability.

#### Effect of Initial Solute Concentration:

Since a given radiation dose yields a definite concentration of reactive radicals which, in turn, react with the solute present it is expected that varying the concentration of solute should not affect the total number of molecules that decompose but rather should alter the proportion of molecules undergoing decomposition at a particular radiation level. If non-radioactive solute is added to a solution of radioactive compound, the non-radioactive solute will compete with its radioactive counterpart in reaction with radiation induced reactive species in solution. Expressed as a fraction of pure radioactive compound remaining, the rate of radiolytic decomposition would decrease with increasing solute concentration as long as indirect decomposition effects were predominant in the system.

Sample solutions were prepared containing 50  $\mu\text{Ci}$  of radioactivity and 3.0 mg of o-iodohippurate per ml along with varying concentrations of free radical scavenger (0.05, 0.1, 0.15, 0.2, 0.3, 0.5 and 1 percent benzyl alcohol). One ml samples were positioned annularly at a distance from the Co-60 source corresponding to an absorbed radiation dose rate of  $1.46 \times 10^4$  Rads/hr. The samples were irradiated for one hour in the dark at 15 degrees C. Immediately after irradiation, the samples were removed and aliquots analyzed in the same manner as described above. The same procedure was carried out with samples irradiated for 2, 3 and 4 hours and the experiment was repeated three times to reduce statistical variation.

### RESULTS

#### Absorbed Radiation Dose Rate:

Figure 1 demonstrates the results obtained when changes in absorbed radiation dose rate occurred. For any given dose rate, radiopharmaceutical decomposition displayed an exponential dependency on the time of irradiation. How-

ever, as the dose rate increased, the rate of I-131-o-iodohippurate decomposition increased. This behavior agreed with theory as expressed in equation 4.

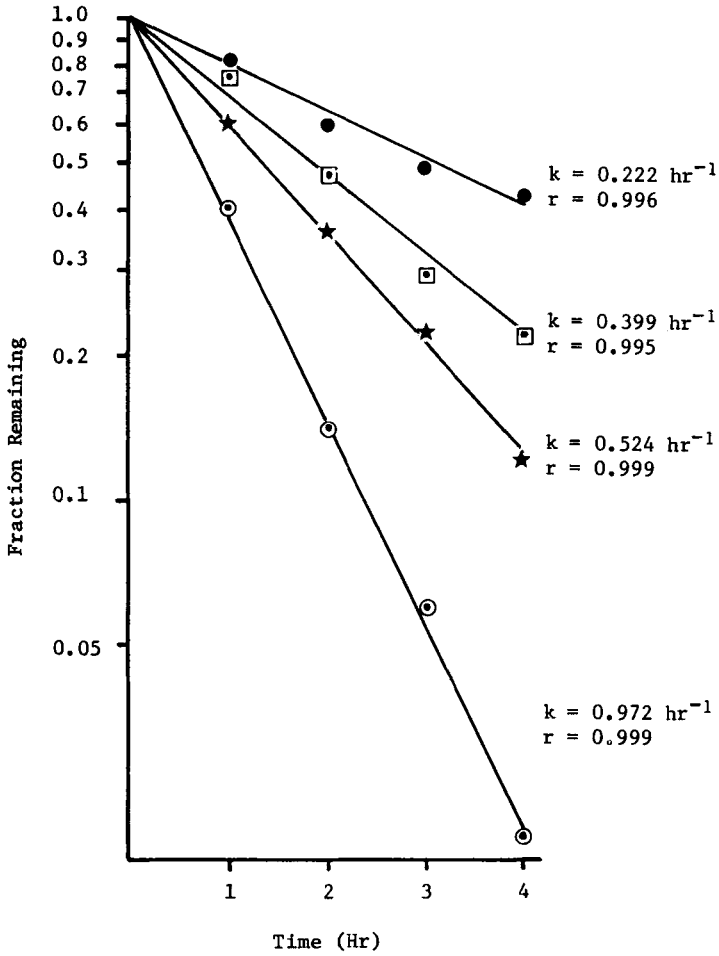


Figure 1: Radiolytic Decomposition of I-131-o-iodohippurate, concentration 1.0 mg/ml.

Key: ● =  $4.4 \times 10^3$  Rad/hr

◻ =  $7.2 \times 10^3$  Rad/hr

★ =  $9.6 \times 10^3$  Rad/hr

⊙ =  $1.46 \times 10^4$  Rad/hr



Initial Solute Concentration:

Figure 2 shows the effects of increasing solute concentration on the rate of decomposition of I-131-o-iodohippurate. The Langmuirian curve is again predicted by the theory developed. In essence, at low  $A_0$ , the expression  $a + b A_0$ , is dominated by the "a" term and the rate appears independent of  $A_0$ . As  $A_0$  increases, however, it exerts more of an effect on the decomposition rate and causes the rate to decrease.

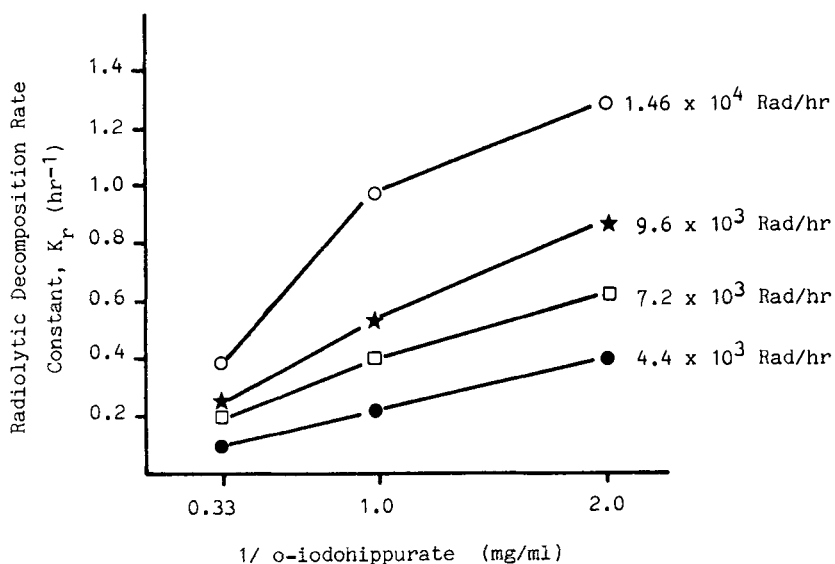


Figure 2.

Effect of initial concentration of o-iodohippurate  
on radiolytic decomposition  
at several radiation dose rates.

Initial Inhibitor Concentration:

Figure 3 shows experimental data which agrees with the theoretical relationship between decomposition rate and the initial concentration of free radical inhibitor. Benzyl alcohol effectively competed with I-131-o-iodohippurate molecules for free radicals. As the effective concentration of free radicals

available for reaction with the radiopharmaceutical decreased, the rate of radiolytic decomposition was shown to diminish.

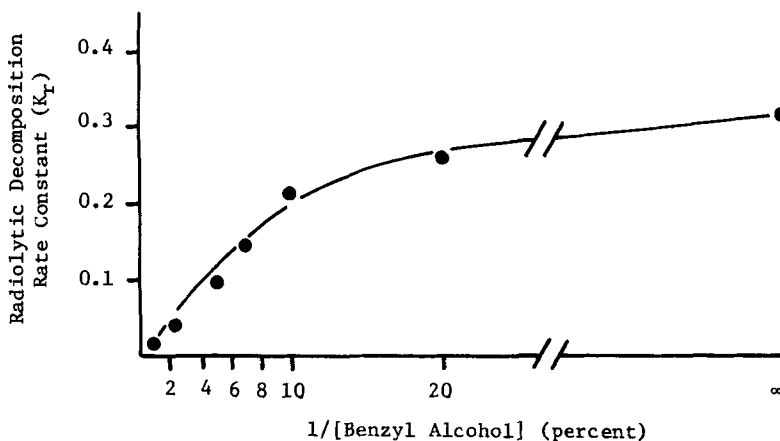


Figure 3: Effect of initial Benzyl alcohol concentration on radiolytic decomposition rate.

#### CONCLUSIONS

Product decomposition due to autoradiolysis or external irradiation appears to be a simple exponential relationship with respect to absorbed radiation dose. However, the absorbed radiation dose rate, initial solute concentration and, if present, the initial concentration of free radical inhibitors are actually involved and their values dictate the relative stability of the compound.

The equation

$$-\ln \frac{A}{A_0} = \frac{1}{a + b A_0 + e I_0} \dot{D} t$$

describes the radiolytic decomposition of I-131-o-iodohippurate in the presence of a free radical inhibitor. This model may be extrapolated to other free-radical inhibitor containing pharmaceuticals to explain product stability.

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