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The decomposition of 5-hydroxymethyl-2-furaldehyde under gamma irradiation

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THE DECOMPOSITION OF 5-HYDROXYMETHYL-2-FURALDEHYDE UNDER GAMMA IRRADIATION

by

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ABSTRACT

An investigation into the effects of gamma irradiation decomposition of 5-hydroxymethyl-2-furaldehyde (HMF) revealed HMF to be resistant to radiation catalyzed decomposition. A 1.0 normal solution of HMF was irradiated at a dose rate of 4.1×10^5 Rads/hr to a total dose of 8.2×10^6 Rads from a 5000 Curie ^{60}Co source. A total of 31 per cent of the HMF decomposed in a zero order reaction, although it was found that HMF decomposed completely under cadmium shielded irradiation in a nuclear reactor with some neutrons present. The reaction rate constant was found to be 4.8×10^{-3} moles liter^{-1} seconds^{-1}.

Formic and levulinic acids, ordinary products of HMF hydroysis, were not detected as constituents of the irradiated liquid phase. The only detectable product was a polymeric material which formed and then decomposed under a continued irradiation.

HMF was analyzed in its free state using gas chromatography with SE-30 10 per cent by weight, column packing and also Carbowax 20M column packing at temperatures between 150 and 200^{o} C.

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I. INTRODUCTION

The use of ionizing radiation to produce chemical changes is fairly well established both in the laboratory and industrial use. Industrial production of ethylene bromide and the gamma irradiation induced polymerization of ethylene are the two best known industrial process examples of gamma ray usage. Food preservation by gamma irradiation is also well represented in the literature as a use for radiation. A synthetic type wood formed by impregnation of wood with various vinyl monomers followed by irradiation gives increased hardness and wearability. These plus other uses show that chemical changes induced by irradiation in materials can be beneficial if they are understood. The general field of radiochemistry and isotope usage has taken a large advance lately - both in research and applied industrial work - which may be attributed to the availability of comparatively cheap radiation sources. With the refueling of the third generation 1000 megawatt - electrical nuclear power plants the abundance of highly radioactive wastes will be a problem. These mixed fission products promise to reduce the costs of radiation sources even lower than they are now.

Presently Cesium^{137} costs about 12.5 cents per curie of activity while the price of ^{60}Co is 50 cents per curie in large amounts. Compact equipment is available from suppliers either for laboratory or industrial use as a stock item, such as the Atomic Energy Canada Limited's gammacell irradiators.

The stability of carbohydrates under gamma irradiation is of concern to the food preservation industry if it plans to preserve food by gamma irradiation. The cost of food irradiation promises to be small, with the large amounts of

gamma producing fission products being produced as by products of the nuclear power industry, with the benefits high especially in underdeveloped areas of the world.(1).

The effect of gamma radiation on carbohydrates has received attention of researchers and been reported in the literature. Certain adverse effects on living cells produced by irradiated sugar solutions have been reported (2). In light of these findings further research is necessary into the products formed by the irradiation process upon carbohydrates.

Since HMF is a product of carbohydrate decomposition under chemical reaction this compound was studied under gamma irradiation and its products examined to see if they might be contributing to the harmful effect noted. This compound has not been detected when sugars are irradiated and it has been suggested that it may be formed but is quickly decomposed by the gamma rays. It has been reported as a product of the irradiation of potato starch.

This investigation was undertaken to determine if 5-hydroxymethyl 2 - furaldehyde (HMF) is radiation sensitive and to determine the kinetics and the decomposition products. From a knowledge of how HMF reacts under gamma irradiation further insight into the reactions of carbohydrates under irradiation is possible.

II LITERATURE REVIEW

The areas covered in the literature review are effects of ionizing radiation on water and aqueous solution, and on carbohydrates. The reactions of the carbohydrates under chemical attack is also covered.

By understanding the carbohydrate reactions induced by chemical means we have a basis for comparison with the results the literature reports for gamma irradiation. The reactions of 5-hydroxymethylfurfural (HMF) are covered in an attempt to determine if any similarities exist between carbohydrates in general under chemical and radiological attack and their chemical product, HMF, also under chemical or radiation induced decomposition.

The chemical and radiation induced decomposition of carbohydrates was reviewed to provide background as to how the parent formation compounds of HMF react under these conditions. There was not found any literature which dealt with the action of HMF under gamma irradiation.

Since the compounds to be considered in the investigation are soluble in water the effect of radiation upon pure water has to be considered to determine what reaction with the solute might be instigated by the water under irradiation.

The Effects of Ionizing Radiation on Water and Aqueous Solutions

The effect of radiation upon compounds dissolved in aqueous solutions depends somewhat upon concentration. At low solute concentrations the yield of products is independent of the solute concentration over a wide range (1). This effect is because the radiation is losing its energy to the water rather than to the solute. The reaction is caused mainly by the OH and H free radicals produced by the radiation interacting with water reacting with the solute.

The exact process' involved are: a) formation of hydrogen atoms and hydroxyl radicals b) free radical and molecular product yields and c) formation of hydrogen and hydrogen peroxide. These reaction mechanisms will be covered in the following paragraphs.

Formation of Hydrogen Atoms and Hydroxyl Radicals. Radiation interacting with matter will produce ionization and molecular excitation. The number of ions produced will depend on the energy of the radiation, and on the physical properties of the substance.

With water, ionization is produced along the primary particle path (3). For a recoil electron from a gamma ray in water the distance between successive ionizations is about 1000 Å (4). At each point of ionization secondary electrons give rise to further ionization, forming a group of ion pairs. This secondary ionization can occur up to 20 Å from the primary particle track. Beyond 20 Å the secondary electrons do not have the required energy to produce ionization but produce electronic excitations of the atom (3). The end

result of this ionization is the formation of H^+ and OH^- radicals and hydrogen atoms.

These process' occur very rapidly being of 10^{-16} to 10^{-18} of a second duration after passage of the primary radiation (3, 4, 5).

$$H_2O + \text{ionizing energy} \rightarrow H_2O^+ + e_1 \qquad (1)$$

where e_1 is the recoil electron formed. The recoil electron may then interact:

$$H_2O + e_1 \rightarrow H_2O^+ + e_2 + e_s \qquad (2)$$

where e_2 is another recoil electron and e_s is the secondary electron which possesses enough energy to initiate its own ionization processes of the type:

$$H_2O + e_s \rightarrow H_2O^+ + e_{s1} + e_s \qquad (3)$$

Reaction (1) is the primary ionization process initiated by a recoil electron.

It is thought that the next step in the process is the conversion of the H_2O^+ ion to the hydroxyl radical within 10^{-12} seconds (3, 5, 6).

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH^- \qquad (4)$$

There are two primary viewpoints concerning the fate of the secondary electron. Samuel and Magee (7, 8) state that the electron does not leave the field of the parent ion and that it forms a hydrogen atom by charge-neutralization with H_2O^+. Platzmann and Fronhlich (9, 10) and Baxendale and Hughes (11) propose that the hydrogen atom is created at a considerable distance from the parent ion - mainly by low energy electrons. The electrons result from the primary ionization of water and lose their energy by inelastic collisions. The electron energy will drop below the excitation level of water and subsequently form a hydrogen atom by combination with an H^+ ion. However, Lea (12) and

Gray (13) propose that the secondary electron is captured by the water molecule to give H_2O^- which produces hydrogen atoms according to the process:

$$H_2O^- \rightarrow H + OH^- \quad (5)$$

The net effect of ionizing radiation on water has been summarized by Allen (12,13), Dainton (3), and Philips (16).

$$H_2O \xrightarrow{\text{Radiation}} H_2O^+ + e^- \quad (6)$$

$$H_2O^+ + H_2O \rightarrow 2H^+ + 2OH \quad (7)$$

$$e^- + H_2O \rightarrow OH^- + H \quad (8)$$

$$OH^- + H^+ \rightarrow H_2O \quad (9)$$

The net result of these processes is the production of H^+ and OH.

$$H_2O \rightarrow H^+ + OH^- \quad (10)$$

The hydrogen and hydroxyl free radicals will be distributed along the tract of the original particle of primary-recoil electron, with the hydroxyl radicals situated near the tract. The hydrogen atoms may be found several Å units away from the site of the electron formation.

Formation of Hydrogen and Hydrogen Peroxide. The formation process for production of hydrogen and hydrogen peroxide is fairly well established and occurs by combination of hydrogen and hydroxyl radicals

$$H + H \rightarrow H_2 \quad (11)$$

$$OH + OH \rightarrow H_2O_2 \quad (12)$$

On the basis of rate studies it has been estimated that about half of the free radicals formed will recombine to form water.

$$H + OH \rightarrow H_2O \quad (13)$$

There is direct competition between combination and diffusion of the free radicals. Mathematical analysis of models for this competition between diffusion and recombination of radicals reacting with a solute agree satisfactorily with experimental results. (8,9,16,17). The secondary combination reactions 11 thru 13 occur within 10^{-7} second after passage of the ionizing particle. If a solute is present in concentration greater than about 10^{-6} molar the hydrogen atom and hydroxyl radicals which escape by diffusion may react with the solute atoms which are located away from the primary ionization track. Pure water under irradiation reaches an equilibrium from the following reactions which remove the hydrogen and hydroxyl radicals.

$$OH + H_2 \rightarrow H_2O + H$$

$$H + H_2O_2 \rightarrow H_2O + OH$$

These recombination reactions serve to limit the buildup of hydrogen and hydrogen peroxide.

Another production method for hydrogen and hydrogen peroxide has been proposed by Johnson and Weiss (19) which is based on the direct interaction of excited water-molecules.

$$2H_2O^* \rightarrow H_2O_2 + H_2$$

$$2H_2O^* \rightarrow H_2 + 2OH$$

Free Radical and Molecular-Product Yields. While the exact mechanisms of formation of free radicals and molecular products are not known the net process resulting from particle interaction is:

$$H_2O \rightarrow aH_2 + bH_2O_2 + c(H) + d(OH)$$

Accurate measurement of the free-radical and molecular-product yields are necessary if reaction rates and mechanism are to be determined. The yields of radio chemical reactions are generally expressed as G values. This G value is defined as the chemical yield in units of molecules formed or consumed per 100 electron volts of energy input.

The chemical methods used for determining the G values of primary products formed in the decomposition of water has been described in the literature (15, 20). The yields of G(OH), G(H), $G(H_2)$, and $G(H_2O_2)$ depend on the reactivity and concentration of the solute (if one is present) and the energy release (ion pairs per path length along the primary track. The effect of solution pH is shown in Figure 1 (15).

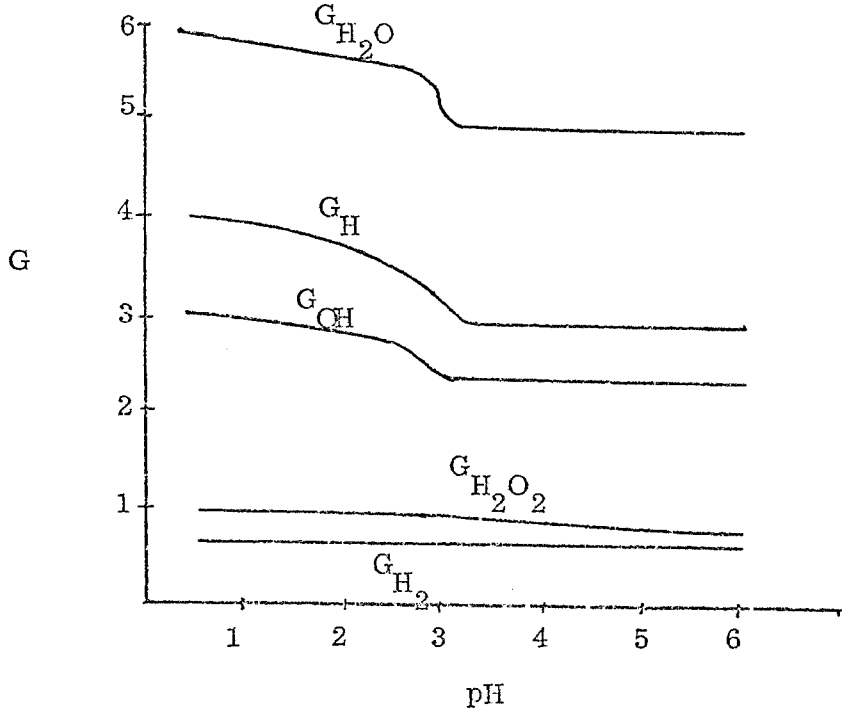

Figure I

The Water Decomposition Yields for Gamma Rays as a Function of pH. (15)

The passage of a light-particle such as gamma ray, x ray or electron thru water results mainly in the production of free radicals. Free radical production is to be expected as the lighter particles are much smaller than the interacting matter and will provide ionization and excertation of the molecules in preference to rupture of the molecular bonds caused by recoil energy from the collision. Heavy particle radiation produces mainly molecular hydrogen and hydrogen peroxide (3, 21). The highest G value is with fission products where $G(H_2)$ is 1.83 with the G(OH) and G(H) almost zero (21).

In acid solution more hydrogen atoms than hydroxyl radicals are formed under gamma irradiation. In 0.8 N H_2SO_4 G(H) is 3.65 and G(OH) is 2.95 (22).

The addition of hydrogen or hydrogen peroxide to a solution before irradiation, effects the yield of hydrogen and hydroxyl radicals. The hydrogen peroxide increases with the concentration of hydroxyl radicals.

$$OH + H_2 \rightarrow H + H_2O$$

$$H + H_2O_2 \rightarrow OH + H_2O$$

The yields $G(H)_2$ and $G(H_2O_2)$ decrease upon increase of the solute concentration. If a solute reacts with hydrogen atoms, the yield of hydrogen decreases as a result of competition between the reactions:

$$H + H \rightarrow H_2$$

$$H + \text{solute} \rightarrow \text{product}$$

When solute concentrations are below 0.1 molar, the chemical change occurs by indirect action. Under these conditions the radiation energy is absorbed by the water and the chemical change results from the effect of the

species produced in the primary radiolysis of water. For higher concentrations the direct-interaction becomes important.

Since this investigation is to be conducted on solutions of 5-hydroxymethyl-furfural a knowledge is necessary of how the products may be formed by interaction with water.

The Radiolysis of Carbohydrates

The earliest work on the effect of ionizing radiation on carbohydrates was performed by Kailan (23, 24). He observed that the radiations from radium induced hydrolysis in sucrose and D-glucose. Later work centered on physical changes which accompany irradiation, including changes in pH, optical rotation, viscosity, and ultraviolet absorption spectra (25-28). Even in the current literature, there is a tendency to report products of carbohydrate irradiation "as an unknown compound having a yellow spot with a R. F. of 2. 5 upon paper chromatography with butan-1-ol-ethanol-water" (2, 36, 37, 45, 47, 53, 64). The reporting of unidentified products is because of the difficult product analysis which requires difficult and laborious separation and identification by paper and gas chromatography, ionophoresis, gas analysis, calorimetry, dialysis, mass spectrometry, infrared and ultraviolet analysis.

Early workers reported that unbranched six-carbon sugars are considerably more stable toward radiation in the solid state than in solution (28-32). The diffusion of radicals formed by irradiation in solids is slower than in solution, and the lattice cage effect helps to reduce the extent of free radical interaction with the solid. The slower diffusion in solids is in contrast to the greater mobility of the free radicals formed in aqueous solution. It was shown that free radicals are still produced in the solid by Williams et al (30) in a paramagnetic-resonance study of sixteen carbohydrates irradiated in solid form.

Free electrons may be formed by action of radiation on a molecule. The

removal of an electron produces an ionized molecule. The ionization energy, if it does not rupture chemical bonds and produce fragments, leaves the molecule with an unpaired orbital electron. The free electrons may recombine with a deficient molecule, excite another molecule or be trapped at imperfections in the lattice. Also formed are ions which react with the electrons to become uncharged free radicals. The free radicals produced may possess a fairly long half-life. The literature (31) reports a half life for the formed radicals of up to 12.5 weeks at 20° C and approximately 8.5 hours at 50° C. A Russian paper (33) presented at the Second All Union Conference of Radiation Chemistry, reported the presence of free radicals (by electron paramagnetic resonance study) in dry sugars and one year after termination of the irradiation. These long-lived radicals are stored in the crystal and on dissolving in aqueous solution, cause the formation of monosaccharides and other substances from polysaccharides. These long lived radicals present a problem to investigators who irradiate solid sugars and then dissolve them in solution to compare the ultraviolet absorption spectra of the irradiated solids with the spectra from an aqueous irradiation. The free radicals are still present in the solid, and upon going into solution gain mobility and interact with the dissolved carbohydrate. These radicals will then react as if they were formed during an aqueous irradiation.

The appearance of the irradiated samples is altered in color, and many samples irradiated fluoresced (32, 34). The change in color along with the observed fluorescence indicates excitation of the molecule along with the possible formation of stable free radicals (32, 34).

Changes in optical rotation, melting point, and acidity show that degradation occurs when D-glucitor, D-glucose, and D-fruitose are irradiated. Indications were obtained from ultraviolet analysis that keto groups are introduced into the molecule (34).

Aldehyde or ketones in the free or hemiacital form generally are the most reactive of the functional groups present in sugars. These groups are called reducing groups and sugars which have these groups are called reducing sugars. The olegosaccharides which have a reducing group at one end of the molecule are called oligosaccharides. When there is not a free aldehyde or ketone group present the compound is nonreducing. Disaccharides like maltose and lactose are reducing, whereas sucrose is nonreducing because the aldehyde and ketone groups of the component fructose and glucose have been combined in the formation of the disacchoridic glycoridic linkage. The reducing power of D-glucose and D-fruitose decreases on irradiation (32).

Phillips and coworkers, after reporting that solid sugars were more stable toward radiation than aqueous solutions, in their publications (34-39) in the late 1950's and early 1960's, suddenly reported in early 1966 that now the solid carbohydrates undergo greater degradation in the solid state than in solution (40, 41). The action of radiation on solid carbohydrate is still not well understood. The effect of radiation on aqueous solutions of carbohydrates has been the subject of various papers in the literature (32-62).

Oxygen has been found to exert an important effect on the nature of the products formed. Different products will be formed because of the secondary

reactions with oxygen. If quantitative measurements are to be taken, it is necessary to maintain either evacuated or oxygenated conditions throughout a particular irradiation. If initially air-equilibrium solutions are used, and no provision is made for replacing the oxygen consumbed during irradiation, the observation cannot be related to either fully oxygenated or to evacuated conditions. The primary products being formed are independent of oxygen content, but the primary products will then take one of several possible reactions under continued irradiation, depending on the presence or absence of oxygen. The method of deaerating the solution by passing nitrogen through has not proved as satisfactory as the complete evacuation process. Bourne et al (52) have shown that there are appreciable differences in yields between sugar solutions irradiated under vacuum and nitrogen.

The results of irradiation of carbohydrates will be considered in detail with much of the following information from an excellent article by Bothner-by et al (47).

Production of Acidic Constituents. In alkaline, buffered, and neutral solutions of glucose, the change in pH indicates that acid is produced during irradiation with gamma radiation. In neutral solutions there is very little difference (0.4 pH unit) in the amount of acid formed between irradiation performed in open beakers and those performed in closed cells. The ratio of acid formed to reducing substance destroyed is not constant, but shifts gradually from 1:3 to 1:2, for an irradiated solution at dosage of 1×10^6 Rads. This shift suggests the formation of intermediates in the reaction. The change in pH

during irradiation indicates a higher yield of acid when the irradiation is performed in open beakers than when closed cell are used. If the sum of the moles of acid formed and the moles of glucose remaining is taken at any point in the course of the irradiations, the total is at no time equal to the original concentration of glucose. It is apparent from the observed deviation that more than one product must be formed during irradiation (47).

Reducing Sugar Content. In irradiated neutral solutions of glucose, the amount of reducing material present decreased exponentially with increasing dosage (47). After exposure to a dose of 3×10^6 rads, 50 per cent of the original ferricynaide-reducing material was still present. The irradiation of an alkaline solution (pH12) of glucose in an open vessel produced a positive deviation from this relationship. At a dose of 2.5×10^6 rads, the solution had 57 per cent of its original reducing power. At this same dose, approximately 25 per cent of the reducing power remained when a closed cell was used. The reduction in reducing power was not affected by deaerating the solution before irradiation.

In irradiated neutral solutions of glucose, the destruction of reducing power as measured by the ferricyanide method appears to follow first-order kinetics. Since acid formation data suggest the presence of an intermediate, the unimolecular reaction is better described as pseudo-unimolecular. Weber and Schuler (62) have observed that pseudo-unimolecular kinetics presumably arise because of competition between the substrate and its reaction products for the free radicals produced in the primary reaction of the solvent. A

consistent picture results only if it is assumed that glucose is the sole substance present which reduces ferricyanide in detectable quantities (47).

Production of Absorbing Substance. When the ultraviolet spectra of irradiated alkaline solutions (pH 12) of glucose were taken at the final pH of the reaction mixture, a very strong absorption maximum was observed at 267 mμ (47). When the solutions were acidified to pH 2 with normal hydrochloric acid, there was a shift of the maximum to 245 mμ accompanied by a decrease of about 40 per cent of the absorption intensity, which could not be attributed to dilution by acidfication. Laurent (63) and Phillips (64) observed the same peaks in alkaline solutions of glucose irradiated with ultraviolet light. The alkaline shift is reversible. Absorption spectra have shown that above pH 7 there is no shift in the wavelength of the absorption maximum, and that the optical density has a constant value.

Sample irradiated at a dose of 2.5×10^6 rads were compared and the absorption maximum of highest intensity was found in the sample which had been irradiated in an open beaker (47). The calculated optical density at 267 mμ was 36.8. Aliquots of an alkaline solution were then irradiated in completely filled, closed cells at a dose of 2.5×10^6 rads. In one case, the solution was deaerated with nitrogen prior to irradiation; in the other, it was not. The difference in the absorption maxima was slight; for the deaerated sample, O.D. 267 mμ = 32.6; for the nondeaerated sample, O.D. 267 mμ = 30.8.

Ultraviolet spectra of irradiated neutral solutions of glucose exhibit no absorption maximum at 267 mμ when readings are taken at the final pH

of 3.7 to 3.1, for the solution. When the solutions are made alkaline to a pH of 10.8 a maximum observed at 275 mμ. In solutions irradiated with a dose of less than 2×10^6 rads, the absorption band is broad and flat and has no sharply-defined maximum. No absorption was reported in samples which had been irradiated at doses of 1 to 10,000 rads (47).

Absorption maxima which are in the range of 200 to 300 mμ and exhibit the alkali acid shift observed here are characteristic of compounds containing an ionizable chromophore group, such as the enediol group of ascorbic acid or the enol group of 4-deoxy-5-keto-3, 6-mannosaccharolactone (47, 63). The decrease in the intensity of the absorption in acid solution would seem to indicate that the compound has the unsymmetrical enol group rather than the enediol group, since the latter gives rise to absorption peaks of equal intensity. In the light of these considerations, the spectroscopic data can be said to support the hypothesis that a larger quantity of a reducing substance other than glucose is formed when alkaline solution of glucose are irradiated through an air layer than when they are irradiated in a closed cell.

Polymer Production. Polymeric material will form from carbohydrates under irradiation only under a vacuum, no polymer formation is noted with oxygen present (49, 50, 51, 56). Baily et al (51) reported the yields of polymers for 21 different carbohydrates . D-glucose under irradiation of 7 M rad (dose rate not reported) produces a polymer material in 45 per cent yield. This polymer contained 51.91 per cent carbon 5.07 per cent hydrogen and the rest oxygen. The comparison of the different carbohydrates shows that polymer production

ccurs more readily through an aldehyde grouping than through a primary alcohol group.

Baker et al (49, 50) investigated the yield of polymer from aqueous glucose solutions. They investigated the effect of dose rate and concentration on polymer yield. The largest amount of polymer material is formed at a total dosage of 7 M rad and the amount formed decreases logarithmically with the dose rate. These results are summerized in the following paragraphs.

Variation of the dose used to produce the polymer from glucose indicates that the optimum yield is obtained when the total dose is 7.05×10^6 rads given over 204 hours. The nature of the polymer produced will vary with the dose as if the polymer formed was suffering radiation damage. The polymer produced from glucose ($C_6H_{12}O_6$) with the lowest dose had the empirical formula $(C_6H_{10}O_{6.8})_n$. The succeeding polymers show a continuous increase in relative carbon content and a concomitant decrease in oxygen content (49), suggesting removal of oxygen-containing functional groups during the ensuing increase in radiation damage. Polymers formed with increasing dose show increasing carboxylic acid contents. It is noticeable that the proportion of these carboxylic acid groups which are unstable and yield carbon dioxide with acid decreases considerably with dose rate. If the initial oxidation in the glucose molecule is at C-1 and C-2 (gluconic acid, 2-ketogluconic acid, and glucosone products have been identified) and dimerization and polymerization are occurring through the C-6 position, this reaction would result initially in the formation of both acid-stable (gluconic acid type) and acid-unstable (2-ketogluconic acid type) groups. The unstable groups are probably eventually decarboxylated

during irradiations so that the proportion of stable groups increases and hence the carbon oxygen ration. In addition, it is likely that oxidation at the C-1 position activates the C-2 position and renders it suitable as a site for dimerization. Such a reaction would compete with the oxidation at C-2 position in the later stages (49).

A molecular weight range of 1200 to 4000 as determined by viacometry was subject to many approximations, mainly because of the behavior of the polymer as a poly-electrolyte and to the difficulty of calibrating the method without suitable standards (49). In discussing the nature of the polymer, it is pertinent to realize that the aggregate of polymeric units which comprises the polymer can vary conceivably from a hexamer upward. In addition, since it is not a rapid-chain polymerization process, different polymer units will have suffered varying amounts of radiation damage, dependent on the length of time of the preparative irradiation period.

At higher dose rates the yield of polymer obtained for a given total dose from 0.1 per cent glucose solutions falls off with increasing dose rate, and conforms to the emperical equation

$$\text{Yield of polymer} = K\ (\text{dose rate})^{.46} \qquad (49)$$

Collinson and Swallow (65) suggested for styrene that the rate of polymer production should become proportional to $(\text{dose rate})^{x}$ where x=0 at the high dose rates. This polymerization has been further studied by Degering and co-workers (66) with gamma-radiation. The dose rate dependency shows an important economic factor in the preparation of such polymers by irradiation techniques, i.e., the desirability of using a low dose rate for maximum yield per M rad

available. The information was primarily obtained from Barker et al (49) article on polymerization of glucose.

General Pattern of Hexose Degradation. The general pattern of hexose degradation has been described by Phillips (56,61) among others.

It has been found that the radiation decomposition is first-order in aldohexose concentration (56). The results of irradiation of D-glucose and D-mannose in both oxygen and vacuum show an identical rate of decomposition. This decomposition similarity indicates that the primary abstraction processes are similar and independent of oxygen. Because initial rates are identical the differing products found in oxygen and in vacuo irradiations must be attributed to differing secondary reactions of identical primary radicals.

Common processes are the formation of hexonic acids and ring scission reactions which lead to two- and three-carbon aldehydic fragments. The presence of oxygen does not appear to have any marked effect in controlling the extent of these processes. Further oxidation occurs but it is a secondary process.

Oxygen influences the product arising from radical attack at C-2. Glucosone (2 oxo-D-arabinoaldehoxose) is formed in vacuo, but ring scission occurs in oxygen to give arabinose and formaldehyde. The carbon-carbon scission which occurs in oxygen is diminished in vacuo (67,68).

No uronic acid is found in vacuo, and no polymer is produced in oxygen. These observations may be rationalized by considering the fate of the primary radical, RCHOH, formed by hydrogen abstraction at C-6 in oxygen and in vacuo. After the initial dimerization step in vacuo, further radicals may be graphed to the growing polymer chain.

The radiation decomposition is first order in aldohexose concentration. On the basis of such kinetics, simple competition between initial hexose and products for the primary species formed during water radiolysis may be envisaged.

The practice of irradiating very dilute solutions of carbohydrates poses a problem. The radiation chemistry of water has been extensively studied (3-22). The products are H_1, OH, H_2O_2, and H_2. In dilute solution, the energy of the radiation is deposited in the water without much energy deposition in the carbohydrate because of the many orders of magnitude greater water molecule concentration. Thus, the only products formed will depend upon the action of the species formed by radiolysis upon the carbohydrate. An irradiation of a concentrated carbohydrate solution could produce products formed by direct action of the radiation on the carbohydrate.

In dilute solution irradiation of aldohexoses the attack is not confined to any particular part of the molecule. The products in oxygen and vacuo demonstrate that all bonds are effected.

Paper chromatographic and radioisotopic methods were used to identify products formed during irradiation of D-glucose solutions in vacuo. By reference to yield-dose curves, the following primary processes were recognized: 1) oxidation at C-1 to give gluconic acid, 2) attack at C-2 to give glucosone, 3) ring scission to yield two- and three-carbon aldehydic fragments, 4) dimerization of radicals of the type RCHOH as an initial step in the formation of polymeric material (56).

Irradiation of the disaccharides sucrose, maltose, and cellobiose, the trisaccharide raffinose, and the polysaccharides dextran and starch demonstrated

that the glycoside bond is espeically sensitive to the action of ionizing radiation and that the order of hydrolysis is quantitatively that found with acid hydrolysis. Hydrolysis of the disaccharides and trisaccharides increased with increasing dosage (32,61).

The general degradation pattern of D-glucose solution is given below (56):

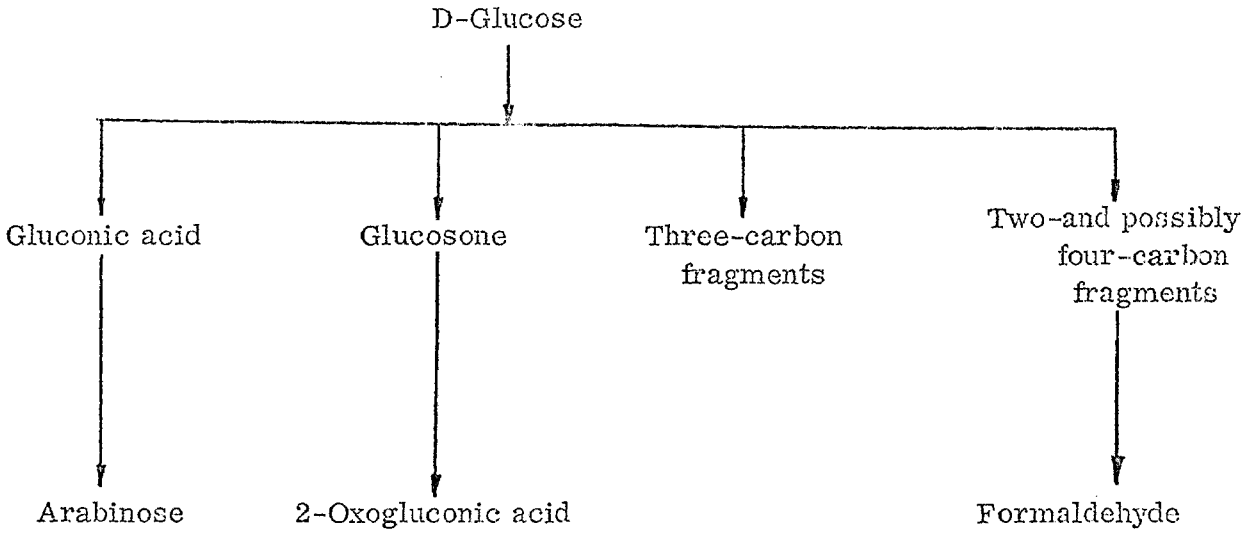

Figure 2
General Degradation Pattern
of D-glucose in Solution

Attack occurs throughout the aldo hexose molecule and is not confined to particular carbon atoms. However the oxidation of an aldohexose in to the corresponding hexuronic acid can be achieved by the specific oxidation of the primary hydroxyl group at C-6 to the carboxyl group (39,52).

The Reactions of Carbohydrates

The reactions of carbohydrates will be considered as they generally produce HMF under dehydration in acid solutions. By examining the reactions of carbohydrates under chemical attack and comparing the results to the action under irradiation, a basis for comparison of chemical and radiation reactions of HMF can be established.

In the Presence of Acids. The mildest reaction of sugars induced by acids, is the interconversion between α and β isomers. This interconversion takes place under very mild conditions of temperature and acidity. Stronger conditions produce dehydration of the carbohydrates. The dehydration may take place by the formation of anhydro rings or of double bonds. This dehydration process serves to remove three molecules of water from the sugar molecule to give the furan nucleus. A wide range of carbohydrates are degraded by acids to the furan compounds. Pentoses produce 2-furaldehyde while hexoses may yield 5 hydroxymethyl-2-furaldehyde (which may react further to yield levulinic and formic acid), the derived difuryl ether, and 2-hydroxyacetylfuran (2-furyl hydroxymethyl ketone).

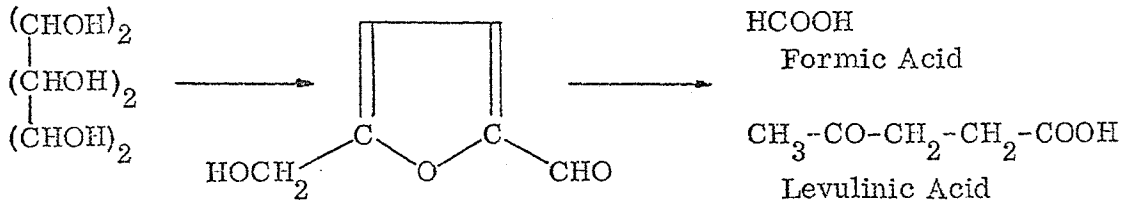

FIGURE 3
The Decomposition of Hexoses To
Formic and Levulinic Acids

Yield of HMF (5 hydroxymethyl-2-furaldehyde) as high as 54 weight per cent and of levulinic acid to 79 weight per cent have been reported using sucrose as the starting material (69,70). Levulinic acid has been prepared commercially from starch by the Moyer patent (71).

Preparation of Levulinic Acid. Mulder, in 1840, reported the formation of levulinic acid upon heating sucrose with mineral acids at high temperature (72). Levulinic acid is produced by the action of acids on most carbohydrates; thus d-glucose (73-76) glasctose (77,78) sucrose (79-81) fructose (82) glucosamine (83) chitose (84,85) sorbose (84,85) desorypentores (86) and hexose sugars as such or joined in disaccharide or polysaccharide configuration (71,82,84,87,88), all produce this acid. McKenzie (87) reported a 22 weight per cent yield by heating sucrose with concentrated hydrochloric acid at atmospheric pressure. Thomas and Schuette (88) showed that if the pressure of the sucrose-hydrochloric acid solution is increased, and allowed to digest at 162^{o} C for one hour, the yield of the acid was 42 weight per cent. The hydrochloric acid concentration for optimum yield was reported to be 4.6 weight per cent. The effect of different acids upon the yield was studied and reported by Wiggins (70). Sulfuric acid yielded 42.3 weight per cent, hydrochloric 60 weight per cent and hydrobromic acid gave 70 weight per cent of levulinic acid.

It has been shown (89) that the concentration of sucrose is a critical factor in the yield of levulinic acid. Highest yields are obtained with dilute solution. Figure 4 shows the relative yields of levulinic acid at different concentration of sucrose heated with hydrobromic acid, all other conditions remaining the same

(89). The highest yield of levulinic acid, based on the weight of the crude product, was 79 weight per cent of the theoretical and was obtained when the sucrose concentration was only 3 weight per cent.

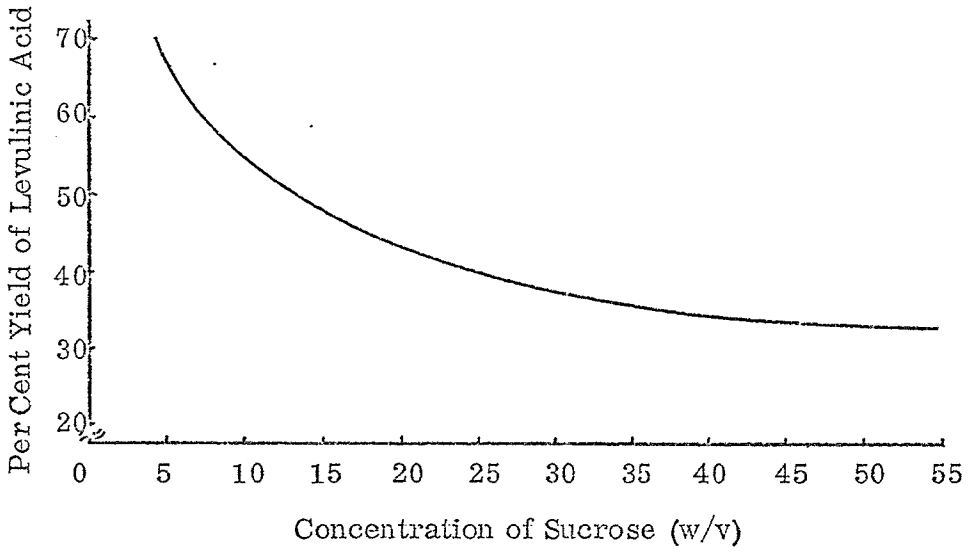

FIGURE 4
Effect of Sucrose Concentration on Levulinic Acid Yield

Ploetz (90) has also used the hydrobromic acid method to make levulinic acid and recorded a yield of 69 weight per cent of the theortical from crude sugar, 75 weight per cent from D-glucose and 64 weight per cent from starch.

The use of hydrobromic acid as a commercial catalyst is ruled out by reason of economics but some improvement in the yield obtained with hydrochloric acid is found by adding a small amount of sodium bromide to the acid solution(91).

For each mole of levulinic acid formed there is an equal amount of formic acid. If appropriate methods of isolation are employed both of these products may be obtained from sucrose. The isolation of levulinic acid can be accomplished either by partial neutralization, filtration of humin or black

polymeric material, and vacuum steam distillation according to the Moyer Process (71) or by solvent extraction. Macollum (95) suggests methylene chloride and also butanol as the solvent. The product obtained is a light yellow liquid and Moyer (96) has proposed a method of decolorizing the acid by treatment with very small amount of sodium chlorite and hydrogen peroxide.

Preparation of HMF. Until 1895 it was assumed that levulinic acid was produced directly from the carbohydrate. Dull (92) then found that fructose when treated with aqueous oxalic acid gave a substance resembling furan. This was later investigated by Kiermayer (93) who found that 3 weight per cent sucrose heated with 0.3 weight per cent aqueous oxalic acid at 120° C was the best source of the compound which was later identified as 5-hydroxymethyl--furfural. Kiermayer also observed that levulinic acid was obtained when HMF was treated with oxalic acid under pressure. Van Ekenstein and Blanksma (94) showed that the complete degradation of hexoses to levulinic acid takes place through the intermediated formation of HMF, the reactions is shown in Figure 3.

5-hydroxymethylfurfural is usually prepared from sucrose using the Kiermayer (93) synthesis. A 30 weight per cent sucrose solution is heated at a temperature of 120-140° for 2.5 hours with 0.3 weight per cent oxalic acid. The resulting solution, which was red-brown colored, is filtered from the black humin material also formed. The HMF was removed from solution by continuous extraction with ethylacetate. When distilled in a high vacuum HMF was obtained in 27 weight per cent of the theoretical yield. The quantity of humin is small. Its composition is unknown beyond its being polymeric. It has been

shown that it is formed from HMF by the action of dilute acids at 130° C (97). Other investigators have found that HMF is formed in neutral solution without the addition of acid (99), and that by heating in an atmosphere of hydrogen instead of air and yield is increased about 30 weight per cent (81).

The pH of the solution has a direct affect on the yield of HMF. The distruction of glucose as a function of pH value has been studied (102,103) and demonstrated the surprising stability of the glucose molecule in the neighborhood of a pH value of three. It appears that ring stability approaches a maximum at a pH of about 2.5 - 3 (84). However, no such minima in the destruction rate of sucrose and D-fructose was found and the rate for these two compounds is greater than glucose (69,97-99). HMF production at a pH of 3 is only one-fifth as much as that formed at pH 1.6 and about one-half as much as neutral solution will produce. The effect of pH on yield of HMF is shown in Figure 5 (102, 103,109).

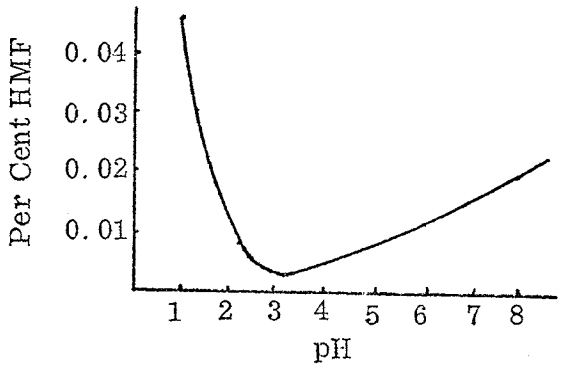

FIGURE 5
Effects of pH on Yield of
5 Hydroxymethyl-2-furaldehyde

It seems that ketoses are more easily degraded than aldoses to HMF and there is no doubt that glucose is more resistant to acidic degradation than fructose (100,102). The resistance of glucose was confirmed by Haworth and Jones (69) who performed many experiments to discover the optimum conditions for HMF formation from sucrose. It was shown that under the conditions present, aqueous solution 0.25 weight per cent oxalic acid under moderate pressure, only the fructose half of the sucrose molecule takes part in the reaction. The glucose was recovered from the residual solution, after the extraction of HMF, in almost quantitive yield as potassium hydrogen D-glucosaccharate (69).

Work has been done on the role of HMF as an important precusor in the formation of brown colors in heated sugar solutions (98,103). It has been found by Scallet and Gardner (99) that aqueous solutions of glucose become highly colored when heated for several hours at 100^{o} C. The coloration was because of the formation of hydroxymethylfurfural followed by its polymerization to form humin substances. The presence of 15 p.p.m. of HMF after 2.5 hours gave a colorless solution but after 7 hours the concentration was up to 170 p.p.m. and the solution was high colored.

In addition to giving a brown colored in food processing it has been stated the HMF inhibits microbial formation (104). Removal of HMF formed during acid hydrolysis of carbohydrate mash by absorption on carbon, or inactivation by addition of sodium bisulfite is necessary for smooth fermentation (105). The suggestion that the relative preservation and germicidal action of various sugars, upon heating in food processing, may very well be because of the ease with which HMF is formed, either during sterilization of the media

or in the heat treatment part of the process after the food has been canned (100,106). The germicidal action seems to be a more reasonable idea when attributed to HMF than to structural or osmatic pressure as has been claimed (107,108).

Mechanism of Formation of 5 Hydroxymethyl-2-furaldehyde and 2-hydroxyacetylfuran. In 1910 (115) Nef suggested the first mechanism for the formation of HMF. His proposal was made at the end of his classical paper on the sacchorinic acids, and was overlooked by subsequent workers and reviewers (110-112). Haworth and Jones (69) in 1944 advanced an identical mechanism for the formation of HMF from D-fructose.

It had been assumed that the dehydration of hexoses, or polysaccharides which contain hexose units, in neutral or acid solution yields only one furan derivative, HMF. However, Miller and Cantor (113) reported the isolation of another furan compound, 2 hydroxyacetylfuran from the acid catalyzed dehydration of sucrose in aqueous solution. This compound appeared to be formed in about two per cent yield duringsynthesis of HMF. Later Moye (114) was to investigate the formation of these two isomeric compounds as interrelated in the decomposition of hexoses.

Nef's mechanism was modified recently by Mednick (116) and also Moye and Krzeminski (117) however, this mechanism was generally rejected (118) and another older mechanism by Hurd and Isenhour (119), Isbell (120) and Wolfrom and coworkers (121,122) continued to be accepted (118). Basically this mechanism is a series of dehydration reactions with the first step being the elimination

of water at C_1 and C_2 giving an intermediate which undergoes a toutomeric change from which the final product is obtained by loss of a third molecule of water. This toutomeric change is based on the well-known elimination of a hydroxyl group in a position beta to a carbonyl group. However, Isbell (120) proposed that the beta elimination proceeds by a consecutive electron displacement involving an enediol intermediate. An extension of this mechanism based on isloation of four intermediates in the formation of HMF from both D-fructose and L-sorbose has been proposed by Anet (118, 123-130). The mechanism is presented in Figure 6.

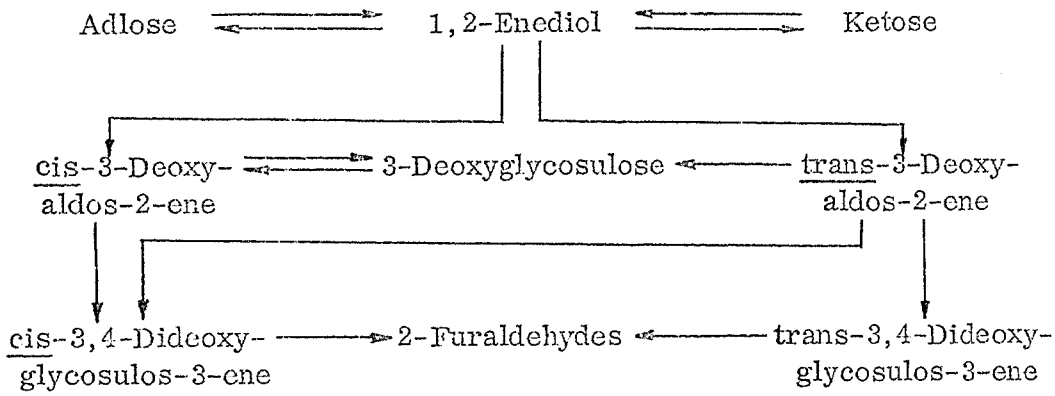

FIGURE 6
Anet's Mechanism for Formation of
2 Furaldehydes from Sugars (118).

It proceeds by, first enolization in the 1, 2, position and elimination of the hydroxyl group from carbon 3 occurs to yield enolic $\alpha\beta$ - unsaturated aldehyde. The enol which is equilibrium with the 3 deosyosone is dehydrated further to the unsaturated osone. The last step involved the loss of the third molecule of water probably from the 2, 5 furanose form of the unsaturated osome to yield the 2 furaldehyde while 2-furyl hydroymethyl ketone is also produced from this common intermediate.

Anet's mechanism is supported by the work of Moye (114) and Krzemenski (117). Since both HMF and 2-furyl hydroxymethyl ketone (2-hydroxyacetylfuran) are formed by the acid catalysed degradation of hexoses, several mechanisms are still valid to explain the formation of these two isomeric furan compounds.

Mechanism for Formation of Levulinic and Formic Acids. The most important aspect of the chemistry of the furan ring in HMF is its scission under the influence of acidic reagents. The mechanism was first studied by Teunessen (131,132) who measured its rate and showed it to be a unimolecular reaction. His proposed scheme for the conversion of HMF to levulinic acid in dilute acid solution is shown in Figure 7.

Pummerer, Guyot and Birkofer (133,134) proposed that the reaction involves opening of the furan ring with the formation of 2, 5-dioro-6-hydroxycaproic aldehyde, followed by elimination of formic acid and oxidation of the aldehyde group by the primary alcohol group.

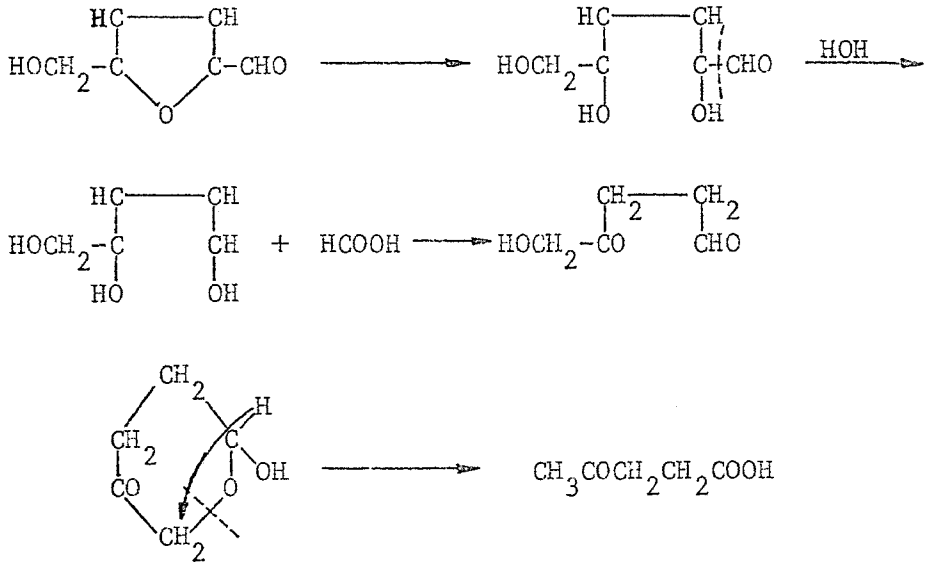

FIGURE 7
Proposed Mechanism For Formation of Levulinic Acid From 5-Hydroxymethylfurfural (131,132)

Snowden (80) offered further proof by using labled 1-C^{14} D-glucose to determine the location of the aldehyde carbon. Using hydrobromic acid and the 1-C D-glucose it was shown that the aldehyde carbon of the hexose eventually becomes the carbon of the formic, while the levulinic acid was devoid of radioactivity.

Reactions of 5 Hydroxymethylfurfural. The reactions of the hydroxymethyl group causes the compound to behave like a normal primary alcohol inasmuch as esters are readily formed. Acetoxymethylfurfural and benzoxymethylfurfural are easily obtained by treating with acetic anhydride and benzoyl chloride respectively.

The hydroxyl group is very easily replaced by any halogen. Using a solution of hydrogen chloride in ether, Reichstein and Zsckokke (135) obtained from HMF the 5-chloromethylfurfural. The ease with which the hydroxyl group is replaced by a halogen atom is not a normal substitution of primary alcoholds and is undoubtedly because of an electronic effect of the furan ring. While these compounds are easily formed they are also very reactive and offer useful intermediates in the formation of derivatives of HMF. In boiling water both 5-chloromethyl and 5-bromomethylfurfural are immediately hydrolyzed to HMF in quantitative yield (136).

Another unusual behavior of the primary alcohol function of HMF is the dehydration which occurs when two molecules of HMF are heated with the ether compound as the product (137).

In the reaction of the aldehyde group there is analogous behavior between furfural and benzaldehyde in many ways. In the presence of alkali furfural

undergoes the Cannizzaro reaction to form furfuryl alcohol and furoic acid (138). With potassium cynaide, furoin, the analog of benzoin, is formed. The aldehyde group attached to the furan nucleus behaves very much as an aromatic aldhyde and this behavior is also true for HMF. However, HMF does not form the corresponding derivative of furoin but rather produces resinous materials (139). It appears that the reactions of the aldehyde group, which in furfural are those of an aromatic aldehyde, are modified by the presence of a substituent at position 5 in the furan ring.

The aldehyde group is subject to reduction reactions with the conversion of this group to the methyl group by hydrazine being reported (135). The reduction may also be carried out by use of Raney nickel at temperatures of 75-130^{o} C with pressures of 70-150 atmospheres (140, 141). It is surprising that reduction of the ring was not achieved under the more vigorour of these conditions. However, Moye (148) states that the aldehyde group is more easily reduced than the ring system. Moye (114) also reduced the aldehyde group of the trimethylsilyl esters of HMF and 2-furyl hydroxymethyl ketose sodium borohydride in an attempt to use gas chromatography as an analysis tool.

The most important reaction of the furan ring itself is the addition of hydrogen. Hydrogenation occurs readily at temperatures above 100^{o} and in the presence of Raney nickel, HMF is converted to 2, 5-dihydroxymethyltetrahydrofuran in good yield (142).

Nuclear substitution in HMF is not of great importance since the most reactive positions in the furan ring, 2 and 5, are already substituted. However,

it is possible to introduce substituents at positions 3 and 4 or to even eliminate one group and replace it with another (143-148).

The scission of the furan ring of HMF under influence to acidic reagents is the reaction usually encountered. The degradation of HMF by scission produces formic and levulinic acids. Teunissen (131,132) studied the reaction and found it to be a unimolecular one.

Analysis Methods

Several analysis methods have been reported in the literature for carbohydrate analysis. The earlier work generally reported on a change of a physical property of the system, such as ultraviolet or optical density. The change was used to follow the course of the reaction. Later chemical analysis methods such as chromatography were used to report the changing composition of individual components rather than of a total system.

Ultraviolet Analysis. The early work on the analysis of irradiated carbohydrates involved primarily ultraviolet analysis of the reaction mixture. The course of the reaction was followed by noting the appearance of absorption peaks in the spectra as the solution was irradiated.

The U.V. spectrum analysis of glucose solution and some of the known products have been published in the literature. Glucose itself does not exhibit any peaks in the U.V. spectrum (149). The major peak for HMF has been reported as 285 mμ ϵ = 16,500 for the major peak and 228 mμ ϵ = 3,620 for the minor peak (121). Other reported values are 283 mμ ϵ = 14,330 (99), and 284mμ ϵ = 16,700 (major peak) and 230 mμ ϵ = 3,080 (102). The two maxima may be because of the possiblity that a molecule containing a carbonyl

group attached to a linear conjugated chain can have more than one electronic transition, one usually more intense than the other (102,150). The appearance of a peak around 280 mμ is usually taken as evidence for the carbonyl group; the intensity of this transition is determined partly by conjugation.

HMF presents a very definite spectra and is easily analyzed because of its high extinction coefficient (102,121,122,149,151) of around 16,000 liters $mole^{-1}cm^{-1}$. By following the appearance of HMF for increasing dosage it was possible to show that it was not formed directly from glucose but rather other absobing substances were produced first which then gave HMF.

The spectra of levulinic acid has been reported (102) with a single maxima at 265 mμ and a much lower molecular absorption coefficient of about 23 liters $mole^{-1}cm^{-1}$.

Two-hydroxyactylfuran has two peaks like its isomeric compound HMF. Its major maxima is at 275 mμ, $\epsilon = 14,000$ and a minor peak at 225 mμ, $\epsilon = 2,790$ (113).

Thin Film and Paper Chromatography. Both of these analysis methods have been used extensively in the study of radiation induced changes in carbohydrates (29,31-39,41,42,44,49,51,54,59,114,151-154). Usually the methods employed two dimensional chromatography with various compounds being used as developing agents for the spots. Some researchers used labeled ^{14}C glucose and dextrose compounds and performed autoradiographys of the chromatograms. The products that were identified were done so by running a known sample on the same chromatogram or by isotropic dilution methods.

Because of the poor resolution of early paper and thin layer methods controversy arose as to the presence or the lack of certain products. The large amount of streaking of the chromatographs appears to be the major reason for this controversy. Phillips et al generally claim a total of ten compounds formed. A recent study of only the head space vapors of irradiated glucose (155) showed at least 15 compounds by gas chromatographic analysis. Gas chromatography seems to be better able to separate the streaking which is common on the thin layer or paper work.

Gas-liquid Chromatography. Gas-liquid chromatography has been developed, refined at a faster rate, and applied more extensively than any other analytical method in the history of chemistry since its inception in 1952. It has been used with practically all classes of organic compounds. A tremendous number of articles, 4338 to October 1961 (156), have appeared in which gas chromatography was used. It is the most sensitive research tool available for organics, and if the separated components are collected from the effluent gas stream and subjected to infrared spectroscopy a positive identification can be made of each component. The greater resolving power and more rapid separations coupled with both quantitatives and qualitative results are distinct advantages of gas-liquid chromatography over other chromatographic techniques.

The use of gas-liquid chromatography for the separation of carbohydrate derivatives was first proposed in 1958 (158). After the article appeared there was a rapid development of the method (156). The process of gas-liquid chromatography involves the introduction of a sample into a column which contains the

liquid phase on an inert support and through which the carrier gas is passed. The sample is then vaporized and acts as the mobile phase which flows over the column packing impregnated with a nonvolatile solvent. The column effluent if then passed through a sensitive detecting device which measures the variation in some property of the gas. Therefore the equipment consists of the following basic units: an injection mechanism, the column, a detector, a gas flow system, an amplifier, and a recorder, in addition to the necessary temperature control elements.

Various components will move through the column at different rates and the detector will show the presence of the components at various times after injection. The effluent can then be collected and melting point determination, infra-red and ultra-violet analysis carried out. Comparison of known compound peaks with unknown peaks also provides identification.

The major limitation of gas-liquid chromatography is the requirement of volatility and thermal stability of the compounds to be separated. It appears that monosaccharides containing more than two free hydroxyl groups may not be sufficiently volatile. The volatility of such compounds can be increased by substitution of the hydroxyl function with methyl, acetyl or trimethylsilyl groups.

Fully methylated glucosides have been used for separation as well as acetates. Both of these methods suffer drawbacks in that proper preparation is difficult and reducing disaccharides, in the case of the acetate, give rather broad peaks. The trimethylsilyl ethers appear to be the answer to this problem in that they are prepared quite easily, allow rapid quantitative measurements, and are sufficiently volative, and readily hydrolyzed (159-165). The advantage

of the ease of hydrolyzation is the fact that their compounds can be separated as their trimethylsilyl esters and then be regenerated unchanged by a very mild hydrolysis, such as with 50 weight per cent aqueous methanol at reflux temperature for 1 to 2 hours (166).

Silyl derivatives prepared by dissolving the sample in pyridine followed by reaction with 1,1,1,3,3,3-hexamethyldisilazane and trimethylchlorosilane. (10:2:1 v/v ratio) (161,163,165). The reaction mixture is then injected directly into the chromatograph.

The application of gas chromatography to separation and identification of HMF is confused. Moye and Krzeminski (117) first employed it in 1963 primarily to observe the existence of the equilibrium: glucose - enedial - fructose, during the conversion of fructose to HMF. Later Moye (114) attempted to determine the yields of 2-furyl hydroxymethyl ketone and HMF from this reaction and failed. It was found that free HMF could not be estimated in this way and that they were further unable to separate the trimethylsilyl derivatives of HMF and 2-furyl hydroxymethyl ketone on a variety of columns.

To reduce the problem of obtaining derivatives of sufficient volatility from irradiation of glucose Herlitz et al (155) used only the head space vapors. They obtained 15 separate compounds formed in the head space vapors of β-glucose using gas chromatography. The number of compounds resolved by gas-liquid chromatography shows the advantage of this type of analysis.

III EXPERIMENTAL DETAILS

The experimental work was carried out in two stages. First was a quantitative survey of analysis procedures and equipment. Second was a detailed qualitative analysis of HMF under irradiation using the experimental procedures from stage one.

Equipment

The major items of equipment were obtained on this campus with the exception of the ^{60}Co facility which is located near the Columbia campus of the University of Missouri system.

Gamma Irradiation Facilities. The preliminary work was performed using the UMR reactor at a power level of 10 kilowatts. This reactor produces both gamma rays and neutron particles while in operation and the various fission products formed will continue to produce gamma rays after shutdown but at a fairly fast decreasing decay rate. The decreasing rate makes irradiation to a known total dosage difficult and prevents entirely the investigation of the effect of dose rates. To reduce the neutron population during irradiation of the samples they were placed inside of a cadmium container. The sample holder was placed away from the reactor core so that almost all neutrons would be thermalized and then absorbed by the cadmium shield because cadmium has a very high absorption cross section for thermal neutrons.

To achieve a known dose rate with a high degree of reproducability a long lived isotope source should be used. The Research Reactor Facility, a systems reactor, located near the Columbia campus has a 5000 curie ^{60}Co source for gamma irradiations. The facility consists of the source which is setting on the floor of an irradiation pool under 15 feet of water. The actual source consists of 10 pencils of ^{60}Co in a ring structure. This configuration provides two advantages: one is the initial shipping of the source is relatively easy since each pencil is only 500 curies which is easier to shield against than the entire source, and second is the ability of the cylindrical geometry to reduce scattering errors for samples irradiated in the center of the ring allowing a better isodose configuration.

The dosage and rate was obtained from the data supplied by the reactor facility and by use of Baush and Lomb Cobalt glass dosimeters which were irradiated along with each sample. The dose rate in the center of the source was 4.1×10^5 Rads/Hr. The Baush and Lomb cobalt dosimeters were read with a Spectronic 20 Colorimeter-Spectrophotomer at 500 mμ.

A dose rate of less than 4.1×10^5 Rads/Hr could be obtained by placing the sample away from the source on the metal grid plate which has a hole drilled to accept sample containers every six inches.

Gas Chromatographs. Two gas chromatographs were used in the analysis. One was a F & M Scientific Corporation Model 810 with a dual flame detector. The partition columns were a matched pair of stainless steel 1/8 inch diameter, six feet long packed with 10 weight per cent SE-30.

The second gas chromatography was a Victoreen Model 4000-1a, serial 184 again with either the 10 per cent SE-30, 6 per cent SE-30, or Carbowax 20m 6 feet long for the partitation columns.

Both gas chromatographs were capable of operation in the temperature programmed mode.

Recorder. The recorder used with the Victoreen instrument was a 10 inch Beckman recorder, Model 1005, serial 100502-1001829 with built in disk integrator.

Ultra Violet Spectrophotometer. The ultra violet spectra was determined by uding a Perkin & Elmer Model 450, U. V. - Visible - NIR spectrophotometer. Slit width was set on automatic scan with 1.001 cm cell path length.

Syringes. The injection syringes used were all Hamilton Microliter syringes. Two were of 10.0 μl capacity, model 701-N, while one was of 1.0 μl capacity, Model 7101-N.

The 701-N have an absolute liquid delivery accurate to within 1 per cent and a repeatable accuracy also within 1 per cent.

The 7101-N has an accuracy of 1 per cent between 0.5 μ1 and 1.0 μl and of 2 per cent down to 0.05 μl delivery volume.

Reagents

The 5-hydroxymethylfurfural (5-hdyroxymethyl-2 furfuraldehyde) used was from two sources. The first used in the preliminary work was purchased from Aldrick Chemical Company Inc. of Milwaukee, Wisconsin, catalogue

number H4080, while the other was supplied by Merck Sharp & Dohme Research Lab., Rahway, New Jersey, lot number L553942-0-41. Each of these was subjected to analysis by gas chromatography to discover any impurities present prior to use.

Levulinic acid was obtained from the Aldrich Chemical Company, catalogue number L200, and again checked for purity prior to use.

The 1,1,1,3,3,3 hexamethyldisilazane and trimethylchlorosilane used in the preliminary work was purchased from Taylor Chemical Company of St. Louis, Missouri. Later the hexamethyldisilazane, catalogue number 82-5001, used was from Varian Aerograph of Walnut Creek, California, while the trimethylchlorosilane, lot number 4031-09-F8, was obtained from Analabs Inc. of Hamden, Conn.

Procedure

The procedures outlined were used both in the initial work and the final investigation. The preliminary work allowed the procedures to be selected and modification to be devised which would facilitate analysis.

Irradiation of Samples. All the irradiated samples were taken from one lot of 1 Normal 5 HMF and when a quantity was prepared for irradiation a similar amount was withdrawn and stored as a blank to show any variation of concentration in the solution.

The irradiated samples were prepared by placing 100 ml of the solution in a plastic screw top bottle. The bottle was then sealed inside of two separate plastic bags with a heat sealer. The sample was placed in a sample holder with

a guide pin on the bottom to enable it to be replaced in the same place each time in the gamma facility. The level of the bottle was adjusted vertically each time to account for the withdrawal of 3 ml of sample solution so as to keep the center line of the liquid on the same isodose curve.

The samples were irradiated for one hour, removed from the gamma facility, a 3 ml sample pipetted out, the bottle resealed in two plastic bags and placed back in the gamma facility. Cobalt glass dosimeter plates were placed with the the sample and a plate was removed along with each sample giving an accurate record of the dosage for that irradiation period.

Nuclear Reactor Irradiation Procedure. The sample was placed in a 100 ml screw top plastic bottle and sealed inside of two plastic bags to insure waterproof containment. This container was placed inside of a cadmium container attached to a plastic sample holder which fits inside of the grid plate of the UMR reactor. The samples were placed in the same location, core position B8 with the container facing toward position A7, for each irradiation.

Analysis Procedure. Each 3 ml sample provided material for several analysis methods. A 10 mg sample was treated with 0.2 ml of hexamethyldisilazane, and 0.1 ml of trimethylchlososilane. The reaction was carried out in a 1.3 ml plastic stoppered vial. The mixture was shaken vigorously for about 30 seconds and then allowed to stand for 1 hour or longer at room temperature prior to chromatography to allow the ammonium chloride to settle. From 0.1 to 1.0 μl of resulting reaction mixture was injected into the gas chromatograph.

Another sample of the liquid was injected into the gas chromatograph without being treated to form the trimethylsilyl derivatives. The sample was

injected into the gas chromatograph to provide data on the compounds which were volatile without being converted to the trimethylsiyl derivatives. Sample sizes ranged from 0.1 μl to 1.0 μl.

For some analysis work the gas chromatograph was operated with temperature programing. Increasing the temperature during analysis allowed the easily volatile substances to be separated at a low temperature to give separate peaks but still enable the higher boiling compounds to be detected in a reasonable time as the temperature is increased linearly as a function of time.

Effect of Total Dosage at a Constant Dose Rate. Samples were irradiated at a constant dose rate of 4.1×10^5 Rads/Hr. A sample was taken every hour up to a total dosage of 8.2×10^6 Rads.

The decomposition of HMF as a function of dosage was determined by plotting the integrated peak response curve as a function of irradiation. The kinetics of the formation of compounds was likewise obtained by plotting each of the peak areas as a function of irradiation dosage.

Gas Chromatography. The analysis was carried out under the following conditions. Isothermal operation, 150° or 200° C; injection port temperature, 265° C; carrier gas flow, 15 ml/min; sample size, 1.0 μl; electrometer sensitivity range, 8×10^{-11} amperes for full scale deflection, 0.5 μl 0.1 N HMF provided 94 per cent of full scale deflection on this range. Temperature programmed operation was used under same conditions except for initial temperature which was 60° with the final temperature of 250° C, 5° C/min temperature rise.

IV DATA AND RESULTS

The data from the investigation is summarized in the following tables. Tables I, II, III show the integrated peak areas for HMF as a function of total received radiation dose, on 6 weight per cent SE-30, Carbowax 20M, and 10 weight per cent SE-30 respectively.

Table IV contains integrated peak area data for the water and formic acid peak.

Table V contains the data obtained by varying the concentration of the injected HMF sample to check the chromatograph linearity.

Table VI contains the data obtained when the injection sample size was varied.

TABLE I

Integrated Peak Area for HMF as a Function of Total Irradiation on 6 Weight Per Cent SE-30 Partition Column Packing

Sample Number	Radiation Dose Rads	Analysis Number	Peak Areas Integrator Units	Peak Areas In^2
1	4.1×10^5	A	895	0.6654
		B	941	0.6996
		C	925	0.6877
		D	899	0.6684
2	8.2×10^5	A	862	0.6408
		B	892	0.6631
		C	897	0.6669
3	1.23×10^6	A	903	0.6713
		B	880	0.6542
4	1.64×10^6	A	819	0.6089
		B	872	0.6483
		C	895	0.6654
5	2.05×10^6	A	842	0.6260
6	2.46×10^6	A	836	0.6215
7	2.87×10^6	A	800	0.5947
8	3.28×10^6	A	799	0.5940
9	3.69×10^6	A	776	0.5769
		B	783	0.5821
		C	803	0.5970
14	5.74×10^6	A	677	0.5033
		B	703	0.5226
		C	717	0.5330
15	6.15×10^6	A	671	0.4988
16	6.56×10^6	A	666	0.4951
17	6.97×10^6	A	640	0.4758
18	7.38×10^6	A	602	0.4475
		B	642	0.4773
19	7.79×10^6	A	594	0.4416
		B	597	0.4438
		C	615	0.4572

TABLE II

Integrated Peak Area for HMF as a Function of Total Irradiation on 10 Weight Per Cent SE-30 Partition Column Packing

Sample Number	Radiation Dose Rads	Analysis Number	Peak Areas Integrator Units	In^2
1	4.1×10^5	A	1069	0.7947
20	8.2×10^6	A	744	0.5531

TABLE III

Integrated Peak Area for HMF as a Function of Total Irradiation on Carbowax 20M Partition Column Packing

Sample Number	Radiation Dose Rads	Analysis Number	Peak Areas Integrator Units	In^2
2	8.2×10^5	A	10,650	7.918
		B	11,230	8.349
5	2.05×10^6	A	9,830	7.308
		B	10,090	7.501
10	4.1×10^6	A	8,830	6.565
		B	9,300	6.914
15	6.15×10^6	A	8,520	6.334
		B	8,600	6.394
19	7.79×10^6	A	7,650	5.687

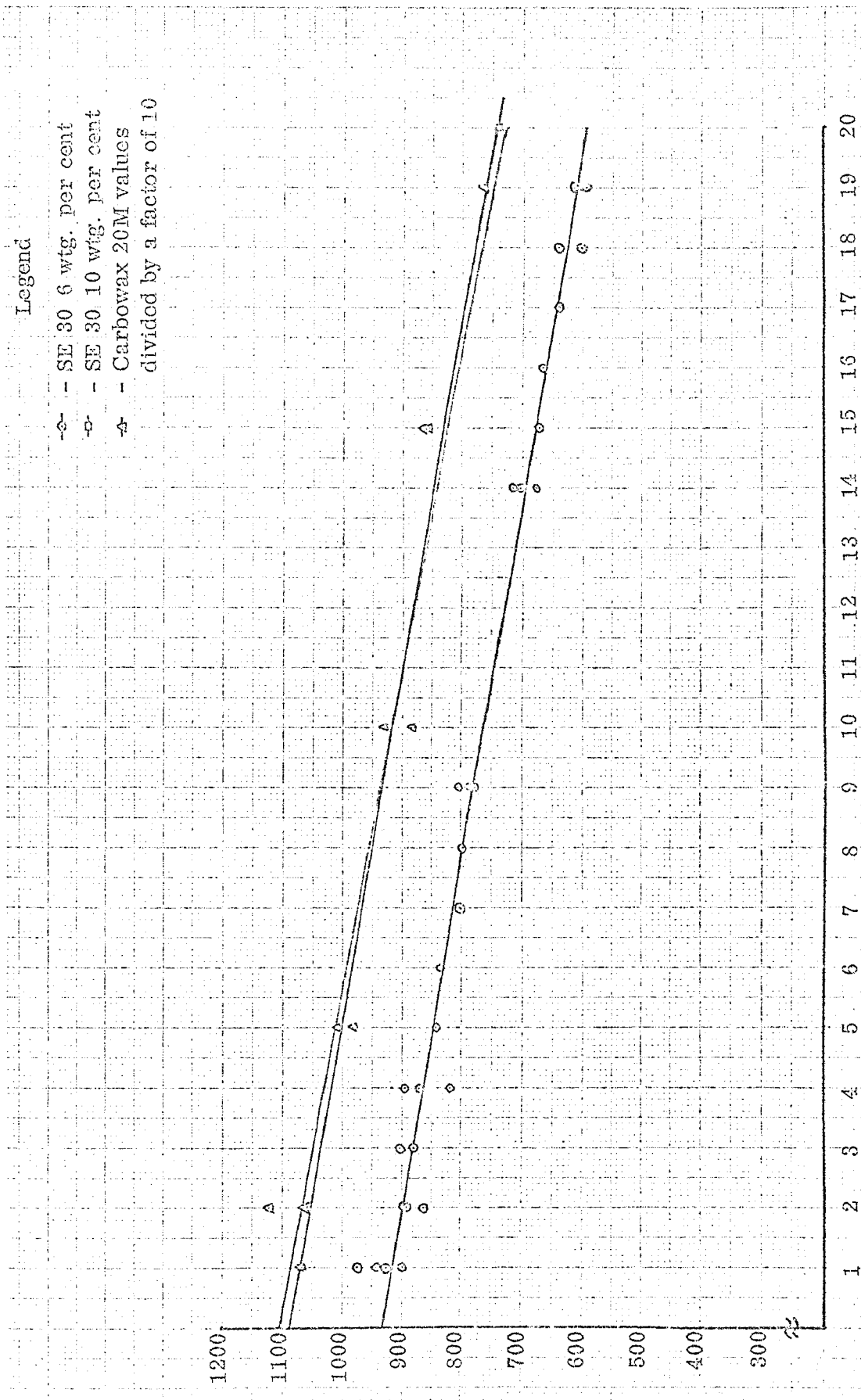

FIGURE 8, Decomposition of HMF as a Function of Gamma Irradiation Dose

TABLE IV

Integrated Peak Area for Water and Formic Acid as a Function of Total Irradiation on Carbowax 20M Partition Column Packing

Sample Number	Radiation Dose Rads	Analysis Number	Peak Areas			
			Major[a] I. U.[b]	In^2	Minor[a] I. U.[b]	In^2
1	4.1×10^5	A	440	0.3271	133	0.0988
		B	435	0.3234	135	0.1003
20	8.2×10^6	A	445	0.3308	135	0.1003

a Major peak occurred in 24 seconds, minor peak in 72 seconds compared to HMF in 2700 seconds.

b Integrator units.

TABLE V

Integrated Peak Area as a Function of HMF Concentration on SE-30 10 Weight Per Cent Partition Column Packing

Sample Number	Concentration Normality	Peak Area Integrator Units	Peak Area In^2
1	1.0	15,902	11.823
2	0.1	1,585	1.178
3	0.05	739	0.549
4	0.01	150	0.111
5	0.005	79	0.058

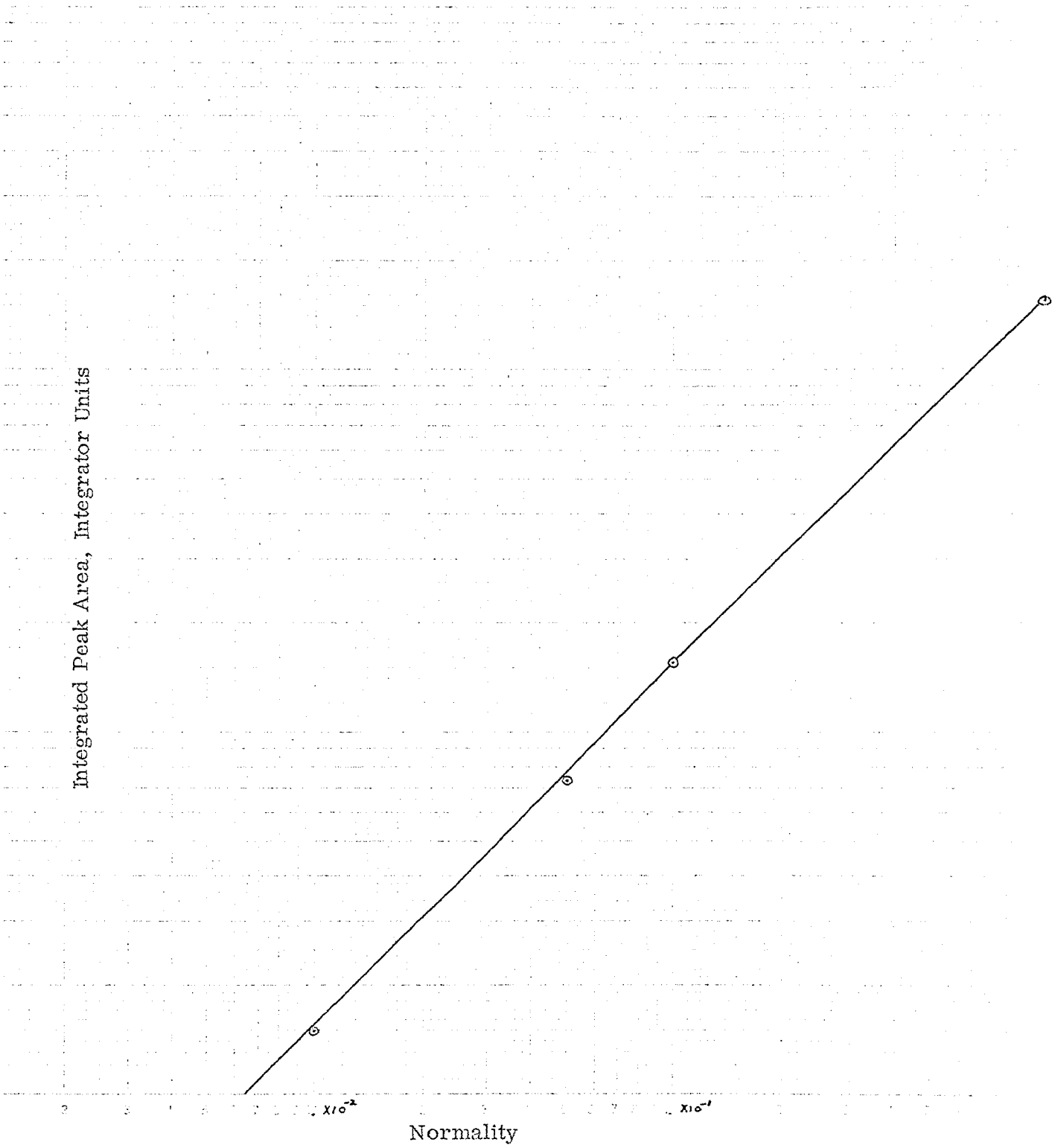

FIGURE 9, Integrated Peak Area as a Function of HMF Concentration

TABLE VI

Integrated Peak Area as a Function of Sample Injection Size for HMF on SE-30 10 Weight Per Cent Partition Column Packing

Sample Number	Injection μl.	Analysis Run	Peak Areas Integrator Units	In^2
1	1.0	A	2481	1.844
		B	2553	1.899
2	0.75	A	1936	1.499
		B	2008	1.493
3	0.50	A	1484	1.103
		B	1417	1.053
4	0.25	A	958	0.712
		B	856	0.636

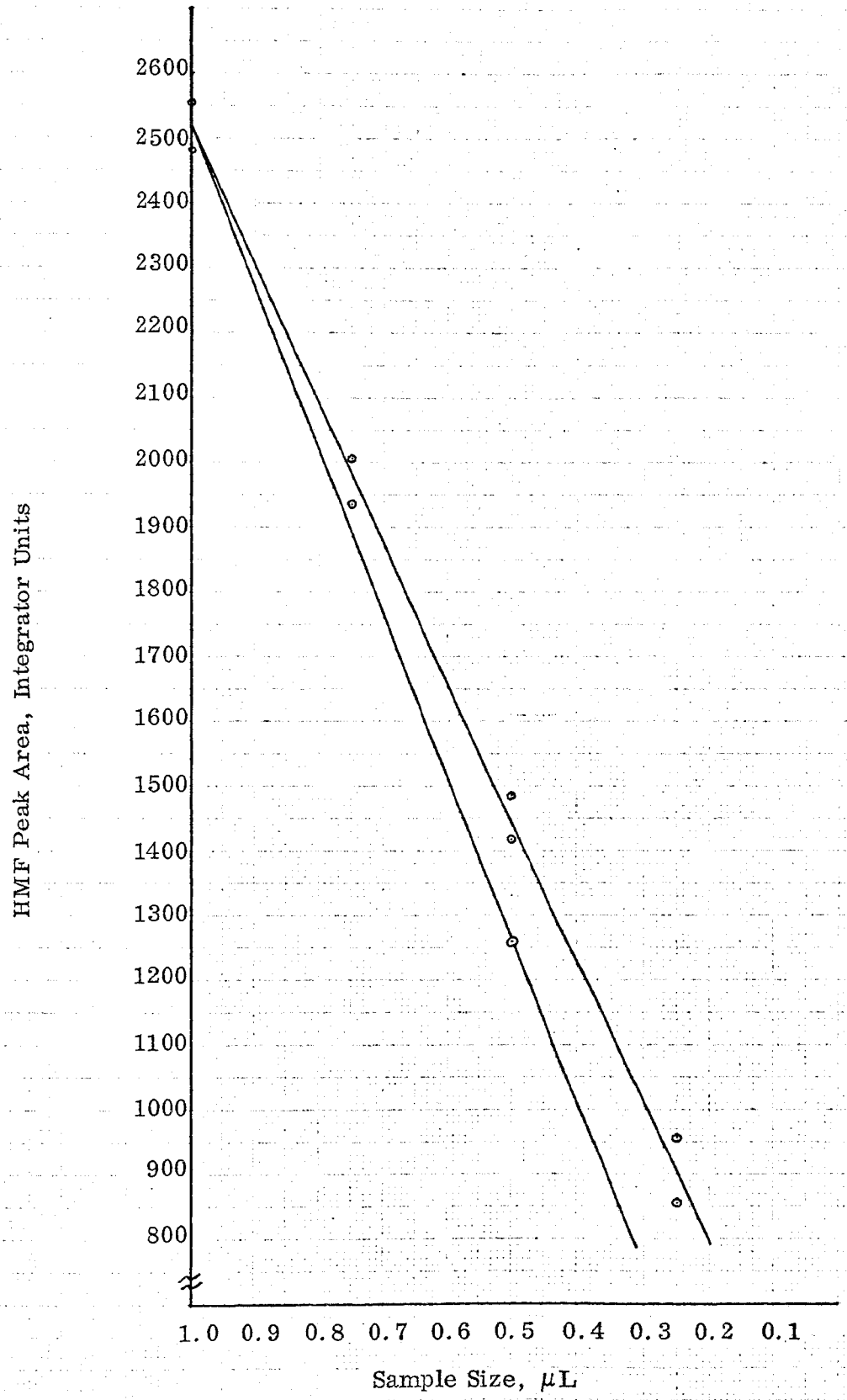

Figure 10, Integrated Peak Area as a Function of HMF Sample Size

V. DISCUSSION

The investigation was divided into two parts, the preliminary work during which the different methods of analysis were tried, instrumentation equipment was obtained and its operation and limitations were determined. The later investigation was planned to give quantitative data using the methods of analysis and procedures which had proved to be the best of the ones tried in the initial work.

Initial Investigation

The initial investigation consisted of determining which methods of analysis had been used for carbohydrates and heterocyclic compounds in the literature. While some methods might offer the desired information, the unavailability of equipment prevented the selection of several methods such as nuclear magnetic resonance spectrometry and mass spectrometry. Of the methods used in the literature, gas-liquid chromatography, ultra-violet and infra-red spectrometry offered to be the usable methods. These analysis procedures were tried and the results discussed in the following sections.

Gas Chromatography. A gas chromatography of F & M manufacture with thermoconductivity detector was initially used to determine if gas chromatography could be used as an analytical tool in detecting HMF. However, the sensitivity obtained was not great enough for an identifiable peak for HMF to be observed. Gas Chromatography should provide the most rapid quantitative and qualitative analysis for HMF provided adequate sensitivity could be obtained.

Another gas chromatography also of F & M manufacture Series 810 with a hydrogen flame ionization detector was then tried. Generally this detector is considered 10,000 times more sensitive, according to Beckman Instruments Inc. information. In addition, in an effort to make the HMF as volatile as possible it was converted to its trimethylsilyl ester (161-163,165). The silylation procedure resulted in very clear well defined peaks for dextrose and other sugars but the peak for HMF was bearly above the baseline. Since pyridine is used as a solvent for the carbohydrates in the silylation procedure it was not used on a sample of HMF. When the pyridine was left out of the silization procedure the HMF formed a strong peak without any tailing, while the sugars still gave well defined peaks.

Ultra-Violet Analysis. Since HMF has a distinct ultra-violet spectra the analysis of it might offer a convenient and accurate method of following the course of a decomposition reaction. Ultra-violet analysis had been used in the literature to follow the formation of HMF as a product of a reaction. To check on the usefulness of the ultra-violet analysis, a sample was prepared and irradiated in the UMR reactor for a two hour period, at a power level of 10 kw, inside of a cadmium container. The cadmium container acted as a partial shield against the large number of thermal neutrons which are present in an operating nuclear reactor. The gamma rays were attenuated only very slightly in passing through the cadmium shield. The unirradiated sample showed the spectra of HMF as reported in the literature (102,121,122,149,151). The irradiated sample showed a smooth ultra-violet spectra in which the absorption

peaks had been completely destroyed. The spectra shifted to a higher absorption in the 230 mμ to 195 mμ region using potassium becarbonate, but again the curve was smooth absorption. The spectra suggested that HMF had been completely changed in composition. The disappearance of HMF under a dose of approximately 1 x 10 Rads/Hr indicates that HMF is quite sensitive either to gamma radiation or possibly to neutron particles since both were present.

Infra-Red Analysis

To check if infra-red analysis would prove to be a useful tool the irradiated sample was extracted with acetone, the acetone was then evaporated to a reduced volume under vacuum and an analysis was run on the IR-4 under the supervision of Dr. Hanna. The results were inconclusive compared to the other methods for the final unseparated irradiated sample. The results were difficult to determine due to the number of radicals present. If the compounds could be separated before the infra red analysis the results would be easier to interpret.

The information from the initial work indicated that flame ionization gas chromatography would be the best analysis tool in that it would detect the presence of HMF quickly and yet with quantitative results.

Detailed Investigation

After it was found that gas chromatography would give separation with the required sensitivity using procedures found in the literature further work was

done to determine if standard procedures could be modified for the quantitative analysis of HMF. Slight modifications of the general carbohydrate analysis procedure might increase the sensitivity or accuracy of the analysis.

Selection of Analysis Procedure. The trimethylsilyl ester of HMF is easily detected on a flame ionization gas chromatograph but it also has several disadvantages which do not occur if the sample is injected without any prior preparation. The sample has to be accurately determined, the two silylation agents have to be measured out volumetrically and trimethylchlorosilane boils near room temperature making accurate volumetric measurement of this liquid with a pipett difficult. The resultant ester with the excess reagents has a tendency to plug the syringe needle presumably with ammonium chloride.

If free HMF could be analyzed on the gas chromatograph there would be less error in the analysis, less possibility of damaging the syringe, and the effluent from the gas chromatography could be condensed out and subjected to an infra-red analysis for each of the peaks. To regenerate the original compound from the trimethylsilyl ester requires refluxing the ester for several hours with 50 per cent aqueous methanol and with the small amount of separated compound available from the gas chromatograph enough of the compound may not be obtained to acquire an infra-red spectra.

Moye (114) reported that estimation of free HMF was not possible under his experimental conditions. He did not report why it was not possible to obtain this data. It is not known if poor peak response prevented quantitative results or if HMF was not detectable on the gas chromatograph apparatus used. The packing

was not specified but it is assumed the same as was reported for the acetates and trimethylsilyl derivatives of HMF and fural-hydroxymethyl-ketone. The packings were Apiezon L and DC Silicone grease. Not having these packings available a direct corrolation of the results has not yet been possible.

A 10 per cent silicone gum rubber type SE-30 proved to be the best of the ones tested for HMF. Tailing was almost non-existant and the peak heights were sharp on the leading edge. The same size injection sample gave within 10 per cent the same peak area on the 10 weight per cent column as it had on the 6 weight per cent column but the sensitivity of the instrument could be reduced by a factor of 4 over that necessary for the lower composition column. The 10 per cent columns had been used extensively where the 6 per cent ones were new and possibly were not as well conditioned.

Carbowax 20M was also used as a column packing and it offered excellent separation of formic and levulinic acids as well as the HMF. While identification was enhanced by this packing the peaks were of a symmetrically nature with both leading and following tailing of the peak. Because of tailing the Carbowax column was used extensively only in a survey task to determine what compounds were formed, but was not used for quantitative data.

For the quantitative analysis the columns were either the 6 or 10 per cent SE-30. The 10 per cent columns allowed a faster analysis than using the carbowax 20M less and a more sharply defined peak was obtained for the disk integrator on the chart recorder to analyze. The sensitivity of the electrometer was normally set so that 8×10^{-11} amperes input would serve to indicated full scale on the 1mv chart recorder. On the range the injection of a 0.5μ l of 0.1 N sample of HMF was

sufficient to give a 94 per cent of full scale indication. When analyzing for trace products the instrument was operated at as sensitive a level as possible, down to 1×10^{-11} amperes or lower.

Carrier gas flow for the analysis was between 10 and 15 ml/min N_2, injection port temperature 265° C and the flame ionization detector temperature was 295° C. The isothermal analysis determinations were either at 150 or 200° C while with the temperature programmed analysis determinations used 60° as the lower limit and 250° C as the upper limit.

Error Analysis. An analysis of the errors which might occur in the investigation is necessary to give a total possible error in the results. There are six sources of error which need to be considered in the irradiation of HMF. They are: (1) error in initial solution concentration, (2) error in delivered radiation dose to the sample, (3) error in sampling the irradiates solution, (4) error in sample injection into the gas chromatograph, (5) error in system response to the sample, (6) operator error in reading the disk integrator output.

The samples and standards were all taken from one lot of 1 Normal HMF. Any variation of normality would be present in all the samples canceling out error. Error could have contributed if the initial solution was not exactly 1 Normal because the decreasing normality of the sample was used for a linearity curve from which the composition of the irradiated samples was derived, however, the reaction was found to be zero order and independent of composition.

The radiation dosage received would be a function of the irradiation time and the position of the sample in the gamma facility. The variation of dosage with position is less in a cylinderical source with the sample in the center than

for a plane source. The isodose curves will vary always in a positive direction an you deviate from the center position in the source, but from the isodose charts supplied by Atomic Energy Canada Ltd., the source suppliers, its appears that the maximum deviation of dosage should be on the order of per cent. The time of irradiation should be accurate to within 1 minute or less. The time variation could have been greatest on samples 9 thru 13 because of a failure of electrical power at the reactor facility which placed the gamma facility in darkness and prevented the use of an electrical timer. Radiation dosage was measured using Baush & Lomb dosimetry plates along with the sample leaving the only real error, that of measuring the radiation dosage. The sampling of the solution by withdrawing a 3 ml sample poses the biggest single source of error. A polyner formed during the irradiation and it produced a fine black powder which settled out. When samples were withdrawn the solution was shaken first to disperse the solid material to give a representative sample of the solution. The solid content of the samples could vary as much as 20 per cent but before chromotography the particles were allowed to settle so as not to clog the syringe since they were assumed nonvolatile and not subject to chromatographic analysis. Therefore the samples consisted only of the liquid which would reduce the error to an estimated 3 per cent.

A series of injections into the gas chromatograph from the same sample were made to check on the degree of reproducibility of the results in order to check on the injection technique and syringe precision. The range of the results were within 6 per cent except for one data point as shown in Figure 8.

The system response to sample concentration was linear from 1 Normal down to 0.005 Normal HMF, however, it is nonlinear in response to sample

size as shown in Figure 8. To remove this error all samples were of the same size, 1μl, for the quantitative analysis of HMF.

Error in operator reading of the disk integrator output on the chart recorder would be within 1 per cent in the range of the integrated peaks encountered.

The total error expected would be on the order of -10 per cent, +15 per cent if all the errors were additive at the same time. The final data points fall within $\pm$ 6 per cent for each sample.

Formation of Formic Acid. Samples of HMF, formic and levulinic acids were analyzed and compared to the results of the irradiates samples. On the Carbowax 20M column at 200^{o} C the small water peak ran concurrent with the formic acid peak. A sample of pure water and a sample of dilute formic acid gave two peaks upon analysis. The major peak started at 24 seconds after sample injection and the minor peak was at 72 seconds after injection. There was not any increase in peak area during irradiation from the sample irradiated at only 4.1×10^{5} Rads to the last one at 8.2×10^{6} Rads. The peak retention times and peak area results are shown in Table IV in the Data and Results section. From these figures it can be stated that formic acid is not one of the products formed by gamma irradiation of HMF at a dose rate of 4.1×10^{5} Rads/Hr, total dosage from 4.1×10^{5} Rads to 8.2×10^{6} Rads at a temperature of 61^{o} F in an air atmosphere.

Formation of Levulinic Acid. A sample of levulinic was analyzed on the Carbowax 20M column to obtain a known peak retention time. When an irradiated sample was analyzed there was not any evidence of a peak above the instrument

noise at the retention time for the known sample. It must therefore, be concluded that levulinic acid is not formed as a product of gamma irradiation at 4.1×10^5 Rads/hr at a dosage of 8.2×10^6 Rads.

Formation of Other Compounds. No other compounds were detected in the irradiated samples. An analysis was conducted using temperature programing over the temperature range of 60° C to 275° C at a high sensitivity setting in an effort to detect any compound present in small quantities. The baseline was unstable because of the high sensitivity but there were not any detectable peaks which could be noted on the recorder. Since about 31 per cent of the HMF disappears during the 20 hours of irradiation other compounds must have been formed but were not detected in the liquid.

The formation of a brown polymeric material was noted in the bottom of the sample bottle during irradiation. After receiving a dose of 4.1×10^5 Rads which corresponded to 1 hour of irradiation there was a brown viscous polymeric material noted in the bottom of the irradiation sample bottle. When the next sample was removed after a dose of 8.2×10^5 Rads the material was not viscous but was of a granular nature. As the irradiation increased the granuals decreased in size until they consisted only of a fine black powder which settled out in the bottom of the withdrawn samples.

Reaction Mechanism. The decomposition of HMF under gamma irradiation proved to be of a zero order reaction. The reaction in this order is unaffected by concentration of the reactant because it is determined by some limiting factor other than concentration, such as the amount of light in a photochemical or amount of catalyst in a catalyst in a catalytic reaction. In the case of the gamma irradiation

of HMF the gamma dosage was the limiting factor. The data points were interpreted by a computerized statistical analysis using the least squares approximation. From Figure 7 the value of k, the reaction rate constant, was determined to be 4.8 $\times 10^{-3}$ moles liters^{-1} seconds^{-1} for 1 N HMF under gamma irradiation at a dose rate of 4.1 x 10^{5} Rads/Hr.

Comparison of Preliminary and Final Analysis. HMF proved to be more stable toward gamma irradiation than the preliminary investigation using the University of Missouri - Rolla Nuclear Reactor indicated. In the initial work HMF was completely destroyed after irradiation at a dose of approximately .5 to 2 M Rads. There is some difference in the irradiating energy between the ^{60}Co source and an operating nuclear reactor. The ^{60}Co supplies gamma rays at a set energy of 1.33 and 1.17 Mev. The gamma radiation produced from an operating reactor is a spectrum of energies up to about 7 Mev. The higher energy gamma rays may produce greater damage than the lower energy ones from ^{60}Co. Since the chemical bonds in the furan ring are of the order of 25 electron volts the gamma energies of ^{60}Co are already on the order of 10^{5} times the chemical energy which should be enough to rupture the bonds if the energy was transferred directly. However, the literature suggests that most of the energy is deposited in the water particularly for dilute solutions and the free radicals then attack the solute.

Comparison of the Radiation Sources Used. While the source gamma energies were different between the ^{60}Co and the nuclear reactor, another particle was present during irradiation in the reactor. In an operating reactor there are present a large number of neutrons of various energies. When solutions of HMF

were irradiated the samples were placed in a cadmium container which readily absorbs thermalized neutrons. The sample container was placed away from the core so that the neutrons which originate in the fission process with an average energy of about 1 Mev would have enough collisions with hydrogen to reduce the initial energy to the thermal energy (0.025 ev). A large amount of these thermal neutrons are then absorbed by the cadmium shield. Not all of the neutrons are reduced in energy or absorbed and these particles can impart their entire energy to a hydrogen atom upon collosion, and up to 28.4 per cent of their energy to a carbon atom. This energy would result in a rupture of one or several chemical bonds in the molecule. Further research will be conducted on this aspect of irradiation by neutrons with a minimum number of gamma rays present.

HMF decomposes to formic and levulinic acids in an acid media but it does not decompose into these compounds under gamma irradiation from ^{60}Co. With the large number of radicals present from the water, OH, H, H_2O^* (activated state) and H_2O_2 it is surprising that no other detectable end products were found. The lack of other compounds would indicate when the HMF does rupture the end products are small gaseous fragements which were not analyzed. There was not any evidence to show the presence of two and three carbon aldehydic fragments as has been found from ring sugars under irradiation.

The literature has stated that HMF may be formed under gamma irradiation of sugars but that it may not have been detected because of its sensitivity to irradiation. This investigation shows that HMF is relatively insensitive that HMF is not formed from sugar under gamma irradiation.

Chromatographic Determination of 5-Hydroxymethylfurfural. It was found that HMF could be determined in its free state in quantitative fashion. Standard solutions were prepared which decreased in normality from 1 to .005 and it was found that the gas chromatograph detected each sample with reproducable results of 5 per cent. The response was linear with respect to decreasing concentration. The irradiated samples were found to have a precision of 6 per cent.

Further work is planned on the irradiation of HMF to determine what compounds are formed during irradiation that may not have been detected in the liquid phase.

VI CONCLUSIONS

The gamma irradiation of 1 Normal 5-hydroxymethyl-2-furaldhyde was studied at a dose rate of 4.1×10^5 Rads/Hr to a total dosage of 8.2×10^6 Rads, using a 5000 curie ^{60}Co source, at a temperature of 55° F.

A nuclear reactor was also used as a mixed source of gamma and neutron radiation for another irradiation at a gamma dose rate of approximately 1×10^6 Rads/Hr at a temperature of 58° F.

The results were determined using a gas chromatograph with flame ionization detector with SE-30, 10 per cent, and Carbowax 20M partitation columns.

From the study of the gamma degradation of HMF the following conclusions were made:

1. The kinetic mechánism of the decomposition of HMF was of zero order.
2. HMF is fairly resistant to radiolysis with 31 per cent being decomposed when irradiated to a dose of 8.2×10^6 Rads over a period of 20 hours.
3. Levulinic acid is not a product of the gamma decomposition of HMF as determined by gas chromatography.
4. Formic acid is not a product of the gamma decomposition of HMF as determined by gas chromatography.
5. Since levulinic and formic acids are not formed the decomposition mechanism for acid hydrolysis and radiation decomposition is not the same.
6. HMF can be quantitively determined on a gas chromatograph with a flame ionization detector using 10 per cent SE-30 column packing.

7. There is 100 per cent decomposition of HMF at a gamma dose rate of only 1×10^6 rads when irradiated in an operating nuclear reactor with neutrons present.

8. Since HMF is radiation resistant it is not formed from sugar irradiation as it has not been detected. If it way very sensitive to gamma irradiation it could have been missed in analysis of its rapid decomposition.

9. A viscous polymeric type material is formed under low dosage to 4.1×10^5 Rads. At 8.2×10^5 Rads it is a grandular material and continues to break down physically under continued irradiation.

VII RECOMMENDATIONS

During the investigation of the irradiation effects on HMF the following ideas are recommended for further study either to add more substance to the knowledge found by this investigation or to extend to an allied field.

1. The radiation sensitivity of levulinic and formic acids should be investigated. The results would offer further information as to the possible mechanisms of decomposition of HMF as these acids may be formed but decompose quickly. The determination of their kinetics of decomposition and products would be valuable.

2. The effect of neutron irradiation of HMF with a minimum amount of gamma irradiation present should be investigated to explain the differing results found under ^{60}Co irradiation and nuclear reactor irradiation.

3. The effect of dose rate should be investigated by irradiating a sample at several different dose rates to the same total dose. By determining the amount of HMF decomposed for the same total dose the K values could be found for different dose rates.

4. The vapor phase over the irradiated solution should be analyzed to determine if any gaseous products are being formed. Since HMF is decomposing the identification of evolved gaseous products would help in understanding the mechanism.

5. A Poropack partition column should be tried for chromatographic analysis. The Poropack series is a new class of packing which have proved to give excellent results on many compounds.

6. The individual separated components in the gas chromatography effluent should be condensed and an infra-red analysis be conducted of these components by washing the condensed effluent into KBr and pelletizing the powder. The pellet can then be subjected to a infra-red analysis which will positively identify the individual separated products.

7. A gas chromatographic analysis of irradiated glucose should be conducted to insure that no HMF is formed as a product. HMF has not been reported as being present in irradiated glucose by thin layer chromatography which shows around six products. A recent analysis of irradiated glucose vapors showed 15 unidentified compounds.

8. The polymeric material should be isolated and that analysis be conducted on this material. Since HMF has several active sites the decomposition noted may be because of the increase in polymer material which then suffers radiation damage itself.

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APPENDIX

```
    1 READ (1,99)
      READ (1,100) N
      SUMT=0
      SUMTSQ=0
      SUMPA=0
      SUMPAT=0
      DO 10 I=1,N
      READ (1,110) T,PA
      SUMT=SUMT+T
      SUMTSQ=SUMTSQ+T*T
      SUMPA=SUMPA+PA
   10 SUMPAT=SUMPAT+T*PA
      DET=N*SUMTSQ-SUMT*SUMT
      SLOPE=(SUMPAT*N-SUMT*SUMPA)/DET
      B=(SUMPA*SUMTSQ-SUMPAT*SUMT)/DET
      WRITE (3,99)
      WRITE (3,200) SLOPE,B
      WRITE (2,99)
      WRITE (2,200) SLOPE,B
      GO TO 1
   99 FORMAT ('
  100 FORMAT (I5)
  110 FORMAT (2E15.8)
  200 FORMAT ('0    SLOPE OF LINE =' E12.5, ' AND INTERCEPT =' E12.5)
      END
1    DATA SET NUMBER  1
   31
                1.            895.
                1.            899.
                1.            925.
                1.            941.
                2.            897.
                2.            892.
                2.            862.
                3.            903.
                3.            880.
                4.            819.
                4.            872.
                4.            895.
                5.            842.
                6.            836.
                7.            800.
                7.            805.
                8.            799.
                9.            776.
                9.            783.
                9.            803.
               14.            677.
               14.            703.
               14.            717.
               15.            671.
               16.            666.
               17.            640.
               18.            602.
               18.            642.
```

```
                19.              594.
                19.              597.
                19.              615.
1       DATA SET  NUMBER 2
     2
                 1.             1069.
                20.              744.
1       DATA SET NUMBER 3
     9
                 2.            10650.
                 2.            11230.
                 5.             9830.
                 5.            10090.
                10.             8830.
                10.             9300.
                15.             8520.
                15.             8600.
                19.             7650.
```

VITA

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