Free Radical Reactions Studied by Means of Luminescence

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Reactions of free radicals are accompanied by release of energy due to bond formation. Some of the energy quanta may primarily or secondarily be converted to light; therefore it is possible to study such radical reactions by luminescence measurements.

In the present study, Kolbe's synthesis of hydrocarbons, where radicals are expected to be involved, and the electrolysis of some aromatic amino acids in dilute salt solution, have been followed by light emission measurements.

The biological effects of ionizing radiation may be ascribed to ionized and possibly also to excited molecules which are the primary products formed on energy absorption. These molecules are subject to fast chemical and physical changes which arise partly in reactions with other molecules. Charged and uncharged free radicals seem to be important intermediates in the production of radiation effects [1].

The oxygen effect is believed to be due to reactions between oxygen and free radicals (or excited triplet states) leading to peroxy compounds.

Irradiated compounds have been shown to have radiomimetic effects in biological systems [2,3].

The reactions of the primarily changed molecules may be studied by physical and chemical methods. In solution, free radicals are extremely short-lived [4] because of rapid recombination, and hence the concentration of radicals is low. Further, if we study free radicals by means of their own properties (magnetic or optical), large amounts of energy must be introduced into the system to produce measurable amounts of radicals. However, if we measure a product of the reactions of radicals, we are independent of the lifetime of the radicals in the system.

The present investigation is an explorative study, employing luminescence measurements, of the reactions of electrolytically produced free radicals.

From a radio-chemical point of view, ionization means that a molecule (charged or uncharged) loses or gains electrons. In this process a free radical is formed (if charged, an ion radical). In principle, identical reactions, although somewhat more specific, occur at the anode and cathode, respectively, in electrolysis. This resemblance is not only valid from physical and chemical
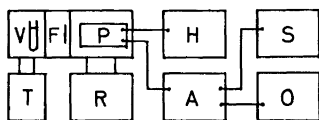
Fig. 1. Photon counting system: V — Reaction vessel; F — Filter; P — Photomultiplier tube EMI 6255 A; H — High voltage supply; S — Scaler; T — Thermostat; R — Refrigerator; A — Amplifier; O — Oscilloscope.
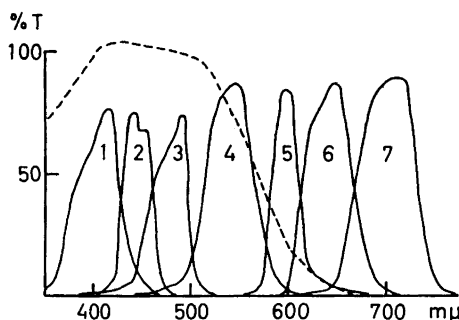

Fig. 2. Transmission curves for Balzers Broad-Band Interference filters Nos. 1 — 7. The broken curve shows arbitrary spectral sensitivity of EMI 6255 A.

points of view, in fact it has been shown that electrolytically produced reactive species are biologically active [5]. Electrolysis of tyrosine in diluted sodium acetate causes chromosome aberrations in barley root tips, thus resembling the indirect action of ionizing radiation.

Among the factors investigated was Kolbe's synthesis of hydrocarbons. For example, electrolysis of sodium acetate in water or methanol gives ethane and carbon dioxide in a reaction between methyl or acetoxyl radicals. In side reactions, some other substances are also formed, such as methane, methanol and methyl acetate [6]. The anode reaction was found to be accompanied by light emission in the visible range.

A few cases described earlier were reactions probably involving radical interaction and accompanied by light emission. In gas reactions of the type $H + NO = HNO$ and $H + Cl_2 = HCl + Cl$, chemiluminescence in the infra-red-visible range has been described [7]. In these cases the compound molecule is vibrationally excited in the ground state.

A luminescence has also been observed to be induced by ultrasonics in liquid systems such as H_2O—CCl_4, where the light emission has also been described as chemiluminescence occurring in the gas phase [8].

EXPERIMENTAL

A schematic diagram illustrating the equipment used for luminescence measurements is presented in Fig. 1.

In the thermostat-controlled vessel the electrolysis is performed in a small test-tube (1 ml) with 0.5 mm Pt-wire as electrodes. During electrolysis and/or luminescence measurements, different chemicals are injected when required into the reaction vessel with a syringe. To a circular plate was fixed a set of interference filters (Fig. 2). When the plate is rotated, these filters are introduced, one at a time, into a position between the test-tube and the photomultiplier tube.

The photomultiplier tube is cooled in a refrigerator to −30°C. The thermal background is hereby reduced by a factor of 100 to 20 counts per second. From the space angle and
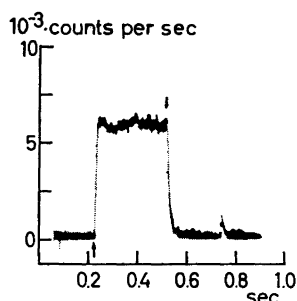
Fig. 3. Electrolysis of 1 M sodium acetate. The arrows show start and stop of electrolysis. The electrolysis current was 150 mA.
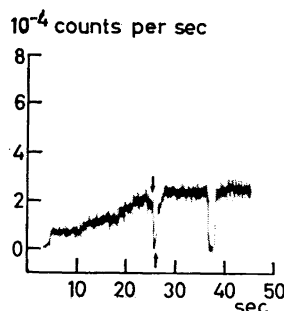
Fig. 4. Electrolysis of 0.001 M phenylalanine in 0.2 M Na_2SO_4. Arrows show stop and start of electrolysis. The current was 100 mA.

the quantum effectiveness of the photomultiplier tube (about 10 % in the range 350 — 550 mμ) it is calculated preliminarily that one photon is counted out of about 2 500 emitted. The photon pulses are either counted on a scaler or recorded on an oscilloscope with a simple rate metre. The time constant of the latter was 0.01 sec.

The following systems were studied.

1. Electrolysis of concentrated ($\geq$1 M) sodium salts of carboxylic acids (Kolbe's synthesis)
2. Electrolysis of different organic compounds in an electrolyte.
3. Electrolysis of Grignard reagents.
4. Wurtz's synthesis (hydrocarbons from alkyl halides and sodium metal).

In the first mentioned type of systems 1 M solutions of for example acetate, propionate, citrate and tartrate were electrolysed. In all cases light emission was observed. In subsequent experiments, acetate was chosen as a model substance.

In series 2, among other substances tested, were several amino acids. Tyrosine and phenylalanine exhibited the brightest luminescence and were used in further experiments.

In the system types 3 and 4 the appearance of luminescence was observed. These experiments are still under progress and hence will not be discussed in the present communication.

RESULTS

Electrolysis of 1 M sodium acetate in water gives a light emission at the anode with maximum intensity in filter 2. When the electrolysis is performed in methanol solution the emission spectrum has its maximum in filter 3—4. At pH $\sim$ 14 in water, the emission spectrum is broadened, the main emission being directed to filter 4. In the latter case, reactions presumably of OH$\cdot$ with $CH_3COO\cdot$ dominate in accordance with the known fact that electrolysis in alkaline solutions leads to alcohol formation. The species giving rise to light emission are formed immediately and are very short-lived as shown in Fig. 3. A time constant of 0.0001 sec. does not change the shape of the curve.

When Cl^- or Br^- is added to the system there is a great increase in the luminescence. The luminescence increases linearly by about 20 000 counts per second per mmole Cl^-. Paramagnetic and peroxide-decomposing ions such as Cu^{++}, Co^{++}, and $Fe^{++} + Fe^{+++}$ have no effect when added in moderate concentrations ($<$0.01 M). Temperature has little effect on the luminescence intensity.
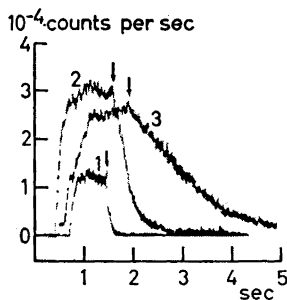
Fig. 5. Electrolysis of 0.001 M phenyl-alanine in 0.2 M Na$_2$SO$_4$ at different tem-peratures. The curves are from the point of maximum intensity in Fig. 4. Curve 1, 80°C; Curve 2, 25°C; Curve 3, 5°C.
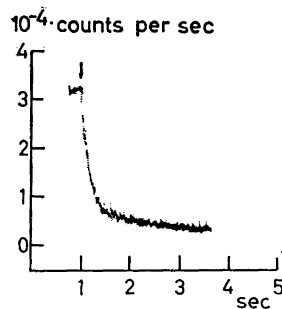
Fig. 6. Long lived luminescence in 0.001 M phenylalanine caused by prolonged electrolysis.

Electrolysis of tyrosine and phenylalanine in 0.2 M sodium sulphate exhibits a light emission having a maximum in filter 3. When electrolysis starts there is a slow increase in light emission intensity, the maximum being reached in about half a minute. As soon as the electrolysis current is broken the luminescence decays in less than one second. In a renewed electrolysis there is no slow build-up, the maximum intensity being reached in one second (Fig. 4). At this maximum intensity level the decay of phenylalanine luminescence was studied as a factor influenced by temperature and concentration of organic material, etc. Fig. 5 shows electrolysis of 0.001 M phenylalanine at 80, 25, and 5°C. The life length of the luminescence decreases at higher temperatures together with a decrease in light emission intensity at 80°C. The intensity and life length also decreases when an increase occurs in the concentration of organic material during electrolysis.

When phenylalanine in sodium sulphate solution is subject to a prolonged electrolysis a new, more long-lived luminescence appears, as shown in Fig. 6.

The effect of some inorganic ions were also studied. Cl$^-$ and Br$^-$, which have an activating effect on the luminescence intensity in the Kolbe reaction, do not affect phenylalanine electrolysis. On the other hand ions such as Cu^{++}, Co^{++}, and Fe^{++} + Fe^{+++} totally quench the last mentioned long-lived luminescence. Fig. 7 shows injection of 0.1 ml 0.01 M Cu^{++}. Renewed electrolysis resulted in the decay being the same as in curve 2, Fig. 5.

As a control experiment, oxygen free water was also injected, resulting in the unexpected increase of the luminescence shown in Fig. 8. The same effect could also be obtained by stirring the solution.

Addition of radical scavengers or radiation protectors (there may be no difference) has a noticeable effect on the intensity, the life length and the emission spectrum of the luminescence in the investigated systems, as shown by electrolysis of acetate in methanol. When the acetate solution contains I$_2$ or diphenylpicryl hydrazyl (a stable free radical), no luminescence is observed. Traces of hydroquinone, p-phenylenediamine and ascorbic acid have the same
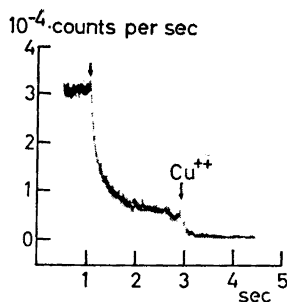
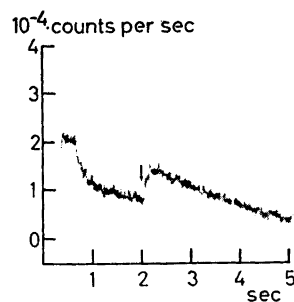*Fig. 7.* Introduction of Cu^{2+} during long lived decay (*cf.* Fig. 6).

Fig. 8. Introduction of oxygen-free water during long lived decay (*cf.* Fig. 6).

effects, but when some amino acids (cysteine and methionine), known as radiation protectors, were introduced there was also a luminescence. However, a spectral analysis shows that the emission is thrown to longer wavelengths with maximum emission in filter 5—6.

DISCUSSION

The discussion of the luminescent reactions in the Kolbe's synthesis are to be based on available knowledge of possible products in this synthesis. As mentioned above the final main products from electrolysis of the acid $C_nH_{2n+1}COOH$ are the hydrocarbons $C_{2n}H_{4n+2}$. However, the Kolbe reaction is accompanied by many side-reactions, the extent to which these occur being governed both by experimental conditions and by the structure of the acid itself. In competing reactions, compounds such as olefins, esters and alcohols are produced, the formation of the latter being specially favoured by alkaline solution. Constituents other than hydroxyl ions may also participate, these being nitrates, nitrites and halogens.

Hydrocarbons of the first mentioned type are probably formed in a primary radical recombination [6], but the pathways involved in side reactions are not very well known. When radicals are formed at a low rate, immediate recombination is lowered and the radical might attack non-radical molecules by abstracting hydrogen. This seems to be the rule when aromatic compounds constitute the primary radicals. It is of interest to note that hydrogen abstraction seems to occur from C—H bonds and not from O—H bonds.[9] The rapid rise (without any build-up), and correspondingly rapid decay, of the luminescence as shown in Fig. 3 indicates a primary reaction, for example a radical recombination, in gas or liquid phase which gives a compound in an excited state (*cf.* H + NO = HNO) capable of emitting light. The available energy in bond formation, 80—100 kcal, in the Kolbe products, is not enough to give electronically excited states in simple molecules such as ethane or methyl chloride, but requires groups with more loosely bound electrons such as a carbonyl group.

Although the reaction $CH_3COO \cdot \rightarrow CH_3 \cdot + CO_2$ is exothermic (12—14 kcal) and occurs spontaneously the possibility that recombination occurs before the splitting off of CO_2 should not be excluded, the compound molecule then containing carbonyl groups where the n—π transition energy might be of the same magnitude as the energy released in bond formation. Another possibility is that the energy released in a radical recombination might excite a third molecule which then emits light.

The Kolbe's synthesis applied to amino acids gives usually aldehydes together with H_3N liberation. This is also true of radiolysis.

The electrolysis of some amino acids in sodium sulphate solution was undertaken to study reactions similar to those occurring in the radiolysis of organic material in water, i.e., where the primarily produced radicals originate from water.

The luminescence exhibited by electrolysis of phenylalanine or tyrosine is produced in a secondary reaction and depends on the production of a secondary product, as shown by the slow build-up of the luminescence intensity.

The effect of concentration and temperature may indicate that the luminescence occurs in different competing reactions with different quantum efficiency. As the increase in life length seems to be accompanied by increasing light emission, it is perhaps worthwhile to elucidate this problem by discussing possible reactions which might increase the life length of the reactive species.

The prolongation of the luminescence in phenylalanine electrolysis may be due either to a physical or to a chemical stabilization. A physical stabilization might consist of a conversion of an excited singlet state with a lifetime of about 10^{-8} seconds to a metastable triplet state with a lifetime that may exceed several seconds. The chemical stabilization may occur by radical chain reactions where the resulting radical is unreactive. The free radical may also combine with oxygen to form a peroxy radical which then may form a peroxide, a potent radical source. Physical and chemical stabilization is supposed to be combined in such a way that the above-mentioned triplet state reacts with oxygen to form a peroxide directly [10].

If oxygen has the above-mentioned effect on the lifetime of the products giving rise to the luminescent reaction, this does not necessarily mean that the light emission efficiency is increased by oxygen. However it is well-known that, in nearly all chemiluminescent reactions, oxygen or peroxides participate in some manner.

The effects of concentration, temperature and stirring may therefore be explained on the basis of oxygen competing with other participants in radical reactions. At a high radical concentration, radicals are expected to react with each other rather than with oxygen, and at high temperature the diffusion velocity of the radicals increases at the same time as the oxygen concentration is lowered in solution. Both effects make oxygen-radical reactions less frequent. Stirring of the solution probably increases the dissolving of oxygen from the oxygen bubbles formed at the anode.

The quenching effect of Cu^{++}, Co^{++}, and $Fe^{++} + Fe^{+++}$ may be ascribed both to their ability to react with radicals and peroxides as well as to their ability to affect the lifetime of the excited triplet state [11].

Information about the luminescent stage, from the shape of the decay curves, is made difficult by the fact that the luminescent species are not uniformly distributed in the solution due to their formation in an electrode process. This affects second and higher order reactions (cf. Fig. 8). However, the curves in Fig. 5 seem to be close to first order and this favours either an excited triplet state or an unimolecular decomposition of an unstable product as the rate determining step in the luminescent decay.

A radical recombination is probably the source of energy in the light emission studied here. It is then obvious that a radical scavenger should affect light emission either by recombination with the primary radical or by affecting the light emitting stage.

A radical scavenger may be a stable free radical such as diphenylpicryl hydrazyl, or NO, or a compound such as I_2 with a very weak bond which is easily attacked by a reactive free radical. Hence the energy released in the reaction of a reactive free radical and a scavenger molecule is usually considerably lower than when two reactive radicals react with each other. This lower energy release, together with the formation of an entirely new configuration, may cause the light emission to disappear. If light is still produced, the maximum of the emission spectrum is shifted to longer wavelengths, possibly out of the sensitive range of the photomultiplier tube. Therefore it is of interest that by introducing, in acetate electrolysis, some amino acids known as radiation protectors, this maximum is displaced to longer wavelengths (filter 5—6).

The above-mentioned method of studying free radical reactions should not unreservedly be transformed to radiation chemistry in solution. This is due to the difficulty in differentiating between the primarily produced excited states and excited states produced in radical reactions. However, by irradiation of a substance in the solid state, the energy is trapped in stable free radicals with a lifetime considerably longer than that of excited singlet and triplet states. By dissolving the irradiated substance in water the radicals are set free to react. This reaction is accompanied by light emission. Observations indicate that in this case at least two different light emitting reactions occur, one of which is short-lived (the lifetime probably shorter than the time necessary to dissolve the compound). This emission might be due to free radical reactions. In addition a rather slow reaction type occurs probably involving peroxides and other reaction products [12] (cf. also Westermark's investigations of irradiated inorganic compounds [13]).

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