

INTRODUCTORY REMARKS ON PROBLEMS OF CHEMILUMINESCENCE IN  
LIQUID SCINTILLATION COUNTING

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I. INTRODUCTION

In certain chemical or biochemical reactions the liberated energy is converted into molecular electronic excitation energy, which by radiative decay results in the emission of light. These reactions are generally known as chemiluminescence or bioluminescence and may be of great interest for users of liquid scintillation counters. In radioactivity measurements chemiluminescence can be quite hazardous and may strongly interfere with the liquid scintillation counting process. On the other hand chemiluminescence and bioluminescence reactions can be used with great benefit for analytical purposes in connection with a liquid scintillation spectrometer.

In our present paper we shall concentrate on chemiluminescence as a problem in liquid scintillation counting. Although chemiluminescence reactions generate only single-photon events, the intensity of these processes may result in such a vast number of photons that the light pulses can pass the coincidence gate of the spectrometer within its resolving time. The increased sensitivity of liquid scintillation spectrometers, predominantly in the low energy range of the spectrum, has consequently increased the possibility of interfering reactions such as chemiluminescence or phosphorescence.

In our earlier papers (Kalbhen, 1967, 1970, 1971, Kalbhen and Rezvani, 1971) we have discussed in detail the possible occurrence and the effects of chemiluminescence reactions in radioactivity measurements in liquid scintillation spectrometers. We have shown that several scintillator solutions,

in combination with certain methods of sample preparation, result in counting samples in which it is possible to demonstrate more or less persistent chemiluminescence reaction. The long duration of the photon emitting process in a high intensity luminescence sample is shown in Figure 1. Even after two days the count rate remained above the normal background level.

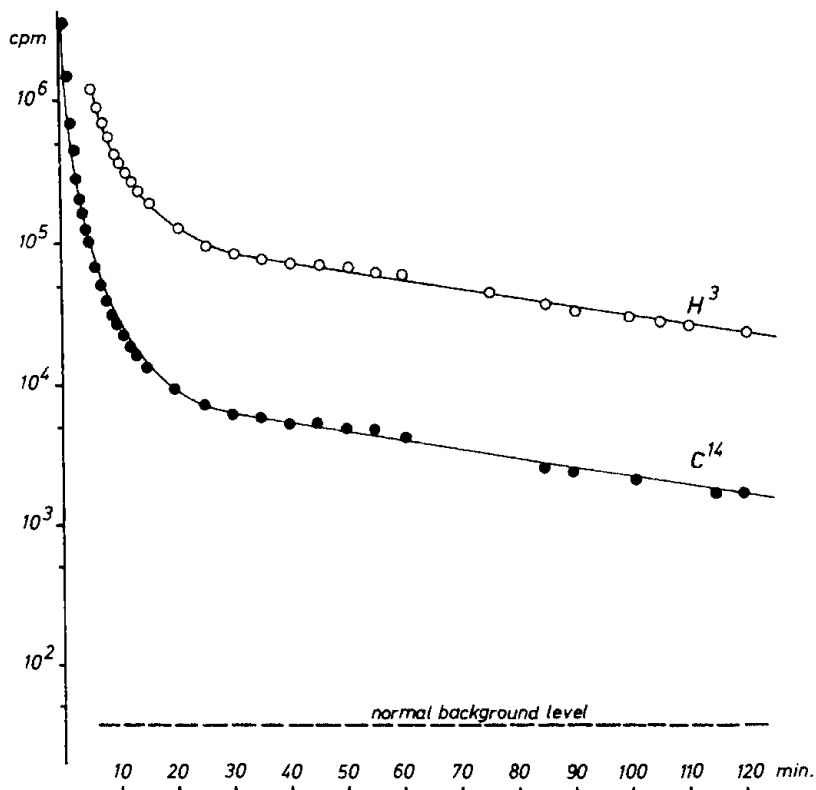


FIGURE 1. Time dependent decrease of count rate of a chemiluminescence reaction measured in the <sup>3</sup>H-channel and <sup>14</sup>C-channel of a liquid scintillation counter.

As it is impossible in most cases to establish a quantitative differentiation between radioactivity and chemiluminescence pulses measured at the same time, this often leads to wrong results in radioactivity measurements. This applies particularly to measurements of tritium because, as will be shown below, the pulses from chemiluminescence reactions are

located predominantly in the energy range of tritium.

Since information on the magnitude and the shape of such spectra may be of interest to users of liquid scintillation counters, we report below the results of our investigation in this field (Kalbhen and Tarkkanen, 1972).

## II. EQUIPMENT AND METHODS

The energy spectra discussed below were obtained with a Packard Tricarb Spectrometer 3380, in combination with a Packard multi-channel analyser Spektrazoon 930. The spectra displayed on the screen of the multi-channel analyzer were photographed with a reflex camera.

The spectra of tritium and of carbon-14 were obtained with unquenched standard samples containing toluene labelled with  $^3\text{H}$  or with  $^{14}\text{C}$  in a PPO-POPOP-toluene cocktail. For inducing chemiluminescence reactions we mixed 10 ml of scintillator solution according to Butler (1961) with 0.5 ml of a saturated benzoyl peroxide solution in toluene and with 0.5 ml Hyamine 10-X.

We also used for the same purpose a mixture of 10 ml emulsion cocktail Aquasol<sup>®</sup> with 0.2 ml benzoyl peroxide solution and 0.3 ml Protosol<sup>®</sup>.

This choice of compounds was purely fortuitous. In principle, chemiluminescence reactions can be induced with all peroxide-containing scintillator solutions and with all emulsion cocktails (e.g., Aquasol, Unisolve, Insta-Gel, and with a cocktail according to Patterson and Greene (1965)) in combination with basic solubilizers (e.g., Hyamine 10-X, Digestin, NCS, Protosol, Soluene 100, KOH, NaOH, phenylethylamine).

All measurements were carried out in the tritium and/or the carbon-14 channel with linear (not logarithmic) amplification.

The designations of measuring ranges within the channels of a liquid scintillation counting instrument differ considerable from one make of instrument to another. In this paper, the terms tritium channel and carbon-14 channel designate the measuring range settings (setting of window, gain, level or discriminator) which result, with an unquenched sample, in the total and optimal determination of the nuclides  $^3\text{H}$  and  $^{14}\text{C}$  respectively.

We induced a phosphorescence reaction by irradiating 10 ml of scintillator solution according to Butler (1961) with an ultraviolet quartz lamp. As this UV-induced phosphorescence decays very rapidly, the scintillator solution must be measured in the liquid scintillation spectrometer immediately after the

irradiation.

The shift of the energy spectrum of an initially very intensive chemiluminescence reaction, shown in Figs. 3A-G, was recorded by photographs taken at intervals of about 1 to 2 minutes.

### III. RESULTS AND DISCUSSION

In the spectra shown in Fig. 2, the intensity of the registered light pulses (ordinates) is recorded versus their relative energy (abscissae).

As can be seen from this figure, the combination of a multichannel analyser with a liquid scintillation spectrometer makes it possible to obtain every spectra of  $^3\text{H}$ ,  $^{14}\text{C}$ , and chemiluminescence for comparison.

Figure 2 clearly demonstrates that, when the tritium channel (or tritium window) is used, the energy spectra of tritium (upper left picture) and of chemiluminescence (middle left picture) are recorded in full whereas, of the carbon-14 energy spectrum (lower left picture), only the lower energy part of the spectrum is recorded within the tritium channel. Accordingly, when the carbon-14 channel (or carbon-14 window) is used, the spectra of tritium (upper right picture) and of chemiluminescence (middle right picture) overlap only with the low energy part of the carbon-14 spectrum.

As already mentioned, the energy spectra of tritium and of  $^{14}\text{C}$  were recorded with unquenched standard samples. It is well known, however, that quenching results in a left shift and deformation of the spectra, so that the number of pulses in the lower energy range increases and that in the higher energy range is reduced correspondingly. This means that, with quenched samples, the overlap of the tritium and  $^{14}\text{C}$  spectra with chemiluminescence spectra increases with the degree of quenching. It should be noted in this connection that all the energy spectra of chemiluminescence reactions shown here were obtained with moderately or strongly quenched samples, and thus correspond fully to situations encountered in practice.

We also compared chemiluminescence and phosphorescence processes and found that the energy spectra of chemiluminescence and of phosphorescence reactions are strikingly similar. Phosphorescence reactions, however, decay completely within 20 to 30 minutes, and thus present no serious problem as a disturbing factor in liquid scintillation counting. It should be stressed, however, that samples for radioactivity measurements should be kept in the dark for about 30 minutes before the start of counting. This may be of decisive importance for samples with

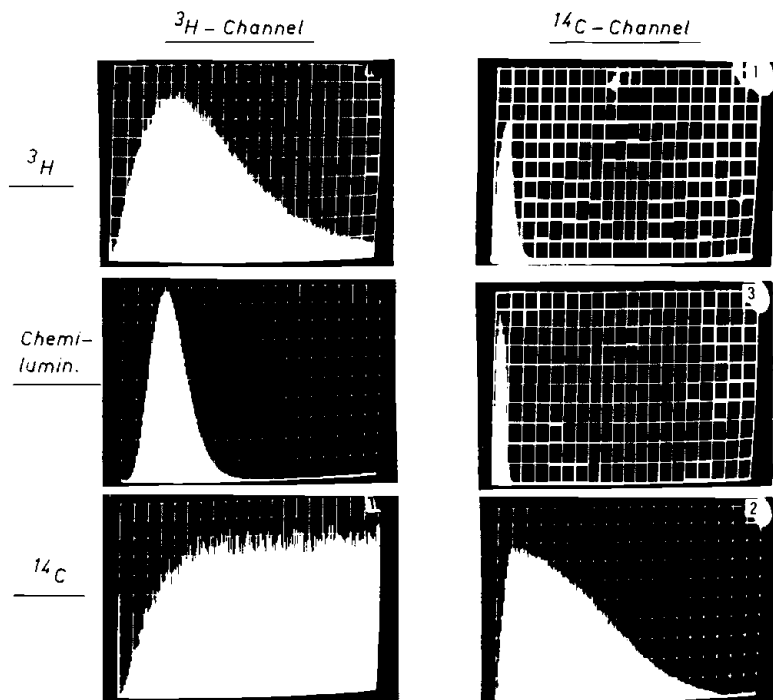


FIGURE 2. Energy spectra of tritium, carbon-14 and chemiluminescence registered in the  $^3\text{H}$ -channel and  $^{14}\text{C}$ -channel of a liquid scintillation counter.

a low activity.

In earlier investigations, we found that, in most cases, the pulse rates of disturbing chemiluminescence reactions in freshly prepared samples amount to between  $10^6$  and  $10^4$  cpm and are therefore measured quantitatively by the spectrometer. With particularly intensive chemiluminescence reactions, however, the initial pulse rate may exceed  $10^8$  cpm. In such cases the resolving time capability of the photo-electronic counting units of the liquid scintillation spectrometer is exceeded. This results in an overloading of the electronic system which is manifested by the so-called "piling up effect". As a result of this overloading of the measuring system, several quasi-simultaneous pulses are recorded as a single pulse with a correspondingly exaggerated energy. If such very intensive chemiluminescence reactions are recorded in a multi-channel analyzer, the spectrum obtained is strongly distorted

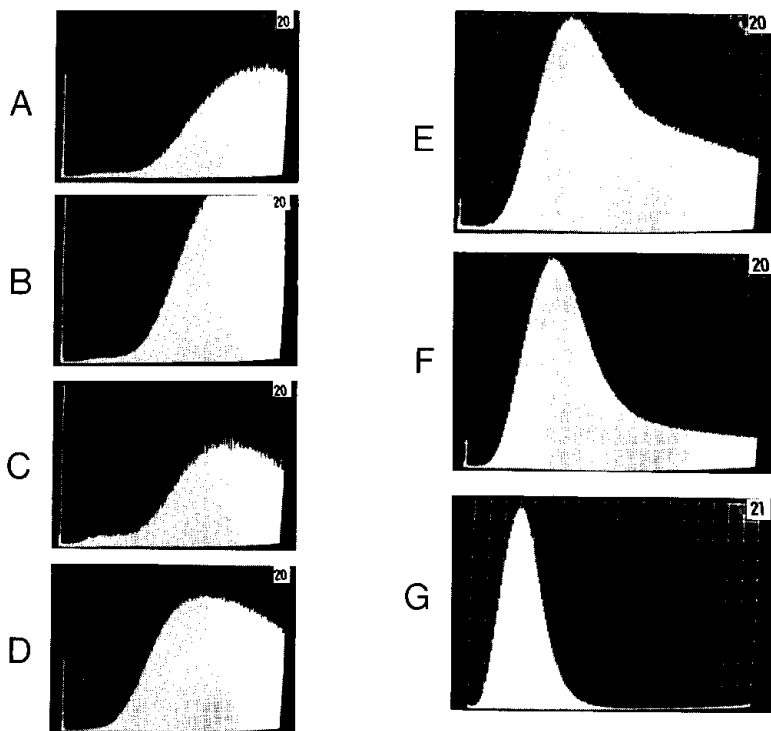


FIGURE 3 A-G:- Energy spectra of an initially highly intensive chemiluminescence reaction, recorded within the tritium channel at intervals of 1 to 2 minutes. The count range was switched over whenever the spectrum exceeded the upper edge of the display tube screen.

towards higher energy ranges. Then, as the intensity of the chemiluminescence reaction drops with time, the piling up effect is also reduced and, consequently, the measured pulses are recorded more and more correctly at their true energies until, when the pulse rate of the chemiluminescence reaction had dropped to less than  $10^6$  to  $10^5$  cpm, the recorded energy spectrum is again "normal". These changes of the recorded energy spectrum of an initially very intensive chemiluminescence reaction, recorded at intervals of about 1 to 2 minutes, are shown in Figs. 3A to 3G. It can be seen that the pulses recorded in the initial phase may reach quite far into the energy range of the  $^{14}\text{C}$  spectrum.

It should be noted in this connection that a piling up effect is observed not only with very intensive chemiluminescence reactions, but also when measuring radioactive samples with count rates exceeding  $10^6$  to  $10^7$  cpm. We have studied this effect in experiments with a tritium sample with an activity of  $4 \times 10^7$  dpm.

The energy spectra recorded in our experiments indicate quite clearly that chemiluminescence reactions may disturb activity measurements of weak beta emitters in a liquid scintillation spectrometer. This well-known effect is strikingly demonstrated by the spectra shown in the figures. These spectra also show that, in tritium measurements in particular, the disturbing effect of chemiluminescence cannot be eliminated by altering the width of the counting window (by adjusting the setting of the discriminator or of the amplifier). In carbon-14 measurements the disturbing effect of chemiluminescence can be eliminated by altering the measuring range (the counting window), but only at the expense of a substantial loss in the count yield, especially when working with quenched samples. With very intensive chemiluminescence reactions, owing to the observed right shift of the energy spectrum of the chemiluminescence, radioactivity measurements may be vitiated even if the measurements are conducted with a raised lower discriminator threshold.

In the past ten years, many efforts have been made to overcome problems of chemiluminescence in liquid scintillation counting. To avoid or suppress counting errors due to chemiluminescence various chemical and physical methods as well as electronic devices have been developed and are excellently reviewed by Peng (1977). These techniques may not be effective in all cases, so it is still necessary to be aware of the possibility of interfering chemiluminescence in liquid scintillation counting.

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