Thiocyanate induced chemiluminescence in dioxane based liquid scintillation fluids

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Received 3 June 2002; revised 2 September 2002.

Dioxane based cocktails are often used in liquid scintillation counting for water soluble biological samples. Phosphorescence is a problem with such cocktails and strong chemiluminescence has also been reported in the presence of a cationic detergent. It has been found that thiocyanate ion produces strong delayed chemiluminescence in dioxane containing peroxides. The luminescence is observed as a pulse in the presence of a scintillator (2,5-diphenyloxazole:POPOP), but not in the presence of toluene or 1,4-bis(5-phenyl-2-oxazolyl)benzene(POPOP) alone. The pulse amplitude and delay time is dependent on thiocyanate concentration. In practice, the observed pulse intensity in a beta counter may be few thousand CPM with cocktails prepared with fresh dioxane to a few lakh CPM in aged cocktails.

Dioxane based cocktails are widely used for beta counting of radioactive aqueous solutions. Background counts due to phosphorescence and chemiluminescence is a problem associated with dioxane based cocktails. Strong chemiluminescence in such cocktails has been earlier reported by Kalhben in the presence of the cationic detergent, Hyamine 10-x hydroxide.

While measuring bound tritium activity of estrogen receptor-estradiol complex by liquid scintillation counting in a dioxane based cocktail, we have observed abnormally high counts. This was traced to the presence of thiocyanate ion in the sample. Thiocyanate along with [1H]-estradiol was used for elution of estrogen receptor from 17β-estradiol-17-hemisuccinate aminopropane-Sepharose affinity chromatography matrix.

We have found that thiocyanate produces strong chemiluminescence in dioxane solutions containing peroxides. Chemiluminescence phenomenon was studied by measuring pulse height and delay time of dioxane solution containing 2,5-diphenyloxazole (POPOP) scintillator in beta counter on addition of different concentrations of thiocyanate ion. We have also found similar chemiluminescence in tetrahydrofuran solution containing peroxides in the presence of thiocyanate.

Materials and Methods

The commercial scintillation cocktail used was cocktail-W(10 g POPOP + 0.25 g of 1,4-bis(5-phenyl-2-oxazolyl)benzene(POPOP) + 100 g naphthalene/litre of dioxane) from Spectrochem, Mumbai, India. Dioxane and tetrahydrofuran were GR grade (99.5%) from E Merck (India) and POPOP and POPOP from SRL, Mumbai, India. Most of the measurements were done in a Model 1409 liquid scintillation counter (Wallac, Turko, Finland), though Monolight 2010 Luminometer (Analytical Luminescence Laboratory, Ann Arbor, MI, USA) was also used to check the measurement in some cases. Scintillation counting was done in glass vials in 2 ml of dioxane (or cocktail) containing 100 μl KSCN solution. The KSCN concentrations given are the final concentrations in the scintillation fluid.

Peroxide concentration of dioxane was increased by continuous bubbling of air under anhydrous conditions for different time periods and then assayed iodometrically by the method of Wibaut et al.

Results and Discussion

Our initial observations came from high beta counts observed while measuring the radioactivity of a tritiated 17β-estradiol bound to estrogen receptor which was eluted from an affinity column with KSCN. The phenomenon was not observed in the absence of KSCN or when it was replaced by KCl. Ammonium thiocyanate could effectively replace the potassium salt and thus thiocyanate ion was clearly implicated in the process.

Chemiluminescence observed in presence of thiocyanate was not instantaneous. In a typical experiment, commercial dioxane containing 5 g/l POPOP gave a peak count of 3000 cpm after about fifty
Table 1—Effect of scintillation fluid components on thiocyanate induced chemiluminescence in dioxane

<table>
<thead>
<tr>
<th>Solvents/Scintillators</th>
<th>Intensity (cpm)</th>
<th>Delay (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioxane</td>
<td>60</td>
<td>—</td>
</tr>
<tr>
<td>Dioxane+naphthalene(100g/l)</td>
<td>42*</td>
<td>—</td>
</tr>
<tr>
<td>Dioxane+POPOP(0.15g/l)</td>
<td>165</td>
<td>44</td>
</tr>
<tr>
<td>Dioxane+PPO(5g/l)</td>
<td>2700</td>
<td>49</td>
</tr>
<tr>
<td>Dioxane+naphthalene(100g/l)+PPO(5g/l)</td>
<td>2800</td>
<td>51</td>
</tr>
<tr>
<td>Dioxane+POPOP(0.15g/l)+PPO(5g/l)</td>
<td>9000</td>
<td>55</td>
</tr>
</tbody>
</table>

*Background count only, no peaks

Fig. 1—Chemiluminescence observed in dioxane containing 5 g/l PPO and 4.76 mM KSCN measured in liquid scintillation counter (A) and Luminometer (B).

Fig. 2—Chemiluminescence observed in dioxane containing 5 g/l PPO in presence of different concentrations of KSCN.

The chemiluminescence intensity and the delay time depended on the thiocyanate concentration used (Fig. 2). With increase in thiocyanate concentration, the luminescence intensity increased and the delay time was shortened. The peroxide concentration of the dioxane used had a similar effect (Fig. 3).

On using tetrahydrofuran in place of dioxane, similar chemiluminescence was observed in presence of thiocyanate, and the chemiluminescence (Fig. 4) was seen in the form of two peaks, the initial peak was sharp and intense. The second peak was less intense and persisted for a longer time. This delayed peak vanishes with the lowering of thiocyanate concentration. In tetrahydrofuran containing the free radical inhibitor quinol(0.1%), low background count was observed in liquid scintillation counter in presence of thiocyanate along with 0.5% PPO.

The maximum chemiluminescence intensity and the delay time was dependent on the pH of the added thiocyanate solution in dioxane. There is a broad pH maximum in the region, 7.0-8.5, the intensity falling sharply at pH 6.5 and 9.0 (Fig. 5).

The effect of thiocyanate on the beta counting of tritiated estradiol was also studied (Fig. 6). The observed count of 100 pmole of estradiol in 2 ml of dioxane containing 0.5% PPO was about 3100 ppm.
Fig. 3—Chemiluminescence observed in dioxane containing different concentrations of peroxide in presence of KSCN (9.52 mM).

Fig. 4—Chemiluminescence seen in tetrahydrofuran containing 5 g/l PPO in presence of different concentrations of KSCN, which showed no decay with time. In presence of thiocyanate, the count was time-dependent and decreased from the maximum of about 10,000 cpm to a steady value of about 2200 cpm which was approximately 26.5% less than that of control. This showed that thiocyanate ion actually quenches count. This was corroborated by the measurement of the radioactivity of the same steroid in freshly distilled dioxane in absence and presence of thiocyanate. The measured count was 44% less in presence of 100 mM thiocyanate.

The interference of the background luminescence of dioxane during liquid scintillation counting is known for a long time. Dioxane is highly susceptible to autooxidation in presence of air, producing hydroperoxides. Decomposition of these peroxides presumably cause the luminescence observed in dioxane based cocktails. This is supported by the luminescence observed in tetrahydrofuran, which is also susceptible to autooxidation forming peroxides. Many peroxides oxidise halides to halogens. As a pseudo-halogen, thiocyanate ion is expected to be similarly oxidised. The reaction can however be more complicated due to further reactions of the

Fig. 5—Effect of pH of KSCN solution (100 μl/2 ml dioxane) on chemiluminescence observed in dioxane. Final KSCN concentration used was 9.52 mM.

Fig. 6—Observed beta count of [3H]-17β-estradiol in dioxane in presence (9.52 mM) and absence of KSCN.
organic product of the initial reaction. The delay time required for chemiluminescence shows that the initial reaction of thiocyanate with peroxide does not lead to emission of radiation. The radiation burst can be explained as due to a free radical chain reaction for which the accumulation of a free radical in sufficient concentration is required for propagation of the chain reaction. The reaction is presumably terminated due to exhaustion of the peroxide.

Kahlben had observed the chemiluminescence in presence of strong base. This indicates the involvement of the peroxide anion in the process. He also observed considerable enhancement of the base-catalysed chemiluminescence in presence of naphthalene. The absence of such an effect in present case indicates that the base induced and thiocyanate induced chemiluminescences are probably produced by different mechanisms.

This interesting reaction occurring in dioxane or in tetrahydrofuran deserves more detailed attention of photochemists. From the practical point of view, the use of thiocyanate may eliminate the problem of luminescence in liquid scintillation counting of dioxane based fluids, if the quenching effect can be tolerated. This may also be a sensitive method for detecting peroxides in dioxane.

References
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