July 23, 1996

Solubilization of Polyacrylamide Gels With Hydrogen Peroxide/Copper Sulphate

Problem:
Observation of about 100 counts of chemiluminescence prompted a researcher to request our assistance. The researcher had prepared 2 mM polyacrylamide gel (4 - 15% tris-glycine), dissolved in 0.4 mL of 30% H_2O_2 with 4 mM $CuSO_4$. The prepared sample was heated to 60 °C for 3.5 hours, then 6 mL of Formula-989 (Packard part number 6NE9899) were added and the chemiluminescence was discovered.

Discussion:
The chemiluminescence observed by this researcher was being caused by the reagent selected for the analysis and the cocktail chosen to facilitate counting. The problem was caused by copper ions in solution with the hydrogen peroxide.

Typically, copper is added to hydrogen peroxide to promote rapid catalytic decomposition (other ions, such as iron and chromium, also promote decomposition). Unfortunately, there is another reaction which can occur in addition to decomposition and this causes luminescence. If the pH is at, or above 7.0, then these metal ions react with peroxide to produce a brown coloration and chemiluminescence. If the medium is kept acidic, then this reaction is suppressed and no chemiluminescence is seen. To confirm this, we prepared the following samples and tested them with various cocktails:

Samples: 0.4 mL 30% hydrogen peroxide heated to 60 °C (two hours)
0.4 mL 30% hydrogen peroxide with 4 mM copper sulphate heated to 60 °C (two hours)

Cocktails: 6.0 mL Formula-989 (Packard part number 6NE9899)
6.0 mL Aquasol®-2 (Packard part number 6NE9526)
6.0 mL Pico-Fluor™ 40 (Packard part number 6013349)
6.0 mL Hionic-Fluor™ (Packard part number 6013319)

These combinations were tested by adding the samples to the cocktails in 20 mL glass vials, then monitoring the background for a two minute count time in a 0 to 18.6 keV window using a Packard Tri-Carb® Model 1900 operated at 19 °C. The results are tabulated below:

		Observed CPM After Lapsed Time			
Cocktail	**Sample**	**2 Minutes**	**15 Minutes**	**30 Minutes**	**Observations**
Formula-989	0.4 mL H_2O_2	56	22	24	Colorless
Aquasol-2	0.4 mL H_2O_2	38	22	24	Colorless
Pico-Fluor 40	0.4 mL H_2O_2	23	17	19	Colorless
Hionic-Fluor	0.4 mL H_2O_2	27	21	23	Colorless
Formula-989	0.4 mL $H_2O_2/CuSO_4$	374	41	33	Yellow/brown
Aquasol-2	0.4 mL $H_2O_2/CuSO_4$	444	135	91	Colorless
Pico-Fluor 40	0.4 mL $H_2O_2/CuSO_4$	273	52	44	Yellow/brown
Hionic-Fluor	0.4 mL $H_2O_2/CuSO_4$	394	69	49	Yellow/brown

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Of the cocktails used, only Aquasol-2 is at a pH less than 6.0, but it is not sufficiently acidic to suppress the chemiluminescence. The other three cocktails have a pH of about 7. This series of experiments shows that the presence of copper ions in a neutral, or slightly alkaline medium is indeed the cause of the chemiluminescence. To confirm this, as well as to try and provide a solution to the researcher's problem, we repeated the sample preparation of the 0.4 mL 30% hydrogen peroxide with 4 mM copper sulphate heated to 60 °C (two hours) and added 0.4 mL 2 M hydrochloric acid to the samples before adding the cocktail. The results obtained were:

		Observed CPM After Lapsed Time			
Cocktail	**Sample**	**2 Minutes**	**15 Minutes**	**30 Minutes**	**Observations**
Formula-989	0.4 mL H_2O_2/$CuSO_4$/HCl	26	17	18	White emulsion
Aquasol-2	0.4 mL H_2O_2/$CuSO_4$/HCl	25	19	23	Clear/colorless
Pico-Fluor 40	0.4 mL H_2O_2/$CuSO_4$/HCl	19	20	18	Clears slowly
Hionic-Fluor	0.4 mL H_2O_2/$CuSO_4$/HCl	19	23	23	Clear/colorless
Hionic-Fluor	0.4 mL H_2O_2/$CuSO_4$/HCl*	32	24	20	Clear/colorless

* Only 0.2 mL 2 M hydrochloric acid was added.

These results showed that the addition of HCl stopped the color formation and suppressed chemiluminescence. Formula-989 could not accept the strong HCl samples and Pico-Fluor 40 only accepted this strong acid after a period of standing.

Recommendation:
If the researcher wishes to stay with this solubilization method, there are two options:

1. Use Hionic-Fluor with the addition of either 0.2 mL or 0.4 mL of 2 M HCl to the digested sample prior to adding 6 mL of Hionic-Fluor.
2. Use Aquasol-2 and add 0.4 mL of 2 M HCl prior to adding 6 mL of Aquasol-2.

The preferred alternative to the two methods cited above, is to consider using a different solubilizer, such as Packard's Soluene®-350 (Packard part number 6003038) with Hionic-Fluor. (See Packard's Sample Preparation Guide, page 61 of the 1997 Chemicals and Supplies catalog for additional information.)

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