INHIBITION OF CHEMILUMINESCENCE BY PHENOL AND SOME OF ITS ANALOGUES

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The phenomenon of chemiluminescence has been known for many years and has been used for chemical analysis via the application of coupled reactions (Kricka & Thorpe 1983). One of the best known chemiluminescence systems involves the oxidation of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) to 3-aminophthalic acid in the presence of hydrogen peroxide and a peroxidase. In addition it has been shown that the luminescence using luminol can be enhanced in the presence of certain substituted phenols such as p-iodophenol and p-phenylphenol (Thorpe et al 1985).

In contrast, this paper reports that phenol and some of its analogues were shown to inhibit the luminescence generated and there was a range over which the concentration of the compound under investigation was linearly related to the intensity of light emission. 200 µl Horseradish peroxidase (30 units), 200 µl luminol (10^{-3} M) and 100 µl of sample all in Sørensens phosphate buffer (pH 8) were pipetted into cuvettes and placed in an LKB 1251 luminometer. The reaction was initiated by the addition of 100 µl of hydrogen peroxide (4×10^{-5} M) and the integrated value of light emission between time zero and 60 seconds was recorded. The temperature of the reaction was 27°C. The results for catechol are shown in Figure 1 and are given as a percentage of the light emission observed with no catechol included. Suppression of the luminescence by catechol is linear over four decades of concentration with a detection limit of 10^{-6} M ($r = 0.9998$).

The results for other phenolic compounds studied are depicted in Figure 2 and the percentage reduction in luminescence for a particular concentration of the inhibiting compound is found to be a function of the oxidation potential of that compound. The phenols compete with luminol for the hydrogen peroxide in the presence of the peroxidase and the analogues which most readily release their hydroxyl-hydrogen cause the greatest suppression. Hydroquinone and pyrogallol both show a marked reduction in light emission at low concentrations whereas phenol and resorcinol which are not such powerful reducing agents, do not lead to such a pronounced suppression.

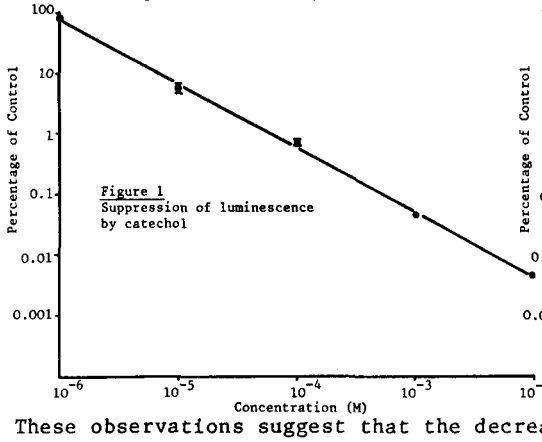

Figure 1
Suppression of luminescence by catechol

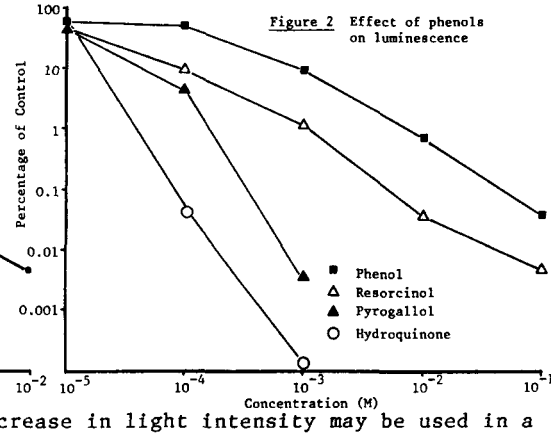

Figure 2 Effect of phenols on luminescence

These observations suggest that the decrease in light intensity may be used in a sensitive assay procedure for the determination of a variety of compounds such as phenols and aromatic amines which contain functional groups capable of donating hydrogen.

Kricka, L.J., Thorpe, G.H.G. (1983) Analyst 108: 1274-1296
Thorpe, G.H.G. et al (1985) Clin. Chem. 31: 1335-1341