Abstract

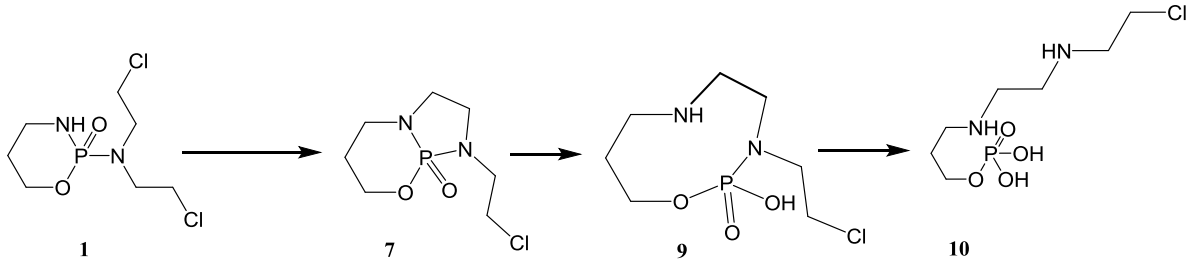
Cytotoxics are drugs used in chemotherapy to prevent growth and replication of cancerous cells via interacting with DNA and other cell processes. This action is non-specific, affecting both cancerous and healthy cells, and hence giving rise to harmful side effects. Cytotoxics have been of increasing concern to environmental chemists because many of these drugs are excreted unchanged and hence released into wastewaters. Their removal by wastewater treatment plants is also often inefficient, resulting in their release into surface waters, where they have the potential to have mutagenic and carcinogenic effects on aquatic organisms. Knowledge of these effects, however, is quite limited. This is in part due to lack of sensitivie analytical methods amenable to real-time, in-situ environmental analysis. Chemiluminescence is a technique with numerous advantages in environmental analysis, however it has had limited use in cytotoxic drug detection. This thesis describes development of chemiluminescence detection for three of the most commonly used cytotoxic drugs in Australia; cyclophosphamide, 5-fluorouracil, and imatinb.

Potassium permanganate, tris-2,2'-bipyridyl ruthenium (II) chloride ($Ru(bipy)_3Cl_2$), manganese dioxide, and cerium sulphate were investigated for their potential as chemiluminescence oxidising reagents of each cytotoxic. $Ru(bipy)_3Cl_2$ when prepared using either PbO_2 or $Ce(SO_4)_2$ produced the most intense chemiluminescence for each analyte. Further investigation into the use of $Ru(bipy)_3Cl_2$ prepared with $Ce(SO_4)_2$ indicated that side reactions were occurring between the cytotoxics and $Ce(SO_4)_2$. This detection method would therefore not offer analytical utility. The use of $Ru(bipy)_3Cl_2$ oxidised with PbO_2 was therefore explored.

While detection of imatinib using this reagent proved unsuccessful, detection methods for both 5-fluorouracil and cyclophosphamide were successfully developed. A new chemiluminescence detection method for 5-fluorouracil using $Ru(bipy)_3Cl_2$ oxidised with PbO_2 was developed using flow injection analysis (FIA). The limits of detection and quantitation were 6.06×10^{-8} M and 1.16×10^{-6} M, respectively. This was a 4-fold decrease in detection limit compared with previously reported methods [1]. Interference from Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{3+}, Cl^-, Br^-, and I^- spiked into 5-fluorouracil solutions was observed, with all but Ca^{2+} resulting in emission quenching. These interferences were found to be removed via sequential strong anion SPE (STRATA-X-A) and strong cation SPE (STRATA-X-C) of the sample prior to analysis. The method was applied to spiked tap and lake water samples. The presence of organic interfering compounds results in recoveries of 27.5 % and 34.1 % from tap and lake water, respectively. However, these compounds could be easily separated from 5-flurouracil

using UHPLC, and hence the developed method has great potential for detection of 5-fluorouracil in surface waters.

A new method for the chemiluminescence detection of cyclophosphamide in aqueous solution was also developed using $Ru(bipy)_3Cl_2$ oxidised with PbO_2. While poor linearity of response was achieved for fresh cyclophosphamide solutions, high correlation between cyclophosphamide concentration and chemiluminescence signal was obtained when analysing 24-hour-old solutions (R^2 of 0.9995). This was found to be due to hydrolysis of cyclophosphamide to compounds 7, 9, and 10 (see below) over 24 hours when stored at room temperature in the absence of light.



In summary, alternative sensitive analysis methods for cytotoxic drugs are required in order to conduct a true risk assessment on their potential harmful effects in surface waters. Here new chemiluminescence detection methods for the common cytotoxics cyclophosphamide and 5-fluorouracil were developed using $Ru(bipy)_3Cl_2$ prepared with PbO_2 as the oxidising reagent. A four-fold decrease in detection limits compared with previous methods was obtained for 5-fluorouracil detection, while chemiluminescence detection of cyclophosphamide proved valuable in monitoring its aqueous degradation. With these new methods further insight into their aqueous stability, and hence fate in surface waters, can be obtained, which would provide valuable information in assessing their effects on aquatic organisms in surface waters.