

## **Abstract**

In recent years, environmental pollution, particularly air pollution, is a subject of great scientific and public interests. The reason is due to the strong impact on human health. Air pollution is due to the placing in the atmosphere of polluting substances coming mostly from fossil fuel use in the various combustion processes, used to power the engine vehicles, industrial machinery and for the production of electrical energy. The primary air pollutants, waste products of combustion are carbon monoxide, sulphur oxides and nitrogen, particulates and aromatic hydrocarbons.

Different scientific studies correlate the presence and increased atmospheric concentrations of aromatic hydrocarbons, in urban canter due to the emission of exhaust gases of vehicular traffic, with arise of a variety of tumors.

Among the aromatic hydrocarbons, benzene is a known carcinogen and is widely demonstrated its toxicity for the blood cells and hematopoietic organs, but it is also the cause of several tumors of the respiratory system. Scientific evidence shows that exposure to high concentrations or prolonged time is responsible of several forms of leukemia, between all acute myeloid leukemia. For these reasons, the emission limit values and the daily exposure are very low, fixed respectively to  $5 \mu\text{g}/\text{m}^3$  and 0.5 ppm.

Actually, conventional analytical methods used for detection of benzene need a preliminary step of sampling on field and then the subsequent laboratory analysis by gas chromatography coupled to mass spectrometry. Consequently, these methods are expensive and time-consuming.

It appears evident that a simple, rapid and sensitive method for detection of benzene that could work in continuous is needed. With this aim we proposed to develop a portable, highly sensitive and economic optical biosensor platform for monitoring in continuous the atmospheric level of benzene.

In particular, we have developed a fluorescent biosensor that use the porcine odorant binding protein (pOBP) as molecular recognition element (MRE). pOBP is a protein that belongs to the Lipocalin superfamily. *In-silico* experiments have showed the pOBP high specificity and affinity for benzene.

To monitor benzene we developed a competitive assay based on FRET principle. In particular, benzene is monitored as competition efficiency between the 1-aminoanthracene (fluorescent probe) and benzene for the pOBP binding site.

The competition efficiency is evaluated as reduction of the fluorescence intensity; the limit of detection of the developed assay is  $3.9 \mu\text{g}/\text{m}^3$ , lower respect to the actual emission limit of benzene as regulated by law.

A next step of the project will be the development of a sensor chip using a silicon wafer for monitor in continuous the atmospheric levels of benzene.

Preliminary results obtained during the PhD project have showed the possibility to immobilize the pOBP on a chip surface. In order to monitor the presence of benzene in large areas it will be necessary to integrate the sensors into an automatic control and wireless communication system (sensor network). In addition, the enormous amount of data collected will require of "big data analysis" through the use of designed software in order to establish a correlation between the onset of cancer and the levels of hydrocarbons present in the atmosphere.