DEVELOPMENT OF A MULTI-ANALYTE FLUORESCENT SENSOR BASED ON IMMOBILIZATION OF 2,3-DIAMINONAPHTHALENE IN A SOL-GEL MATRIX

Mª José Martínez-Tomé, Rocío Esquembre, Ricardo Mallavia and C. Reyes Mateo
Instituto de Biología Molecular y Celular. Universidad Miguel Hernández, Elche. E-03202, Alicante (Spain)
rmateo@umh.es

The development of new sensor devices is presently the subject of extensive research in areas such as clinical diagnostic, food technology, biomedical and environmental analysis. One of the major trends in the current research of optical chemical sensor technology is the multi-parameter functionality on a single platform (so-called multi-analyte sensing) in order to provide analytical devices that are rapid, sensitive, specific, non expensive, and suitable for real-time on-site detection. In this work, we have developed a dual-analyte optical chemical sensor platform based on the immobilization of the fluorescent compound 2,3-diaminonaphthalene (DAN) in a sol-gel matrix.

DAN reacts with nitrite to yield the fluorescent product 2,3-naphthotriazole anion (NAT), with excitation/emission maxima ~365/415 nm [1]. DAN also reacts with Se (IV) yielding the complex 4,5-benzopiazselenol, which fluoresces at 550 nm [2]. Thanks to this reactivity, the compound has been successfully used to detect and quantify nitrite or selenium in biological and environmental samples. However, despite these assays offer great sensitivity and versatility, they show several drawbacks such as the poor solubility of DAN in water and its toxicity. Moreover, there is a great difficulty in employing the method to detect traces of nitrite and Se (IV) in certain biological samples because of interference by inherent biological substances and colorimetric chemicals, which often complicate the accurate detection of the analytes. In addition, due to the low fluorescent quantum yield of 4,5-benzopiazselenol in water, determination of selenium with DAN requires the extraction of the final product with organic solvents. Most of these problems could be avoided by using an efficient procedure for immobilization of the indicator on an appropriate polymer matrix. Encapsulation of DAN in a solid support, such as sol-gel matrix, should largely reduce its toxicity, providing additional advantages including easy probe handling and enhanced stability of the encapsulated dye. Moreover, due to the tunable porosity of the sol-gel glasses, the matrix could act as a filter, avoiding the interferences by biological components.

In this study we have encapsulated the DAN reagent in a porous silicate glass matrix, via previous incorporation of the dye in 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). Changes in fluorescence intensity were used to characterize the inclusion complex and determine the association constant and stoichiometry of the process. Fluorescence spectrum of this complex was also used to monitor its immobilization within the sol-gel matrix. Reactivity of the immobilized complex was evaluated with increasing concentrations of nitrite and Se (IV). A calibration curve was built for each analyte which relates the fluorescence increasing with analyte concentration. The response was linear in the range studied with a detection limit around 20 nM (Fig. 1). Results show that sol-gel immobilization does not modify the reactivity of the dye against these analytes and serves to prepare a highly sensitive ready to use fluorescence-based sensor to simultaneously quantify nitrite and selenium in aqueous samples, with no further sample pretreatment.

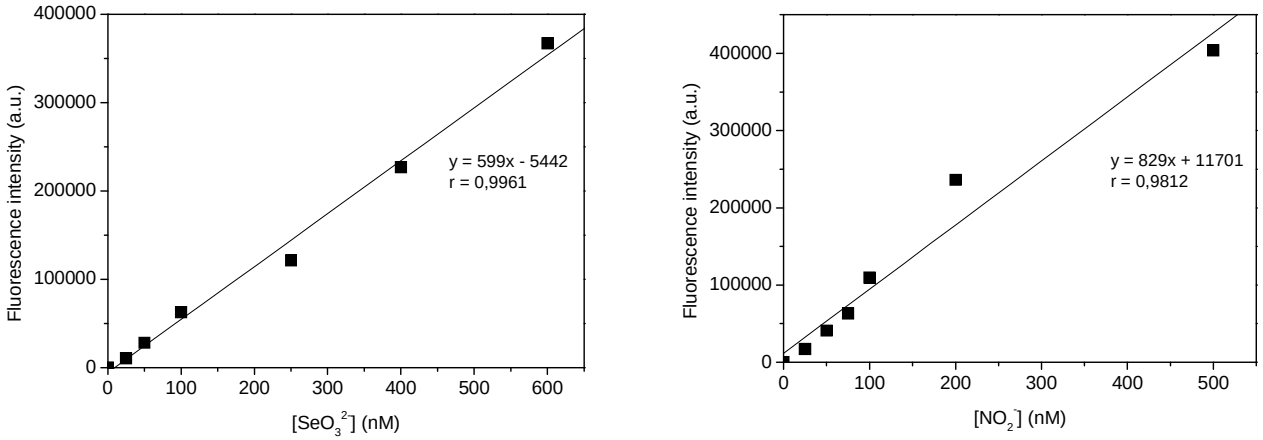

Figure 1: Dependence of the fluorescence intensity of 4,5-benzopiazselenol (left) and NAT (right) as a function of Se (IV) (left) and nitrite (right) concentrations.

[1] T. P. Misko, R. J. Schilling, D. Salvemini, W. M. Moore and M. G. Currie, Anal. Biochem. **214** (1993) 11-16.

[2] J. Pedro, F. Andrade, D. Magni, M.Tudino, A. Bonivardi, Analytica Chimica Acta, **516** (2004) 229-236.