ARRAY OF FLUOROPHORES USING A SILICA GEL-BASED, HIGH-THROUGHPUT COMBINATORIAL PLATFORM

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Non-covalent interactions in molecular environments produce the following effects on fluorescence parameters [1]: i) specific interactions between analytes and fluorophores provoke fluorescence quenching; ii) changes in both quantum yield and wavelengths are often simultaneously observed; and iii) we have demonstrated in previous works [2-4] that non-specific interactions (ion or dipole-induced dipole) enhance fluorescence intensity without producing changes in emission wavelength. However, since most of the models only consider wavelength shifts and no changes in intensity, no systematic analytical use of non-covalent interactions for quantitative measurements, with the exception of the quenching effect, can be found in the literature.

The effect of non-specific interactions has been demonstrated to be general [5], and includes non-absorbing analytes, such as saturated hydrocarbons, which produce an increase in the emission of fluorophores. Therefore, practically almost all analytes have a response when interacting with fluorophores. Sensitivity of this response is particularly high in silica gel medium. Consequently, it seems realistic to develop an array of fluorophores and screen their interaction with analytes in a high-throughput format, using a silica gel-based combinatorial platform. This system may take advantage of the combined response of its components to create specific pattern responses or fingerprints for analyte discrimination [6]. Likewise, it may be help to understand non-covalent interactions involved in analyte-fluorophore molecular environments; and to select fluorophores as revealing agents for Thin-Layer Chromatography (TLC) analytical applications [7].

Here we present the preliminary results obtained concerning the viability of this technique.

Arrays have been prepared using 53 fluorophores which have been immobilized by spray-on spotting on HPTLC silica gel plates through non-covalent adsorption. Fluorophores have been uniformly applied at a constant density on silica gel plates as 4x2 mm bands by previous solvent nebulization using N_2 as impulsion gas (3 bar). In-situ UV spectra of fluorophore sprayed bands have been recorded and fluorescence measurements performed at five different wavelengths using scanning densitometry (λ_{exc}= 230, 313, 365, 406 y 436 nm). Subsequent spray spotting of the corresponding target analyte on fluorophore bands, and further fluorescence measurements have been done. Cholesterol and sphingomielin, which are not fluorescent and had poor spectroscopical properties, have been used as analytes.

Parameters affecting repeatability of the procedure have been studied concerning sample application, scanning densitometry, intra- and inter-plate runs, as well as the effect of time on fluorophore signal. A multivariable analysis of signals has been performed.

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