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A novel FRET probe for determination of fluorescein sodium in aqueous solution: Analytical application for ophthalmic sample

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Fluorescent pyrene nanoparticles (PyNPs) have been prepared by a reprecipitation method in the presence of sodium dodecyl sulphate (SDS) as a stabilizer. The formation of PyNPs has been confirmed by dynamic light scattering (DLS), UV-visible absorption spectroscopy, fluorescence spectroscopy and excited state lifetime measurements. DLS results of PyNPs shows a narrow size distribution with average particle size of 77.4 nm and negative zeta potential. The systematic FRET experiments performed by measuring fluorescence quenching of PyNPs with successive addition of FL-Na analyte exploited the use of PyNPs as nanoprobe for detection of FL-Na in aqueous solution. The fluorescence of PyNPs has been quenched by Fl-Na and quenching has been in accordance with the Stern-Volmer relation. The distance r between the donor (PyNPs) and acceptor (FL-Na) molecules has been obtained according to the fluorescence resonance energy transfer. The fluorescence quenching results have been used further to develop an analytical method for estimation of fluorescein sodium from ophthalmic samples available commercially in the market.

Keywords: Fluorescent pyrene nanoparticles, Fluorescein sodium, Fluorescence resonance energy transfer

Fluorescein sodium (FL-Na), also called uranine, is a non-toxic, low molecular weight and highly water-soluble dye, shows the physical property of fluorescence and commonly used as a quantitative fluorophore for studying different tissues of the eye[1-3]. Fl-Na shown in Fig. 1 is extensively used as a diagnostic tool in the field of ophthalmology and optometry. It is available as sterile single use sachets containing lint-free paper applicators soaked in Fl-Na[4]. It has a pK_a of 6.4 and its ionization equilibrium leads to pH-dependent absorption and emission over the range of 5 to 9. It can exist in seven prototropic forms, each of which possesses its own distinct spectral properties[5]. In neutral solutions, such as water and methanol (which also act as polar solvents) it exists mainly as dianion. It is widely used as fluorophore in the biosciences and as a fluorescent tracer for many applications[6]. Few methods have been used for detection and estimation of dyes[7-9]. A direct fluorimetric method requires separating the analyte from interfering constituents in the samples and having absorption in the region of analyte molecule. By contrast the fluorescence quenching/enhancement methods have high sensitivity and more simple detection and do not need separation of analyte molecules from other interfering constituents[10-13]. Therefore, the development of sensitive and selective sensors for FL-Na is of current interest.

Fluorescent organic nanoparticles (FONs) of low molecular weight functional compounds found special interest because of high variability and flexibility in materials and method of nanoparticles preparation[14-15]. Organic nanoparticles (ONs) occupy the intermediate state between isolated molecules and the bulk crystal. It is observed that most of the fluorescent organic materials belonging to the class of polynuclear aromatic hydrocarbons (PAHs) are water insoluble and gives their monomer emission in lower wavelength regions. PAHs are used as a fluorescent probe for the fluorescence quenching process[16-18]. Among the PAHs, Perylene and Pyrene are popular because of their large lateral π-orbital stacking between molecules and are most widely used probes

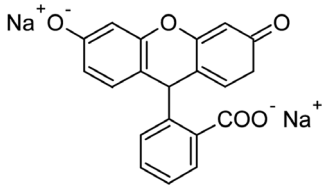

Fig. 1 — Structure of fluorescein sodium.