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Steady-State and Time-Resolved Spectroscopy of Ion-Sensitive Fluorescent Probes

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The present work is focused on the study of the spectroscopic and complexometric behavior of different ion-sensitive fluorescent probes. Three of the four types of probes studied follow a conventional principle of signal generation and their molecular constitution corresponds to either a *fluorescent ligand* (BP(OH)_2), so-called *intrinsic* charge transfer (ICT) fluorescent probes (donor-acceptor-substituted chalcones), or *electron transfer* (ET) fluorescent probes (substituted triaryl- Δ_2 -pyrazolines). The latter two systems are designed in a modular way and thus, both the signal generating fluorophore and the complexing receptor can be carefully tuned. Whereas in the case of the ICT probes both units are electronically conjugated, these moieties are more or less electronically decoupled in the ET probes and signal generation is based on the modification of a long range electron transfer process. The fourth type of probe investigated, a boron dipyrromethene derivative (BDP), is also designed in a modular way. But here, due to the highly pretwisted constitution of the molecule, fluorophore and receptor are only "virtually" decoupled. Besides employing this alternative mechanism of signal generation, control of the selectivity by carefully directed receptor design and improvement of the selectivity and sensitivity of fluorometric metal ion analysis by recording time resolved emission spectra (TRES) were the main aims of these investigations.

Furthermore, with the aid of various model compounds, NMR spectroscopy as well as quantum chemical calculations, a fundamental understanding of the photophysical behavior of the different classes of dyes was obtained.

In accordance with its parent compound 2,2'-bipyridyl, the fluorescent ligand BP(OH)_2 shows complexation induced changes of its absorption and emission behavior in the presence of heavy and transition metal ions. Whereas paramagnetic ions such as Cu_{II} or Ni_{II} (and also Hg_{II}) lead to static fluorescence quenching, the diamagnetic ions Zn_{II} and Cd_{II} form strongly fluorescent complexes with ion specific fluorescence lifetimes in the ns time range. Both complexes are stable in the excited state which is manifested by a strongly reduced rate constant of non radiative deactivation compared to that of the free ligand. Both the absorption and emission spectra of the complexes largely overlap and a discrimination with steady-state fluorometry is very limited. Nevertheless, with time resolved fluorometry a separation of both components is possible and for a series of synthetic $\text{Zn}_{\text{II}}/\text{Cd}_{\text{II}}$ containing samples this gain in selectivity by global analysis of the time resolved emission spectra (TRES) could be demonstrated.

The intrinsic fluorescent probes of chalcone type contain a cation selective receptor in the donor part of the molecule and show a strong decrease of the intramolecular charge transfer process due to complexation induced weakening of the donor. Depending on molecular probe design and solvent polarity, the fluorescence of the uncomplexed molecule occurs either from a polar directly excited (E^*) or a highly polar charge transfer state (A^*) which is populated via an excited state reaction involving single bond twisting. Here, competing processes include population of a non emissive polar (K^*) or weakly polar (P^*) transient state. Furthermore, the fluorescence of these dyes can be quenched by energetically close lying $n\pi^*$ states (in apolar solvents) or by enhanced internal conversion due to the decreasing energy gap between ground and excited state (in highly polar solvents). As mentioned above, the donor strength is reduced upon cation complexation in polar solvents and a moderate fluorescence enhancement occurs. Besides increased fluorescence quantum yields, this effect results in ion specific fluorescence lifetimes of the complexes. Upon exchanging the aza-oxa for an aza-thia macrocycle in the receptor part of the system, the cation selectivity could be tuned from alkali and alkaline earth metal ions to the thiophilic metal ions Ag_{I} and Hg_{II} . Here, especially the fluorescence enhancement in the presence of the well-known quencher Hg_{II} is analytically valuable.

Upon cation binding the ET probes of the triaryl- Δ_2 -pyrazoline type show very small spectral shifts but much larger fluorescence enhancement compared to the ICT probes. Again, by problem specific use of different receptors, the selectivities can be controlled. Based on a detailed investigation, for the 5-*p-N,N*-dialkylanilino-3-benzthiazol-1-phenyl- Δ_2 -pyrazolines, the intramolecular processes which lead to fluorescence quenching in the unbound state and "switching on" of the fluorescence in the complexed state could be identified. For these molecules, the intramolecular charge transfer in the basic chromophore occurs in the sub ps time range and is quenched by an electron transfer process from the largely electronically decoupled receptor to the acceptor part of the basic chromophore on the ps time scale in highly polar solvents.

“Virtually” decoupling fluorophore and receptor in the BDP derivative finally makes it possible to yield extremely high complexation induced fluorescence enhancement signals. For these highly pretwisted donor acceptor biaryls, the directly excited emissive state (LE) forms a highly polar, twisted charge separated charge transfer state (CT) in polar solvents. Emission of the latter is strongly forbidden and, depending on solvent polarity, is largely red shifted compared to the LE fluorescence. Thus, a largely separated dual fluorescence is already observed in solvents of medium polarity and in highly polar solvents both fluorescence bands are nearly completely quenched. Metal ion complexation blocks off the CT process and leads to a “switching on” of the LE emission yielding fluorescence enhancement factors > 1000. This process is so sensitive that for most of the cation complexes studied, two emissive complex conformers with strongly overlapping absorption and emission spectra occur which could be distinguished by their fluorescence lifetimes.