Summary

Drugs are used for therapeutic, diagnostic or preventive purposes and their transport towards the intracellular region occurs through interfaces. Hence, the importance of microheterogeneous media resides on the possibility of modeling the behavior of drugs in confined spaces. A wide number of techniques have been used to address this problem; however, their main drawbacks are associated with the lack of sensitivity and reproducibility or a complicated workup. Recently, laser flash photolysis (LFP) has been used for the study of ligand-biomolecule systems. It has been demonstrated that the triplet excited state is very sensitive to the environment and, therefore, it can be used as a probe in drug-protein complexes.

Whit this background, a new methodology has ben developed, based on the use of LFP and fluorescence techniques, in order to obtain relevant information about the nature of the supramolecular interactions that take place between drugs and a variety of entities. Thus, transient species generated from selected substrates have been studied, using their properties as quantitative parameters that report on the characteristics of the medium.

First, photophysical and photochemical properties of the new calcimimetic agent (*R*)-cinacalcet (CIN) have been studied. The main deactivation processes of its excited states have been identified, and their lifetimes, as well as the quantum yields of the involved photophysical processes have been determined. Specifically, the triplet excited state of CIN is highly sensitive to the environment, and its lifetime in the presence of human serum albumin (HSA) is considerably

longer than in solution. Moreover, the rate constant of triplet quenching by oxygen in the protein is two orders of magnitude lower than in solution. Thus, the protein microenvironment protects (*R*)-CIN from attack by oxygen; this prevents the phototoxic effects caused by the generation of singlet oxygen ($^1O_2$) and results in an enhanced photosafety of the drug.

After characterizing the lowest singlet and triplet excited states of (*R*)-CIN, further effort has been devoted to gain a more precise knowledge of drug-protein interactions. Thus, the study has been extended to other drugs, which also contain a naphthalene (NP) chromophore like (*S*)-naproxen ((*S*)-NPX) and (*S*)-propranolol ((*S*)-PPN). To detect a possible stereodifferentiation in the binding process, parallel studies have been conducted with their corresponding enantiomers. For this purpose, non-commercial (*S*)-CIN has been synthesized from 3-(trifluoromethyl)cinnamic acid. Subsequently, the interactions between different substrates and binary systems, with serum albumin (SA) and α-acid glycoprotein (AAG) simultaneously present in the same media, have been studied. Experiments have been performed in both human and bovine proteins. Fitting the decays of the triplet excited states generated by LFP has revealed that SA is the main carrier for NPX, whereas AAG plays this role for PPN; CIN constitutes an intermediate case, as it binds efficiently to both proteins. No significant stereodifferentiation has been found for any NP derivatives.

Another interesting field of application of LFP has been the study of drug-drug interactions in a common protein binding site of a carrier protein (human and bovine AAG). In the PPN/CIN/AAG system, it has been observed that the first triplet excited state of PPN interacts with

CIN, which results in a substantial decrease in the lifetime of the former. This deactivation can be explained by a triplet-triplet energy transfer (TTET) within the only binding site available within the AAG, which is in good accordance with the relative triplet energy values determined for both drugs. Moreover, theoretical calculations for the PPN/CIN system have confirmed that the spatial arrangement of both chromophores within the protein is compatible with TTET. Similar results have been obtained for nabumetone (NAB) in NAB/CIN/AAG systems.

Finally, singlet and triplet excited states have been used as probes to investigate the encapsulation of (*R*)-CIN, (*S*)-PPN, (*S*)-NPX, as well as (*S*)-NPXMe (methyl ester of (S)-NPX) within mixed micelles (MM). For this purpose, fluorescence and triplet excited state quenching experiments with iodide and nitrite, respectively, have been carried out, both in solution and in MM. The decrease by one order of magnitude in the quenching rate constants found for PPN, CIN and NPXMe in MM is associated with an efficient encapsulation, whereas this is not the case for NPX. The observed phenomenon can be due to the different hydrophobicity of the substrates, since NPX has a free carboxylic acid in its chemical structure and exhibits higher solubility in aqueous medium.