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POLYAMINE RECEPTORS AS FLUORESCENT PROBES FOR ANIONS

Polyamine-based receptors can be exploited as fluorescent probes to detect emerging polluting ionizable compounds in aqueous media. Receptors L1 and L2 in their protonated forms have been used to detect the emerging pollutants ketoprofen and perflurooctanoic acid. The salt bridge interaction between the carboxylate group of the targets and the ammonium groups of the receptors results in a change of L1 and L2 emission properties.

Introduction

Due to the significant role that anions play in a variety of biological and environmental processes, the study of anion recognition and sensing by synthetic molecular receptors has emerged as one of the most crucial fields of supramolecular chemistry. However selective recognition of anions may be a hard task, especially in water **[1, 2]**. An efficient and selective anion receptor is based on the co-operative action of weak non-covalent interactions including hydrogen bonding, electrostatic contacts, hydrophobic effects, and π-staking interactions. In water at neutral pH, many anions can typically exist in several deprotonated states with different negative charges. For these reasons, a good receptor should be positively charged and equipped with H-bond donor and acceptor groups **[3]**. In this context, polyamines scaffolds provide the proper properties needed for the design and development of anion receptors. Polyamines protonation at a neutral pH in water solution results in the formation of polyammonium cations, which can bind anionic substrates *via* salt bridging (simultaneous H-bonding and electrostatic) interactions with the negatively charged functionalities of the anion **[4-6]**. The inclusion of fluorogenic units, which responds to the presence of the analyte by changing its photophysical properties, within the receptor structure, may lead to selectivity and

Equilibrium	L1	L2
$L + H^+ = HL^+$	6.76(8)	9.44(2)
$HL^+ + H^+ = H_2L^{2+}$	5.84(7)	7.67(1)
$H_2L^{2+} + H^+ = H_2L^{3+}$	-	3.64(2)

Tab. 1 - Protonation constants (log K) of L1 and L2 in H_2O/EtOH (50:50 v/v) ([L1] = [L2] = $5x10^{-4}$ M, 0.1 M NMe_4Cl, 298.1±0.1 K)

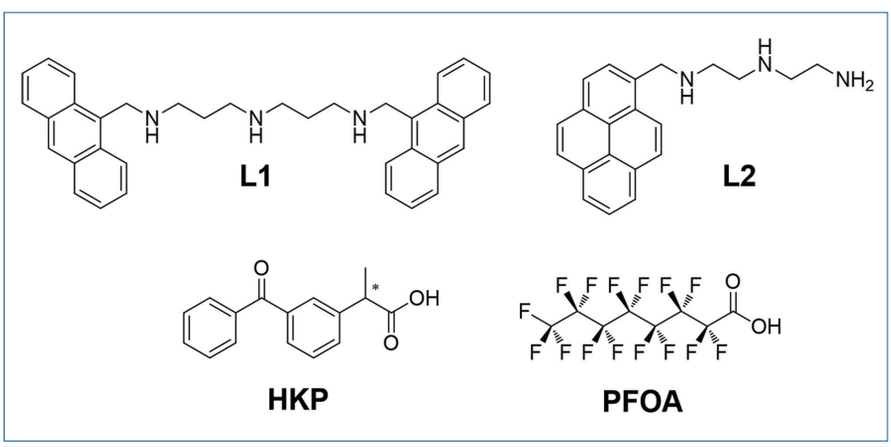

Fig. 1 - Molecular structure of polyamine-based fluorescent receptors, L1 and L2, and target anions, HKP and PFOA

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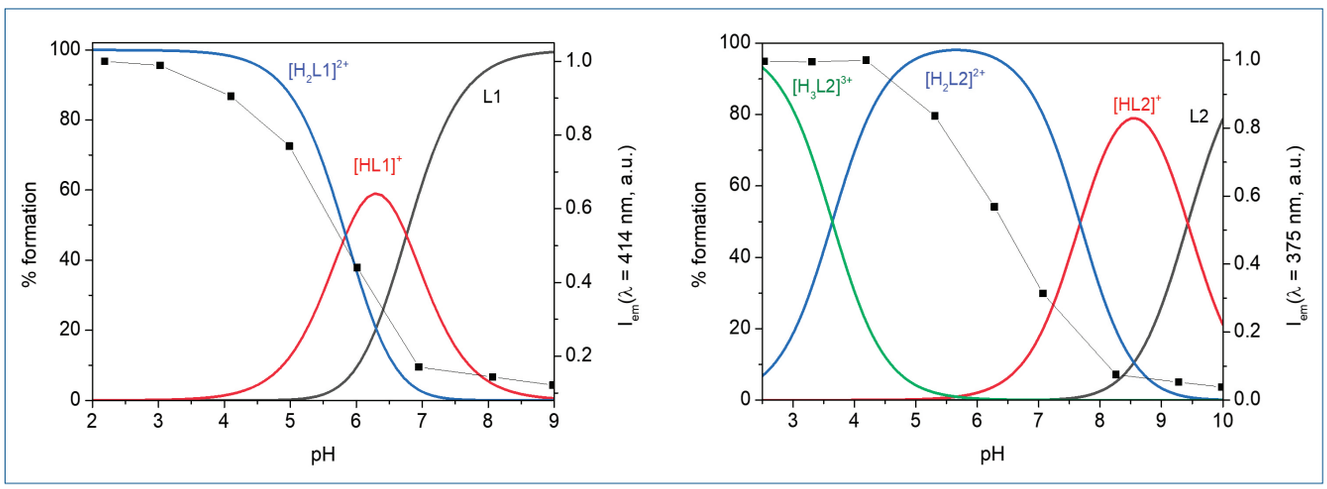

Fig. 2 - Fluorescence emission at 414 and 375 nm (black squares) of L1 (left) and L2 (right) superimposed to the distribution diagrams of the protonated species present in solution ([L1] = [L2] = $5x10^{-4}$ M), calculated on the basis of the equilibrium constants in Tab. 1 (H_2O/EtOH 50:50 (v/v), 0.1 M NMe_4Cl, 298.1±0.1 K)

optical sensing of a target anion **[7, 8]**. Nowadays there is a growing interest in the development of new chemical sensors for "Emerging Pollutants" (EPs), substances whose environmental concern is recently emerged and often not yet regulated by the Italian or European rules. They include several substances, among which pharmaceutics, such as non-steroidal anti-inflammatories drugs (NSAIDs) and antibiotics, waterproofing agents, plasticizers etc., whose use and release in the environment can lead to a continuous intake from living beings, with possible long-term toxic effects **[9-11]**. Most of these pollutants present a general chemical structure, composed of one or more ionizable sites, in most cases a carboxylic group, which is in its deprotonated form at neutral pH in aqueous solution. Here we briefly report the results obtained for two triamine-based receptors (L1 and L2 in Fig. 1), bearing two anthracene units and one pyrene unit respectively, as fluorescent probes for the recognition of ketoprofen (HKP) **[12]** and perfluorooctanoic acid (PFOA) **[13]** in aqueous solution.

Receptors protonation in water/ethanol mixture

Potentiometric titrations have been used to analyse the acid-base behaviour of the two receptors L1 and L2, identifying the species formed in solution and their protonation constants (Tab. 1). As shown by the distribution diagrams of the species in solution (Fig. 2), at neutral pH, L1 is mainly in its monoprotonated and not protonated forms, whereas, in the case of L2, $[H_2L2]^+$ is the most abundant species in solution.

In L1, the central amino group undergoes the first protonation equilibrium, while, in the second protonation step, the two protons are likely localized on the two lateral amine groups to minimize electrostatic repulsion, as also confirmed by ^1H NMR measurements. In L2, the quite high constant relative to the first protonation equilibrium can be attributed to the protonation of the terminal primary amine group, while the in the H_2L2^{2+} species, the two acidic protons can be localized on the primary amine group and on the benzylic one, adjacent to the pyrene unit. The fluorescence emission properties of both receptors show a marked pH dependence (Fig. 2) and the progressive deprotonation of the triamine chains induces a considerable decrease of the emission. In the not protonated polyamine receptors, the emission is likely inhibited by a photoinduced electron transfer (PET) process from the benzylic nitrogen atom to the excited fluorophore.

Equilibrium	Log K
$L + KP^- = [L(KP)]^-$	2.95(4)
$HL^+ + KP^- = [HL(KP)]$	2.47(7)
$H_2L^{2+} + KP^- = [H_2L(KP)]^+$	2.98(4)

Tab. 2 - Formation constants of the L1 adducts with HKP in H_2O/EtOH (50:50 v/v) ([L1] = $5x10^{-4}$ M, 0.1 M NMe4Cl, 298.1±0.1 K)

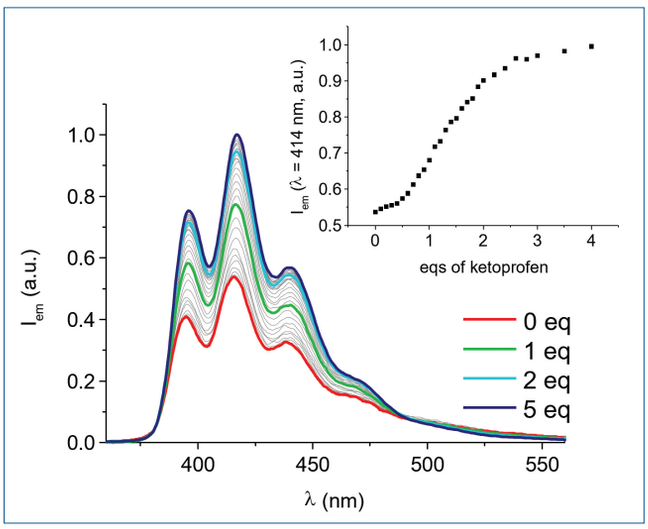

Fig. 3 - Fluorescence emission spectra of L1 at pH 7 (0.001 M TRIS buffer) in H_2O/EtOH 50:50 (v/v) and (inset) emission intensity at 414 nm in the presence of increasing amounts of KP (λ_{exc} = 340 nm, [L1] = 10^{-5} M, 298 K)

Binding and fluorescence sensing of ketoprofen by L1

Potentiometric titrations shows that the protonated forms of receptor L1 can bind HKP, in its anionic form, leading to the formation of 1:1 complexes (Tab. 2), at least in the condition of potentiometric measurements (NMe_4Cl 0.1 M). The adducts formation is most likely due to hydrophobic forces and salt bridging between the anionic site of KP^- and the positively charged amine groups. As shown in Tab. 2, the charge of the receptor does not significantly affect the complex stability, and the neutral receptor can also bind neutral HKP, implying that hydrophobic interactions are strongly involved in complex formation.

To investigate the receptor ability to optically signal HKP, acting as fluorescent probe in aqueous solution, we performed fluorescence titrations by adding increasing amounts of HKP (in its anionic form at pH 7) to a solution of L1, buffered at pH 7 with TRIS. The addition of HKP induces a 2-fold increase of the fluorescence emission of the anthracene (Fig. 3). The emission at 414 nm increases almost linearly up to the addition of 2 equiv. of HKP (inset of Fig. 3) to achieve an almost constant value for molar ratio greater than 3. This could suggest the formation of complexes with 1:1 and 2:1 stoichiometry between HKP and L1, at least in the condition of fluorimetric measurements (without ionic medium). The emission enhancement in the presence of HKP

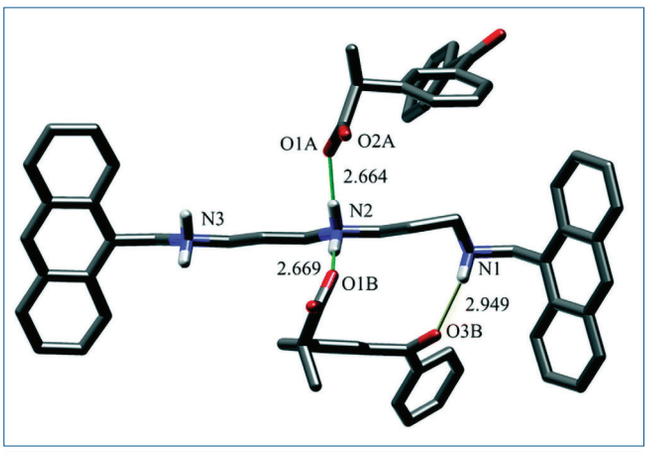

Fig. 4 - Asymmetric unit of the $[H_2L1](KP)2{\cdot}0.75EtOH{\cdot}2.75H_2O$ complex, comprising one ligand molecule and 2 KP^- anions, KP_A and KP_B [12]

may be ascribed to the mechanism of interaction between the protonated receptor and the carboxylate group of KP^-, as clarified by ^1H NMR experiments. The coordination of KP^- induces a proton transfer process from the protonated central amine group to the not protonated benzylic nitrogen, inhibiting the PET process and partially restoring the fluorescence emission. KP^- adduct stabilization by salt bridging is also supported by the crystal structure of the $[H_2L1](KP)_2{\cdot}0.75EtOH{\cdot}2.75H_2O$ complex. The asymmetric unit (Fig. 4) contains two anionic molecules of HKP, KP_A and KP_B, bound to the ligand *via* 2 salt bridges. KP_B is further stabilized by an additional H-bond between the not-protonated amino group and KP_B carbonyl function.

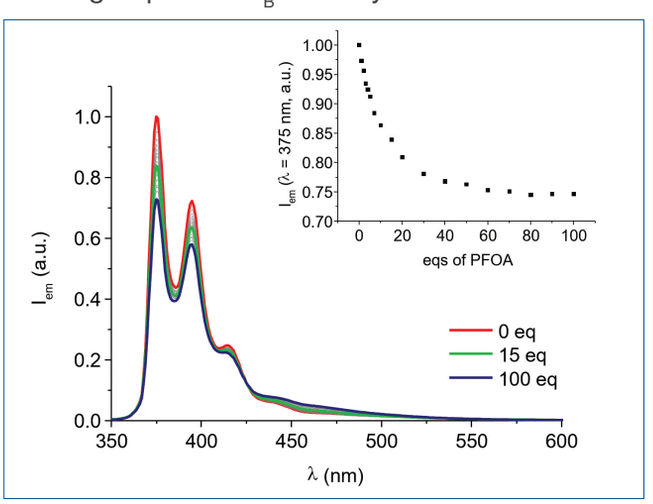

Fig. 5 - Fluorescence emission spectra of L2 at pH 7 (0.005 M TRIS buffer) in H_2O/EtOH 50:50 (v/v) and (inset) emission intensity at 375 nm in the presence of increasing amounts of KP (λ_{exc} = 340 nm, [L2] = 10^{-5} M, 298 K)

Fluorescence sensing of PFOA by L2

The same experimental conditions have been chosen for the investigation of receptor L2 as fluorescent probe for PFOA in neutral water/ethanol solution. The addition of 10 equivs. of PFOA to the solution of L2 at pH 7 induces an almost linear slight decrease of the emission (ca. 10%, Fig. 5); a higher amount of PFOA (more than 80 equivs.) gives rise to a smoother emission decrease (ca. 25%). The low emission decrease observed may be related to the presence in solution of the poorly emissive species $[HL2]^+$. To clarify the relevance of the protonation state of the polyamine chain in the formation of the complexes and the consequent changes in the emission properties of the fluorophore, a fluorescence emission titration at pH 4 have been also performed. At this pH value the fully protonated and emissive species of the receptor is present in solution. The increasing concentration of PFOA in solution induces an overall 80% quenching of the pyrene emission. These results suggest that the interaction involves salt bridging contacts between the ammonium groups of the receptors and the carboxylate group of PFOA. This interaction mode can also explain the quenching observed upon PFOA binding. In fact, the interaction of the protonated amine groups with the anionic PFOA *via* salt bridging implies a partial sharing *via* H-bonding of the acidic proton located on the amine group, thus favouring a PET process from the nitrogen atom of the polyamine chain to the excited fluorophore.

Conclusions

The presented results highlight how the presence of multiple H-bond donor group, together with the ability of polyamines to be protonated in solution at neutral pH are two fundamental features in anion coordination. The protonated triamine scaffold of receptors L1 and L2 can efficiently bind the target molecules *via* formation of electrostatic contacts. Moreover, the coupling of the polyamine chain with fluorescent molecules, like anthracene or pyrene, leads to the development of fluorescent molecular probes able to optically sense anionic species, such as ketoprofen and PFOA, in solution. The performance of the chemosensors in anion detection can be, in perspective, enhanced by their incorporation in nanostructured materials, in which energy transfer cooperative effects may occur between closely placed fluorescent probes.

Acknowledgments

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Recettori poliamminici come sonde fluorescenti per anioni

I chemosensori triamminici L1 e L2, funzionalizzati con unità fluorescenti di antracene e pirene, sono in grado di coordinare specie anioniche come ketoprofene e PFOA tramite interazioni elettrostatiche tra il gruppo carbossilato degli analiti e il gruppo amminico protonato del recettore, segnalandone la presenza in soluzione acquosa tramite variazione delle loro proprietà di emissione.