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Photosensing by a fluorescing probe covalently attached to the silica.

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Abstract: Starting from aminopropyl silicagel, an anthracene fluorophore has been covalently grafted on the surface of silica, the secondary amino group being left in the linking arm. When one fifth of the initial amino functions are grafted, the silica behaves as a pH probe, the fluorescence of which switches on when the amino groups are protonated. On the contrary, if the silica loading is almost complete, the pH probing is no longer possible. In this case, an excimeric versus monomeric emission can be used in sensing the solvent water content. Copyright © 1996 Elsevier Science Ltd

Besides its interest in photochemistry, Photoinduced Electron Transfer (PET) is a process largely used in the design of fluorescent ion sensing molecules [1-3]. One of the simplest systems is based on fluorescent aromatic compounds linked to amino groups and proposed as possible fluorescent proton sensors [4,5]. In this case, the fluorescence of the fluorophore "lights on" when the amino group is protonated. In the absence of protons, the fluorescence is quenched by a PET originating from the nitrogen lone pair. Various ways of immobilization of indicators have already been tested, using mainly physical entrapment in porous matrices of polymer [6] or sol-gel glasses [7] and cases of entrapment of fluorescent pH probes are known [8-10]. When probes are trapped in SiO_2 films, their use is often limited by leaching problems and if the substrate is too much immobilized by longer equilibration times [11]. This problem will probably be avoided if the probe is covalently attached to its support and this has already been done on aminoethyl cellulose substrate [12]. The goal of our work is to prepare and use a fluorescing pH probe covalently attached to silica.

The chosen molecule was 9 N-propylaminomethylanthracene which was obtained by reacting aminopropyl silica ($PA) with chloromethylanthracene (CMA). This leads to the grafted $PAMA in which the intramolecular PET is expected to be efficient. The PET process leads to an aromatic

radical anion - amino radical cation pair and is expected to be traced by the extinction of the anthracene fluorescence. An intramolecular excited state PET quenching has already been shown to be effective in the case of a photoreaction sensitized by a benzophenone attached to silica through a secondary amino group [13], in a very similar arrangement. Two $PAMA grafted silica were prepared with different anthracene loadings: $PAMA$_l$ with 21% and $PAMA$_h$ with 95% of grafted amino groups.

$PA   **CMA**   **$PAMA**
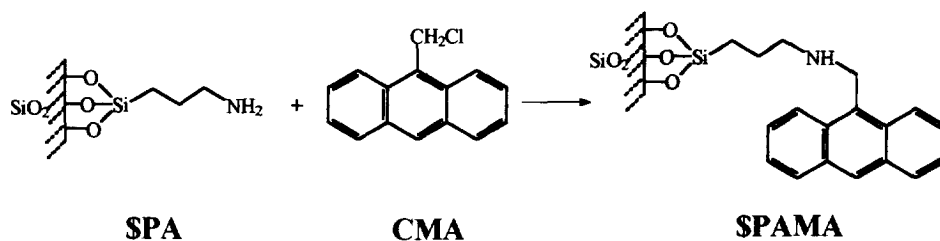
The ratio between emission intensity from excited $PAMA$_l$ protonated (HCl 0.1N) or not (with added Na$_2$CO$_3$), is 15:1 in acetonitrile-water (4-1) and 11:1 in water (fig 1). The addition of carbonate is necessary since it has been shown that the acido-basic properties of grafted silica are dependent on the presence of silanol groups: Bayer *et al.* have observed that amino groups are always at least partially protonated, that the water is the necessary proton carrier and that the silanol groups are the proton donors [14]. That the partial protonation of amino groups is related to silanols has been confirmed by trimethylsilylating the grafted $PAMA$_h$ with trimethylsilyl chloride: the fluorescence level is lowered by this treatment.

One of the advantage of grafting fluoroprobes on a solid is that they can be used in solvents where they are unsoluble. This is the case here for water in which a neutralization curve has been established using the fluorescence intensity (measured at 420 nm) against pH (fig. 2). The response time of $PAMA$_l$ suspended in the water solution is very short (less than half a second) and the reversibility of the system has been verified by several successive changes between pH 3 and 10.

If $PAMA$_h$ is considered, important differences, when compared with $PAMA$_l$, are to be noticed. The main one is a much longer response time to pH (more than 15 minutes), rendering this grafted silica unable to function as a pH probe. With a high loading of fluorophores, more deeply buried anthracenic probes are certainly present: this may lead, through protonation, to conformational changes around nitrogens bearing bulky alkylanthracenic groups and some slow reorganization of the silica may ensue. Another point is the high density of electrical charges when protonating the amines and this also may help some further slow rearrangement of the silica.
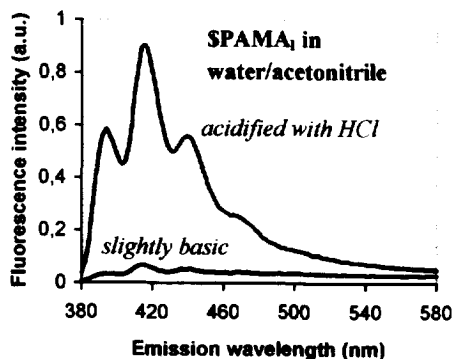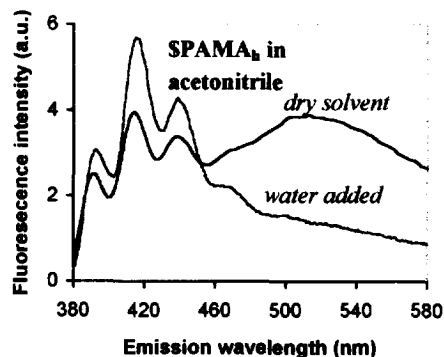
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Fig 1

Fig 3

On the other hand, silica bearing a large number of probes have some other interesting features. If the fluorescence of $PAMAh is observed in dry acetonitrile or methanol, an important emisssion between 480 and 600 nm shows up, corresponding to excimer (or exciplex?) fluorescence. The interesting point is that the excimer-like emission completely and rapidly (response time of less than 1 s) disappears when a small amount of water is added to these solvents: the ratio between the two emissions is very sensitive to aliquots of 1 µl of water in 3 ml of solvent. Therefore $PAMAh is a possible fluorescent water probing system (fig 3). Further experiments are under progress in order to check this quantitatively.

One possible explanation for the double fluorescence is that the status of the amino groups varies, as explained before[14] from free (in dry solvent) to protonated (in the presence of water): the free amino groups may give rise to exciplex formation.
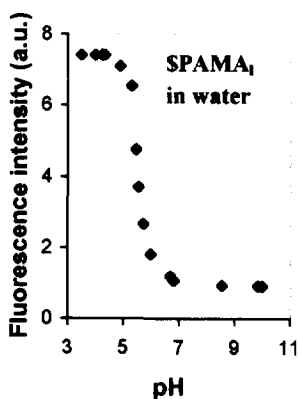

Fig 2

However, if the excitation spectra measured in dry solvent at the monomeric or excimer like emission wavelengths are compared, significant profile changes are seen. This means that the excimer-like emission arises from ground state associations. On the other hand, the adsorbed water molecules on the silica surface have been reported to create a well defined structure made of regular layers of adsorbed water molecules [15]. The mobility of the grafted anthracenic molecule may be strongly reduced if the covalently attached probes are maintained by the hydrophobic interactions like in holes drilled in the water layers. This may be an explanation for the excimer like emission disappearance

That some mobility of the anthracenic probe is necessary for the observation of excimer is reinforced by the absence of any excimeric emission in cyclohexane solvent. In this case, despite the

high surface density, encounters between neighbouring anthracenes is strongly reduced by the strong adsorption to the surface of the silica in this very apolar solvent.

Experimental:
Grafting of 9-chloromethylanthracene on $PA :

$PAMA$_h$: In a round-bottomed flask, 2 g of $PA (1.1 10^{-3} mole of aminopropyl chain per g of silica, dried under vacuum at 60 °C for 12 h), were suspended in 10 ml of dry acetonitrile containing 370 μl of dry triethylamine. After 20 min of stirring, a solution of 0.5 g of CMA dissolved in 50 ml of acetonitrile was added over 45 min and the reaction mixture refluxed under N_2 for 4 days. The silica is washed with methanol-water, ethyl ether and separated by centrifugation. Then, the silica was again washed with refluxing methanol and ether in a Sohxlet apparatus. Finally it was dried under vacuum at 115°C during 14 h. The weight was 2.22g. Elemental analysis (%) C, 18.66; N, 1.29.

$PAMA$_l$: the same recipe was used with 0.1g of CMA. Elemental analysis (%) C, 9.64; N,1.81.

Surface modification of $PAMA :

0.5 g of $PAMA$_h$ (dried under vacuum during 2 days at 110°C), were suspended in 15 ml of dry toluene in the presence of 1ml of triethylamine. After adding 0.5 ml of trimethylsilylchloride diluted in 15 ml of dry toluene, the mixture was kept under stirring at 40°C during 5 h. The modified silica was successively washed under refluxing with MeOH, MeOH-H_2O, MeOH and diethylether in a Soxhlet apparatus. Elemental analysis (%): C,16.99 ; N,1.20

Neutralization curve of $PAMA$_l$ in water suspension:

Measurements were done in fluorescence cells whose top was sealed to an enlarged cuvette in which the pH electrode was dipped. 3.5 mg of $PAMA$_l$ was used, under magnetic stirring. The water volume was around 9 ml. (λ_{ex} = 372 nm; λ_{em} = 420 nm). All measurements were made on non degassed samples.

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