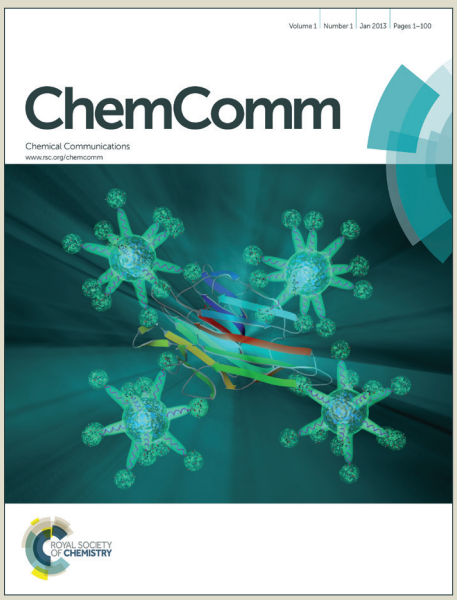
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

RSCPublishing

COMMUNICATION

Fluorescent polymeric aggregates for selective response to Sarin surrogates

Cite this: DOI: 10.1039/xoxxoooooox

Anca D. Rusu*[a,c], Ioana A. Moleavin[a,b], Nicolae Hurduc[c], Matthieu Hamel[a] and Licinio Rocha[a]

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Abstract: By combining the sensitivity of fluorescent units with the response of "smart" polymers to environment changes, we propose a new approach for chemical detection applications. The system proved to be sensitive to 12 ppb of diethylchlorophosphate (DCP), a Sarin surrogate and to discriminate between the interfering molecules.

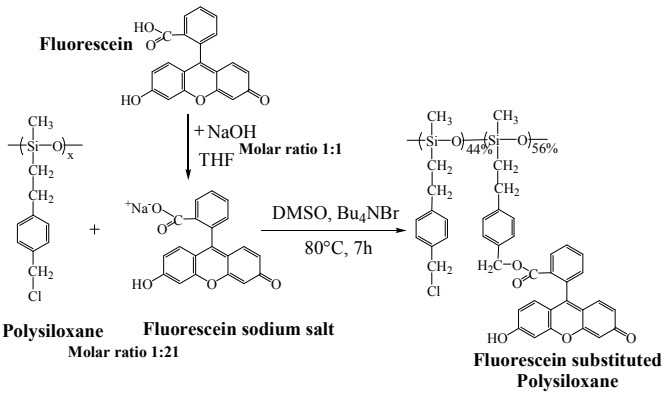
The detection of nerve agents, especially organophosphates, has gained a lot of interest after the Second World War. Nerve agents' toxicity relies on the reactive phosphate group able to interact with hydroxyl function present onto a critical nervous system enzyme, the acetylcholinesterase, responsible of the acetylcholine neurotransmitter hydrolysis.[1]

Due to their very high toxicity and because they share similar chemical structure with pesticides [1] widely used in agriculture these past 50 years, nerve agents detection requires the development of sensitive, but also selective materials.

To improve the selectivity while maintaining high sensitivities, we propose an approach based on a dual signal generation from responsive polymers also known as "smart" materials. The aim is to couple sensitive fluorescent units with "smart" polymers able to experience large structural changes triggered by the interaction of the fluorescent units with specific chemical molecules. Besides the high sensitivity inherent to fluorescence variations, the structural changes undergone by the polymer chains may be used to improve the detection properties[2,3] and obtain additional information about the involved interactions.

Fluorescein and derivatives are known to interact with phosphoryl halides leading to modifications of their fluorescent properties. [4] Sensitivity to 16 ppb of DCP was previously reported by some of us for Fluorescein in DMSO solution.[5] On the other hand, smart polymeric micelles can change their shape and properties as a response to an external stimulus (temperature, light, pH, chemical molecule, etc.).[6] Here we propose to combine the sensitivity of the Fluorescein emission properties with the response of polymeric 3D architectures, which organisational properties in aqueous solvent are sensitive to target chemical molecules. The generation of a new material sensitive to Sarin surrogates with an improved selectivity between the interfering molecules is investigated here to illustrate the potential of the proposed approach for chemical sensing applications.

Fluorescent polymers were obtained by starting from Polysiloxane main chains with chlorobenzyl groups in the side chain [7] with a molecular weight value M_n = 6,000-6,400. Following a nucleophilic substitution mechanism, a given percentage of chlorobenzyl groups were substituted by Fluorescein sodium salt (Scheme 1, Fig. S1, 56% substitution degree in that example). The polymer synthesis is detailed in supporting information.



Scheme 1 The chemical synthesis of the fluorescent polymers

These polymers are able to self-assemble in water solutions as fluorescent polymeric aggregates with a polysiloxanic hydrophobic core exhibiting the Fluorescein groups to the solution medium as a constitutive part of the shell.

In Fig. 1 is given the size distribution by number a) and the emission spectrum b) of an aqueous solution made from a 56% substituted polysiloxane. In water, the fluorescent polymer self-assembles in aggregates with an average size of 50 nm. A possible explanation of the present statistical polymers aggregation capacity

may be found on the polysiloxane chain flexibility facilitating the chemical functions arrangement.

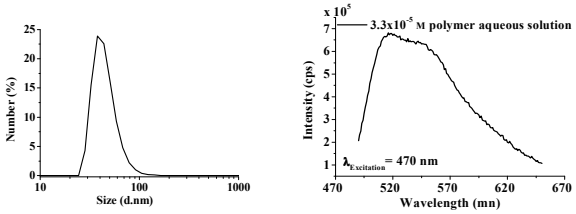


Fig. 1 Polymeric aggregates size distribution by number a) and fluorescence spectrum b) for 3.3×10^{-5} M polymer aqueous solution.

An emission spectrum of the solution was recorded with an excitation wavelength of 470 nm. The fluorescence spectrum shape corresponds to the monoanion form of Fluorescein in a low acid environment with a maximum of intensity at 516 nm [8] which is consistent with the distilled water pH 5.4-6 (pK_a of Fluorescein in aqueous solution is 6.3-6.8[9]). The obtained spectrum is also confirming a bonding of the Fluorescein to the polymer chains through the carboxylic group which does not modify the spectral properties. [10]

A. Song *et al.* demonstrated previously that protonation or alkylation of the Fluorescein hydroxyl function lead to strong modifications of the molecule's spectral properties.[10] The phosphorylation of a hydroxyl group is a common mechanism in organophosphates detection, the inactivation of acetylcholinesterase enzyme (which leads ultimately to the death) being the result of phosphate ester formation.[11] Based on this we expect an interaction with organophosphates through the hydroxyl group to modify the Fluorescein spectral properties. Simultaneously as the bonding of the Fluorescein units onto the polysiloxane chains is leading to the formation of amphiphilic polymeric aggregates, their interaction with the target molecules is expected to change the balance governing the aggregation process. As a result of the aggregates reorganisation a modification of the solution diffusion properties was expected.

The detections properties of the aggregates water solutions have been tested using diethyl chlorophosphate (DCP) as a surrogate molecule of Sarin, a model compound with a similar reactivity but with a lower toxicity.[12] To check the selectivity of the synthesized materials, side-tests with dichlorvos (DDVP) and dimethyl methylphosphonate (DMMP) have also been performed (Fig. 2).

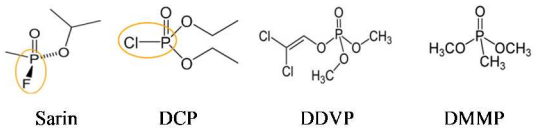


Fig. 2 Chemical structure for Sarin and organophosphates used for detection experiments.

As well the fluorescence of Fluorescein being dependent of the pH environment, [8,10,13] and the DCP undergoing hydrolysis in water with as a result HCl production, the effect of HCl has also been investigated. Comparative measurements were performed using Dynamic Light Scattering (DLS) and fluorescence spectroscopy techniques.

In this paper we present the results obtained with 56% Fluorescein substituted Polysiloxanes.

The experiments were performed in 3.3×10^{-5} M aqueous aggregates solution, with average size of 50 nm. Fluorescence emission spectra were recorded at two different excitation wavelengths in order to check the sensitivity limit to DCP, as well as the different responses of the sample to the other organophosphates. The fluorescence experiments were made in $1 \times 1 \times 3$ cm^3 quartz cells using a 2 mL sample volume. The pollutants were added under magnetic stirring using a 10 µL syringe and measurements were recorded immediately after addition. At low concentrations the pollutants were diluted in dry acetonitrile (10 to 10,000 dilutions factors) taking into account the molecular mass of the added pollutant. All the results are given in pollutant concentrations in part per million (ppm). Adding similar volumes (dilution factor) of pure acetonitrile into the polymer solution did not reveal any change in fluorescence or in aggregates organization.

Fig. 3 shows the evolution of the emission of Fluorescein when exposed to DCP. A 470 nm excitation wavelength is used. The addition of a very small amount of DCP (12.4 ppb) provides a significant decrease of the fluorescence response of the sample. For DCP concentrations of 6 ppm, a decrease close to 80% of the fluorescence is observed (I/I_0=0.23) tending slowly to zero for higher concentrations.

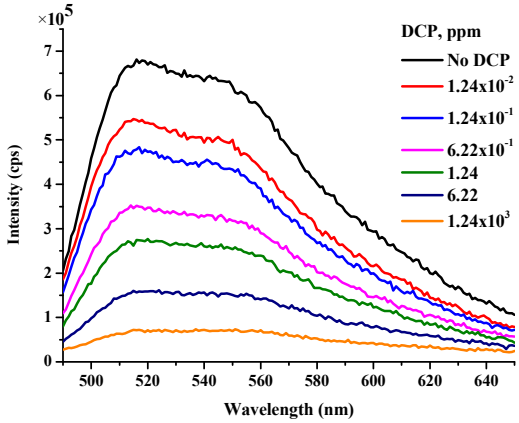


Fig. 3 Emission spectra of an aqueous agregates solution before and after DCP addition, excitation wavelength 470 nm.

Comparative tests with DDVP and DMMP are reported in Fig. 4 (Fig. S7, Fig. S8), showing the intensity variations recorded at 516 nm for two excitation wavelengths. As can be observed in Fig. 4 a) where a 470 nm excitation wavelength is employed, the sample is less reactive to DDVP and not reactive at up to 8 ppm of DMMP. For high quantities of pollutant added, the quenching in fluorescence (the ratio I/I_0) becomes similar.

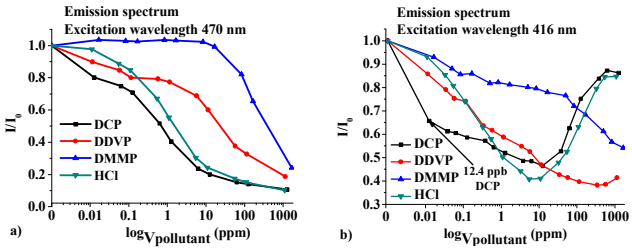


Fig. 4 Steady-state fluorescence response of aqueous agregates solutions to DCP and to interfering samples additions when excited to a) 470 nm and b) 416 nm. I/I_0: Division between fluorescence maximum intensity with and without pollutant.

Instead, if the sample is excited at 416 nm (Fig. 4(b)), bigger differences between DCP and the interfering molecules can be observed below 100 ppb. At 12.4 ppb of DCP the response in

fluorescence (I/I_0) quenching is 35% compared with a variation of 7 to 14% in the case of the others pollutants. After 37 ppm of DCP, the maximum of the emission intensity (when excited at 416 nm) starts to increase without any modification of the fluorescence emission spectrum shape. This coincides however with a change of the excitation spectrum shape, with a maximum of intensity shifted from 442 nm to 436 nm (see supporting information Fig. S6). It was previously found that a chemical interaction with the hydroxyl group of Fluorescein is leading to spectral modification.[10] A small increase in intensity can also be observed after 573 ppm DDVP addition. An increase of the signal intensity is also observed in the case of HCl for concentrations equivalent to DCP, when the excitation spectra shape coincides with cationic form of Fluorescein. The cation emission spectrum can be observed just in high acidity environment and that is why no change of the spectrum shape could be noticed on emission.[13a]

The increase in fluorescence intensity in the case of DCP and DDVP addition coincides with the amphiphilic aggregates reorganization observed by DLS measurements (see Fig. 5). Besides the fluorescence quenching, the interaction with phosphoryl halides appears to increase the hydrophobicity of the system leading to clusters formation followed by a precipitation of the polymer.

The evolution of the aggregates size and solutions turbidity evidences significant differences between the pollutants. For 37 ppm of DCP the aggregates size increases and the solution starts to become turbid. The changes in turbidity can be seen with the naked eye but are also proved by the increase of the intensity of the diffused light (DCR) measured by DLS experiments (Fig. 5). After 124 ppm DCP addition the big size aggregates separate from the solution, leading to a decrease in turbidity (DCR decreases while Z-average continues to increase). In time, a thin layer of polymer can be observed on the top of solution: the polymer is removed from the solution. The separation process can be accelerated by stirring; in this case the polymer remains stuck on the stirrer. The sample revealed a low sensitivity to DDVP and no size changes up to 1,600 ppm of DMMP could be observed in the polymeric aggregates solution, while a strong decrease of the sample fluorescence was visible for these DDVP and DMMP concentrations.

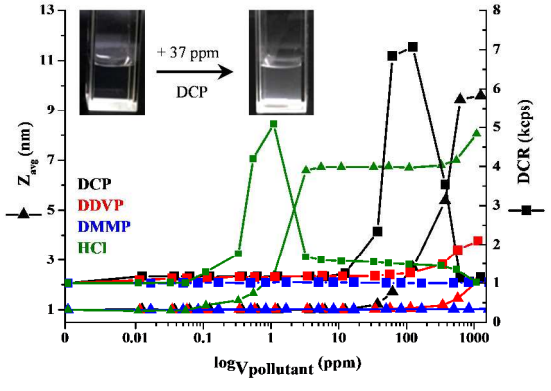
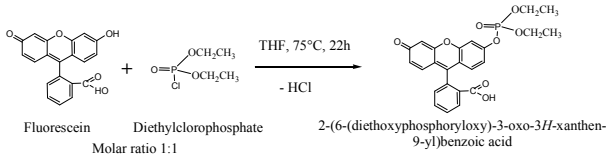


Fig. 5. Size (Zavg) and turbidity (DCR) variation with pollutant addition.

Analysing the results obtained for HCl, no correlation could be found between fluorescence and DLS measurements. The DLS experiments revealed indeed an increase in size and turbidity just after 109 ppb of HCl, well below the concentration of DCP or HCl required (> 37 ppm) to produce an increase of the fluorescence intensity observed with a 416 nm excitation. This reveals a different interaction process between HCl and organophosphates. In the first case the response may be due to the pH environment changes; fluorescein being sensitive to pH [10,13] and also to the fact that a small acidity of the medium can favor the polymer precipitation. While the sample response to phosphoryl halides can be due to physical interactions as Hydrogen bounding or a chemical interaction with the Fluorescein moieties on the Polysiloxanic chain.

To verify the possibility of a chemical interaction between Fluorescein and DCP leading to a new compound with different fluorescent and absorption properties, a synthesis was performed in THF in dry environment at 75°C for 22 h (

Scheme **2**). ^{1}H, ^{13}C and ^{31}P NMR spectra revealed the presence of diethyl phosphoryl moiety bonded to Fluorescein molecule. The chemical synthesis and the NMR spectra are detailed in supporting information (Fig. S2, S3, S4).



Scheme 2. The chemical reaction between Fluorecein and DCP.

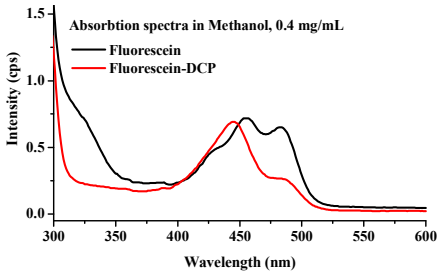


Fig. 6. Absorption spectra of Fluorescein before and after the chemical interaction with DCP.

Conclusions

The response of fluorescent polymeric aggregates aqueous solutions is investigated towards warfare agents' detection. The different response of the system to DCP (a Sarin surrogate) and interfering molecules (DDVP, DMMP, HCl) was monitored by DLS and Fluorescence spectroscopy. In the presence of DCP, the fluorescence system is quenched and the solution becomes turbid due to aggregates reorganization which leads, in time, to complete removing of the polymer from the solution. The system was found to be sensitive to 12 ppb of DCP. Along with fluorescence quenching based sensitivity of the polymer, the aggregates reorganisation following interaction with the DCP confers to the system good discrimination properties between the interfering molecules being thus a good candidate for Sarin detection.

During the experiments we noticed an increase of the agregates sizes occurring for HCl concentrations smaller by two orders of magnitude than for DCP while a stronger fluorescence decrease is observed for bigger HCl volumes addition. The different sample responses to HCl and DCP lead to the conclusion that the response to DCP is not mainly due to the HCl resulting from DCP hydrolysis in water.

Next step will concern the improvement of the polymeric aggregates solutions stability and the investigation of amphiphilic aggregates with different sensitive fluorescent molecules. Aqueous aggregates properties with different fluorescent units' substitution degrees are currently investigated.

It is noteworthy the fluorescence and turbidity evolution of the amphiphilic aggregates solution, when a pollutant is added, can also be monitored simultaneously, thus opening possibilities for real time measurements. The preliminary tests made for these purposes are very optimistic, the sample response in fluorescence quench is instantaneous and the fluorescence intensity signal is stabilized after around three minutes. Also, the aggregates reorganisation after interacting with DCP is increasing the sample turbidity (see supporting information, Fig. S11 and S12).

These investigations are part of a generic approach which may be applied to other classes of chemical molecules.

Financial support this research from ANCS (Grant CEA–C1–01/2010) is gratefully acknowledged.

Notes and references

[a] CEA, LIST, Laboratoire Capteurs et Architectures Electroniques, 91191, Gif-sur-Yvette, Cedex, France
E-mail: rusuancadaniela@yahoo.com
[b] Centre of Advanced Research in Bionanoconjugates and Biopolymers, Petru Poni Institute of Macromolecular Chemistry, Ghica Voda Alley 41A, 700487, Iasi, Romania
[c] Department of Natural and Synthetic Polymers, ''Gheorghe Asachi'' Technical University of Iasi, Prof. D. Mangeron Street 73, 700050 Iasi, Romania

Electronic Supplementary Information (ESI) available: Experimental procedures and data, Fig. S1-S10 (PDF). See DOI: 10.1039/c000000x/

1 K. Kim, O. G. Tsay, D. A. Atwood and D. G. Churchill, *Chem. Rev.* 2011, **111**, 5345.
2 J. Lee, S. Seo and J. Kim, *Adv. Funct. Mater.* 2012, **22,** 1632.
3 T. H. Kim, D. G. Kim, M. Lee and T. S. Lee, *Tetrahedron 2010*, **66**, 1667.
4 S. Bencic-Nagale, T. Sternfeld and D. R. Walt, *J. Am. Chem. Soc.* 2006, **128,** 5041–5048.
5 M. Hamel, J. Hamoniaux, L. Rocha and S. Normand in *Proc. of SPIE,* Vol. 8710 87100H-1, Baltimore, Maryland, USA, 2013, doi:10.1117/12.2011332
6 a) A. D. Rusu Hodorog, C. Ibanescu, M. Danu, B. C. Simionescu, L. Rocha and N. Hurduc, *Polym. Bull.* 2012, **69,** 579; b) I. A. Moleavin, Ibanescu, A. Hodorog-Rusu, E. Peptu, F. Doroftei and N. Hurduc, *Cent. Eur. J. Chem.* 2011, **9,** 1117; c) E.S. Gil and S. M. Hudson, Prog. *Polym. Sci.* 2004, **29,** 1173.
7 K. Kazmierski, N. Hurduc, G. Sauvet and J. J. Chojnowski, *Polym. Sc.i Part. A: Polym. Chem.* 2004, **42,** 1682.
8 a) N. Klonis and W. H. Sawyer, *J. Fluoresc.* 1996, **6,** 147; b) M. Ali, V. Kumar and S. Pandey, *Chem. Commun.* 2010, **46**, 5112; c) A. Bhagi, S. Pandey, A. Pandey and S. Pandey, *J. Phys. Chem. B,* 2013, **117,** 5230.
9 L. D. Lavis, R. J. Rutkoski, R. T. Raines, *Anal. Chem.* **2007**, 79, 6775-6782.
10 A. Song, J. Zhang, M. Zhang, T. Shen and J. Tang, *Colloids and Surfaces A: Physicochem. Eng. Aspects,* 2000, **167**, 253.
11 a) S.-W. Zhang and T. M. Swager, *J. Am. Chem. Soc.* 2003, **125,** 3420; b) T. J. Dale and J. Rebek, *Angew. Chem. Int. Ed.* 2009, **48,** 7850.
12 a) T. J. Dale and J. Rebek, *J. Am. Chem. Soc.* 2006, **128,** 4500; b) L. Ordronneau, A. Carella, M. Pohanka and J.-P. Simonato, *Chem. Commun.* 2013, **49**, 8946.
13 a) R. Sjoback, J. Nygren and M. Kubista, *Spectrochimica Acta Part A,* 1995, **51,** 7; b) S. Biswas, S. C. Bhattacharya, P. K. Sen and S. P. Moulik, *J. Photochem. Photobiol. A-Chem.* 1999, **123,** 121.

