CHEMPLUSCHEM
COMMUNICATIONS

ChemPubSoc
Europe

DOI: 10.1002/cplu.201402157

Ultraviolet-Induced Fluorescence of Polydopamine-Coated Emulsion Droplets

Sandrine Quignard,[a] Marco d'Ischia,[d] Yong Chen,[b] and Jacques Fattaccioli*[c]

Polydopamine (PDA), a multifunctional biomaterial with strong adhesion and coating properties, exhibits melanin-like optoelectronic properties but is virtually devoid of intrinsic fluorescence. Herein we disclose the first PDA-based system that can develop fluorescence without chemical manipulation. The polymerization of dopamine in the presence of oil microdroplets results in the formation of smooth PDA-coated core–shell droplets, which develop a bright and persistent fluorescence upon UVA illumination. The fluorescence induction depends on the excitation wavelength, the nature of the oil, and oxidation and buffer conditions, suggesting photo-oxidation of PDA components within the oil matrix, which shields the developing fluorophores from water quenching. Peroxyl radical scavengers enhance the fluorescence, whereas hydrogen peroxide induces quenching. The new system holds potential interest for imaging and tocopherol sensing applications.

Polydopamine (PDA) has become the focus of increasing interest in materials sciences[1] owing to its unique adhesion properties. Oxidative polymerization of dopamine results in the formation of a black insoluble melanin-type material, which adheres strongly on virtually any type and shape of material to form a thin film,[2,3] and which can be functionalized covalently by biomolecules thanks to its numerous functional groups.[4] PDA coatings have recently been used to modify the surfaces of different types of colloidal particles.[5–8] Furthermore, PDA is biocompatible and displays interesting properties in optics and electronics.[1,9–11] Like most melanins, PDA exhibits a broadband absorbance profile but no detectable fluorescence owing to its very low quantum yield, which is attributed to structural and chemical disorder.[12–14] Despite its inherently low emission properties, PDA may generate detectable fluorescence either by chemical oxidation using oxygen peroxide for several hours to develop PDA capsules or particles[15,16] or by multiphoton excitation[17].

Herein, we disclose the first PDA-based system developing fluorescence by simply illuminating PDA-coated oil-in-water liquid droplets in the UVA range (365 ± 40 nm) without chemical manipulation. Since the nature of the oil can be changed freely,[18] emulsions provide an efficient tool to explore the effects of this liquid interface on the PDA properties under illumination. We demonstrate that the fluorescence induction depends on the chemical nature of the oil, the polymerization conditions, and the presence of light-induced radical species in the medium.

In this study, we use oil-in-water emulsion droplets obtained as described previously[19] using oils of different types, from vegetable oils with various degrees of unsaturation (olive or soybean oil) to more inert oils such as mineral or silicone oil. Figure 1A provides a schematic representation of the method used to prepare the PDA-coated core-shell emulsion droplets. In brief, PDA coating of the microdroplets is performed by incubating the emulsions with dopamine solutions under oxidative alkaline conditions in water (see Supporting Information for details) for 1–24 h. After rinsing, the droplets are injected into observation chambers and studied by epifluorescence microscopy. Classically, PDA coatings are prepared in Tris-HCl buffer at pH 8.5, with the dioxygen dissolved in air-equilibrated

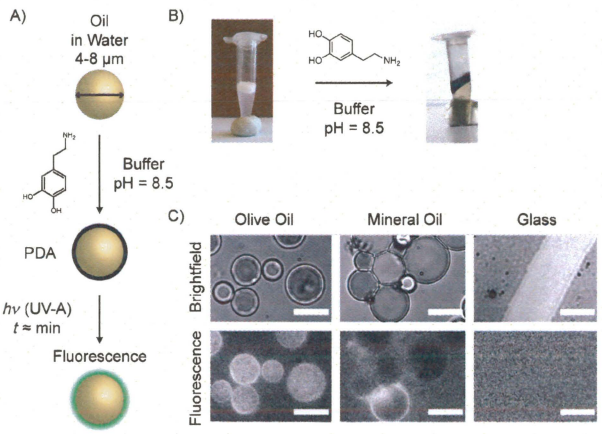


Figure 1. A) Polydopamine coating of oil microdroplets and fluorescence induction upon UVA illumination. B) Images of bare and PDA-coated olive oil emulsions after centrifugation. C) Fluorescence induction upon UVA illumination of PDA coated on different substrates (PDA deposition in borate buffer, $KMnO_4$, optimum illumination time): olive and mineral oil droplets or glass coverslips (scratched PDA layer). Bright-field and fluorescence microscopy images (DAPI filter, scale bar 5 µm).

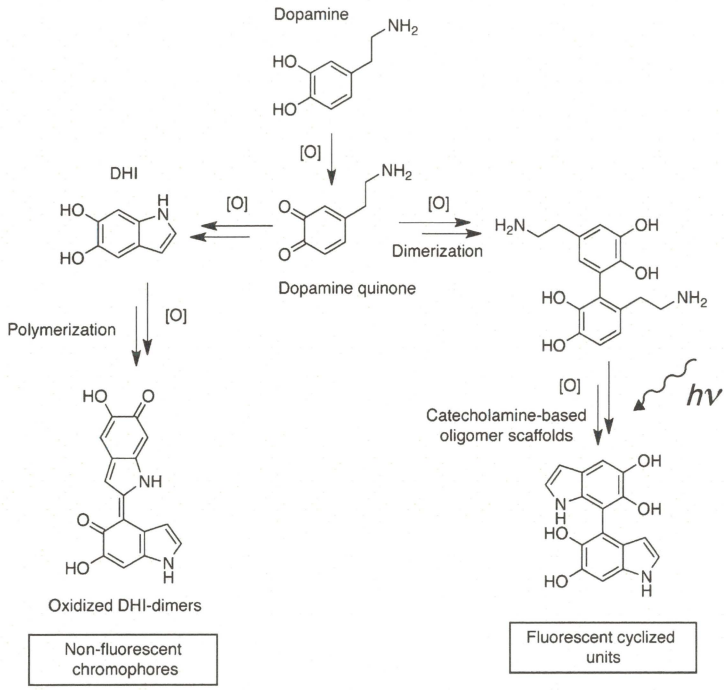
[a] Dr. S. Quignard
Ecole Normale Supérieure - PSL Research University,
Département de Chimie, 24, rue Lhomond, 75005 Paris (France)

[b] Prof. Y. Chen
Sorbonne Universités, UPMC Univ. Paris 06
PASTEUR, 75005, Paris (France)

[c] Dr. J. Fattaccioli
CNRS, UMR 8640 PASTEUR,
75005, Paris (France)
Fax: +(33) 1 44 32 34 38
E-mail: jacques.fattaccioli@ens.fr
Homepage:: http://www.chimie.ens.fr

[d] Prof. M. d'Ischia
Department of Chemical Sciences„ University of Naples "Federico II"
Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126, Naples (Italy)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cplu.201402157.



Scheme 1. Scheme of PDA polymerization and pathways leading either to nonfluorescent or fluorescent moieties.

water as the oxidizing agent.[2] Oxidation of dopamine leads to dopamine quinone, which undergoes intramolecular cyclization to give leucodopaminochrome and then dopaminochrome. Isomerization leads to 5,6-dihydroxyindole (DHI); this polymerizes to give PDA, which consists of a highly heterogeneous mixture of cross-linked oligomeric species (representative structures are shown in Scheme 1).[20,21] The Tris buffer has been reported to interact with dopamine quinone and be incorporated into the polymer film,[18] so reactions were also performed in phosphate and borate buffers to compare their effects on fluorescence induction. Whereas phosphate would not be expected to participate actively in the polymerization reaction, borate forms stable complexes of the catechol moieties of dopamine,[22] inhibiting oxidation processes in air.[19] Accordingly, PDA formation in borate buffer requires a strong oxidant such as $KMnO_4$, which induces much faster reaction times without any apparent influence on the physicochemical properties of the PDA coating. Whereas dioxygen, a mild oxidant, would hardly induce any degradation of reactive oil cores, the use of $KMnO_4$ would raise the issue of whether a more or less extensive degradation of the unsaturated fatty acid components in soybean or olive oils might occur. However, $KMnO_4$ is not introduced in excess, and hence, is fully consumed by the dopamine with none left for reaction with the oil.

Figure 1 B shows that the polymerization of dopamine is accompanied by a marked darkening of the emulsion suspension. Notably, no black sediment is observed at the bottom of the reaction tube in the presence of microdroplets and no significant PDA aggregates are detected in the continuous phase by microscopy at the end of the reaction.[19] Conversion of dopamine to PDA thus occurs only at the droplet interface, regardless of the nature of the oil used, and without any effect on the size distribution of the droplets suspension (Figure S2).

Figure 1 C shows that, upon illumination in the UVA range, olive oil and mineral oil droplets coated with PDA become fluorescent, on the surface and/or in their volume, whereas PDA-coated glass under the same conditions does not. Once induced, the fluorescence is irreversible and persists for several days (Figures S3 and S4, Supporting Information). In addition, the fluorescence spans from 400 to 650 nm depending on the excitation wavelength (Figure S5).[23,24] Illumination at longer wavelengths also leads to fluorescent droplets but with slower kinetics and lower efficiency. Comparison of different buffer/oxidant combinations for olive oil and mineral oils (Figure 2) shows that $KMnO_4$ in borate buffer leads to the strongest fluorescence, whereas the same oxidant in phosphate buffer leads to little or no fluorescence, as in the case of mineral oil (the same trend is observed for soybean oil, as shown in the Supporting Information). Furthermore, using O_2 as the oxidant leads to droplets with poorer fluorescence properties either in terms of intensity (e.g., no fluorescence for mineral oil droplets) or in terms of the photostability of the in-

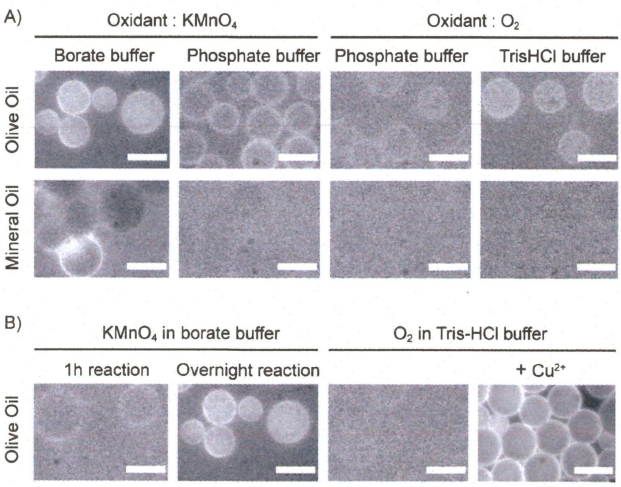


Figure 2. Fluorescence microscopy images (DAPI filter) of PDA-coated droplets upon 5 min UVA illumination, A) with different PDA-coating conditions (buffer and oxidant) for olive and mineral oil droplets. B) Influence of the presence of metallic cations during PDA deposition on olive oil droplets on fluorescence induction. Fluorescence microscopy images after 5 min UVA illumination at 365 nm (DAPI filter, scale bar 5 μm).

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

duced fluorescence. In addition, PDA-coated olive oil droplets produced with either oxidant in phosphate buffer show fluorescence in both cases, suggesting that this effect is not the result of the degradation of the oil by $KMnO_4$ but is indeed the result of the photogeneration of fluorescent species within the PDA layer. Finally, our results show that the buffer plays a role in fluorescence development, as borate acts as a stabilizer in the $KMnO_4$-induced fluorescence, whereas phosphate does not.

On the basis of the available data, it can be concluded that UVA irradiation can induce the photo-oxidation of PDA components, probably oligomers in the uncyclized state (Scheme 1, right path). Chromophore excitation occurs following stimulation in the low-energy part of the catecholamine absorption maximum (UVA) and becomes negligible if visible light is used, implying the oxidation of simple reduced chromophores, for example, catecholamine and 5,6-dihydroxyindole (DHI), but not of visible light-absorbing quinones or aggregates. Photo-oxidation of catecholamines leads to aminochromes, which then isomerize to 5,6-dihydroxyindoles, whereas UVA irradiation of DHI leads to dimers and oligomers in oxidized forms (Scheme 1).[25] Of all these species, only DHI and its dimers in the reduced form exhibit significant emission properties.[26] It is therefore likely that uncyclized catecholamine units in PDA undergo UVA-induced oxidation, leading to cyclized fluorescent units. The stabilizing effect of borate on fluorescence induction can be attributed to complexation of the catechol groups preventing photochemical reoxidation of fluorescent DHI units. This would be indirect proof that the fluorescence comes from reduced catechol systems in DHI oligomers. The lower fluorescence observed in Tris-HCl buffer (Figure 2A) can be attributed to the addition of Tris to dopamine quinone, which would lead to adducts preventing cyclization and the formation of fluorescent units.[20]

Figure 2A shows that PDA deposited by $KMnO_4$ oxidation in phosphate buffer exhibits a higher fluorescence than PDA deposited using O_2 as the oxidant. To assess whether metal cations interact with the PDA layer and modify its fluorescence properties, we compared the induction of fluorescence of olive oil droplets coated in borate/$KMnO_4$ for 1 h or overnight and in Tris-HCl supplemented with 1 mM Cu^{2+}. Figure 2B shows that in both cases, higher fluorescence is observed in the presence of the metal cations, suggesting that the cations affect the fluorescence process, possibly by promoting aminochrome isomerization to DHI or DHI dimerization leading to fluorescent species.[25] A much smaller effect after 1 h is observed in the case of Mn^{2+}, suggesting slow incorporation of the cations into the polymer layer.

The finding that induction of fluorescence is observed with both vegetable oils (soybean or olive) and inert mineral oils, but not with silicone oil or PDA coated on a glass coverslip (Figures 1C and S3) suggests that partial dissolution of PDA components into the hydrophobic oil matrix is essential for fluorescence development, probably through effects relating to the exclusion of water as a quencher,[27] as it is known that the fluorescence intensity in 5,6-dihydroxyindoles depends on the solvent and is lower in water, as excited-state lifetimes are slower.[28] In addition, DHI is much more soluble in organic solvents than dopamine,[29] suggesting that better solubilization of photochemically generated DHI in the oil component would favor the development of fluorescence by a UVA-induced photo-oxidative process in a water-free environment. Depending on the relative polarity of the oil, photogenerated PDA structural components can penetrate the droplet and produce fluorescence that is shielded from the quenching effects of water. It is possible that the lack of PDA fluorescence in silicone oil and mineral oils is caused by the poor penetration of PDA components in these relatively more hydrophobic and apolar media. It is also likely that uncyclized dopamine oligomers penetrate the oil droplet, becoming inaccessible to chemical oxidants (oxygen is too weak), but are available for UVA-induced oxidative cyclization and conversion to DHI units in oligomer form. Thus, droplets with suitable physicochemical characteristics offer a water-free liquid environment that allows fluorescence development in the absence of significant quenching. This could explain why PDA coated on other interfaces reported so far does not exhibit fluorescence as seen in the present system.[1,12]

UV illumination of PDA under aerobic conditions would trigger photoreactions able to produce hydrogen peroxide and superoxide anions by electron-transfer processes.[30] To check whether reactive oxygen species (ROS) can affect UV-induced fluorescence, we investigated the effects of ROS scavengers and hydrogen peroxide. Figure 3 compares the fluorescence of

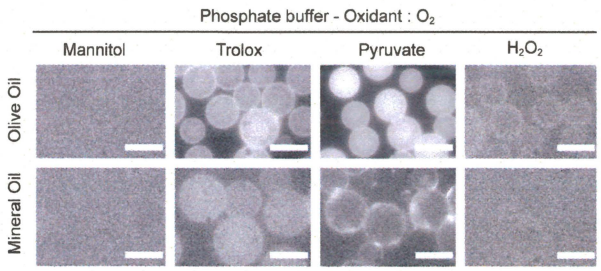


Figure 3. Fluorescence microscopy images (DAPI filter) of PDA-coated droplets upon 5 min UVA illumination with different additives: ROS-scavenging species such as mannitol (20 mM, $HO^{\bullet}$), trolox (10 μM, $ROO^{\bullet}$), and pyruvate (10 mM, H_2O_2), or ROS-promoting species such as H_2O_2 (100 mM). Scale bar 5 μm.

PDA-coated olive oil droplets in phosphate buffer, in the presence of a H_2O_2 scavenger (pyruvate), a peroxyl radical ($ROO^{\bullet}$) scavenger (trolox), or a $HO^{\bullet}$ radical scavenger (mannitol), as well as in the presence of additional hydrogen peroxide. The effects on fluorescence were studied systematically under conditions in which $ROO^{\bullet}$, $HO^{\bullet}$, $^1O_2$, H_2O_2 or $O_2^{\bullet-}$ were scavenged efficiently (Table S6). The lack of effect from $HO^{\bullet}$ and singlet oxygen ($^1O_2$) scavengers indicated that these species had little effect on the fluorescence emission. Conversely, H_2O_2 is detrimental for the fluorescence, as apparent from both direct incubation experiments and the enhancing effects of pyruvate. Likewise, scavengers of $ROO^{\bullet}$ and $O_2^{\bullet-}$ species lead to stronger fluorescence, indicating that these species can also cause fluo-

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

rophore destruction. It appears that UVA-induced fluorescence is derived from photo-oxidation reactions leading to the formation of superoxide. The latter disproportionates to give hydrogen peroxide, which in turn can reoxidize and oxidatively cleave quinonoid DHI units, thus destroying the fluorescence. This provides further evidence that the fluorescence is caused by reduced cyclized units embedded in the oil matrix. The poor effect of OH radical and singlet oxygen scavengers clearly indicates that single electron transfer from an excited state of catecholamine units to oxygen, leading to superoxide, is the main photochemical process.

In conclusion, we have demonstrated that UVA can cause a remarkable induction of fluorescence in PDA, probably owing to photochemical processes converting nonfluorescent moieties into emitting ones. The liquid organic interface and the deposition conditions provide a water-free environment as well as oriented polymerization, both of which favor fluorescence development. The droplets described here could provide a means of rendering PDA shells strongly fluorescent for imaging purposes, without the need for chemical manipulation and with enhanced emission properties compared to PDA coating. The noticeable increase in fluorescence in the presence of a micromolar concentration of trolox could be used to develop a tocopherol sensor.

Acknowledgements

We acknowledge Prof. Ludovic Jullien and Dr. Laurence Grimaud (Département de Chimie, ENS) for discussions.

Keywords: emulsions · fluorescence · interfaces · photoactivation · polydopamine

[1] Y. Liu, K. Ai, L. Lu, *Chem. Rev.* **2014**, *14*, 5057–5115.
[2] H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science* **2007**, *318*, 426–430.
[3] S. M. Kang, J. Rho, I. S. Choi, P. B. Messersmith, H. Lee, *J. Am. Chem. Soc.* **2009**, *131*, 13224–13225.
[4] H. Lee, J. Rho, P. Messersmith, *Adv. Mater.* **2009**, *21*, 431–434.
[5] Q. Ye, F. Zhou, W. Liu, *Chem. Soc. Rev.* **2011**, *40*, 4244–4258.
[6] W. Wang, Y. Jiang, Y. Liao, M. Tian, H. Zou, L. Zhang, *J. Colloid Interface Sci.* **2011**, *358*, 567–574.
[7] J. Cui, Y. Wang, A. Postma, J. Hao, L. Hosta-Rigau, F. Caruso, *Adv. Funct. Mater.* **2010**, *20*, 1625–1631.
[8] H. Xu, X. Liu, D. Wang, *Chem. Mater.* **2011**, *23*, 5105–5110.
[9] C. Xu, K. Xu, H. Gu, R. Zheng, H. Liu, X. Zhang, Z. Guo, B. Xu, *J. Am. Chem. Soc.* **2004**, *126*, 9938–9939.
[10] S. H. Ku, J. Ryu, S. K. Hong, H. Lee, C. B. Park, *Biomaterials* **2010**, *31*, 2535–2541.
[11] M. E. Lynge, R. Ogaki, A. O. Laursen, J. Lovmand, D. S. Sutherland, B. Städler, *ACS Appl. Mater. Interfaces* **2011**, *3*, 2142–2147.
[12] S. Nighswander-Rempel, J. Riesz, *J. Chem. Phys.* **2005**, *123*, 194901.
[13] M. L. Tran, B. J. Powell, P. Meredith, *Biophys. J.* **2006**, *90*, 743–752.
[14] M. D'Ischia, A. Napolitano, A. Pezzella, P. Meredith, T. Sarna, *Angew. Chem.* **2009**, *121*, 3972–3979; *Angew. Chem. Int. Ed.* **2009**, *48*, 3914–3921.
[15] X. Chen, Y. Yan, M. Müllner, M. P. van Koeverden, K. F. Noi, W. Zhu, F. Caruso, *Langmuir* **2014**, *30*, 2921–2925.
[16] X. Zhang, S. Wang, L. Xu, Y. Ji, L. Feng, L. Tao, S. Li, Y. Wei, *Nanoscale* **2012**, *4*, 5581–5584.
[17] J. Kerimo, M. Rajadhyaksha, C. A. DiMarzio, *Photochem. Photobiol.* **2011**, *87*, 1042–1049.
[18] F. Leal-Calderon, V. Schmitt, J. Bibette, *Emulsion Science: Basic Principles*, Springer, New York, **2007**.
[19] G. M. Nocera, K. Ben M'Barek, D. G. Bazzoli, G. Fraux, M. Bontems-Van Heijenoort, J. Chokki, S. Georgeault, Y. Chen, J. Fattaccioli, *RSC Adv.* **2014**, *4*, 11564.
[20] N. F. Della Vecchia, R. Avolio, M. Alfè, M. E. Errico, A. Napolitano, M. d'Ischia, *Adv. Funct. Mater.* **2013**, *23*, 1331–1340.
[21] J. Liebscher, R. Mrówczyński, H. Scheidt, C. Filip, N. D. Hădade, R. Turcu, A. Bende, S. Beck, *Langmuir* **2013**, *29*, 10539–10548.
[22] K. T. Yasunobu, E. R. Norris, *J. Biol. Chem.* **1957**, *227*, 473–482.
[23] S. P. Nighswander-Rempel, J. Riesz, J. Gilmore, J. P. Bothma, P. Meredith, *J. Phys. Chem. B* **2005**, *109*, 20629–20635.
[24] K. Teuchner, J. Ehlert, W. Freyer, *J. Fluoresc.* **2000**, *10*, 275–281.
[25] M. d'Ischia, A. Napolitano, A. Pezzella, E. J. Land, C. A. Ramsden, P. A. Riley, *Adv. Heterocycl. Chem.* **2005**, *89*, 1–63.
[26] A. Corani, A. Huijser, A. Iadonisi, A. Pezzella, V. Sundström, M. d'Ischia, *J. Phys. Chem. B* **2012**, *116*, 13151–13158.
[27] J. Oshima, T. Yoshihara, S. Tobita, *Chem. Phys. Lett.* **2006**, *423*, 306–311.
[28] M. Gauden, A. Pezzella, L. Panzella, M. T. Neves-Petersen, E. Skovsen, S. B. Petersen, K. M. Mullen, A. Napolitano, M. d'Ischia, V. Sundström, *J. Am. Chem. Soc.* **2008**, *130*, 17038–17043.
[29] P. Luliński, D. Maciejewska, M. Bamburowicz-Klimkowska, M. Szutowski, *Molecules* **2007**, *12*, 2434–2449.
[30] P. Meredith, T. Sarna, *Pigment Cell Res.* **2006**, *19*, 572–594.

Received: May 30, 2014
Published online on July 2, 2014

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim