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Widening the View on Dispersant−Pigment Interactions in Colloidal Dispersions with Saturation Transfer Difference NMR Spectroscopy

Agnieszka Szczygiel,[†] Leo Timmermans,[‡] Bernd Fritzinger,[†] and José C. Martins*,[†]

Ghent University, NMR and Structure Analysis Unit, Krijgslaan 281 S4, B-9000 Gent, Belgium, and AGFA Materials, Septestraat 27, B-2640 Mortsel, Belgium

Received July 8, 2009; E-mail: Jose.Martins@UGent.be

The properties of colloidal dispersions critically depend on the nature and strength of particle−dispersant interactions.[1] These intervene during the formulation and determine the stability of dispersions but are also important to tune the dispersion properties toward those desired for their application.[2] Fundamental and in-depth knowledge of these interactions in situ remains therefore a primary requirement in colloid chemistry research.[3] Many of the analytical techniques and surface characterization methods that are commonly applied often perturb the dispersion and the chemical equilibria involved and/or provide a macroscopic view on the interaction.[4] These studies must therefore be completed with information from techniques that provide a direct and detailed view upon the underlying interactions at the molecular level.

Solution NMR spectroscopy is a method of choice to provide such a view, due to its noninvasive character and unique ability to monitor each species individually. Intermolecular interactions can be studied through their effect on the chemical shift, relaxation times, and translational diffusion coefficient of the various species and their sensitivity to variables such as concentration, solvent, and temperature.[5,6] A host of specific NMR approaches are routinely applied to screen for ligand binding to biomolecular targets, such as protein receptors, providing detailed information about the binding process and conformation.[6] These have recently inspired NMR based approaches for the in situ characterization of the interaction between capping ligands and semiconductor nanocrystals.[7]

Saturation Transfer Difference (STD) spectroscopy is a powerful technique to investigate ligand binding to biomacromolecules ranging from proteins to viral assemblies or membrane embedded receptors.[8,9] Working with an excess of ligand, it consists in saturating some resonances of the protein target in a so-called on resonance experiment taking care not to affect the ligand resonances. This selective saturation subsequently spreads throughout the entire network of dipolar-coupled protons in the protein via spin diffusion. When a ligand binds to the protein, part of the saturation is transferred onto its protons at a rate that is determined by the dissociation rate k_{off} and the protein−ligand complexation constant K_d. Since each protein undergoes multiple binding events during the saturation time, a sizable fraction of the ligands is affected, leading to a reduction of the ligand resonance intensity to $I_{sat,on}$. This is most easily characterized by subtracting a reference, so-called off resonance experiment wherein saturation is applied outside the frequency range where ligand and protein resonances occur, leaving the resonance intensities unperturbed at $I_{sat,off}$. The difference spectrum yields nonzero intensities $I_{STD} = (I_{sat,off} - I_{sat,on})$ only for resonances of binding ligands.[8,10] As ligand protons in close contact with the protein receive more saturation than more distant ones, the relative I_{STD} values of the ligand resonances can be interpreted in terms of a binding epitope.[8b,11]

Here, we demonstrate the potential of STD NMR for the investigation of interactions between dispersant molecules and molecular nanoparticles composed of water insoluble organic compounds. Our investigations were performed using a dispersion of pigment red 122 (PR122) particles and sodium dodecylsulfate (SDS) as dispersant in D_2O as a model. In the ^1H NMR spectrum of an initial 7%/5% (w/w) SDS-PR122 dispersion, two overlapping sets of SDS signals with different line-width characteristics are present that can most easily be distinguished by the α-methylene SDS resonance (Figure 1).

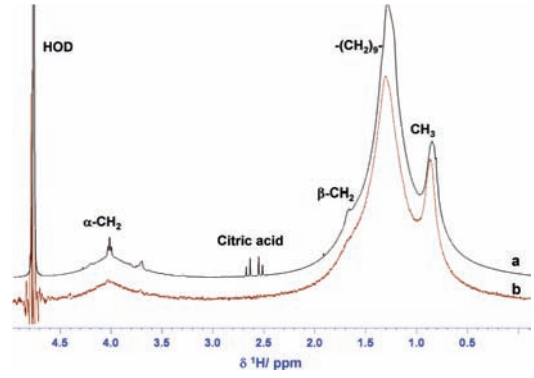

Figure 1. (a) Reference ^1H NMR spectrum of an SDS-PR122 dispersion containing 7% w/w SDS and 5% w/w PR122 in D_2O. The SDS resonances occur as indicated. The presence of micellar SDS is most easily recognized from the sharp α-methylene triplet riding atop a much broader peak from the same moiety in SDS molecules that interact with the pigment surface. (b) STD NMR spectrum obtained by alternate 2 s selective irradiation at 20 ppm (on-resonance) and 300 ppm (off-resonance). Only the broadened resonances appear, simultaneously demonstrating the adsorption of SDS molecules on the pigment and the lack of interaction with micellar SDS and citric acid.

The narrow line-width set can be attributed to SDS in micellar form, based on its diffusion coefficient and the fact that it disappears as the SDS concentration is reduced below the CMC. The second set shows strong, concentration dependent line broadening consistent with a fast exchange process involving a fraction of pigment surface bound dispersant molecules (vide infra). Additional resonances are contributed by citric acid, present for preservation reasons in the SDS starting material. The pigment particles consist of two types of quinacridone molecules present in a 3:1 ratio and regularly stacked into box shaped particles of nanoscale dimensions (10 by 30 by 100 nm on average). The solid like environment leads to efficient line broadening due to transverse relaxation processes, thereby eliminating a measurable contribution to the ^1H spectrum. At the same time it provides the dense network of dipolar coupled

[†] Ghent University.
[‡] AGFA Materials.

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protons required for saturation transfer. Figure 1b shows the result of an STD experiment to the dispersion shown in Figure 1a. The response in the STD spectrum is limited to those of the broadened set of SDS resonances. This validates the assumption of fast exchange with the pigment surface for this SDS species and also demonstrates that micellar SDS does not participate in direct interactions with the pigment. Also, no response can be seen for the resonances of citric acid showing that it acts as a nonligand, as expected. Direct irradiation of SDS resonances has been excluded as no STD response was obtained in the absence of the pigment. These results demonstrate the efficacy of STD to screen for pigment surface binding species in the dispersion. For further investigations, more dilute dispersions consisting of 0.25% (w/w) PR122 and 0.1 to 0.5% SDS were prepared, as these afforded considerable line narrowing of the SDS resonances.

The intensity of the STD response critically depends on not saturating the dispersant (ligand) or pigment molecules in the off-resonance experiment.[10] As no resonance contribution is apparent for the pigment, we initially set the off-resonance frequency to 30 ppm. The signal envelope contributed by the molecules in the solid pigment particles was subsequently established to extend from 60 to −70 ppm using the procedure described by Rademacher et al.[12] For all subsequent experiments, the on and off-resonance frequency was therefore set to 20 and 300 ppm respectively, providing 2.5-fold intensity enhancement. Additional parameters that were determined to obtain optimal results include the T_1 relaxation time of the saturated pigment protons (∼1.7 s) as well as the T_1 values of the dispersant molecules at the various concentrations used,[13] leading to an interscan delay of 20 s for total relaxation.

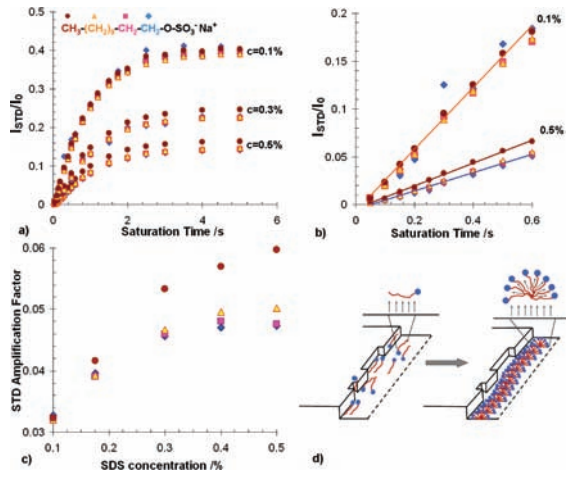

Figure 2. (a) Buildup of the normalized STD intensity I_{STD}/I_0 as a function of the saturation time for dispersions of 0.1%, 0.3%, and 0.5% (w/w) SDS with 0.25% PR122. (b) Detailed view of the first 600 ms of the initial buildup for the 0.1% and 0.5% SDS dispersions, which clearly shows the higher buildup rate for the 0.1% and the significant differentiation for the methyl STD response for the 0.5% dispersion, as supported from the regression fit to the data. (c) STD amplification factors as a function of SDS concentration. The plot is obtained by multiplying the initial slope of the buildup curves (b) with the dispersant weight percentage. (d) Schematic representation of the reorganization of the SDS molecules at the pigment surface, proposed to explain the differentiation observed in STD amplification factors in (c).

The effect of the duration of the saturation time on the normalized STD intensity I_{STD}/I_0 is shown in Figure 2a for dispersions containing 0.1, 0.3, and 0.5% (w/w) SDS and features the typical profile well-known from the protein−ligand case.[14] For each individually resolved resonance, the initial rise in normalized STD intensity, determined by k_{off} and the dispersant concentration, levels off into a plateau, indicating that a steady state is reached due to competition with T_1 relaxation induced loss of saturation of the SDS molecules. In protein−ligand interactions, the so-called group-epitope map is obtained by interpreting differences in saturation transfer intensities for the various ^{1}H resonances as the result of protein−ligand proximity, with higher intensities reflecting a closer proximity of a ligand hydrogen to the saturated binding surface. To take care of the possible interference of variations in T_1 relaxation times in the dispersant, the initial slopes for saturation times up to 0.6s of the STD intensity buildup (Figure 2b) rather than the STD intensities at a specific saturation time were used for our analysis of the PR122:SDS dispersion. For the 0.1% SDS sample, the initial slopes for all resonances are seen to be identical within error, indicating equally efficient transfer of saturation to the SDS chain from the pigment. As expected, the slope of the STD intensity buildup steadily decreases with concentration, since the fraction of noninteracting dispersant also decreases when constant binding site concentration is assumed. Interestingly, this decrease is less pronounced for the methyl group which becomes clearly differentiated from the various methylene chain resonances. To directly compare the STD responses of samples with different ligand concentrations, the slope of the normalized STD intensity buildup is multiplied by the ligand to binding site concentration ratio to yield the amplification factor A_{STD} (Figure 2c). Since the total pigment binding surface is unknown we multiplied I_{STD} with the SDS concentration expressed as weight percent. For all resonances, A_{STD} rises with SDS concentration, eventually leveling off toward a maximum value, which is similar to the typical protein−ligand case. This is interpreted as the result of a Langmuir-type absorption behavior: beyond a certain SDS concentration, the surface will be completely covered preventing any further increase in A_{STD}. Second, the methyl group is confirmed to behave distinctly from the remainder of the methylene chain as the SDS concentration increases. At the lowest SDS concentrations (up to 0.25%), the various A_{STD} are identical throughout the SDS chain. From 0.30% SDS and onward, the methyl group A_{STD} value increases more rapidly, resolving it from the methylene groups. At the highest concentration studied A_{STD} is 25% larger for the methyl than for the methylenes. Since initial slopes of the I_{STD} buildup are used, concentration dependent changes in the ligand ^{1}H T_1 relaxation rate are an unlikely source for this. Finally, we note that no resonances for micellar SDS species are apparent throughout the concentration range studied, excluding possible interference from this species which in any case acts as a nonligand as presented in Figure 1.

A major difference with respect to the protein−ligand paradigm for the interpretation of STD NMR data comprises the fact that we do not have a 'one molecule binds one protein binding site' relationship, but rather a hydrophobic surface area that is large with respect to the individual molecules, providing many locations and modes for binding. Considering this we propose that the data indicate an evolution in the saturation transfer efficiency that could be linked to a reorganization of the SDS molecules on the pigment surface (Figure 2d). At low concentrations, the available hydrophobic surface area on the pigment is large and flat-on adsorption of SDS molecules occurs. The entire chain interacts directly with the saturated surface leading to identical I_{STD} buildup slopes. With increasing concentration the SDS molecules reorganize into hemicylindric shaped micellar structures, with sulfonate directed toward the solvent. Thus, the methyl chain ends obtain their saturation mostly from direct contact with the surface while most methylene chain protons receive their saturation indirectly via intra- and interchain spin diffusion pathways. Such concentration dependent reorganization is in line with the work of Wanless et al.,[15] who

demonstrated the concentration dependent emergence of hemicylinder SDS micelles on hydrophobic graphite surfaces using AFM, for SDS concentrations above 0.08%. Unfortunately, a direct demonstration of such structures on the surface of PR122 using AFM has so far proven elusive due to the challenges to obtain high quality images of such systems in situ.

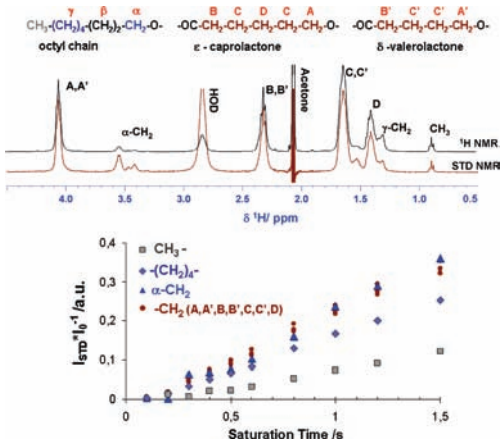

Figure 3. (a) Reference ^{1}H NMR (top) and corresponding STD NMR (saturation time 2 s) spectrum (bottom) of a PR122 dispersion (2% w/w) in acetone stabilized with 2% (w/w) octyl terminated poly-ε-caprolactone-δ-valerolactone. (b) Normalized STD intensity I_{STD}/I_0 plotted as a function of the saturation time for individual polymer resonances.

The application potential of STD NMR was also investigated beyond this simplified model system, using a dispersion of PR122 with low molecular weight copolymers of ε-caprolactone (12 units) and δ-valerolactone (2 units) terminated by octyl chains (1 unit), both present in 2% w/w in acetone-d_6. The polyester macromonomer represents the pigmentophilic part, whereas the octyl chain should prefer the solvent environment, based on Hansen solubility parameters.[16] This prediction is confirmed from the STD NMR spectrum where a clearly weaker STD response is obtained for the octyl chain ends compared to the polyester unit and αCH_2 of the octyl chain (Figure 3). This holds for all concentrations investigated and is in line with the expectation that the polyester chain should directly interact with the surface, while the octyl chains should extend into the solvent, thus receiving far less saturation than the polyester unit.

In conclusion, we have shown that STD NMR is a valuable addition to the in situ solution characterization techniques available for the characterization of particle−dispersant interactions. It allows detection of binding ligands and discrimination of ligand from nonligand and can provide a qualitative view on the organization of the ligand on the surface. The approach should be generally applicable as long as the particle is comprised of a dense network of hydrogens, implicating almost all organic molecular nanoparticles, and provides a novel investigative window on dispersions that is highly complementary to existing ones.

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Supporting Information Available: Experimental details regarding the preparation of samples, the setup of STD NMR measurements, and relaxation properties. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Napper, D. H. *Polymeric Stabilization of Colloidal Dispersions*; Academic Press: New York, 1983. (b) Hiemenz, P. C.; Rajagopalan, R. *Principles of Colloid and Surface Chemistry*; Marcel Dekker: 1997; pp 578−585. (c) Miller, C. A.; Neogi, P. *Interfacial Phenomena: Equilibrium and Dynamic Effects*; CRC Press: 2007. (d) Tharwat, T. *Adv. Colloid Interface Sci.* **2003**, *104*, 191–226.
(2) (a) *Technological Applications of Dispersions*; McKay, R. B., Ed.; Surfactant Science Series 52; Marcel Dekker: 1994. (b) Clayton, J. *Pigm. Resin Technol.* **1998**, *27* (4), 231–239.
(3) (a) Yin, Y.; Alivisatos, P. *Nature* **2005**, *437*, 664–670. (b) Griffiths, P. C.; Cheung, A. Y. F.; Davies, J. A.; Paul, A.; Tipples, C. N.; Winnington, A. L. *Magn. Reson. Chem.* **2002**, *40*, 40–50.
(4) *Dispersions. Characterization, Testing, and Measurements*; Kissa, E., Ed.; Surfactant Science Series 84; Marcel Dekker: 1999.
(5) (a) Cosgrove, T.; Griffiths, P. C. *Adv. Colloid Interface Sci.* **1992**, *42*, 175–204. (b) Soederman, O.; Stilbs, P. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 445–482. (c) Schoenhoff, M.; Soederman, O. *J. Phys. Chem. B* **1997**, *101*, 8237–8242. (d) Brand, T.; Cabrita, E. J.; Berger, S. *Prog. Nucl. Magn. Reson. Spectrosc.* **2005**, *46*, 159–196. (e) Grzesiek, S.; Allan, M.; Cordier, F.; Haussinger, D.; Jensen, P.; Kahmann, J.; Meier, S.; Sass, H. J. *Pure Appl. Chem.* **2005**, *77* (8), 1409–1424. (f) Houliston, R. S.; Jacobs, B. C.; Tio-Gillen, A. P.; Verschuuren, J. J.; Khieu, N. H.; Gilbert, M.; Jarrell, H. C. *Biochemistry* **2009**, *48* (2), 220–222.
(6) (a) Cameron, K. S.; Fielding, L. *J. Org. Chem.* **2001**, *66* (21), 6891–6895. (b) Lucas, L. H.; Larive, C. K. *Concepts Magn. Reson. A* **2004**, *20A* (1), 24–41. (c) Fielding, L. *Prog. Nucl. Magn. Reson. Spectrosc.* **2007**, *51*, 219–242. (d) Peters, T. *Carbohydrates in Chemistry and Biology, Vol. 2*; Wiley-VCH: 2000; pp 1003−1023.
(7) (a) Majetich, S. A.; Carter, A. C.; Belot, J.; Mccullough, R. D. *J. Phys. Chem.* **1994**, *98*, 13705. (b) Aldana, J.; Wang, Y. A.; Peng, X. G. *J. Am. Chem. Soc.* **2001**, *123*, 8844. (c) Ribot, F.; Escax, V.; Roiland, C.; Sanchez, C.; Martins, J. C.; Biesemans, M.; Verbruggen, I.; Willem, R. *Chem. Commun.* **2005**, 1019. (d) Hens, Z.; Moreels, I.; Martins, J. C. *ChemPhysChem* **2005**, *6*, 2578. (e) Moreels, I.; Martins, J. C.; Hens, Z. *ChemPhysChem* **2006**, *7*, 1028. (f) Steckel, J. S.; Yen, B. K. H.; Oertel, D. C.; Bawendi, M. G. *J. Am. Chem. Soc.* **2006**, *128*, 13032. (g) Van Lokeren, L.; Maheut, G.; Ribot, F.; Escax, V.; Verbruggen, I.; Sanchez, C.; Martins, J. C.; Biesemans, M.; Willem, R. *Chem.−Eur. J.* **2007**, *13*, 6957. (h) Liu, H. T.; Owen, J. S.; Alivisatos, A. P. *J. Am. Chem. Soc.* **2007**, *129*, 305. (i) Moreels, I.; Fritzinger, B.; Martins, J. C.; Hens, Z. *J. Am. Chem. Soc.* **2008**, *130*, 15081. (j) Fritzinger, B.; Moreels, I.; Lommens, P.; Koole, R.; Hens, Z.; Martins, J. C. *J. Am. Chem. Soc.* **2009**, *131* (8), 3024–3032.
(8) (a) Mayer, M.; Meyer, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1784–1788. (b) Mayer, M.; Meyer, B. *J. Am. Chem. Soc.* **2001**, *123*, 6108–6117. (c) Meyer, B.; Peters, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 864–890.
(9) (a) Mari, S.; Serrano-Gómez, D.; Cañada, F. J.; Corbí, A. L.; Jiménez-Barbero, J. *Angew. Chem., Int. Ed.* **2004**, *44* (2), 296–298. (b) Claasen, B.; Axmann, M.; Meinecke, R.; Meyer, B. *J. Am. Chem. Soc.* **2005**, *127* (3), 916–919. (c) Pellecchia, M. *Chem. Biol.* **2005**, *12*, 961–971. (d) Haselhorst, T.; Blanchard, H.; Frank, M.; Kraschnefski, M. J.; Kiefel, M. J.; Szyczew, A. J.; Dyason, J. C.; Fleming, F.; Holloway, G.; Coulson, B. S.; Von Itzstein, M. *Glycobiology* **2006**, *17* (1), 68–81.
(10) Rademacher, C.; Peters, T. *Top. Curr. Chem.* **2008**, 273.
(11) Shin, S. Y.; Gaethje, H.; Schwardt, O.; Gao, G. P.; Ernst, B.; Kelm, S.; Meyer, B. *ChemBioChem* **2008**, *9* (18), 2946–2949.
(12) Rademacher, C.; Rama Krishna, N.; Palcic, M.; Parra, F.; Peters, T. *J. Am. Chem. Soc.* **2008**, *130* (11), 3669–3675.
(13) Yan, J.; Kline, A. D.; Mo, H.; Shapiro, M. J.; Zartler, E. R. *J. Magn. Reson.* **2003**, *163* (2), 270–276.
(14) Blume, A.; Berger, M.; Benie, A. J.; Peters, T.; Hinderlich, S. *Biochemistry* **2008**, *47* (49), 13138–13146.
(15) Wanless, E. J.; Ducker, W. A. *J. Phys. Chem.* **1996**, *100*, 3207–3214.
(16) Hansen C. M. *Hansen Solubility Parameters*; CRC Press: 2007.

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