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A New Nanocomposite: L-DOPA/Laponite

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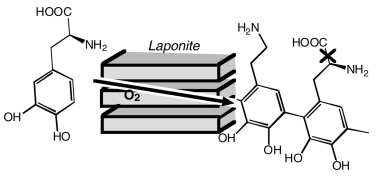
ABSTRACT A simple procedure is reported to obtain original nanocomposite materials combining the smectite Laponite and melanin-like polymers. Aqueous polymerization of L-DOPA is considerably accelerated in the presence of suspended Laponite particles, and its mechanism is significantly modified with respect to homogeneous systems. The resulting hybrid materials have been characterized by solid-state NMR, thermogravimetric analysis, and electron microscopy.

SECTION Nanoparticles and Nanostructures



L-DOPA (3,4-dihydroxyphenylalanine) is a fascinating molecule that plays important roles in biochemistry and medicinal chemistry and shows promise for nanocomposite materials preparation. It is a nonessential amino acid as well as a precursor of the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine. In opposition to these transmitters, it can cross the blood-brain barrier, which has made it the most effective drug for the symptomatic control of Parkinson's disease. It also plays a crucial role in the biosynthesis of natural melanins,[1] which are among the most important pigments in human beings and are involved in the functioning of the central nervous system and probably in melanome carcinogenesis.[2] And finally, it shows promise to develop bioplatforms based on L-DOPA polymerization on a number of substrates,[3] especially inorganic oxides.[4−7]

We have undertaken a systematic study of L-DOPA interaction with a variety of oxides, either to achieve controlled DOPA delivery with pharmaceutical applications (in cases where the molecule does not polymerize) or to obtain organic/inorganic nanocomposites (when it does polymerize). The L-DOPA/Laponite system has shown an interesting behavior, which we will underline here.

Laponite belongs to the smectite family, one of the largest and most important classes of the phyllosilicate clay minerals group. To the best of our knowledge, no composites of smectite with L-DOPA have been reported so far, even though many other clay mineral− and organoclay−polymer nanocomposites have been described in the past decade.[8] Smectites are abundant in nature, which makes them a logical choice as ingredients in pharmaceutical products, both as excipients and active substances[9] (the use of clay minerals for curative and protective purpose is as old as mankind itself).[10] However, the low purity and highly variable composition of natural materials have led to the developement of synthetic smectites, which can be more useful as initial models for academic research. Laponite is a synthetic layered silicate similar in structure and composition to the natural smectite hectorite. Each Laponite platelet is composed of three sheets: a central sheet of magnesium ions in octahedral coordination with oxygen anions and hydroxyl groups, and two outer tetrahedral silica sheets. The isomorphic substitution of some magnesium cations with lithium in the central sheet, as well as the presence of some vacant positions, gives rise to a partial negative charge, which is balanced by sodium cations; the reported Laponite empirical formula is $Na+^{0.7}[Si_8Mg_{5.5}Li_{0.3}]-O_{20}(OH)_4]^{0.7-}$. The finite dimensions of the clay platelets are responsible for the occurrence of silanol groups at the edges of the sheets. The average dimensions of the Laponite sheets (25 nm diameter, 0.92 nm height) confer to Laponite a quite high edge-to-surface ratio (0.07).[11]

In our DOPA/Laponite syntheses (see details in below), the initially translucent suspensions turned pink 3 min after contact and black after at most 30 min. The black suspensions gelified after 5 h of aging; while gelation has also been observed in pure laponite suspensions, that was only for laponite concentrations higher than 3% (w/w),[12] thus significantly higher than in our conditions. For comparison, L-DOPA was dissolved in a buffer at the same pH value but without Laponite. The same sequence of colorations was observed but took at least 24 h to complete. Eventually, a black solid began to form.

What happens in the buffered solution is relatively well-understood (at least regarding the first stages).[1,13,14] The pink coloration is due to the oxidation of the catechol function of L-DOPA to dopaquinone ("DQ") by atmospheric O_2 (other oxidants can be effective as well). The resulting molecule can be involved in a series of redox isomerizations and O_2 oxidations that result in formation of the indole ring. The successive reactions are summarized in Figure 1.

Alternatively, at several stages of this scheme, 1-electron oxidation to the semiquinone can lead to polymerization through the formation of a C−C bond between the aromatic nuclei of different monomers. The resulting polyaromatics are responsible for the black coloration. This sequence of reactions has been studied a lot by biochemists, as it is involved in the classical picture of eumelanin biosynthesis, known as the

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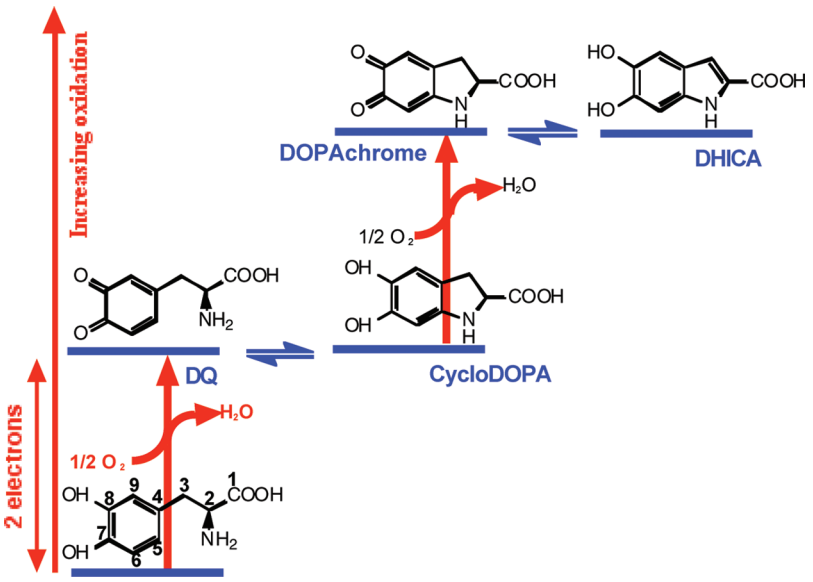

Figure 1. Redox reactions involving L-DOPA. DQ = dopaquinone; DHICA = dihydroxyindolecarboxylic acid.

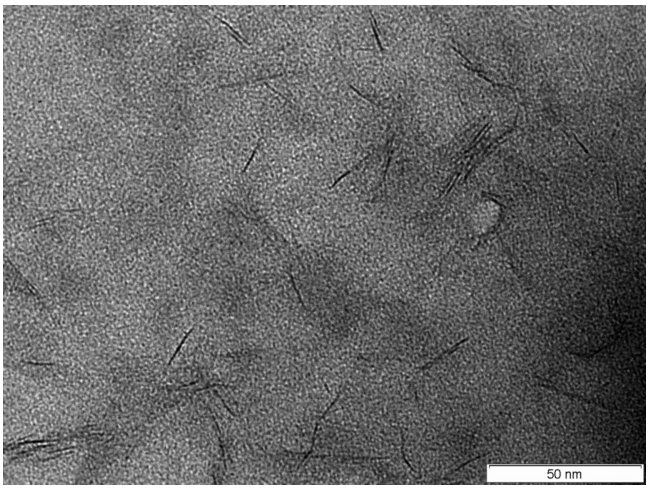

Figure 2. TEM micrograph of the L-DOPA/Laponite nanocomposite.

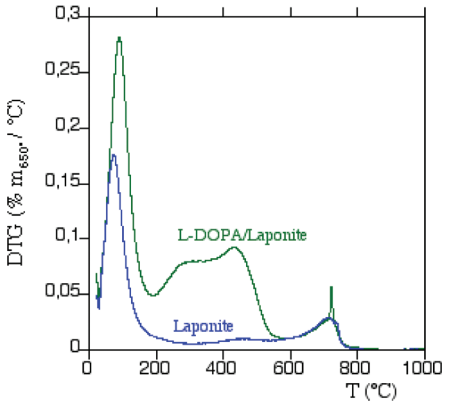

Figure 3. Differential thermogravimetric analysis in flowing air of L-DOPA/Laponite nanocomposite compared to pure Laponite.

Raper−Mason scheme; in biochemical systems though, the successive steps are of course under the control of specific enzymes.

Thus, direct observation suggests that a pattern of reactions similar to melanogenesis can occur in the presence of inorganic surfaces, and indeed be catalyzed by them. The first claim is not unprecedented. Messersmith et al. have obtained polymeric organic coatings on different supports by simple dipping into aqueous solutions of L-DOPA and the related dopamine. Their interpretation has varied somewhat: early works (and their reviews) conveyed the impression that the polymeric material was akin to the adhesive proteins of mussels,[5,15] which contain L-DOPA in the proteine framework, polymerized by peptide links; the most recent papers clearly state that "polymerization of dopamine occurs in a manner that is reminiscent of melanin formation".[16] The second interpretation appears much more likely, since peptide bond formation is thermodynamically very unfavorable in the absence of the appropriate cell machinery.[17]

We have submitted the dark solids obtained after DOPA/Laponite reaction to further characterization in order to determine if they were indeed a kind of melanin/Laponite composite material.

The X-ray diffraction (XRD) pattern (not shown) of the black powder exhibits broad diffraction peaks characteristic of the Laponite clay structure The *d*(060) value at 0.152 nm is typical of trioctahedral clays such as Laponite. The (001) reflection is large, indicating either the presence of several types of layers with different interlayer distances or a low distance of coherence in the corresponding direction. Its maximum at about 0.125 nm corresponds to the "one-water layer" form of the smectite, observed at intermediate degrees of hydration.

Transmission electron microscopy (TEM) images (Figure 2) show that the L-DOPA/Laponite contains delaminated clay

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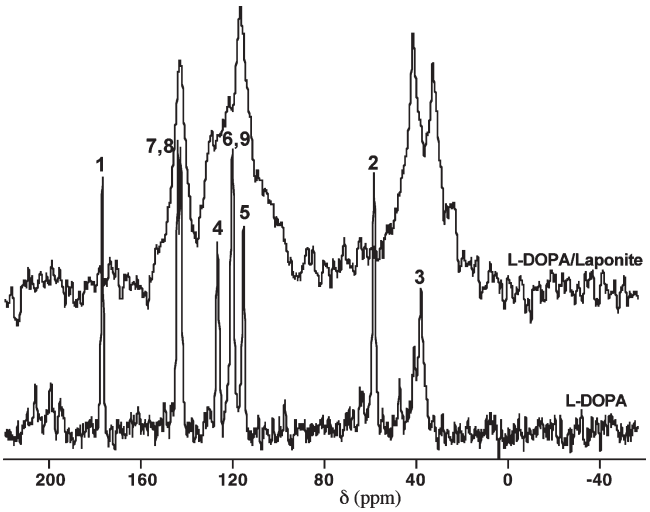

Figure 4. ^{13}C CP-MAS NMR spectra of crystalline L-DOPA and of the L-DOPA/Laponite nanocomposite. The numbering is the same as in Figure 1.

layers with 20−30 nm length as well as stackings of a few layers. At places, they seem inbedded within a dark material, which is, however, hard to distinguish from the underlying carbon grid.

The derivative thermogravimetric (DTG) curves (Figure 3) show a first loss of 8% in the 50−100 °C range associated to an endothermic phenomenon and corresponding to the departure of physisorbed water. The second weight loss in the 230−550 °C range, due mostly to the elimination of the organic component (with a minor event also present in the Laponite blank as a result of the dehydroxylation of the edge groups), corresponds to the loss of about 25% of the mass remaining at 650 °C, which is close to the total organic matter introduced in the initial suspension. A third, strongly exothermal weight loss is observed in the 650−750 °C temperature range, corresponding, as confirmed by XRD, to the phase transition from Laponite to enstatite. Therefore, the Laponite component of the material is stable up to high temperatures, while the organic part is eliminated earlier. It can also be noted that no thermal event is observed in the 160−200 °C region, where an eventual peptide bond condensation event would be expected.[18]

The solid-state ^{13}C NMR spectrum of pure crystalline L-DOPA is comparable to the one published by Park et al.[7] with sharp resonances for all nine carbons of the molecule (compare numbering on Figures 1 and 4). The spectrum of the L-DOPA/Laponite exhibits broader peaks, but most of them can be matched to those of L-DOPA indicating that many elements of the basic structure are preserved upon polymerization, including part of the side chain (carbons 2 and 3).

Significant differences are also apparent between the L-DOPA/Laponite composite and the starting molecule. First, the resonance corresponding to the carboxylate groups (carbon 1) disappears in the composite or is at least strongly decreased. This could be an artifact of the CP method due to the often poor polarization transfer to the nonprotonated −CO− carbons. However, we also observe that the resonance of neighboring carbon 2 has been shifted upfield by several parts per million. This suggests instead that the carboxylate moiety has indeed been eliminated in the composite. Decarboxylation is known to occur in biological pathways, although mostly after formation of the indole ring, while in our materials indole rings do not seem to be formed to a large extent: pyrrolic carbons are expected to resonate around 120 ppm and might be present under the broad aromatic resonance, but the carbons of the aliphatic side chain are still predominant (as opposed to synthetic melanins),[19] which means that cyclization has not occurred to a large extent.

Finally, the appearance of a shoulder around 110 ppm on the signal of the aromatic carbons might indicate the formation of carbon−carbon bonds between aromatic cycles that is expected in the semiquinone polymerization pathway; in model compounds, the corresponding carbons have been observed at 112 to 118 ppm.[20]

Thus, while solid-state NMR of the composite confirms the basic idea that the chemistry involved in L-DOPA polymerization in the presence of Laponite is similar to melanogenesis, this technique also underlines significant differences, namely, (1) polymerization before indole ring formation, and (2) extensive decarboxylation of the amino acid moiety. Furthermore, those differences are due to the reactivity of molecules in the "adsorbed phase" since we also recorded the liquid-state ^{13}C NMR spectrum of the supernatant solution in contact with the inorganic solid: no significant difference with the starting L-DOPA spectrum could be observed along the reaction course.

In contrast with composites, the solid-state NMR spectra of "synthetic melanins" obtained by precipitation from homogeneous solutions (in the absence of Laponite) by ourselves and others showed peaks corresponding to a pyrrolic carbon, indicating that the cyclization of DOPA has occurred in those cases.

In summary, our initial experiments on L-DOPA/Laponite systems indicate that they can lead through a simple procedure to interesting composite materials in which well-dispersed Laponite layers are held together by organic material, which helps in the reticulation of gels, and is composed of an original polymer similar to eumelanin, but not identical with it, which might be called "pseudomelanin". The effect of the Laponite component is both to accelerate the polymerization by a catalytic effect and to direct the oxidation and polymerization to a different outcome than in solution. In the final product, the laponite and pseudomelanin components are interspersed at the nanometric scale and show cooperative properties.

Additional research is currently underway in order to better characterize this organic material and the physical properties of the nanocomposites it forms with Laponite. In addition, preliminary experiments with other inorganic supports—saponite, a natural phyllosilicate, nonporous and porous silicas, and zeolites—indicate that reaction with L-DOPA has very different outcomes, eventually resulting in nanocomposites with diverse chemical compositions and chemical properties.

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Nanocomposite preparation procedure: A 2% (w/w) Laponite Rockwood suspension was prepared in a 2.70×10^{-2} mol·L^{-1} aqueous solution of L-DOPA, and the resulting suspension was stirred for 24 h. The pH of the medium was 8.2. The solid was then dried for 72 h at 60 °C under air.

Characterization: Prior to thermal analysis, the sample was dehydrated by oven-drying (303 K) and then rehydrated under controlled moisture (80%). Thermogravimetric curves were then recorded on a SDT Q600 apparatus (TA Instruments) under 100 mL/min air flow, with a 5 °C/min temperature ramp. Solid-state NMR spectra were obtained on a Bruker Avance 500 spectrometer operating at $\omega_L = 500$ MHz (^{1}H) and 100.62 MHz (^{13}C). Proton cross-polarization magic angle spinning (CP-MAS) was applied with a contact time optimized at 5 ms. For the spectra discussed here, samples were spun at the magic angle at a frequency of 10 kHz; some spectra were also acquired at 14 kHz to check that there was no interference from spinning side bands (SSBs). The ^{13}C pulse length was 5 μs.

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