

Supporting information

Fluorescent Labeling and Tracking of Nanoclay

Carlos A. Diaz,^{†,‡,□} Yining Xia,^{†,‡} Maria Rubino,^{†,*‡} Rafael Auras,^{†,*‡} Krishnamurthy Jayaraman,[§] and Joseph Hotchkiss[†]

[†] School of Packaging, [§] Chemical Engineering, Michigan State University, East Lansing, Michigan 48824, United States

*E-mail: (M.R.) mariar@msu.edu; (R.A.) aurasraf@msu.edu.

Supporting Information

The current text describes in further detail the experimental methods used to (S1) label the nanoclay, (S2) detect and characterize fluorescence from labeled nanoclay, (S3) determine the thermal stability of the labeled nanoclay, (S4) incorporate the labeled nanoclay in the polymer matrix, and (S5) track labeled nanoclay that migrated from the polymer matrix into a solvent.

S1. Labeling of nanoclay

MATERIALS

Nanoclay (Nanomer® I.44P), containing 35–45% quaternary ammonium compounds, bis(hydrogenated tallow alkyl)dimethyl, chlorides; 55–65% montmorillonite (MMT), was purchased from Nanocor (Arlington Heights, IL). Linear polypropylene (PP, Profax 6523) was used as the model matrix and maleic anhydride-grafted-PP (MA-g-PP or MAPP, Bondyram® 1001, 1 wt% bound maleic anhydride) was used as the compatibilizer. Silane (3-mercaptopropyl)-trimethoxysilane (>95%), purchased from Sigma-Aldrich (St. Louis, MO), was

used to functionalize the nanoclays. Two fluorescent dyes, tetramethylrhodamine-5-maleimide (rhodamine) and fluorescein-5-maleimide (fluorescein), were used for tagging; single isomers were purchased from Molecular Probes (Eugene, OR). Table S1 lists the properties of the fluorescent dyes. Thiol reactive dyes were selected for selective reaction with the functionalized nanoclay. Amine reactive dyes were avoided to prevent the reaction of the fluorescent tag with amino groups in the surfactant used to modify the nanoclay.

Table S1. Properties of fluorescent probes as reported by Molecular Probes

	tetramethylrhodamine-5-maleimide	fluorescein-5-maleimide
Molecular formula	$C_{28}H_{23}N_3O_5$	$C_{24}H_{13}NO_7$
Molecular weight	481.51	427.37
Absorption maximum, nm	541	592
Emission maximum, nm	567	515

METHODS

Silylation. A mercaptosilane was selected for the functionalization of the nanoclay. The silylation treatment was carried out following the procedure of Chaudhary.¹ Organomodified montmorillonite (o-MMT) (15 g) was dispersed in 500 mL of a solvent (80 wt% methanol + 20 wt% deionized water) using a magnetic stirrer. In a separate flask, 4.5 g of silane was diluted with 200 mL of the same solvent. This diluted silane solution was slowly added to the clay dispersion and stirred for 6 h at 23 °C. The clay suspension was filtered and washed at least 3 times using the original solvent to remove any unreacted silane. The resulting clay cake was

dried for 24 h under vacuum at 80 °C, 20" Hg pressure (6.8×10^{-4} Pa, abs). The cured clay was then powdered using a mortar and pestle and shaken through a No. 200 (75 μ m) sieve.

Conjugation. The conjugation procedure for both fluorescent labels was carried out in a phosphate buffered saline (PBS) solution: 10 mg of the fluorescent dye was dissolved in 240 mL PBS. The fluorescein-based dye was dissolved directly in PBS, whereas the rhodamine-based dye was first dissolved in methanol (10 mL) and then the stock solution was dissolved in PBS. Modified nanoclay (treated with mercaptosilane, 0.25g) was dissolved in 100 mL ethanol and carefully added to the PBS solution containing the dye. The solution was shaken and stirred with a magnetic stirrer for 1 h (incubation time). The clay was separated from the solution by centrifuging the mixture at 2500 rpm for 5 min. To remove unreacted dye, the clay pellets were washed in ethanol and centrifuged; this washing cycle was repeated at least 3 times until a clear solution was obtained.

S2. Fluorescence characterization

Fluorescence detection and characterization of emission spectra was done via confocal laser scanning microscopy (CLSM). The images were acquired using an Olympus FluoView FV1000 CLSM configured on a fully automated inverted IX81 microscope. For rhodamine the fluorescence was excited using the 543 nm line of the Helium Neon laser and the fluorescence signal in red emission was captured using a 560 long pass filter. For fluorescein, the fluorescence was excited using the 488 nm line of the Argon laser and the green emission was captured using a 505–525 nm band pass filter. The transmitted light image was generated in a brightfield mode. To record the emission spectra from the rhodamine-labeled nanoclay samples, excitation was

provided by the 559 nm solid state diode laser, with the emission recorded from 565–720 nm in 5-nm increments. The fluorescein-labeled nanoclay was excited at 488 nm and the emission spectra recorded from 500–740 nm in 10-nm increments.

The Olympus FluoView FV1000 Advanced Software was used to analyze each emission spectrum. Regions of interest were traced around particles, and the average fluorescence intensity of each region was calculated and plotted for each wavelength.

In addition, confocal microscopy-aided autofluorescence characterization (spectrophotometric analysis) of the unlabeled clay was carried out. Autofluorescence characterization can assist in the selection of a fluorescent dye by providing information of scattered emissions that can interfere with the fluorescent tag. At the excitation wavelengths from 405 to 488 nm, the unlabeled clay emission peaked at 500 nm, which overlapped with the fluorescein-tagged clay (emission peak at 515 nm when excited at 488 nm). However, there was no interference with the rhodamine-tagged clay, which usually excited at a wavelength of 543 nm and had an emission peak at 567 nm beyond the autofluorescence of the unlabeled clay. Depending on the different components in the nanocomposite that may produce autofluorescence, one can select which dye to use. In the case mentioned in this study, the use of fluorescein may have some limitations due to the similarity of the emission from the tag and the autofluorescence of the nanoclay.

S3. Thermal stability test

Thermal stability tests were performed using a thermogravimetric analysis (TGA) instrument (TA Instruments model Q50). Labeled nanoclay (2 to 5 mg) was placed in an aluminum pan and heated to the desired temperature (i.e., 220 or 250 °C) and held at that temperature for 15 min. A

continuous purge of nitrogen (60 mL/min) was maintained during the experiment. After heating, the samples were mounted on a glass microscope slide for confocal analysis.

Multiple emission spectra were recorded for each sample and the relative integral fluorescence emission (*RIFE*) was calculated as:

$$RIFE = I_t / I_o \quad \text{Eq. S1}$$

where I_t is the integral of fluorescent intensity vs. wavelength for each emission spectra, and I_o is the integral for the average intensity of the unheated sample.

S4. Incorporation of labeled nanoclays into a polymer matrix

The polypropylene was blended with 3 wt% nanoclay and 12 wt% compatibilizer (maleic anhydride modified polypropylene) to enhance dispersion of the nanoclay. Labeled and unlabeled nanocomposites were prepared. The labeled nanocomposites included 15 wt% of either fluorescent-labeled clay based on the total amount of clay (3 wt%). The mixing was performed with an internal mixer (CW Brabender, Duisburg, Germany) heated at 180 °C for 6 min at 80 rpm under a nitrogen atmosphere. The melt-mixed batch was used to produce films of ~ 100 μm in thickness, prepared via compression molding (175 °C, 10 tons) using a Teflon mold. The contrast between the fluorescent-labeled and the unlabeled nanocomposite films when analyzed by CLSM at the same conditions is shown in Figure S2 for rhodamine and in Figure S3 for fluorescein.

<Figure S1 & S2>

In addition, videos S1 and S2 show the emission intensity as a function of z-position for the rhodamine-labeled and fluorescein-labeled nanocomposite films.

<Video S1 & S2>

S5. Tracking of labeled nanoclay from the polymer matrix into a solvent

In addition to the migration results discussed in the main text, a two-sided migration test was performed following ASTM D4754 where PP-clay nanocomposite films with and without rhodamine-labeled nanoclay were exposed to 100% (v/v) ethanol. PP-clay nanocomposites were thoroughly rinsed with 100% ethanol before the migration study. Using a temperature bath set at 80 °C, the samples were exposed to the solvent at the elevated temperature for 4 h. The solvent was collected before and after the 4-h migration period, placed in cuvettes and allowed to evaporate inside a fume hood. The residue left in the cuvette was analyzed through CLSM. Figure S3 shows the contrast in the migrated residue from labeled and unlabeled nanocomposite films analyzed via CLSM for rhodamine labeled films.

<Figure S3>

REFERENCE

(1) Chaudhary, A. K. Rheology modification and foaming of polypropylene - clay nanocomposites with coupling agents, Ph.D. Dissertation. Michigan State University, 2010.

FIGURES

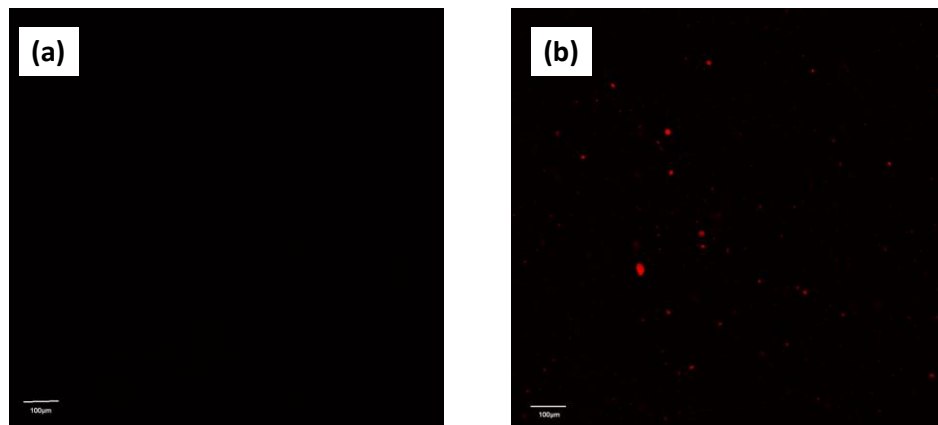


Figure S1. Detection of fluorescence on rhodamine-labeled nanocomposite film. (a) Control nanocomposite film without labeled clay. (b) Nanocomposite film with labeled clay. Both micrographs were taken at the same conditions.

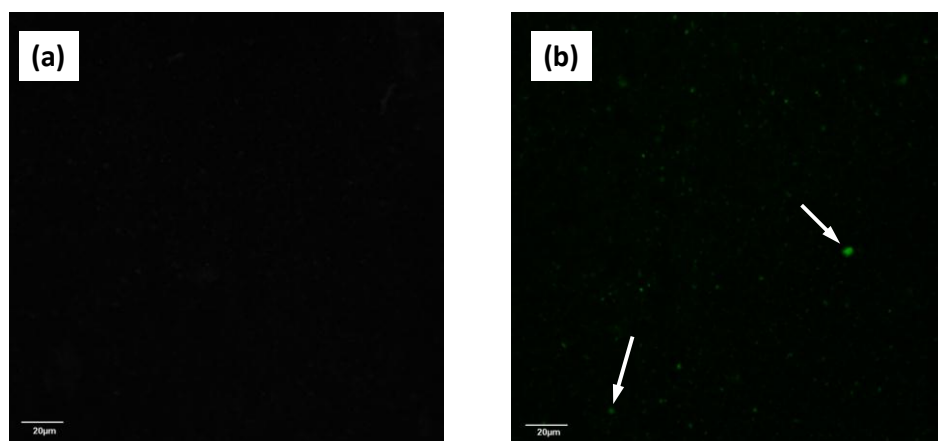


Figure S2. Detection of fluorescence on fluorescein-labeled nanocomposite film. (a) Control nanocomposite film without labeled clay. (b) Nanocomposite film with labeled clay. Both micrographs were taken at the same conditions.

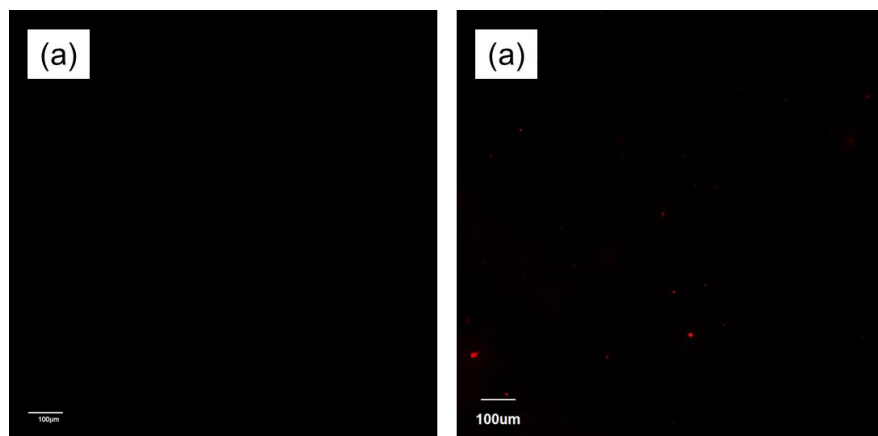


Figure S3. Fluorescence of the migrated precipitate. Both micrographs were taken at the same conditions. (a) Migrated residue from nanocomposite without labeled nanoclay. (b) Migrated residue from nanocomposite with rhodamine-labeled nanoclay.

VIDEOS

Video S1. Fluorescent intensity of rhodamine-labeled nanocomposite film as a function of z-position (i.e., depth of the film) via CLSM.

Video S2. Fluorescent intensity of fluorescein-labeled nanocomposite film as a function of z-position (i.e., depth of the film) via CLSM.