Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supporting Information for

Chelating DTPA Amphiphiles: Self-Assembly Structures and Gadolinium Complexes

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Materials Synthesis

N-mono-(carboxy- *cis*-9-Octadecenyl)- Diethylene triamine-N-N′,N″-tetraacetic acid (DTPA-MO), and N,N″-bis -(carboxy- *cis*-9-Octadecenyl)- Diethylene triamine-N-N′,N″- triacetic acid (DTPA-BO)

DTPA-dianhydride (3.54 g, 10 mmol) was added to 40 mL anhydrous DMF and was heated to 105 °C until dissolved. The reaction mixture was removed from the heat and oleyl alcohol (4.28 g, 16 mmol), dissolved in 10 mL of tetrahydrofuran (THF), was added to the reaction mixture. It was stirred for 3h at ambient temperature, followed by addition of water (5 mL) and additional stirring for 1 h. The solvents were removed under reduced pressure and the oily residue was re-dissolved in ethanol/Na-acetate solution. The insoluble material was filtered and the filtrate, mostly the mono conjugates as was shown by analytical HPLC and MS, was purified by a C18 preparative HPLC column (50 mm X 200 mm, Waters, Madison, USA). The pure conjugates were obtained by a stepwise gradient method from buffer A: H_2O/ethanol 90/10, to buffer B: ethanol. The mono oleyl conjugate eluted at 80% buffer B and the bis-oleyl conjugate eluted at 100% buffer B. The insoluble material, mostly the bis-conjugate was dissolved in DCM and purified by flash chromatography using a pre-packed silica column (Büchi). A gradient solvent system from 100% dichloromethane (DCM) to DCM/methanol (70/30 by volume) was used to elute the material. Alternatively, the crude mixed sample was dissolved in excess Na-acetate and Ethanol solution and was loaded on the preparative HPLC column to separate the mono- and bis-conjugates. The pure title compounds were dried under reduced pressure to obtain 0.7 g and 3.6 g of the mono- and bis-conjugate respectively with an overall yield of 60%.

DTPA-MO; MS: 642 (negative ion), ^{1}H NMR (400MHz, D_2O, ref. HOD at δ=4.8) 5.38 (s, 2H, $CH{=}CH$), 4.15 (s, 2H, α CH_2-O), 3.64 (s, 8H, N- CH_2–CO), 3.35 (s, 2H, N- CH_2 –CO), 3.33(s, 4H, N- CH_2), 3.17 (s, 2H, N- CH_2), 3.03 (s, 2H, N- CH_2), 2.05(s, 4H, CH_2-CH=CH), 1.67 (s, 2H, β CH_2), 1.32 (24H, CH_2), 0.92 (3H, CH-CH_3).

DTPA-BO; MS: 893.4 (negative ion), ^{1}H NMR in CD$_3$OD (δ): 5.36 (dt, 4H, J= 5.7, 16 Hz, C*H*=C*H*), 4.14 (t, 4H, J= 6 Hz, αCH_2-O), 3.96 (s, 2H, N- CH_2 –CO), 3.66 (s, 4H, N- CH_2 –CO), 3.60 (s, 4H, N- CH_2 –CO), 3.49 (t, 4H, J=6 Hz, N- CH_2), 3.20 (t, 4H, J= 5 Hz, N- CH_2 –CO), 2.05 (dd, 8H, J= 5, 6 Hz, CH_2-CH=CH), 1.66 (m, 4H, βCH_2), 1.33 (m, 48 H, CH_2), 0.92 (t, 6H, CH_3).

N-mono-(carboxy- 3,7,11,15-tetramethylhexadecanyl)- Diethylene triamine-N-N′,N″-tetraacetic acid (DTPA-MP), and N,N″-bis -(carboxy-3,7,11,15-tetramethylhexadecanyl)- Diethylene triamine-N-N′,N″- triacetic acid (DTPA-BP)

The above title compounds were synthesized using a similar method as described above with an overall yield of 51%.

DTPA-MP; MS: 672.9 (negative ion), ^{1}H NMR in DMSO-d6 (δ): 4.07 (m, 2H, αCH_2-O), 3.52 (s, 2H, N- CH_2 –CO), 3.47 (s, 2H, N- CH_2 –CO), 3.42 (s, 6H, N- CH_2 –CO), 2.92 (t, 4H, *J*= 6 Hz, N- CH_2), 2.84 (t, 4H, *J*= 6 Hz, N- CH_2 –CO), 1.65-0.95 (m, 24 H, C*H*-CH$_2$, O-CH$_2$-CH_2 , CH-CH_2), 0.8-0.9 (m, 15 H, CH-CH_3).

DTPA-BP; MS: 953.9 (negative ion), ^{1}H NMR in CD3OD (δ): 4.19 (m, 4 H, CH_2-O), 3.95(s, 2H, N- CH_2 –CO), 3.66 (s, 4H, N- CH_2 –CO), 3.59 (s, 4H, N- CH_2 –CO), 3.49 (t, 4H, N-CH_2), 3.20 (t, 4H, N- CH_2 –CO), 1.84 -0.94 (m, 48H, C*H*-CH$_2$, O-CH$_2$-CH_2 , CH-CH_2), 0.94-0.72 (m, 30H, CH_3).

Gd-DTPA-MO

To a solution of DTPA-MO (500 mg, 0.778 mmol) in 10 mL of water, was added 3 mL of 2M Na-acetate solution. Gadolinium acetate (262 mg, 0.784 mmol), dissolved in 4 mL water, was subsequently added and the reaction mixture stirred for 30 min at ambient temperature. ESI/MS revealed the completion of the reaction mixture, showing the disappearance of the peak at 642 due to the non-complexed sample and the appearance of a peak at 797 due to the complexed amphiphile. The sample was purified on a preparative HPLC to obtain 273 mg of the pure sample in 44% yield.

Gd-DTPA-BO

To a solution of DTPA-BO (892 mg, 1 mmol) in 10 mL Ethanol, was added 4 mL of 2M Na-acetate solution. Gd-acetate (334 mg, 1 mmol), dissolved in 5 mL water, was added and the mixture was stirred for 30 minutes. The MS analysis showed the 100% chelation of the Gd by disappearance of the peak at 892 and emergence of two new peaks at 1047 and 1070 (the Na-salt) in the positive ion mode. The solvents were evaporated to dryness and the residue was re-dissolved in DCM and purified on a flash chromatography silica column using DCM and Methanol as solvent A and B respectively. 636 mg of pure sample was obtained with an overall yield of 60%. The NMR spectrum of the sample showed very broad peaks for the proton NMR of the DTPA headgroup. The broad peak is consistent with the properties of paramagnetic materials.

Gd-DTPA-MP and -BP

The title compounds were synthesized according to the methods used for GD-DTPA-MO and –BO respectively.   The MS showed one single peak at 827 for Gd-DTPA-MP and two peaks at 1108 and its related Na-salt at 1131 respectively for Gd-DTPA-BP. The yield of the reaction was 47% and 52% for the respective complexes.

Methods

High Performance Liquid Chromatography (HPLC)

Analytical HPLC was performed on Waters HPLC equipment (Waters Corporation, Milford, MA, USA), comprising of a 600 solvent delivery system with a 600 automated gradient controller and an Alltech 2000 Evaporative Light scattering Detector (ELSD). A gradient run with a flow rate of 1 mL/min was used to elute samples on a Phenomenex Gemini C18 column (5 μM, 4.6 X150 mm), starting with 80% solvent A to 100% solvent B within 8 min. The total time for one run was 14 min. The mobile phases consisted of A) Acetonitrile (ACCN)/H_2O (50/50) and 0.05% (v/v) trifluoroacetic acid (TFA) and B) THF/ACCN (60/40) and 0.05% TFA by volume. The ELSD nebulizer temperature and nitrogen gas flow were set at 103 $^{\circ}$C and 2.9 L/min respectively. No TFA was used in the mobile solvents when analyzing the Gd complexed amphiphiles.

Preparative HPLC

Separation of the mono- and bis-DTPA-amphiphile conjugates were performed either on Waters preparative LC 4000 system using a PrePak C_{18} reverse phase column (100X 40 mm) or the Reveleris® Flash System (Grace, Australia) using a C_{18} reverse phase cartridge (12g). Both systems were eluted with a linear gradient with water and ethanol as the mobile phase and at flow rates of 40 ml/min and 36 ml/min respectively. Detection of the peaks were performed on a Waters 2487 Dual λ absorbance UV detector, with the wavelength set at 220 nm. Purification on Reveleris® Flash system was tracked with a dual λ absorbance UV detector and an ELSD detector.

Preparative Flash Chromatography

Purification of the bis- conjugates and their complexes were conducted by a Sepacore Büchi flash chromatography system composed of two pumps (module C-60) and a pump manger (C-615). Samples were loaded on Büchi pre-packed silica columns, using Dichloromethane (DCM) and methanol as solvent A and B, respectively. A linear gradient from 0-50% Methanol was used to obtain the pure samples. The pure fractions were identified by TLC, analytical HPLC and MS analyses.

Electro-spray ionization mass spectroscopy (ESI-MS)

A thermo Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) equipped with an ESI interface was used for MS analyses of the neat amphiphiles and their Gd complexes. Samples were infused using a syringe pump. Negative or positive ions were detected at an ion spray voltage of 4.5 kV, the capillary temperature of 250 °C and the capillary voltage of 10V.

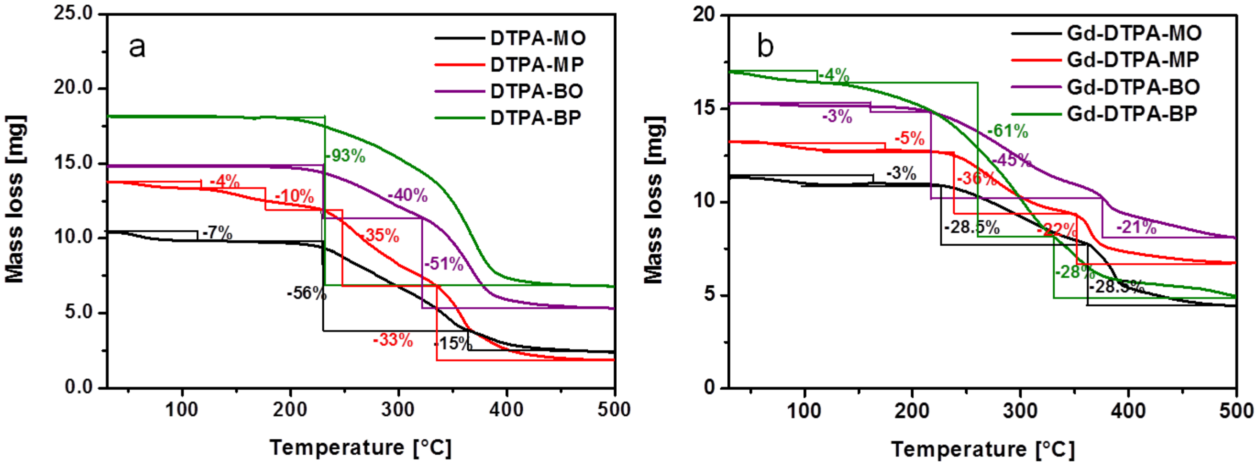


Figure 1S. TGA of (a): DTPA amphiphiles and (b): their Gd complexes

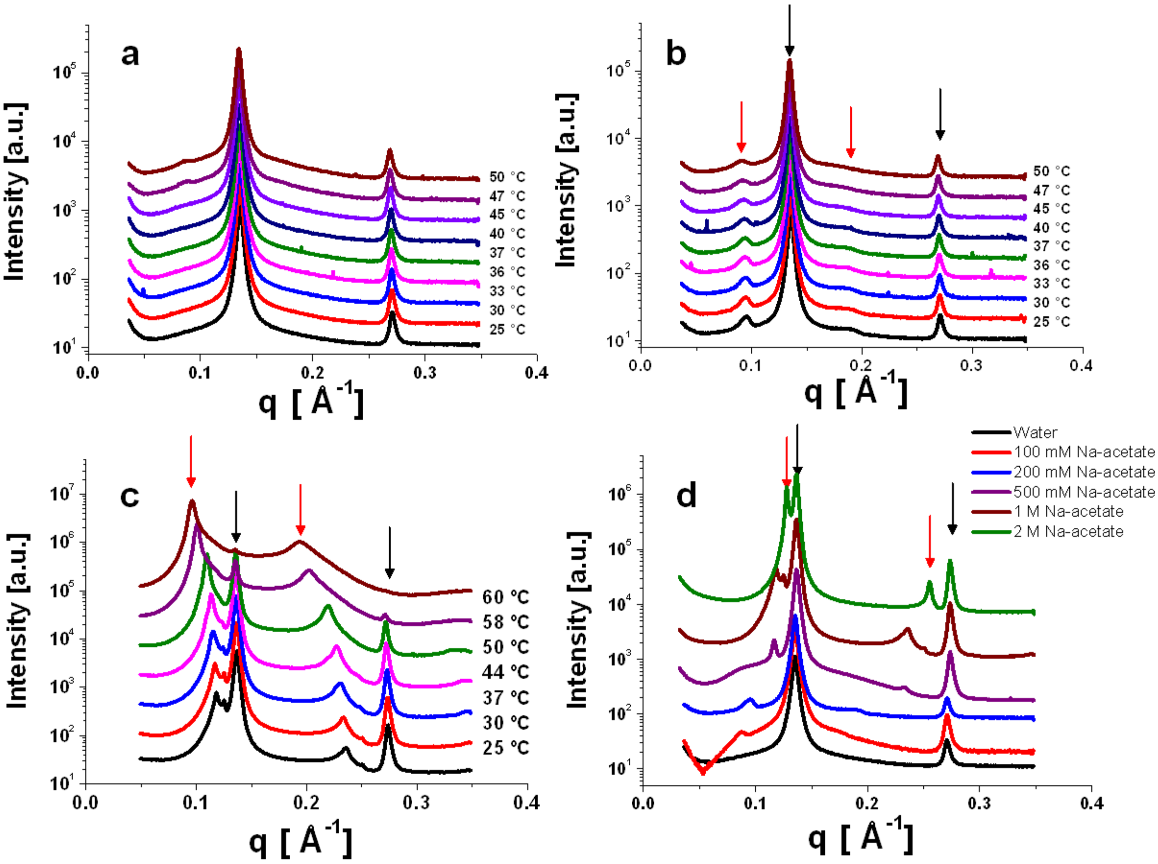
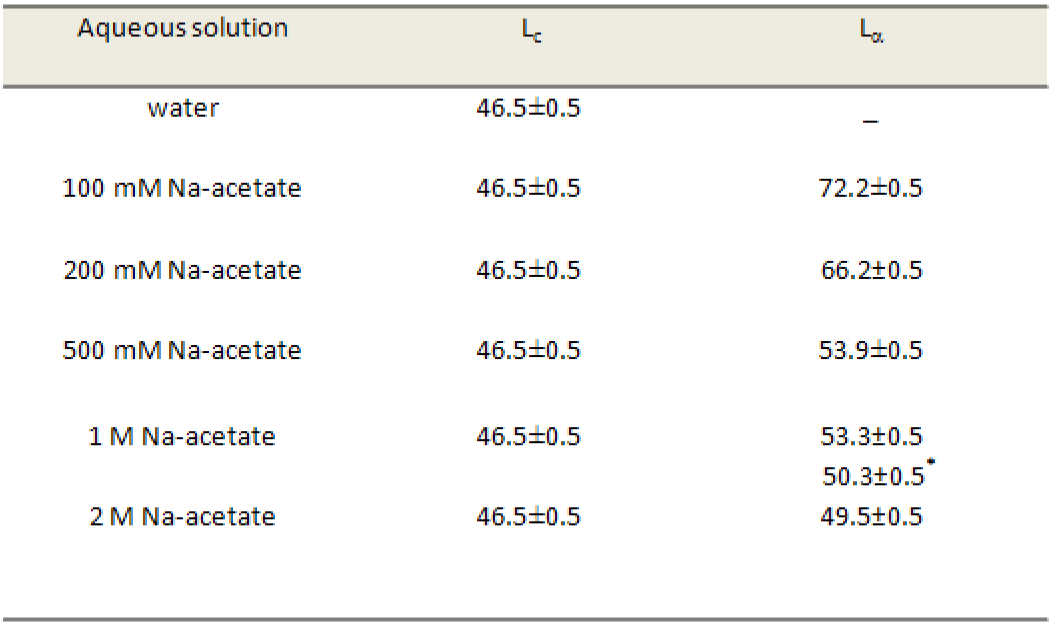


Figure 2S. 1-D diffraction pattern of bulk phases DTPA-BO lyotropic liquid crystalline structures hydrated with 70 weight % of aqueous solutions; a. water, b. 200 mM Na-acetate, c. 1 M Na-acetate, and d. hydration at varying ionic state at 25 °C. black and red arrows show the scattering peaks of L_α phase 1 and 2 respectively.



Aqueous solution	L_c	L_α
water	46.5±0.5	_
100 mM Na-acetate	46.5±0.5	72.2±0.5
200 mM Na-acetate	46.5±0.5	66.2±0.5
500 mM Na-acetate	46.5±0.5	53.9±0.5
1 M Na-acetate	46.5±0.5	53.3±0.5 50.3±0.5*
2 M Na-acetate	46.5±0.5	49.5±0.5

Table 1. Repeat distance of the lamellar lyotropic phases of DTPA-BO amphiphile formed at 70% by weight water and Na-acetate solutions with varying concentrations at 25 °C.(*) shows the lattice parameter of the third lamellar phase at 1 M Na-acetate solution.

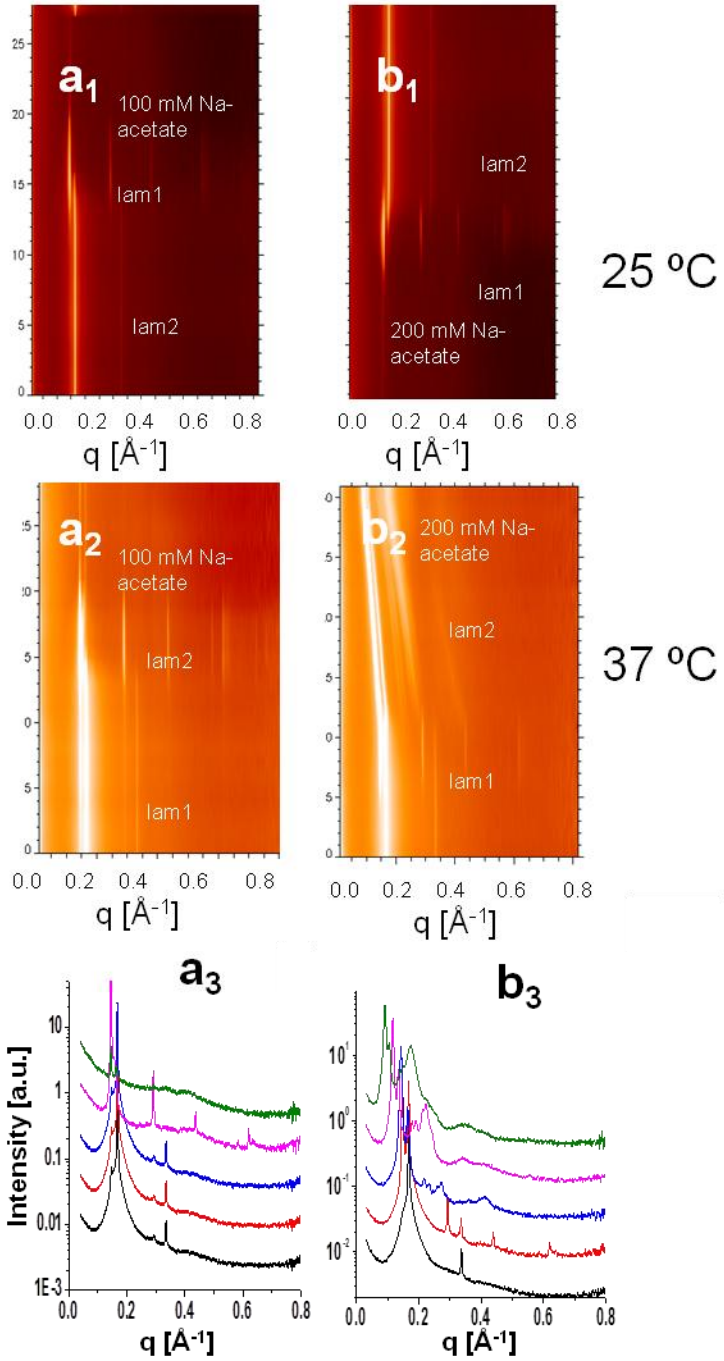


Figure 3S. Contour X-ray diffraction plots of DTPA-BP flooding with aqueous solutions of 100, and 200 mM Na-acetate solutions and at two different temperatures 25 ºC (a_1 and b_1) and 37 ºC (a_2 and b_2). 1D scattering patterns of the representative bands at 37 ºC are plotted in a_3 and b_3.

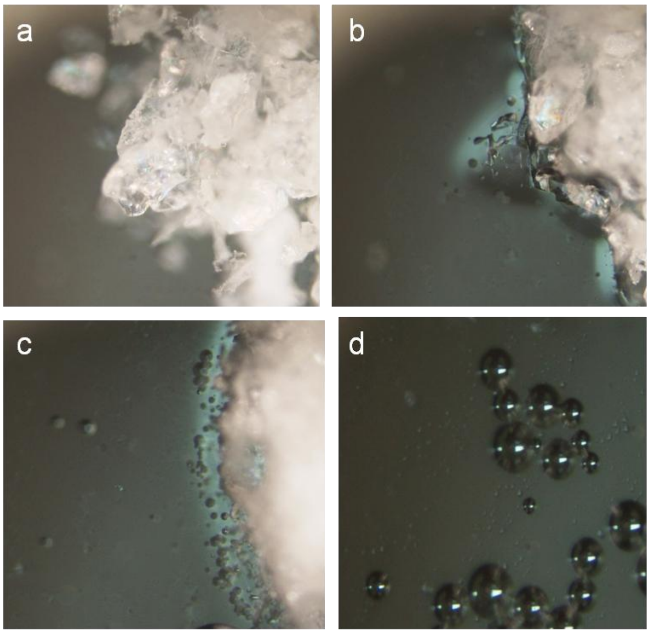


Figure 4S. POM images of water penetration into Gd-DTPA-MO at 25 ºC; (a) Water penetrated very fast into the neat amphiphile and an isotropic phase, typical for micellar phase was formed in the boundary (b, c). After 10 min water penetrated into inner phase of the neat amphiphile and only an isotropic dark band with many round flowing bubbles was remained (d).

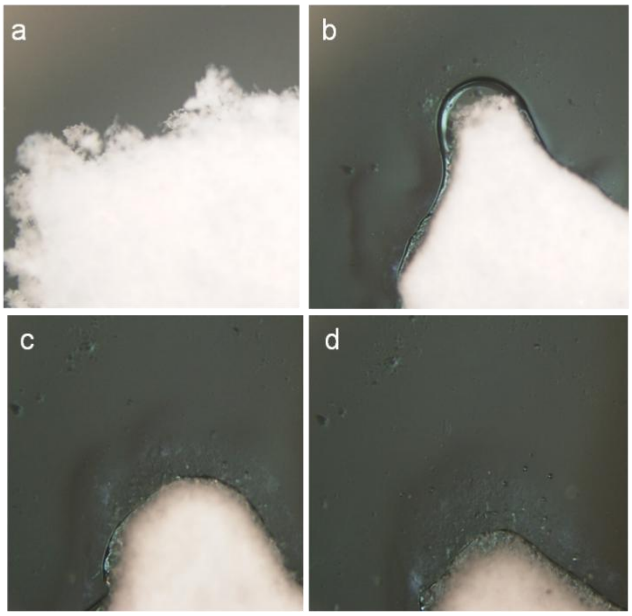


Figure 5S. POM images of flooding Gd-DTPA-MP (a) at 25 ºC. Water penetrated very fast into the neat amphiphile and generated an isotropic band of micellar phase (b, c and d).

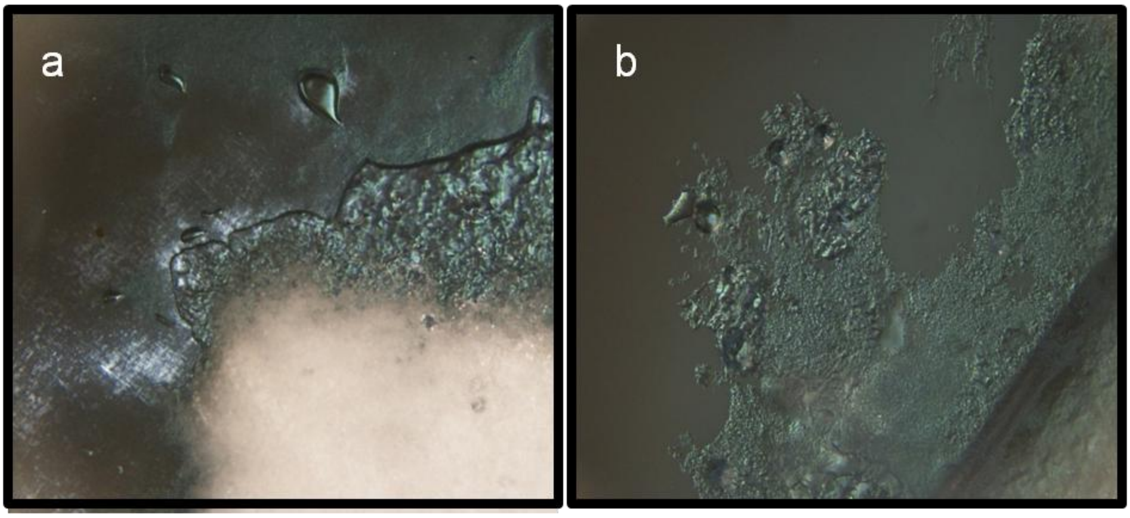


Figure 6S. POM images of water penetration into a. Gd-DTPA-BO, b. Gd-DTPA-BP