Supporting information for

Influence of electrostatic interactions on the release of charged molecules from lipid cubic phases

*Renata Negrini, Antoni Sánchez-Ferrer, Raffaele Mezzenga**

ETH Zurich

Food & Soft Materials Science, Institute of Food Nutrition & Health, Department of Health Science & Technology, Schmelzbergstrasse 9, LFO E23, 8092 Zürich, Switzerland

*Corresponding author: email: raffaele.mezzenga@hest.ethz.ch

Pyridin-4-ylmethyl linoleate (PML) Synthesis

Pyridin-4-ylmethyl linoleate was prepared following the Steglich esterification (DCC coupling reaction) between linoleic acid (LA) and pyridinemethanol. In a 500 mL two-necked round-bottomed flask equipped with a magnetic stirrer, condenser, and nitrogen inlet, 10 g (35.66 mmol, 1 eq) of linoleic acid (LA), 3.892 g (35.66 mmol, 1 eq) of pyridinemethanol, 1.3 g (10.64 mmol, 0.3 eq) of 4-dimethylaminopyridine (DMAP) and 8.1 g (39.26 mmol, 1.1 eq) of *N*,*N*'-dicyclohexylcarbodiimide (DCC) were mixed together and purged with nitrogen for 10 min. Freshly distilled dichloromethane (150 mL) was added and the mixture cooled in an ice bath while stirring for 12 h. Afterwards, the solvent was removed under vacuum, and the residue was dissolved in *n*-hexane and separate from the impurities by washing three times with water. Finally, the solvent was removed under vacuum obtaining a yellowish oil compound. Yield: 9.65 g (72.8 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.58 (2H, dd, Ar, *J* = 4.4 Hz, *J* = 1.6 Hz), 7.23 (2H, dd, Ar, *J* = 4.4 Hz, *J* = 1.6 Hz), 5.34 (4H, m, C*H*=C*H*), 5.11 (2H, s, ArCH_2OCO), 2.75 (2H, CH=CH-CH_2-CH=CH, Al, *J* = 6.4 Hz), 2.39 (2H, t, CH_2COO, *J* = 7.5 Hz), 2.03 (4H, q, CH_2-CH_2-CH=CH, *J* = 6.9 Hz), 1.65 (3H, quint, CH_2CH_2COO, *J* = 7.4 Hz), 1.30 (18H, m, CH_2), 0.87 (4H, t, CH_3, *J* = 6.9 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.2 (CO), 150.1 (2 ArCH), 145.2 (ArC), 130.3-130.1 (*C*H=CH-CH_2-CH=*C*H), 128.2-128.0 (CH=*C*H-CH_2-*C*H=CH), 122.0 (2 ArCH), 64.2 (ArCH_2), 34.2 (*C*H_2COO), 31.0, 29.7, 29.5, 29.3, 29.2, 29.2 (CH_2), 27.3 (2 *C*H_2-CH=CH), 25.8 (CH=CH-*C*H_2-CH=CH), 25.0 (*C*H_2CH_2COO), 22.7 (*C*H_2CH_3), 14.2(CH_3) ppm. FTIR (ATR-diamond): *v* = 3009 (st, ArC-H), 2926 (st, C-H), 2854 (st, OC-H), 1742 (st, C=O), 1604 (st, Pyr), 1160 (st, C-O), 993 (δ, ArC-C), 796 (γ, ArCH_2), 724 cm^{-1} (γ, CH_2). UV (EtOH, 25 °C): λ_{max} = 256 nm (ε = 1760 L·mol^{-1}·cm^{-1}).

Characterization Techniques

^{1}H and ^{13}C NMR experiments were carried out at room temperature on a Bruker Avance Spectrometer operating at 400 and 100 MHz, respectively, and using $CDCl_3$ as solvents and as the internal standard. Fourier-transform infrared (FTIR) spectra of solid samples were recorded at room temperature with a Varian 640 FTIR spectrometer and a MKII Golden Gate single attenuated total reflection (ATR) system.

Small Angle X-ray Scattering data.

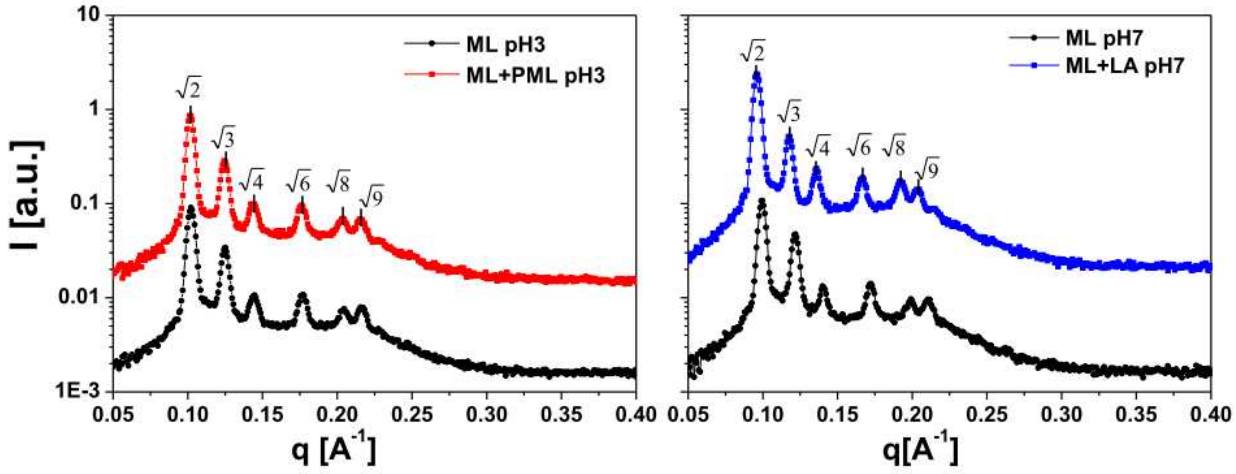

Figure S1: 1D SAXS profiles for the reverse bicontinuous cubic phases of the pH responsive LLCs at pH 3 (A) and 7 (B). The different patterns represent ML – the neutral mesophase (black) – and the two phases containing 2 wt% PML (red) at pH 3, and 0.5 wt% LA (blue) at pH 7. All the three systems maintained their structure throughout the performed experiments as indicated by the SAXS diffraction peaks in the ratio: √2, √3, √4, √6, √8, √9.

Values calculated from all the models employed.

Table S1: Fitting parameters and the corresponding apparent diffusion coefficient obtained from the Higuchi and the Ritger-Peppas models.

	Higuchi			Ritger-Peppas			
SYSTEM/pH	k_H (x10^{-3}) (h^{-1})	D (cm^2h^{-1}) (x10^{-4})	R	k_{RP} (x10^{-3}) (h^{-1})	n	D (cm^2h^{-1}) (x10^{-4})	R
P(+)D/pH7	8.8 ± 0.4	6.4 ± 0.3	0.98	12.4 ± 0.3	0.59 ± 0.01	9.0 ± 0.2	0.9991
P(+)LA/pH7	5.1 ± 0.2	3.7 ± 0.2	0.991	7.4 ± 0.4	0.59 ± 0.02	5.4 ± 0.3	0.9991
P(+)D/pH3	14.8 ± 0.4	10.8 ± 0.3	0.98	16 ± 1	0.53 ± 0.02	12 ± 1	0.995
P(+)PML/pH3	16.6 ± 0.4	12.1 ± 0.3	0.990	17 ± 1	0.51 ± 0.02	13 ± 1	0.998
A(-)D/pH3	6.7 ± 0.4	4.9 ± 0.3	0.97	11 ± 1	0.63 ± 0.02	7.7 ± 0.4	0.997
A(-)PML/pH3	2.2 ± 0.2	1.6 ± 0.1	0.95	5.0 ± 0.3	0.66 ± 0.02	3.7 ± 0.2	0.9994
A(-)D/pH7	6 ± 1	4.7 ± 0.4	0.97	11.7 ± 0.5	0.69 ± 0.02	8.5 ± 0.3	0.997
A(-)LA/pH7	10.6 ± 0.5	7.7 ± 0.4	0.97	14 ± 1	0.58 ± 0.02	10 ± 1	0.997
C(n)D/pH7	51 ± 1	37 ± 1	0.996	57 ± 4	0.53 ± 0.02	42 ± 3	0.998
C(n)LA/pH7	50 ± 1	36.1 ± 0.5	0.996	54 ± 2	0.52 ± 0.01	39 ± 2	0.998
P(+)LA/pH7	5.1 ± 0.2	3.7 ± 0.2	0.991	7.4 ± 0.4	0.59 ± 0.02	5.4 ± 0.3	0.9991
P(+)LA/pH7/ 150	8.4 ± 0.4	6.1 ± 0.3	0.96	12.0 ± 0.4	0.62 ± 0.01	8.6 ± 0.3	0.995

Table S2: Fitting parameters and the corresponding apparent diffusion coefficient, maximum concentration at the equilibrium and initial velocity obtained from the Weibull and the Peleg models.

	Weibull						Peleg					
SYSTEM/pH	*A*	*B*	k_W (x10^{-3}) (h^{-1})	*D* (x10^{-4}) (cm^2h^{-1})	C_{max}	*R*	k_1 (h)	k_2	*D* (x10^{-4}) (cm^2h^{-1})	C_{max}	V_0 (x10^{-2}) (h^{-1})	*R*
P(+)D/pH7	0.90 ± 0.03	0.73 ± 0.02	30 ± 2	22 ± 2	0.91 ± 0.03	0.9996	22 ± 1	1.08 ± 0.03	33 ± 2	0.93 ± 0.03	4.5 ± 0.2	0.998
P(+)LA/pH7	0.7 1± 0.03	0.80 ± 0.05	29 ± 3	21 ± 3	0.72 ± 0.03	0.998	35 ± 3	1.2 ± 0.1	21 ± 2	0.81 ± 0.03	2.9 ± 0.2	0.996
P(+)D/pH3	1.09 ± 0.03	0.68 ± 0.01	30 ± 2	22 ± 2	1.11 ± 0.03	0.9997	16 ± 1	0.93 ± 0.03	46 ± 3	1.07 ± 0.03	6.3 ± 0.4	0.998
P(+)PML/pH3	1.12 ± 0.02	0.65 ± 0.01	31 ± 2	22 ± 1	1.14 ± 0.02	0.9998	14 ± 1	0.94 ± 0.03	52 ± 4	1.06 ± 0.03	7.1 ± 0.5	0.996
A(-)D/pH3	0.82 ± 0.01	0.80 ± 0.01	28 ± 1	21 ± 1	0.83 ± 0.01	0.9998	30 ± 1	1.07 ± 0.01	24 ± 1	0.94 ± 0.01	3.3 ± 0.1	0.9993
A(-)PML/pH3	0.58 ± 0.01	0.79 ± 0.02	20 ± 1	14 ± 1	0.58 ± 0.01	0.9995	59 ± 2	1.54 ± 0.03	12 ± 1	0.65 ± 0.01	1.70 ± 0.05	0.9990
A(-)D/pH7	0.97 ± 0.05	0.80 ± 0.04	20 ± 3	15 ± 2	0.98 ± 0.05	0.9990	35 ± 1	0.90 ± 0.02	21 ± 1	1.11 ± 0.03	2.9 ± 0.1	0.9990
A(-)LA/pH7	1.06 ± 0.02	0.70 ± 0.01	24 ± 1	17 ± 1	1.07 ± 0.02	0.9998	22 ± 1	0.91 ± 0.02	32 ± 2	1.10 ± 0.03	4.4 ± 0.2	0.998
C(n)D/pH7	1.02 ± 0.01	0.66 ± 0.01	112 ± 4	82 ± 3	1.07 ± 0.01	0.99990	4.6 ± 0.3	0.96 ± 0.02	158 ± 10	1.04 ± 0.02	22 ± 1	0.995
C(n)LA/pH7	1.09 ± 0.02	0.63 ± 0.01	92 ± 4	66 ± 3	1.15 ± 0.02	0.99990	4.6 ± 0.3	0.98 ± 0.03	159 ± 12	1.02 ± 0.03	22 ± 1	0.995
P(+)LA/pH7	0.71 ± 0.03	0.80 ± 0.05	29 ± 3	21 ± 3	0.72 ± 0.03	0.998	35 ± 3	1.2 ± 0.1	21 ± 2	0.81 ± 0.03	2.9 ± 0.2	0.996
P(+)LA/pH7/ 150	0.88 ± 0.02	0.77 ± 0.02	30 ± 2	22 ± 1	0.89 ± 0.02	0.9998	25 ± 1	1.03 ± 0.02	29 ± 1	0.97 ± 0.02	4.0 ± 0.2	0.9990