

Initiation of Lipid Autoxidation by ABAP at pH 4 – 10 in SDS micelles.

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Supplementary Material

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Table S1. Concentration of PMHC, rates of non-inhibited autoxidation (R_{ox}) and induction times (τ_{ind}) for peroxidation of methyl linoleate-SDS micelles initiated by 10 mM ABAP at pH 4.

[PMHC] mM	R_{ox} % O ₂ min ⁻¹	$R_{ox} \times 10^7$ mol O ₂ dm ⁻³ s ⁻¹	τ_{ind} minutes
0	6.8232	3.57	
	6.1293	3.21	
	6.0364	3.16	
	6.318	3.31	
	6.0189	3.15	
	4.5238	2.37	
	$R_{ox}=3.13$		
0.0043	6.4524	3.38	13
	6.1757	3.23	15
	5.9511	3.11	13
	5.4421	2.85	14
	$R_{ox}=3.14$	$\tau_{ind}=13.25$	
0.0086	6.5489	3.43	27
	6.2053	3.25	29
	4.8109	2.52	27
	6.289	3.29	29
	5.7634	3.02	25
	6.1735	3.23	24
	$R_{ox}=3.12$	$\tau_{ind}=26.83$	
0.0173	5.1564	2.70	50
	4.9498	2.59	51
	5.436	2.84	51
	5.7868	3.03	50
	4.7793	2.50	64
	5.7139	2.99	64
	$R_{ox}=2.78$	$\tau_{ind}=55.00$	

Table S2. Concentration of PMHC, rates of non-inhibited autoxidation (R_{ox}) and induction times (τ_{ind}) for peroxidation of methyl linoleate-SDS micelles initiated by 10 mM ABAP at pH 5.

[PMHC] mM	R_{ox} % O ₂ min ⁻¹	$R_{ox} \times 10^7$ mol O ₂ dm ⁻³ s ⁻¹	τ_{ind} minutes
0	4.594	2.40	
	5.6796	2.97	
	6.8368	3.58	
	6.5674	3.44	
	6.4389	3.37	
	5.2588	2.75	
	$R_{ox}=3.09$		
0.0043	4.8501	2.54	18
	4.8159	2.52	17
	5.5774	2.92	22
	5.5905	2.93	19
	$R_{ox}=2.73$	$\tau_{ind}=19.00$	
0.0086	5.42596	2.84	43
	6.4575	3.38	42
	4.9359	2.58	43
	4.6699	2.44	55
	5.375	2.81	45
	5.4174	2.84	45
	$R_{ox}=2.82$	$\tau_{ind}=45.50$	
0.0173	4.25573	2.23	81
	6.01647	3.15	76
	4.5665	2.39	99
	4.2833	2.24	98
	4.2732	2.24	90
	4.8523	2.54	96
	$R_{ox}=2.46$	$\tau_{ind}=89.67$	

Table S3. Concentration of PMHC, rates of non-inhibited autoxidation (R_{ox}) and induction times (τ_{ind}) for peroxidation of methyl linoleate-SDS micelles initiated by 10 mM ABAP at pH 6.

[PMHC] mM	R_{ox} % O ₂ min ⁻¹	$R_{ox} \times 10^7$ mol O ₂ dm ⁻³ s ⁻¹	τ_{ind} minutes
0	5.4359	2.84	
	3.7468	1.96	
	4.4979	2.35	
	3.3608	1.76	
	4.8616	2.54	
	6.971	3.65	
	7.5561	3.95	
	5.1979	2.72	
	6.2296	3.26	
	$R_{ox}=2.78$		
0.0043	4.1614	2.18	29
	4.5539	2.38	33
	4.8085	2.52	37
	4.7983	2.51	33
	5.6624	2.96	31
	6.7532	3.53	36
	6.2974	3.30	31
	6.7843	3.55	35
		$R_{ox}=2.87$	$\tau_{ind}=33.57$
0.0086	5.3236	2.79	67
	2.8749	1.50	59
	5.743	3.01	65
	4.486	2.35	64
	6.6154	3.46	70
	6.1921	3.24	74
		$R_{ox}=2.72$	$\tau_{ind}=66.5$
0.0173	5.90429	3.09	144
	6.29868	3.30	142
	6.3122	3.30	140
	5.9855	3.13	156
	6.0654	3.17	152
	6.2318	3.26	153
		$R_{ox}=3.07$	$\tau_{ind}=148.0$

Table S4. Concentration of PMHC, rates of non-inhibited autoxidation (R_{ox}) and induction times (τ_{ind}) for peroxidation of methyl linoleate-SDS micelles initiated by 10 mM ABAP at pH 7.

[PMHC] mM	R_{ox} % O ₂ min ⁻¹	$R_{ox} \times 10^7$ mol O ₂ dm ⁻³ s ⁻¹	τ_{ind} minutes
0	7.77618	4.07	
	7.1846	3.76	
	8.9147	4.67	
	7.3838	3.86	
	$R_{ox}=3.27$		
0.0043	8.9647	4.69	26
	7.244	3.79	28
	9.399	4.92	29
	7.7538	4.06	29
	6.1632	3.23	28
	$R_{ox}=4.14$	$\tau_{ind}=28.00$	
0.0086	9.2105	4.82	56
	8.8736	4.64	54
	10.2867	5.38	53
	8.9645	4.69	46
	9.7194	5.09	56
	9.1703	4.80	53
	$R_{ox}=4.90$	$\tau_{ind}=52.50$	
0.0173	10.3345	5.41	110
	8.26	4.32	96
	10.4218	5.45	97
	7.5846	3.97	101
	9.1533	4.79	111
	7.5406	3.95	114
	$R_{ox}=4.65$	$\tau_{ind}=104.83$	

Table S5. Concentration of PMHC, rates of non-inhibited autoxidation (R_{ox}) and induction times (τ_{ind}) for peroxidation of methyl linoleate-SDS micelles initiated by 10 mM ABAP at pH 8.

[PMHC] mM	R_{ox} % O ₂ min ⁻¹	$R_{ox} \times 10^7$ mol O ₂ dm ⁻³ s ⁻¹	τ_{ind} minutes
0	6.4098	3.35	
	6.3211	3.31	
	6.2581	3.28	
	6.7647	3.54	
	6.8939	3.61	
	6.4581	3.38	
	6.7787	3.55	
	8.043	4.21	
	$R_{ox}=3.53$		
0.0043	6.1975	3.24	32
	6.5368	3.42	31
	6.4385	3.37	26
	6.6308	3.47	29
	7.5418	3.95	26
	7.2312	3.78	29
	6.8436	3.58	31
		$R_{ox}=3.55$	$\tau_{ind}=39.14$
0.0086	6.3288	3.31	60
	6.5859	3.45	60
	6.5956	3.45	65
	7.2009	3.77	61
	5.842	3.06	64
	5.3134	2.78	65
		$R_{ox}=3.30$	$\tau_{ind}=62.50$
0.0173	6.2929	3.29	106
	6.6266	3.47	100
	6.3885	3.34	118
	6.3536	3.33	118
	5.1662	2.70	136
	5.7355	3.00	133
	4.5226	2.37	141
	5.0622	2.65	131
	$R_{ox}=3.02$	$\tau_{ind}=122.9$	

Table S6. Concentration of PMHC, rates of non-inhibited autoxidation (R_{ox}) and induction times (τ_{ind}) for peroxidation of methyl linoleate-SDS micelles initiated by 10 mM ABAP at pH 9.

[PMHC] mM	R_{ox} % O ₂ min ⁻¹	$R_{ox} \times 10^7$ mol O ₂ dm ⁻³ s ⁻¹	τ_{ind} minutes	
0	12.3976	6.49		
	10.0605	5.26		
	12.3236	6.45		
	12.9200	6.76		
	12.2600	6.42		
	$R_{ox}=6.28$			
0.0043	8.96	4.69	30	
	10.4491	5.47	28	
	10.7127	5.61	30	
	10.7709	5.64	28	
	11.1782	5.85	31	
	11.1606	5.84	31	
	11.0079	5.76	34	
	14.9048	7.80	25	
	12.6182	6.60	28	
	11.875	6.21	25	
	$R_{ox}=5.95$	$\tau_{ind}=29.00$		
0.0086	10.5131	5.50	67	
	11.0405	5.78	67	
	10.5633	5.53	71	
	13.4693	7.05	47	
	10.2855	5.38	50	
	10.9867	5.75	55	
	9.4782	4.96	57	
		$R_{ox}=5.71$	$\tau_{ind}=59.14$	
0.0173	7.2407	3.79	127	
	7.8742	4.12	121	
	9.0903	4.76	120	
	8.9473	4.68	124	
	11.263	5.89	97	
	7.9685	4.17	101	
	9.2236	4.83	98	
	9.0927	4.76	102	
		$R_{ox}=4.62$	$\tau_{ind}=111.25$	

Table S7. Concentration of PMHC, rates of non-inhibited autoxidation (R_{ox}) and induction times (τ_{ind}) for peroxidation of methyl linoleate-SDS micelles initiated by 10 mM ABAP at pH 10.

[PMHC] mM	R_{ox} % O ₂ min ⁻¹	$R_{ox} \times 10^7$ mol O ₂ dm ⁻³ s ⁻¹	τ_{ind} minutes
0	11.440	5.99	
	11.582	6.06	
	12.358	6.46	
	$R_{ox}=6.28$		
0.0043	13.070	6.84	31
	12.530	6.56	30
	14.310	7.49	28
	8.578	4.49	32
	12.609	6.60	32
	11.610	6.08	23
	$R_{ox}=6.34$	$\tau_{ind}=29.3$	
0.0086	11.49	6.01	55
	10.97	5.74	56
	12.75	6.67	55
	11.69	6.12	
	$R_{ox}=6.14$	$\tau_{ind}=55.3$	
0.0173	10.02	5.24	104
	9.36	4.90	100
	10.3	5.39	98
	10.002	5.23	95
	$R_{ox}=5.19$	$\tau_{ind}=99.3$	

Table S8. Calculation of kinetic chain length.

pH	$R_{ox} \times 10^7$ / Ms ⁻¹	$R_{inh} \times 10^9$ / Ms ⁻¹	R_{ox}/R_i
4	3.04	10.60	29
5	2.78	6.76	41
6	2.86	4.16	69
7	4.24	5.36	79
8	3.35	4.74	71
9	5.64	4.99	113

Table S9. Measurements of [PMHC] uptake during decomposition of ABAP in SDS micelles at 37°C, [Initiator]₀=4.81mM.

time / s	[PMHC] / M	time / s	[PMHC] / M	time / s	[PMHC] / M	time / s	[PMHC] / M
pH=4		pH=5		pH=6		pH=7	
0	1.90E-03	0	1.74E-03	0	1.77E-03	0	1.95E-03
22800	1.79E-03	22800	1.66E-03	21900	1.72E-03	46800	1.87E-03
72000	1.45E-03	72000	1.45E-03	40200	1.67E-03	87600	1.82E-03
122400	1.10E-03	122400	1.15E-03	85800	1.61E-03	133200	1.76E-03
		165600	9.67E-04	107400	1.49E-03	169200	1.63E-03
				127200	1.46E-03	254400	1.49E-03
	R_f= 1.32E-08 Ms⁻¹		R_f= 9.59E-09 Ms⁻¹		R_f= 4.84E-09 Ms⁻¹		R_f= 3.64E-09 Ms⁻¹
pH=8		pH=9		pH=10			
0	1.83E-03	0	1.81E-03	0	1.78E-03		
40200	1.64E-03	32100	1.67E-03	35100	1.45E-03		
85800	1.45E-03	77400	1.49E-03	81000	1.02E-03		
127200	1.23E-03	113400	1.23E-03	110700	9.03E-04		
172200	9.99E-04	163800	1.06E-03				
		250200	7.03E-04				
	R_f= 9.64E-09 Ms⁻¹		R_f= 9.02E-09 Ms⁻¹		R_f= 8.18E-09 Ms⁻¹		

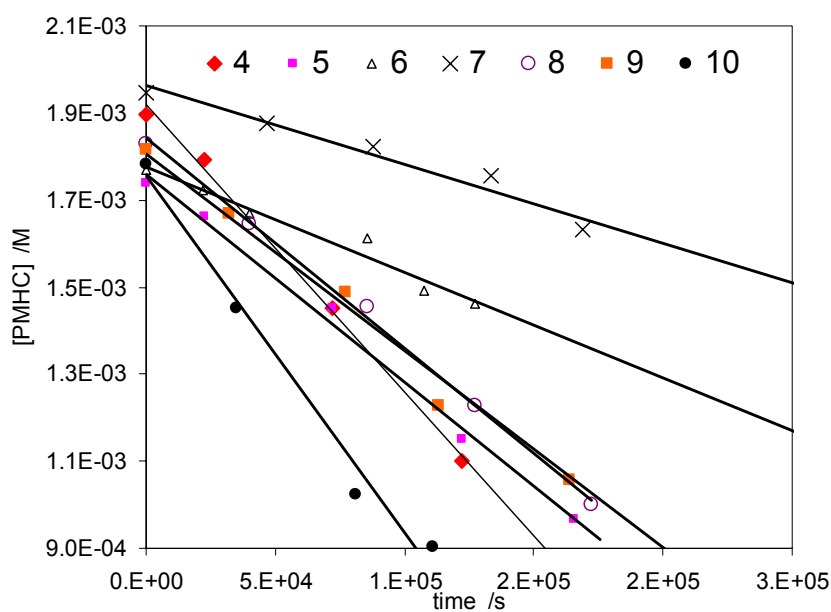


Table S10. Measurements of [PMHC] uptake during decomposition of AIBN in SDS micelles at 37°C, [Initiator]₀=9.88 mM.

time / s	[PMHC] / M	time / s	[PMHC] / M	time / s	[PMHC] / M	time / s	[PMHC] / M
pH=4		pH=5		pH=6		pH=7	
0	1.68E-03	0	1.68E-03	0	1.69E-03	0	1.69E-03
3600	1.67E-03	22800	1.59E-03	24000	1.66E-03	22800	1.66E-03
7200	1.66E-03	72000	1.39E-03	64200	1.57E-03	72000	1.57E-03
14400	1.64E-03	93600	1.31E-03	93000	1.54E-03	93600	1.53E-03
18000	1.62E-03	158400	1.19E-03	150600	1.47E-03	158400	1.43E-03
21600	1.62E-03	360600	8.34E-04				
R_f= 6.37E-09		R_f= 4.52E-09		R_f= 2.95E-09		R_f= 3.40E-09	
pH=8		pH=9		pH=10			
0	1.68E-03	0	1.68E-03	0	1.68E-03		
24000	1.64E-03	24000	1.61E-03	24000	1.55E-03		
64200	1.55E-03	64200	1.46E-03	64200	1.24E-03		
93000	1.52E-03	93000	1.37E-03	93000	1.00E-03		
150600	1.46E-03	150600	1.26E-03	150600	7.80E-04		
R_f= 2.96E-09		R_f= 5.64E-09		R_f= 1.25E-08			

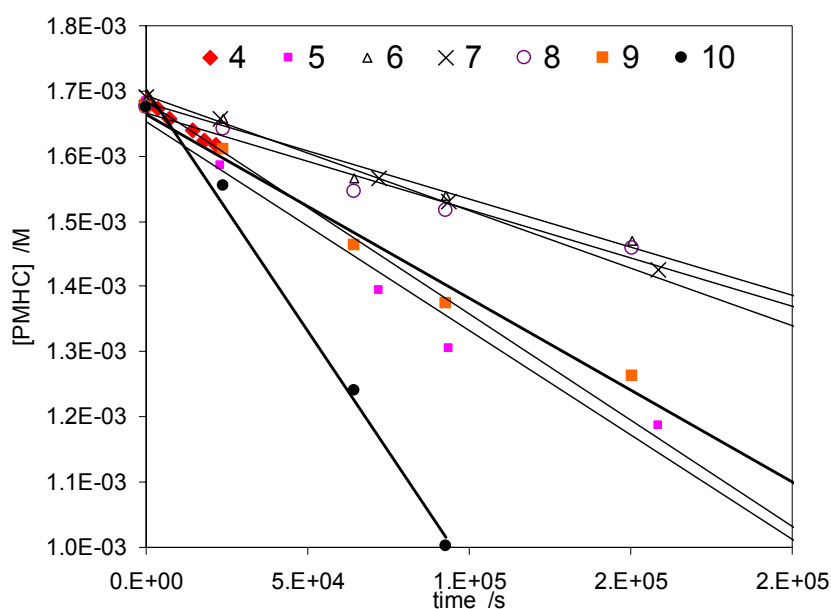
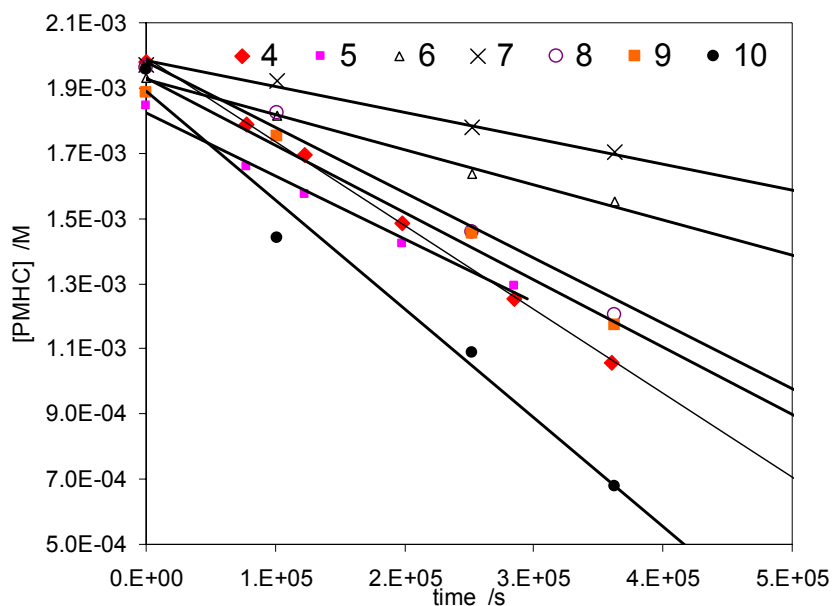


Table S11. Measurements of [PMHC] uptake during decomposition of AMEP in SDS micelles at 37°C, [Initiator]₀=68.1 mM.

time / s	[PMHC] / mM	time / s	[PMHC] / mM	time / s	[PMHC] / mM	time / s	[PMHC] / mM
pH=4		pH=5		pH=6		pH=7	
0	1.98E-03	0	1.85E-03	0	1.93E-03	0	1.97E-03
78000	1.79E-03	78000	1.66E-03	100800	1.82E-03	100800	1.92E-03
123000	1.69E-03	123000	1.57E-03	252600	1.64E-03	252600	1.78E-03
198600	1.48E-03	198600	1.42E-03	362400	1.55E-03	362400	1.70E-03
285000	1.26E-03	285000	1.29E-03	600000	1.28E-03	600000	1.51E-03
360600	1.06E-03	360600	1.13E-03				
R_f = 5.15E-09		R_f = 3.85E-09		R_f = 2.15E-09		R_f = 1.58E-09	
pH=8		pH=9		pH=10			
0	1.96E-03	0	1.89E-03	0	1.96E-03		
100800	1.83E-03	100800	1.75E-03	100800	1.44E-03		
252600	1.46E-03	252600	1.46E-03	252600	1.09E-03		
362400	1.20E-03	362400	1.17E-03	362400	6.80E-04		
600000	8.03E-04	600000	6.74E-04		R_f = 6.68E-09		
R_f = 4.01E-09		R_f = 4.13E-09					



EXPERIMENTAL DETAILS

Preparation of SDS emulsions. Methyl linoleate in acetone was put in glass ampoule and acetone was gently removed under nitrogen flow. Buffered 0.5 M SDS (5 ml) was added and the mixture was shaken on Vortex for two minutes. For experiments with inhibitor (method 1), at that moment the appropriate amount (25-100 μ L) of inhibitor (PMHC) dissolved in acetone was added with microliter syringe.

Oxygen uptake measurements (methods 1 and 2). The emulsion was then transferred into the vessel in thermostated bath of 5300A Biological Oxygen Monitor (Yellow Springs Instruments) equipped with magnetic stirrer. An aeration was conducted during 5 minutes. After that time the initiator stock solution was injected and Clark type electrode was immediately placed in the vessel and the concentration of oxygen was recorded. The electrode was calibrated for air saturated (100%) and degassed (0%) solutions of SDS emulsion.

In Method 1 inhibited autoxidation was monitored in order to determine induction time (see Figure 1 in manuscript), however, when induction period was over, the autoxidation rate was the same as for non-inhibited process. In Tables S1-S7 R_{ox} (rate of non-inhibited process) and induction times are listed.

In Method 2 no inhibitor was used and rate of non-inhibited autoxidation (R_{ox}) was measured.

PMHC uptake measurements (method 3). Buffered and aerated 0.5 M SDS emulsions (volume 10 mL, buffers were the same as described previously) containing PMHC and initiator were incubated at temperatures 37 during 40 hours with magnetic stirring. The initial concentrations of ABAP, AMEP, and AIBN (and [PMHC]₀ in parentheses), were 4.81 (1.93) mM, 68.1 (2.0) mM, and 9.88 (1.7) mM, respectively. Because these emulsions did not contain lipid, all phenol molecules reacted with peroxy radicals generated during initiator decomposition (equation 1 in the text). 50 μ L samples were periodically collected, diluted with methanol and analyzed by HPLC using NovaPack C18 column (3.9 x 150 mm) and Waters 486 UV detector (290 nm). A mixture of acetonitrile-water (80:20 v/v) containing 0.1% acetic acid was used with flow rate 0.3 mL/min. Retention time for PMHC was 6.5 min. The plots of [PMHC] vs time gave excellent straight lines (See Fig. S1-S3), the slopes of these lines gave rates of PMHC uptake, which equals $0.5R_i$.

Buffers. The buffers used for experiments were:

for pH 4.0	18 mL 0.2 M CH ₃ COOH + 82 mL 0.2 M CH ₃ COONa,
for pH 5.0	70.5 mL 0.2 M CH ₃ COOH + 29.5 mL 0.2 M CH ₃ COONa,
for pH 6.0	12.1 mL 0.05 M Na ₂ HPO ₄ + 87.9 mL 0.05 M K ₂ HPO ₄ ,
for pH 7.0	61.2 mL 0.05 M Na ₂ HPO ₄ + 38.8 mL 0.05 M K ₂ HPO ₄ ,
for pH 8.0	100 mL 0.05 M TRIS-HCl + x mL 0,025M NaOH to obtain pH 8
pH 9.0	100 mL 0.05 M TRIS-HCl + x mL 0,025M NaOH to obtain pH 9
pH 10.0	50 mL 0.025 M Na ₂ B ₄ O ₇ + 18.3 mL 0.1 NaOH + 31.7 mL H ₂ O.

The pH was measured and adjusted at 30°C to have ± 0.05 units precision (combined glass electrode). Increase of temperature to 37°C results in 0.02 units of pH change (see Handbook of Chemistry and Physics, 67th Ed., CRC Press, Boca Raton). Before use, all buffers and SDS solutions were kept over

Chelex100 to remove traces of metals. Calculated ionic strength of solutions (0.5M SDS+ buffer+ initiator) was within the range from 0.6 to 0.7.