Supporting information for:

Precipitation of calcium phosphates and calcium carbonates in the presence of differently charged liposomes

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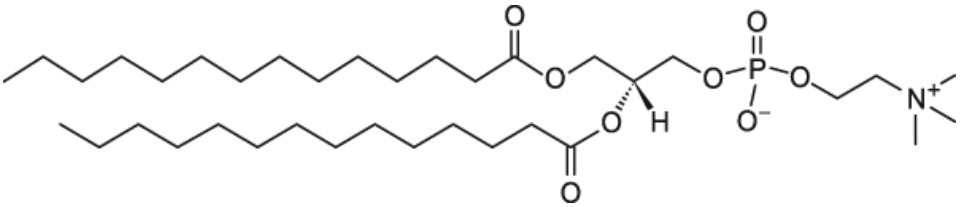
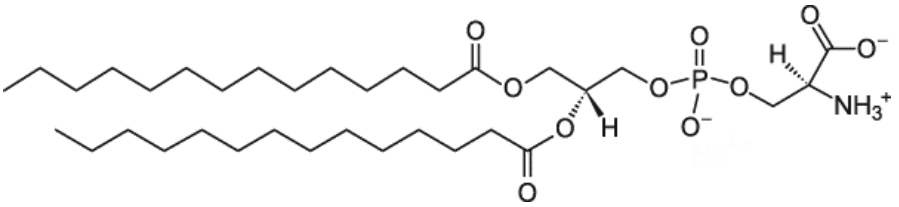
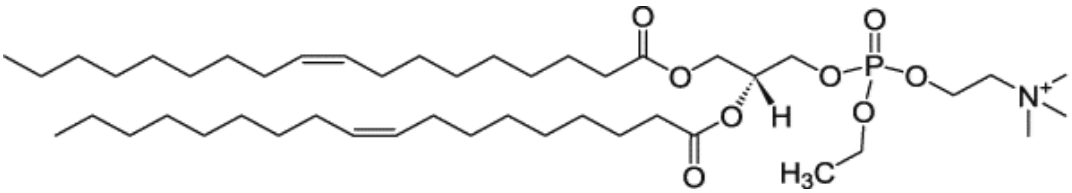
Figure S7. PXRD patterns of the precipitate formed after 10 minutes reaction time in $CaCO_3$ spontaneous precipitation experiments.



DMPC - 1,2-dimyristoyl-sn-glycero-3 phosphocholine



DMPS - 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine



EPC - 1,2-dioleoyl-sn-glycero-3ethylphosphocholine

Figure S1. Molecular structure of the lipids used in this study for the preparation of the liposomes with different surface charge: DMPC is zwitterionic, DMPS is negatively charged and EPC is positively charged at given experimental conditions.

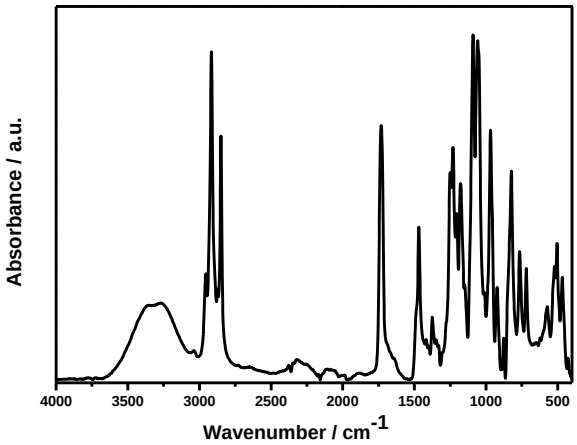


Figure S2. FTIR spectrum of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC).

Table S1. Assignment of IR bands in the FTIR spectrum of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC).

Wavenumber / cm^{-1}	**Band assignment[a]**
3659 – 3040	O-H stretching mode
2960	C–H asymmetric stretching (CH_3)
2914	C–H asymmetric stretching (CH_2)
2851	C–H symmetric stretching (CH_2)
1728	C=O stretching mode
1471	C–O–H deformation in plane
1377	CH_3 symmetric deformation
1251	C–O stretching mode
1229	P=O stretching mode
1178	C–N stretching mode
1099, 1055, 967	P–C–O asymmetric stretching
921	C–O–H out of plane deformation
821, 763	P–C–O symmetric stretching
715	CH_2 rocking deformation

[a]P. J. Larkin, IR and Raman spectroscopy: Principles and Spectral Interpretation, Elsevier, Amsterdam, 2011.

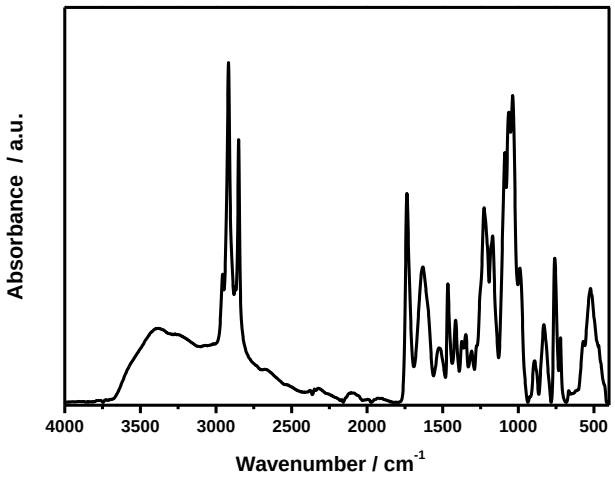


Figure S3. FTIR spectrum of 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (DMPS).

Table S2. Assignment of IR bands in the FTIR spectrum of 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (DMPS).

Wavenumber / cm^{-1}	**Band assignement[a]**
3671 – 3129	O-H stretching mode
3282 – 3129	N-H stretching mode
2955	C–H asymmetric stretching (CH_3)
2913	C–H asymmetric stretching (CH_2)
2850	C–H symmetric stretching (CH_2)
2657	N-CH_2 stretching mode
1734	C=O stretching mode
1628	NH_2 scissors deformation
1524	CH_3 asymmetric deformation
1470	C–O–H deformation in plane
1413	CH_2 scissoring deformation
1370	CH_3 symmetric deformation
1307	CH_2 wagging deformation
1229	P=O stretching
1171	C–O stretching
1093	C–N stretching
1056, 1040, 994	P–C–O asymmetric stretching
894	C–O–H out of plane deformation
836	NH_2 wagging deformation
757, 721	P–C–O symmetric stretching

[a]P. J. Larkin, IR and Raman spectroscopy: Principles and Spectral Interpretation, Elsevier, Amsterdam, 2011.

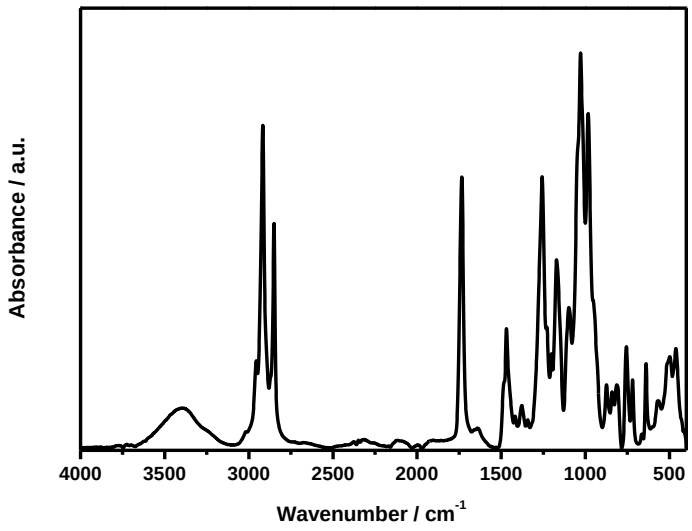


Figure S4. FTIR spectrum of 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (EPC).

Table S3. Assignment of IR bands in the FTIR spectrum of 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (EPC).

Wavenumber / cm^{-1}	**Band assignment**[a]
3674 – 3130	O-H stretching mode
3018	=C–H stretching mode
2956	C–H asymmetric stretching (CH_3)
2918	C–H asymmetric stretching (CH_2)
2849	C–H symmetric stretching (CH_2)
1734	C=O stretching mode
1638	C=C stretching mode
1460	CH_2 scissoring deformation
1419	=C–H deformation in plane
1377	CH_3 symmetric deformation
1340	CH_2 wagging deformation
1245	C–O stretching
1171	P=O stretching
1092	C–N stretching
1020, 983	P–C–O asymmetric stretching
878, 834, 815	P–C–O symmetric stretching

[a] P. J. Larkin, IR and Raman spectroscopy: Principles and Spectral Interpretation, Elsevier, Amsterdam, 2011.

Table S4. Hydrodynamic diameter (d_h) and zeta potential (ζ) of liposomes dispersed in phosphate ($c(Na_2HPO_4)$ = 7 mmol dm^{-3}) or carbonate ($c(NaHCO_3)$ = 40 mmol dm^{-3}) solution at 25 °C, γ(lipid) = 50.0 ppm.

Liposomes	PO_4^{3-}			CO_3^{2-}		
	d_h / nm	Vol / %	ζ / mV	d_h / nm	Vol / %	ζ / mV
DMPC	1610.6 ± 304.8 4877.8 ± 952.4	80 20	–0.6 ± 0.4	1773.2 ± 204.9 5193.8±263.1	87.5 12.5	–1.8 ± 0.6
DMPS	231.0 ± 80.8	100	–100.6 ± 3.4	1093.7 ± 155.5	100	–84.4 ± 8.9
EPC	44.8 ± 14.6 308.1 ± 35.7	40.4 59.6	56.6 ± 3.9	69.1 ± 17.3 240.2 ± 25.0	38.8 62.2	37.3 ± 4.5

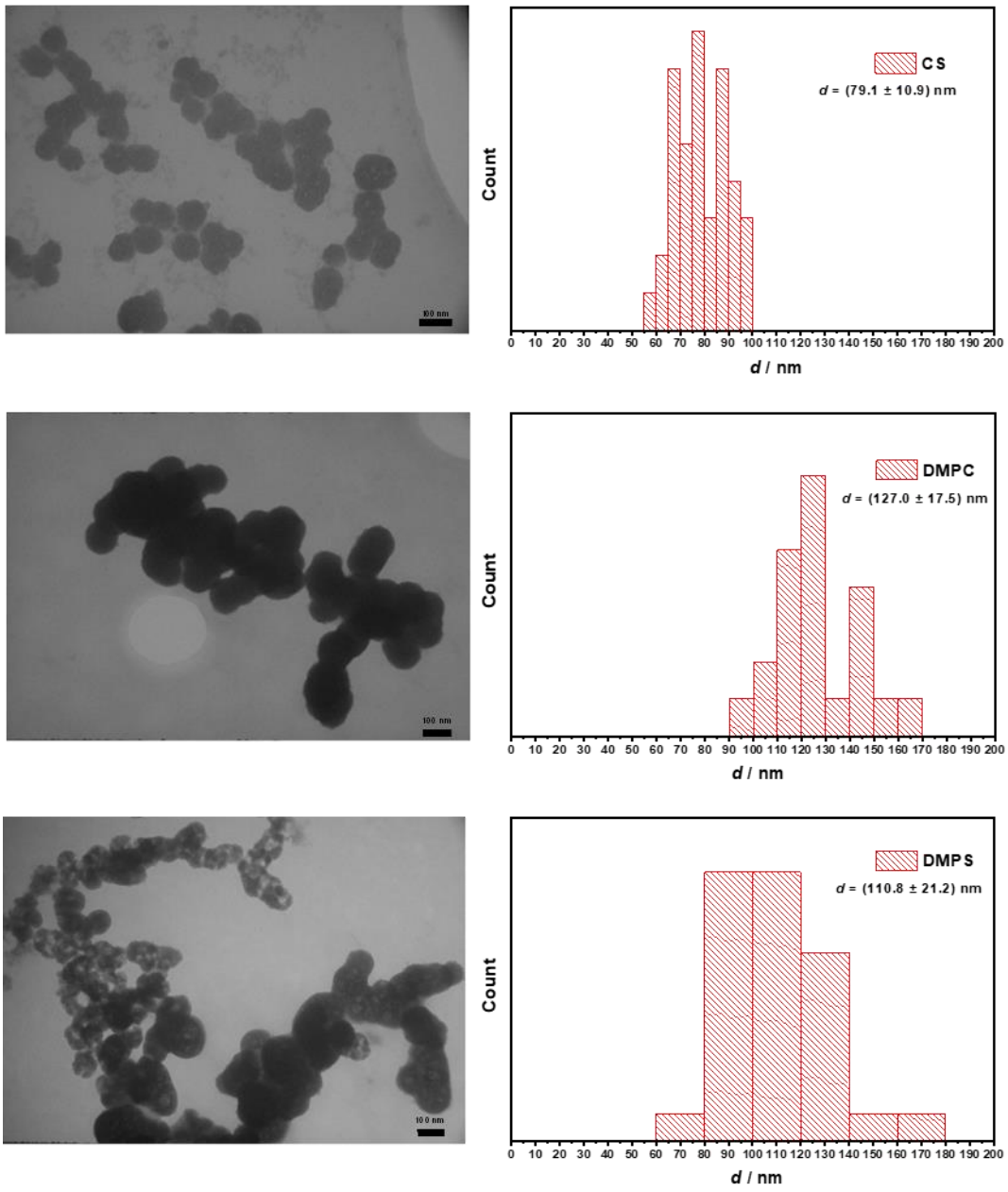


Figure S5. TEM micrographs and size distribution of ACP particles formed in control system and in the presence of DMPC and DMPS liposomes (γ(lipid) = 25.0 ppm). c_i(Ca) = c_i(PO_4) = 3.5 mmol dm^{-3}, pH_i = 7.4, ϑ/ °C = 25 ± 0.1.

Table S5. Assignment of IR bands in the FTIR spectra of precipitate formed after 60 min in calcium phosphate control system. $c_i(Ca) = c_i(PO_4) = 3.5$ mmol dm^{-3}, $pH_i = 7.4$, ϑ/ °C = 25 ± 0.1.

Wavenumber / cm^{-1}	Band assignment	Reference
3665 – 2546	O-H stretching mode of H_2O	a
2337	CO_2	b
1650	O-H-O bending of H_2O	a
1282	HPO_3^{2-}	
1114	ν_{3a} triply degenerate asymmetric stretching mode of PO_4^{3-} (P–O bond)	a
1020	ν_{3c} triply degenerate asymmetric stretching mode of PO_4^{3-} (P–O bond)	a
956	ν_1 nondegenerate symmetric stretching mode of PO_4^{3-} (P–O bond)	a
856	HPO_4^{2-}	c
594	ν_{4a} triply degenerate bending mode of PO_4^{3-} (O–P–O bond)	a
553	ν_{4b} triply degenerate bending mode of PO_4^{3-} (O–P–O bond)	a
469	ν_2 doubly degenerate bending mode of PO_4^{3-} (O–P–O bond)	a

[a]Koutsopulos, S.; *J. Biomed. Mater. Res*., 2002, 62, 600 – 612
[b]Gremlich, H. In: *Infrared and Raman Spectroscop*y, VCH Publishers, New York, 2008.
[c]Mochales, C; Wilson, R. M.; Dowker, S. E.; Ginebra, M. P.; *J. Alloy Compd*., 2011, 509, 7389-7394

Table S6. Assignment of IR bands in the FTIR spectra of precipitate formed after 60 min in calcium phosphate system containing DMPC liposomes. $c_i(Ca) = c_i(PO_4) = 3.5$ mmol dm^{-3}, $pH_i = 7.4$, ϑ /°C = 25 ± 0.1.

	γ (DMPC) / ppm				
	2.5	**6.25**	**12.5**	**25.0**	**Band assignment**
Wavenumber / cm^{-1}	3685 - 2600	3700 - 2585	3678 - 2632		O-H stretching mode of H_2O
	2352	2341	2357	2357	CO_2
	1654	1659	1649		O-H-O bending of H_2O
	1283	1283	1278		HPO_3^{2-}
	1130	1119	1114	1113	v_{3a} triply degenerate asymmetric stretching mode of PO_4^{3-} (P–O bond)
	1018	1024	1019	1024	v_{3c} triply degenerate asymmetric stretching mode of PO_4^{3-} (P–O bond)
	961	950	966	962	v_1 nondegenerate symmetric stretching mode of PO_4^{3-} (P–O bond)
	866	866	860	870	HPO_4^{2-}
	601	595	601	596	v_{4a} triply degenerate bending mode of PO_4^{3-} (O–P–O bond)
	559	564	559	553	v_{4b} triply degenerate bending mode of PO_4^{3-} (O–P–O bond)
	468	469	474	469	v_2 doubly degenerate bending mode of PO_4^{3-} (O–P–O bond)

Table S7. Assignment of IR bands in the FTIR spectra of precipitate formed after 60 min in calcium phosphate system containing DMPS liposomes. $c_i(Ca) = c_i(PO_4) = 3.5$ mmol dm^{-3}, $pH_i = 7.4$, ϑ / °C = 25 ± 0.1.

	γ (DMPS) / ppm				
	2.5	**6.25**	**12.5**	**25.0**	**Band assignment**
Wavenumber / cm^{-1}	3664 - 2643	3635 - 2547	3669 - 2621	3642 – 2590	O-H stretching mode of H_2O
	2364	2352	2363	2357	CO_2
	1649	1648	1643	1649	O-H-O bending of H_2O
	1289		1283	1279	HPO_3^{2-}
	1114	1120	1109	1114	ν_{3a} triply degenerate asymmetric stretching mode of PO_4^{3-} (P–O bond)
	1019	1019	1021	1020	ν_{3c} triply degenerate asymmetric stretching mode of PO_4^{3-} (P–O bond)
	966	961	966	950	ν_1 nondegenerate symmetric stretching mode of PO_4^{3-} (P–O bond)
	860	870	866	860	HPO_4^{2-}
	601	595	601	603	ν_{4a} triply degenerate bending mode of PO_4^{3-} (O–P–O bond)
	553	559	559	559	ν_{4b} triply degenerate bending mode of PO_4^{3-} (O–P–O bond)
	464	474	474	474	ν_2 doubly degenerate bending mode of PO_4^{3-} (O–P–O bond)

Table S8. Assignment of IR bands in the FTIR spectra of precipitate formed after 60 min in calcium phosphate system containing EPC liposomes. $c_i(Ca) = c_i(PO_4) = 3.5$ mmol dm^{-3}, $pH_i = 7.4$, ϑ / °C = 25 ± 0.1.

	γ (EPC) / ppm				
	2.5	**6.25**	**12.5**	**25.0**	**Band assignment**
Wavenumber / cm^{-1}	3648 - 2621	3674 - 2645	3670 - 2710	3638 - 2621	O-H stretching mode of H_2O
	2357	2353	2352		CO_2
	1643	1650	1654	1655	O-H-O bending of H_2O
	1282		1287	1277	HPO_3^{2-}
	1120	1109	1110	1110	ν_{3a} triply degenerate asymmetric stretching mode of PO_4^{3-} (P–O bond)
	1014	1021	1020	1010	ν_{3c} triply degenerate asymmetric stretching mode of PO_4^{3-} (P–O bond)
	963	956	952	952	ν_1 nondegenerate symmetric stretching mode of PO_4^{3-} (P–O bond)
	856	872	868	872	HPO_4^{2-}
	595	599	606	595	ν_{4a} triply degenerate bending mode of PO_4^{3-} (O–P–O bond)
	553	548	557	553	ν_{4b} triply degenerate bending mode of PO_4^{3-} (O–P–O bond)
	458	469	464	458	ν_2 doubly degenerate bending mode of PO_4^{3-} (O–P–O bond)

Table S9. Assignment of IR bands in the FTIR spectra of precipitate formed in control calcium carbonate system. $c_i(Ca) = c_i(CO_3) = 20$ mmol dm^{-3}, $pH_i = 8.15$, ϑ / °C = 25 ± 0.1.

Wavenumber / cm^{-1}	**Band assignement**[a]
1446	ν_3, asymmetric C-O stretching mode
1089	ν_1, symmetric C-O stretching mode
877	ν_2, CO_3 out of plane deformation mode
746	ν_4, O-C-O bending (in plane deformation) mode

[a] F. A. Andersen, Lj. Brečević: Infrared spectra of amorphous and crystalline calcium carbonate, *Acta Chim. Scand.* **45** (1991) 1018-1024.

Table S10. Assignment of IR bands in the FTIR spectra of precipitate formed in calcium carbonate system containing DMPC liposomes. $c_i(Ca) = c_i(CO_3) = 20$ mmol dm^{-3}, $pH_i = 8.15$, ϑ / °C = 25 ± 0.1.

Wavenumber / cm^{-1}				
γ (DMPC) / ppm				
2.5	**6.25**	**12.5**	**25.0**	**Band assignment**
3680 - 3075	3675 - 3100	3630 - 3095	3610 - 3095	O-H stretching mode of H_2O
		2912	2908	C–H asymmetric stretching (CH_2)
		2847	2851	C–H symmetric stretching (CH_2)
1468	1467	1470	1469	ν_3, asymmetric C-O stretching mode
1086	1087	1088	1087	ν_1, symmetric C-O stretching mode
875	876	877	876	ν_2, CO_3 out of plane deformation mode
746	746	746	746	ν_4, O-C-O bending (in plane deformation) mode

Table S11. Assignment of IR bands in the FTIR spectra of precipitate formed in calcium carbonate system containing DMPS liposomes $c_i(Ca) = c_i(CO_3) = 20$ mmol dm^{-3}, $pH_i = 8.15$, ϑ / °C = 25 ± 0.1.

Wavenumber / cm^{-1}				
γ (DMPS) / ppm				
2.5	**6.25**	**12.5**	**25.0**	**Band assignment**
3691 - 3104	3688 - 3102	3675 - 3099	3687 - 3100	O-H stretching mode of H_2O
	2917	2913	2912	C–H asymmetric stretching (CH_2)
	2849	2845	2851	C–H symmetric stretching (CH_2)
1467	1471	1469	1469	ν_3, asymmetric C-O stretching mode
1087	1085	1082	1081	ν_1, symmetric C-O stretching mode
873	877	875	875	ν_2, CO_3 out of plane deformation mode
746	746	746	746	ν_4, O-C-O bending (in plane deformation) mode

Table S12. Assignment of IR bands in the FTIR spectra of precipitate formed in calcium carbonate system containing EPC liposomes. $c_i(Ca) = c_i(CO_3) = 20$ mmol dm^{-3}, $pH_i = 8.15$, ϑ / °C = 25 ± 0.1.

Wavenumber / cm^{-1}				
γ (EPC) / ppm				
2.5	**6.25**	**12.5**	**25.0**	**Band assignment**
3665 - 3091	3670 - 3095	3669 - 3099	3672 - 3088	O-H stretching mode of H_2O
1468	1468	1468	1469	ν_3, asymmetric C-O stretching mode
1085	1087	1085	1085	ν_1, symmetric C-O stretching mode
876	873	875	876	ν_2, CO_3 out of plane deformation mode
746	746	746	746	ν_4, O-C-O bending (in plane deformation) mode

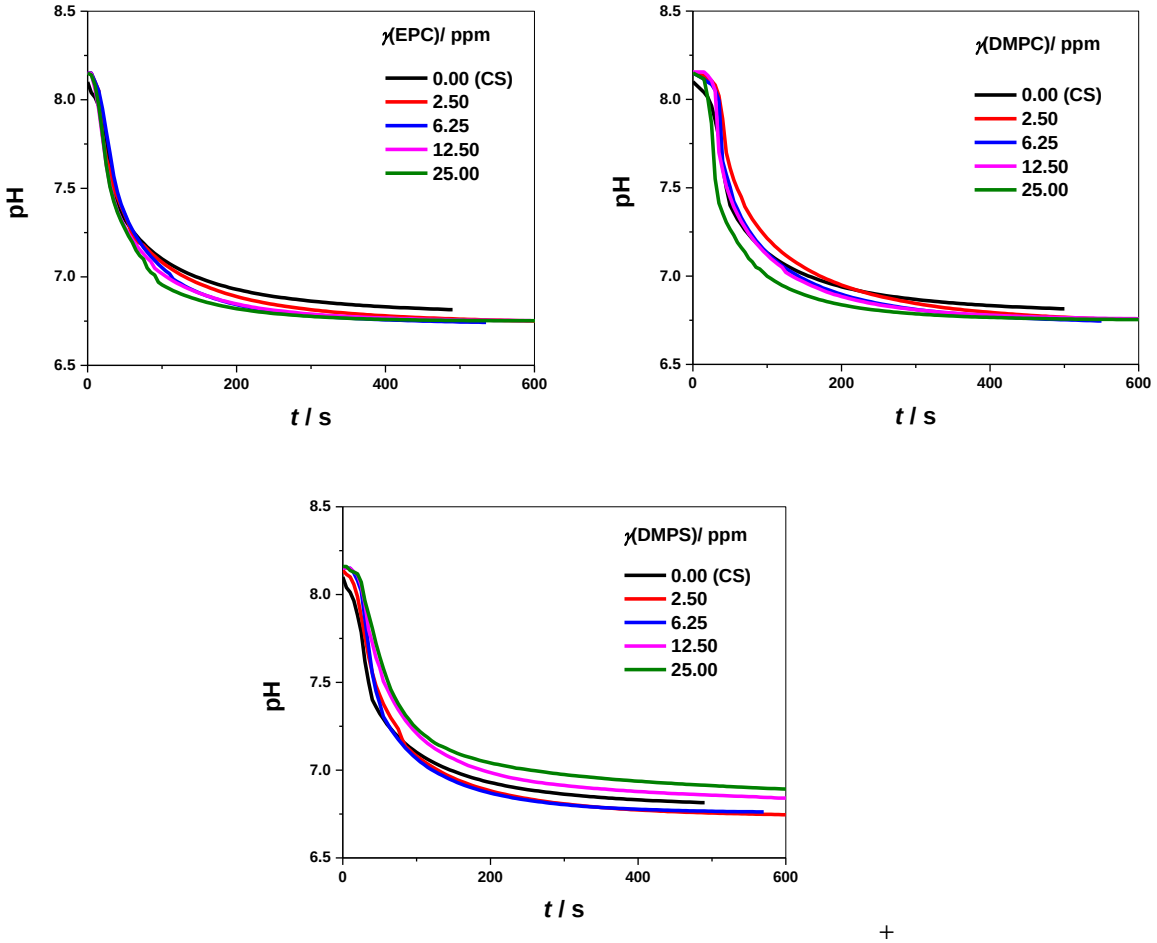


Figure S6. Representative pH vs. time curve of $CaCO_3$ spontaneous precipitation experiments at different concentration of liposomes: 2.5 ppm < γ (lipid) < 25.0 ppm. $c_i(Ca) = c_i(CO_3)$ = 20 mmol dm^{-3}, pH_i = 8.15, ϑ / °C = 25 ± 0.1.

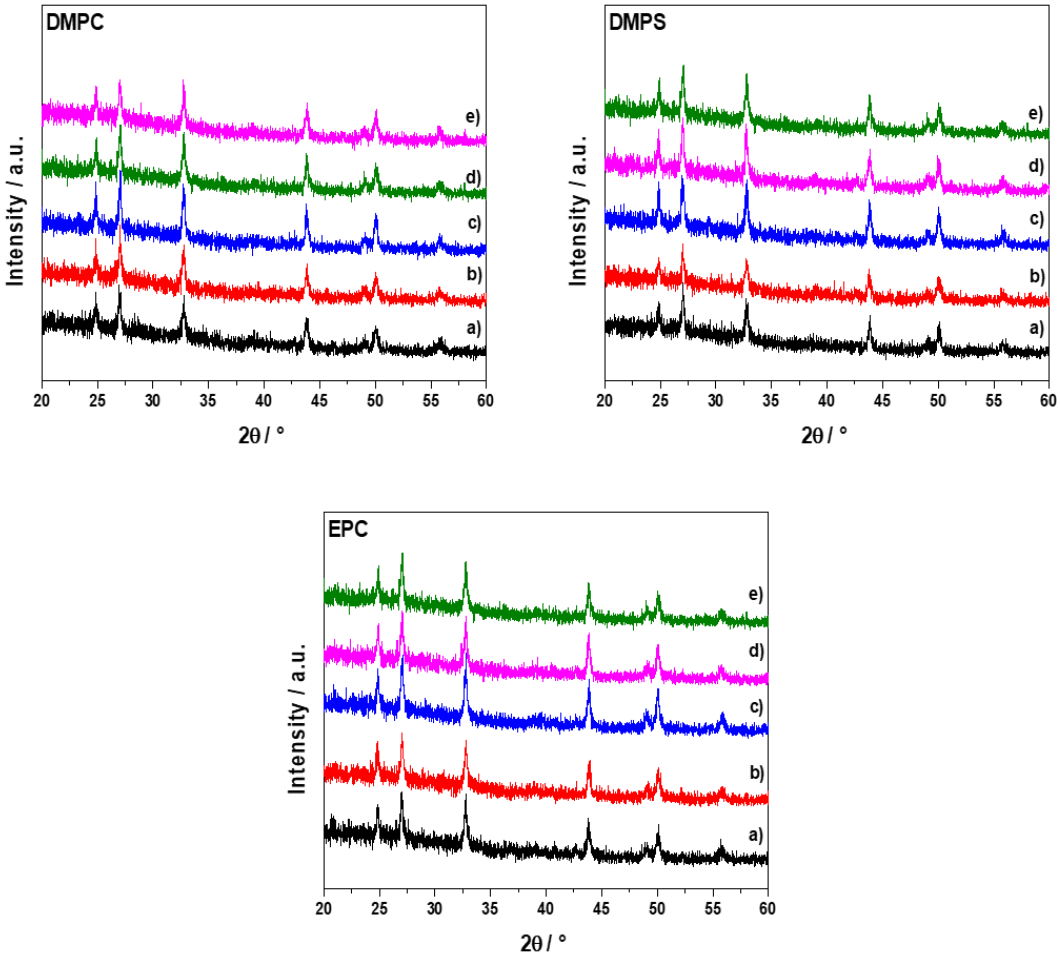


Figure S7. PXRD patterns of the precipitate formed after 10 minutes reaction time in the absence (a) and presence of different concentration of liposomes (expressed as lipid concentration): b) 2.50 ppm, c) 6.25 ppm d) 12.50 ppm e) 25.00 ppm. $c_i(Ca) = c_i(CO_3) = 20$ mmol dm^{-3}, $pH_i = 8.15$, ϑ / °C = 25 ± 0.1.