Interaction of N-acetylcysteine with DPPC liposomes at different pH: a physicochemical study

Juan Marcelo Arias[1], Rafael A. Cobos Picot[1], María Eugenia Tuttolomondo[1], Aida Ben Altabef[1*] and Sonia Beatriz Díaz[1*]

[1]INQUINOA-CONICET, Cátedra de Fisicoquímica I, Instituto de Química Física, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Lorenzo 456, T4000CAN S. M. de Tucumán, R. Argentina.

Supporting Information

Table S1. Effect of NAC on the CH_2, CH_3 vibrational bands and CH_2 deformation band in gel (A) and liquid crystalline phase (B), at pH 2 and at 30 °C and 50 °C respectively by FTIR measurements.

(A)

NAC:DPPC Molar ratio (30 °C)	**ν_a CH_3 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_s CH_3 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_a CH_2 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_s CH_2 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**δ_s CH_2 /cm^{-1}**	**$\Delta\delta$ /cm^{-1}**
0.00:1.0	**2956**	**0.0**	**2873**	**0.0**	**2919**	**0.0**	**2850**	**0.0**	**1468**	**0.0**
0.37:1.0	2957	0.6	2875	2.6	2919	0.1	2851	0.1	1468	0.2
1.10:1.0	2957	0.5	2873	0.4	2919	-0.1	2851	0.2	1468	-0.1
1.47:1.0	2957	1.3	2873	0.5	2918	-0.4	2851	0.4	1468	0.2
2.93:1.0	2957	0.8	2874	1.1	2919	0.0	2851	0.2	1468	0.1

(B)

NAC:DPPC Molar ratio (50 °C)	**ν_a CH_3 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_s CH_3 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_a CH_2 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_s CH_2 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**δ_s CH_2 /cm^{-1}**	**$\Delta\delta$ /cm^{-1}**
0.00:1.0	**2956**	**0.0**	**2874**	**0.0**	**2923**	**0.0**	**2853**	**0.0**	**1468**	**0.0**
0.37:1.0	2958	1.3	2873	-0.4	2923	-0.3	2853	-0.1	1468	0.5
1.10:1.0	2958	1.7	2874	0.5	2923	0.2	2852	-1.1	1468	-0.4
1.47:1.0	2958	1.7	2873	-0.5	2923	0.0	2853	0.0	1467	-0.8
2.93:1.0	2958	1.4	2873	0.0	2923	-0.1	2853	-0.2	1467	-0.5

Table S2. Effect of NAC on the CH_2, CH_3 vibrational bands and CH_2 deformation band in gel (A) and liquid crystalline phase (B), at pH 7 and at 30 °C and 50 °C respectively by FTIR measurements.

(A)

NAC:DPPC Molar ratio (30 °C)	**ν_a CH_3 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_s CH_3 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_a CH_2 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_s CH_2 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**δ_s CH_2 /cm^{-1}**	**$\Delta\delta$ /cm^{-1}**
0.00:1.0	**2956**	**0**	**2873**	**0**	**2919**	**0**	**2850**	**0**	**1468**	**0**
0.37:1.0	2957	1	2874	1	2918	-1	2850	0	1468	0
1.10:1.0	2957	1	2873	0	2919	0	2851	1	1468	0
1.47:1.0	2956	0	2872	-1	2919	0	2851	1	1469	1
2.93:1.0	2957	1	2874	1	2919	0	2851	1	1468	0

(B)

NAC:DPPC Molar ratio (50 °C)	**ν_a CH_3 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_s CH_3 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_a CH_2 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**ν_s CH_2 /cm^{-1}**	**$\Delta\nu$ /cm^{-1}**	**δ_s CH_2 /cm^{-1}**	**$\Delta\delta$ /cm^{-1}**
0.00:1.0	**2956**	**0**	**2874**	**0**	**2923**	**0**	**2853**	**0**	**1468**	**0**
0.37:1.0	2958	2	2874	0	2923	0	2853	0	1467	-1
1.10:1.0	2957	1	2873	-1	2921	2	2853	0	1467	-1
1.47:1.0	2957	1	2873	-1	2923	0	2853	0	1467	-1
2.93:1.0	2957	1	2872	-2	2922	-1	2852	0	1467	-1

Table S3. Bandwidth of the CH_2 antisymmetric and symmetric stretching modes as a function of the molar ratio of NAC: DPPC in gel and liquid crystalline states.

pH 2

NAC:DPPC Molar ratio	Band width at 30 ºC		Band width at 50 ºC	
	ν_a CH_2	ν_s CH_2	ν_a CH_2	ν_s CH_2
0.00:1.0	19	11	24	14
0.37:1.0	16	9	22	12
1.10:1.0	18	10	22	13
1.47:1.0	17	10	23	14
2.93:1.0	16	10	21	13

pH 7

NAC:DPPC Molar ratio	Band width at 30 ºC		Band width at 50 ºC	
	ν_a CH_2	ν_s CH_2	ν_a CH_2	ν_s CH_2
0.00:1.0	19	11	24	14
0.37:1.0	**18**	**11**	**24**	**15**
1.10:1.0	**19**	**11**	**24**	**15**
1.47:1.0	**19**	**12**	**24**	**15**
2.93:1.0	**19**	**12**	**24**	**15**

Table S4. Effect of NAC on the CH_3, CH_2 stretching vibrational bands, CH_2 deformation and torsion bands and C-C stretching vibration bands by Raman measurements in gel state**.**

NAC:DPPC Molar ratio *pH 2*	**ν N-$(CH_3)_3$**	**ν_a CH_3**	**ν_s CH_3**	**ν_a CH_2**	**ν_s CH_2**	**ν S-H**	**δ CH_2**	**τ CH_2**	**ν(C-C)$_T$**	**ν_a(C-C)$_G$**	**ν (C-C)$_T$**	**ν C-N**	**ν C-S**
0.00:1.0	**3042**	**2961**	**2935**	**2885**	**2850**	**-**	**1441**	**1299**	**1131**	**1099**	**1066**	**718**	**-**
0.37:1.0	3039	2964	2933	2881	2847	-	1437	1295	1128	1097	1063	716	-
1.10:1.0	3039	2961	2934	2881	2847	-	1437	1296	1128	1096	1063	716	-
1.47:1.0	3035	2961	2935	2881	2847	2586	1438	1295	1128	1098	1062	716	665
2.93:1.0	3033	2960	2936	2881	2846	2584	1438	1296	1128	1097	1063	716	684-665
1.00:0.0	-	-	-	-	-	2583	-	-	-	-	-	-	684

NAC:DPPC Molar ratio *pH 7*	**ν N-$(CH_3)_3$**	**ν_a CH_3**	**ν_s CH_3**	**ν_a CH_2**	**ν_s CH_2**	**ν S-H**	**δ CH_2**	**τ CH_2**	**ν(C-C)$_T$**	**ν_a(C-C)$_G$**	**ν (C-C)$_T$**	**ν C-N**	**ν C-S**
0.00:1.0	**3042**	**2961**	**2935**	**2885**	**2850**	**-**	**1439**	**1297**	**1131**	**1099**	**1064**	**718**	**-**
0.37:1.0	3032	2956	2930	2879	2845	-	1433	1292	1124	1091	1060	713	-
1.10:1.0	3030	2956	2930	2879	2845	2568	1433	1293	1126	1094	1060	714	667
1.47:1.0	3030	2958	2932	2879	2846	2568	1436	1295	1126	1094	1060	714	667
2.93:1.0	3036	2955	2932	2878	2845	-	1438	1296	1128	1097	1063	713	667
1.00:0.0						2580						-	684

Table S5. Intensity ratio of the bands corresponding to the stretching methyl, methylene and carbon-carbon, as a function of the molar ratio of NAC:DPPC in gel state, at pH 2 and 7.

pH 2

NAC:DPPC Molar ratio	I_{2885}/I_{2851}	I_{1099}/I_{1066}	I_{2935}/I_{2851}
0.00:1.0	1.1	0.6	0.5
0.37:1.0	1.2	0.6	0.5
1.10:1.0	1.2	0.6	0.5
1.47:1.0	1.2	0.6	0.5
2.93:1.0	1.2	0.5	0.6

pH 7

NAC:DPPC Molar ratio	I_{2885}/I_{2851}	I_{1099}/I_{1066}	I_{2935}/I_{2851}
0.00:1.0	1.18	0.45	0.30
0.37:1.0	1.14	0.55	0.52
1.10:1.0	1.10	0.58	0.80
1.47:1.0	1.12	0.56	0.75
2.93:1.0	1.2	0.5	0.6

Table S6. C=O stretching mode by FTIR measurements in gel (30ºC) and liquid crystalline (50ºC) states.

pH 2					
NAC:DPPC Molar ratio / 30 ºC	**ν CO_{bond}**	**ν CO_{free}**	**NAC:DPPC Molar ratio / 50ºC**	**ν CO_{bond}**	**ν CO_{free}**
0.00:1.0	1739	1731	**0.00:1.0**	1741	1730
0.37:1.0	1744	1732	**0.37:1.0**	1746	1733
1.10:1.0	1743	1732	**1.10:1.0**	1745	1732
1.47:1.0	1743	1731	**1.47:1.0**	1745	1730
2.93:1.0	1744	1731	**2.93:1.0**	1745	1731

pH 7					
NAC:DPPC Molar ratio / 30 ºC	**ν CO_{bond}**	**ν CO_{free}**	**NAC:DPPC Molar ratio / 50ºC**	**ν CO_{bond}**	**ν CO_{free}**
0.00:1.0	1739	1731	**0.00:1.0**	1741	1730
0.37:1.0	1743	1732	**0.37:1.0**	1745	1732
1.10:1.0	1744	1732	**1.10:1.0**	1743	1734
1.47:1.0	1744	1733	**1.47:1.0**	1745	1734
2.93:1.0	1744	1733	**2.93:1.0**	1743	1732

Table S7. Effect of NAC on the C=O stretching mode by FTIR measurements in gel (30ºC) and liquid crystalline (50ºC) states.

	pH 2		**pH 7**	
NAC:DPPC Molar ratio / 30ºC	**Δν CO_{bond}**	**Δν CO_{free}**	**Δν CO_{bond}**	**Δν CO_{free}**
0.00:1.0	0	0	0	0
0.37:1.0	5	1	4	1
1.10:1.0	4	1	5	1
1.47:1.0	4	0	5	2
2.93:1.0	5	0	5	2
NAC:DPPC Molar ratio / 50ºC	**Δν CO_{bond}**	**Δν CO_{free}**	**Δν CO_{bond}**	**Δν CO_{free}**
0.00:1.0	0	0	0	0
0.37:1.0	5	3	3	2
1.10:1.0	4	2	2	4
1.47:1.0	4	0	3	4
2.93:1.0	4	1	2	2

Table S8. PO_2^- stretching mode by FTIR measurements and effect of NAC on the PO_2^- stretching modes in gel (30ºC) and liquid crystalline (50ºC) states.

pH 2

NAC:DPPC Molar ratio 30ºC	$\nu_a PO_2^-$	$\nu_a PO_2^-$	$\nu_s PO_2^-$	$\nu_s PO_2^-$
0.00:1.00	1228	0	1089	0
0.37:1.00	1232	4	1090	1
1.10:1.00	1232	4	1091	2
1.47:1.00	1232	4	1091	2
2.93:1.00	1234	4	1090	1
NAC:DPPC Molar Ratio 50 ºC	$\nu_a PO_2^-$	$\nu_a PO_2^-$	$\nu_s PO_2^-$	$\nu_s PO_2^-$
0.00:1.00	1232	0	1088	0
0.37:1.00	1232	0	1088	0
1.10:1.00	1232	0	1089	1
1.47:1.00	1232	0	1089	1
2.93:1.00	1233	1	1089	1

pH 7

NAC:DPPC Molar ratio 30ºC	$\nu_a PO_2^-$	$\nu_a PO_2^-$	$\nu_s PO_2^-$	$\nu_s PO_2^-$
0.00:1.00	1228	0	1089	0
0.37:1.00	1226	-2	1089	1
1.10:1.00	1234	6	1090	1
1.47:1.00	1234	6	1090	1
2.93:1.00	1235	7	1091	2
NAC:DPPC Molar ratio 50 ºC	$\nu_a PO_2^-$	$\nu_a PO_2^-$	$\nu_s PO_2^-$	$\nu_s PO_2^-$
0.00:1.00	1232	0	1088	0
0.37:1.00	1232	0	1088	0
1.10:1.00	1234	2	1089	1
1.47:1.00	1234	2	1089	1
2.93:1.00	1236	4	1089	1

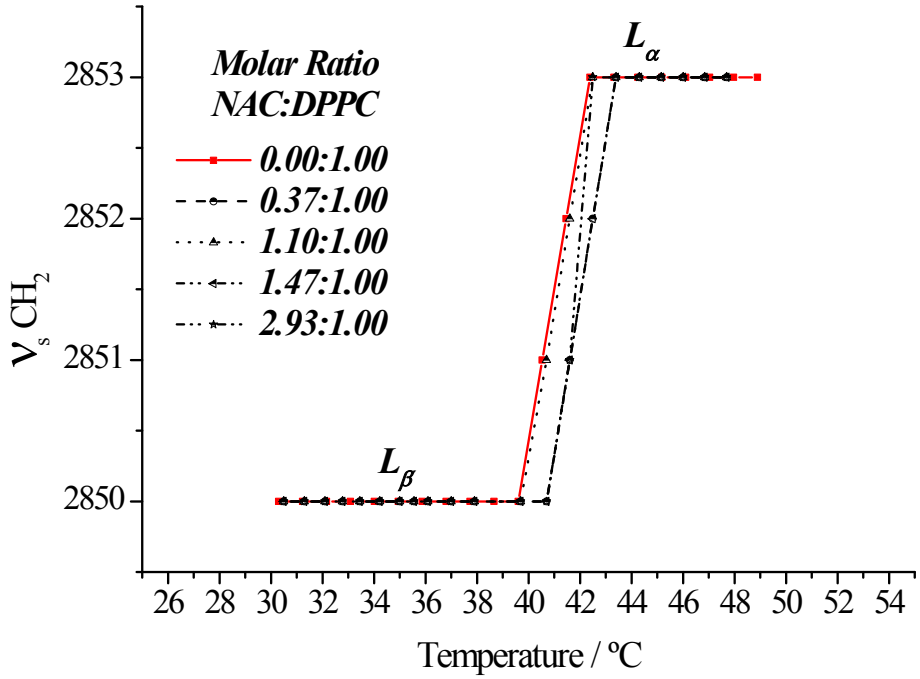
Table S9. PO_2^- stretching mode by FTIR measurements and effect of NAC on the PO_2^- stretching modes.

Molar ratio NAC:DPPC	**pH 2**		**pH 7**	
	T_p	T_m	T_p	T_m
0.00:1.00	36.3	41.00	36.3	41.10
0.37:1.00	35.88	42.84	34.86	39.60
1.10:1.00	32.16	41.93	36.52	45.40
1.47:1.00	32.16	42.88	37.33	43.74
2.93:1.00	30.00	41.93	38.18	43.73

Table S10. Calorimetric parameters analyzed for the different samples studied; T_m: transition temperature; ΔH_{cal}: transition enthalpy; $\Delta T_{1/2}$: mean height width of the principal transition; T_p: pretransition temperature and ΔHT_p: pretransition enthalpy.

pH 2								
NAC:DPPC Molar ratio	**T_m / ºC**	**ΔHcal / J.mol^{-1}**	**ΔS/J mol^{-1}**	**$\Delta T_{1/2}$ / ºC**	**T_p / ºC**	**ΔHT_p / J.mol^{-1}**	**ΔH_{vH} /J mol^{-1}**	**C.U.**
0,00:1,0	41.53	10174.20	32.35	0.70	36.17	2571.39	81900.55	8.05
0,37:1,0	42.30	33003.31	104.67	0.96	35.74	1472.12	61954.17	1.88
1,10:1,0	42.09	34745.19	110.27	1.77	32.26	1106.48	33269.45	1.49
1,47:1,0	41.75	36891.34	117.21	1.77	32.22	774.07	32734.12	0.96
2,93:1,0	41.40	34908.68	111.03	2.35	29.93	311.95	24243.42	0.89

pH 7								
Molar ratio NAC:DPPC	**T_m / ºC**	**ΔHcal / J.mol^{-1}**	**ΔS/J mol^{-1}**	**$\Delta T_{1/2}$ / ºC**	**T_p / ºC**	**ΔHT_p / J.mol^{-1}**	**ΔH_{vH} / J mol^{-1}**	**C.U.**
0.00:1.0	41.53	10186.43	32,35	0.7	36.17	2571.39	81900.55	8.05
0.37:1.0	41.49	24648.74	78,38	1.46	34.92	2697.53	39191.78	1.59
1.10:1.0	42.25	24625.48	78,11	1.05	37.1	2203.15	56509.98	2.29
1.47:1.0	42.52	26179.45	82,97	0.82	38.05	4675.92	73288.14	2.80
2.93:1.0	42.42	28730.70	91,09	1	37.41	5832.88	59813.93	2.08



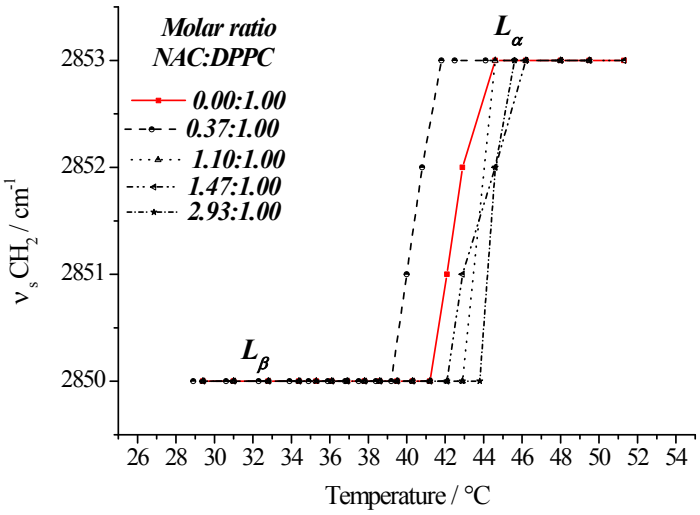
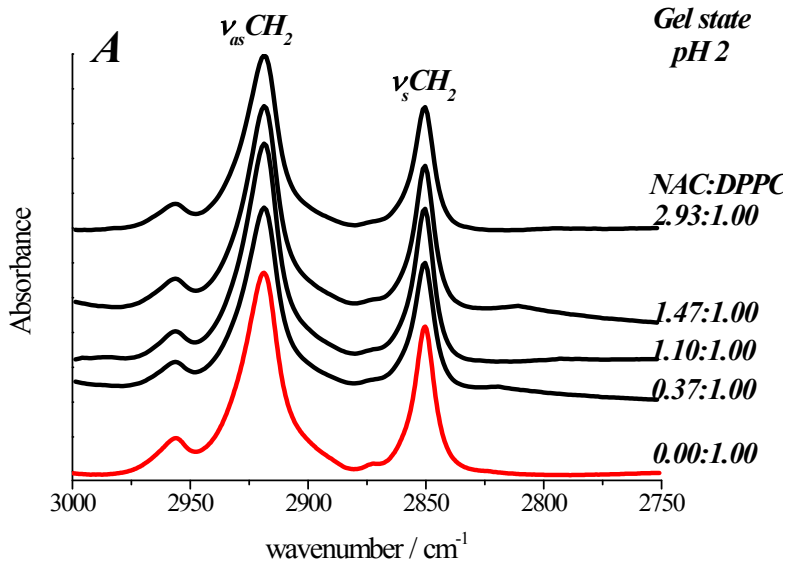
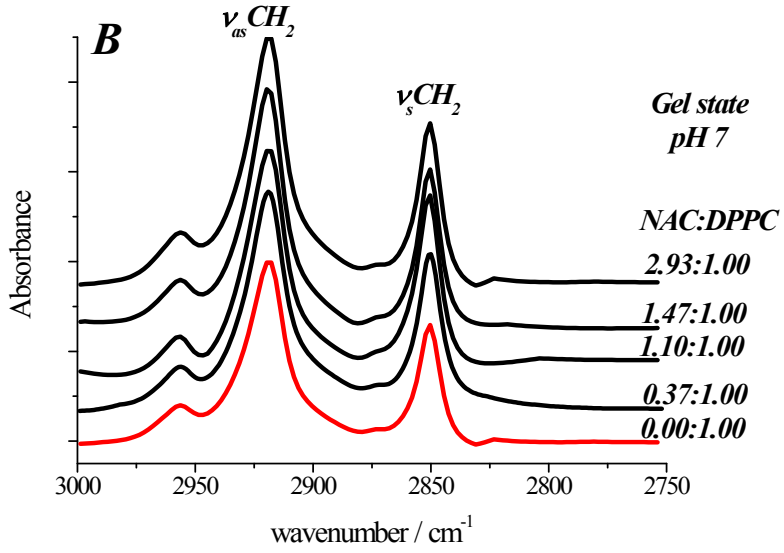


Figure S1. Changes in the wavenumber of the CH_2 symmetric stretching in NAC: DPPC (at different molar ratios) liposomes as a function of the temperature (at pH 2 (upper) and 7 (down)).


A
$\nu_{as}CH_2$
ν_sCH_2
Gel state
pH 2
NAC:DPPC
2.93:1.00
1.47:1.00
1.10:1.00
0.37:1.00
0.00:1.00
Absorbance
3000
2950
2900
2850
2800
2750
wavenumber / cm^{-1}


B
$\nu_{as}CH_2$
ν_sCH_2
Gel state
pH 7
NAC:DPPC
2.93:1.00
1.47:1.00
1.10:1.00
0.37:1.00
0.00:1.00
Absorbance
3000
2950
2900
2850
2800
2750
wavenumber / cm^{-1}

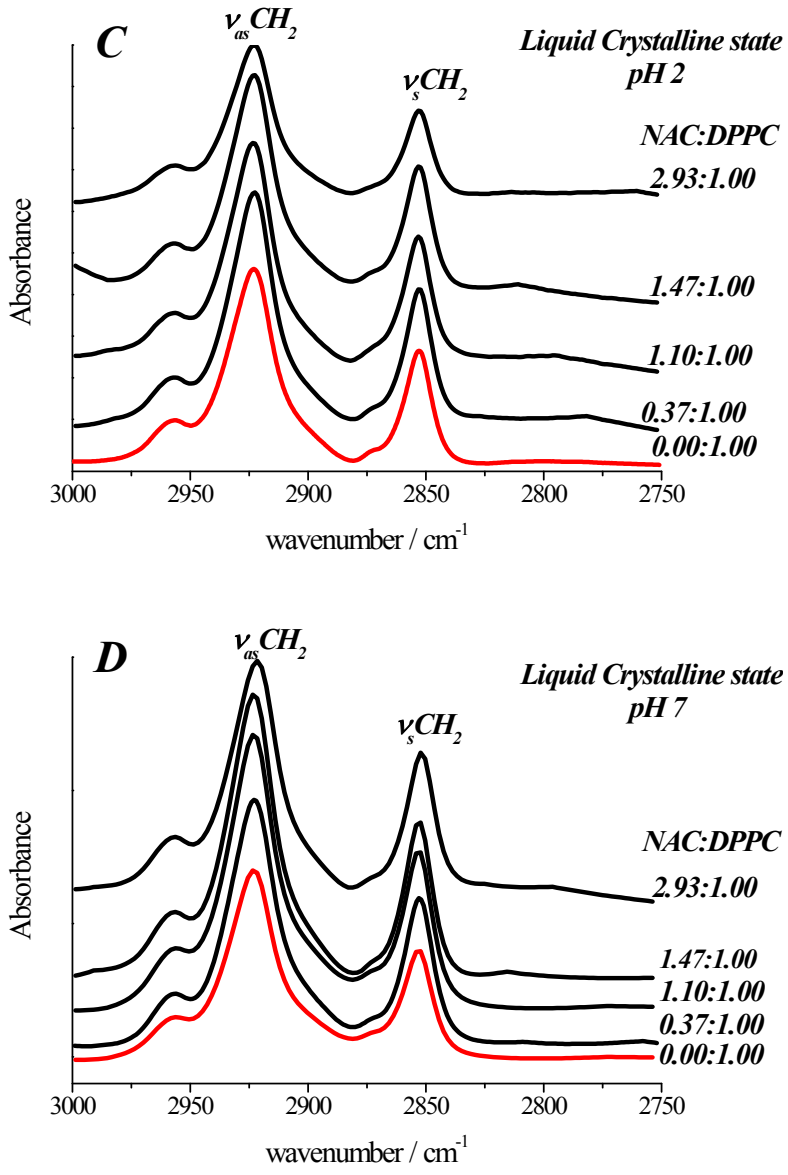
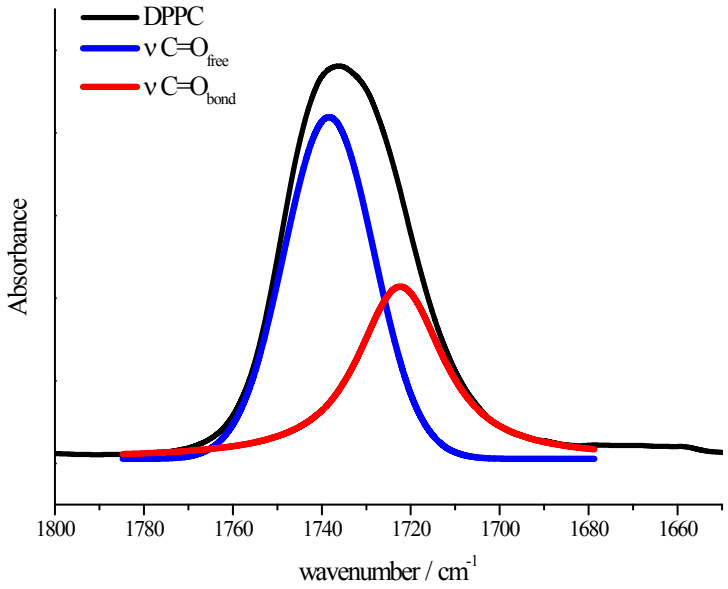
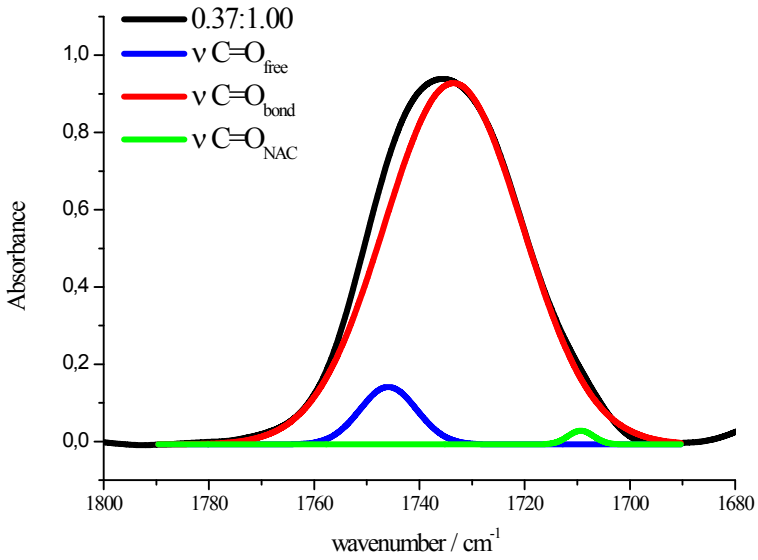
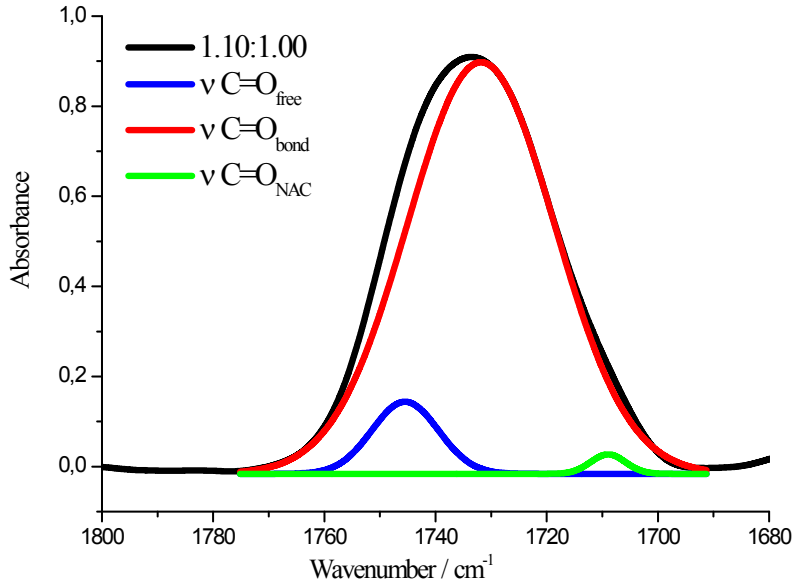


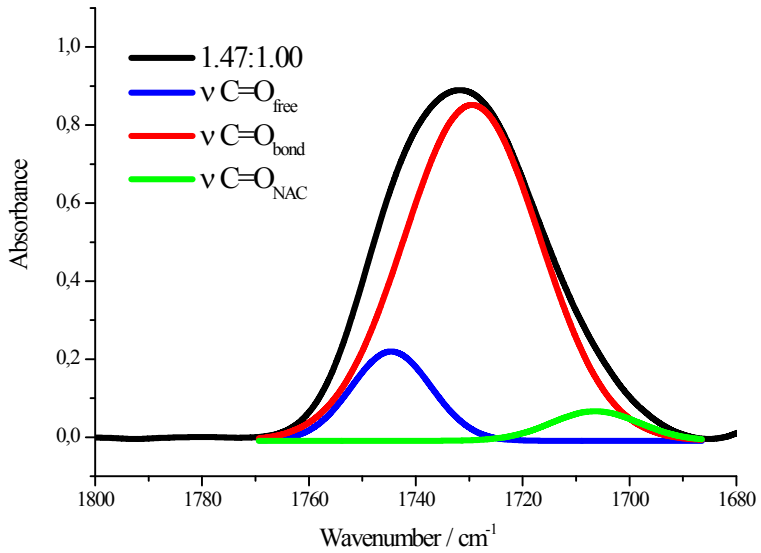
Figure S2. FTIR spectra for the stretching modes of the ν_{as} CH_2 and ν_s CH_2 groups, for pure DPPC and NAC:DPPC (all molar ratio), in the gel (A;B) and liquid crystalline (C;D) states.

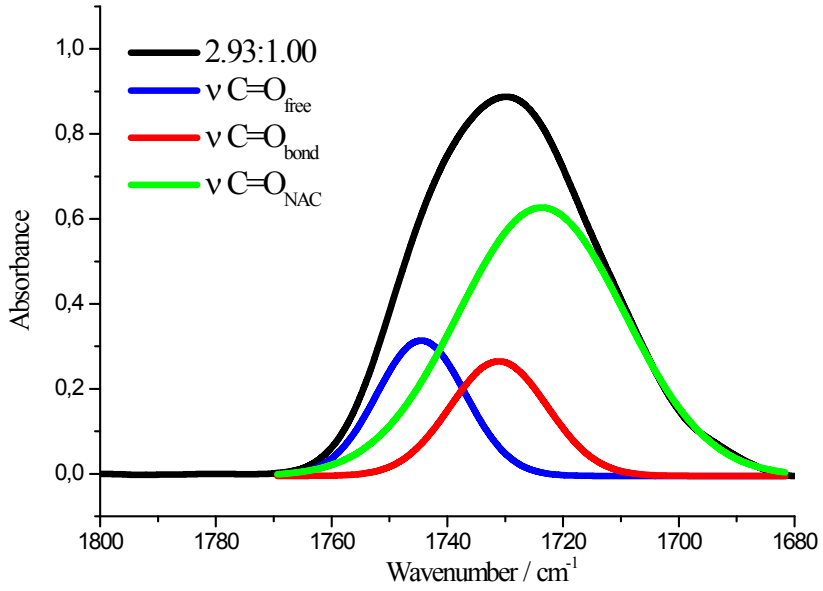

DPPC
ν C=O free
ν C=O bond
Absorbance
1800
1780
1760
1740
1720
1700
1680
1660
wavenumber / cm-1

A (pH 2)

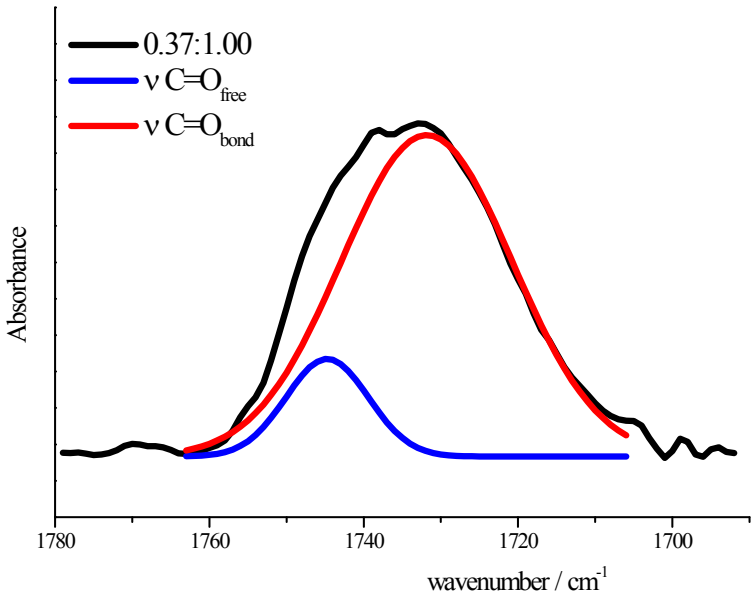

0.37:1.00
ν C=O free
ν C=O bond
ν C=O NAC
Absorbance
1,0
0,8
0,6
0,4
0,2
0,0
1800
1780
1760
1740
1720
1700
1680
wavenumber / cm-1

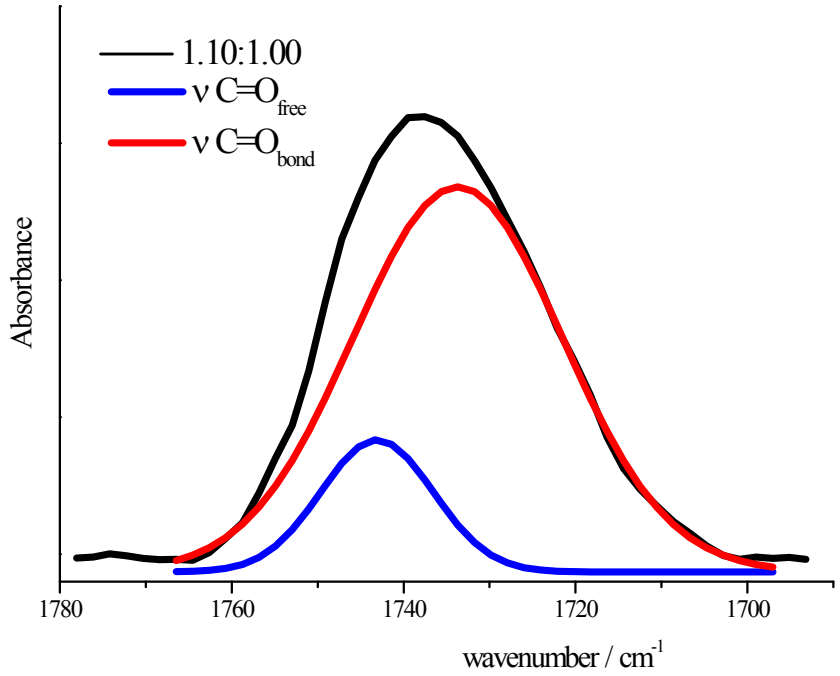

1,0
0,8
0,6
0,4
0,2
0,0
Absorbance
1.10:1.00
ν C=O free
ν C=O bond
ν C=O NAC
1800
1780
1760
1740
1720
1700
1680
Wavenumber / cm-1

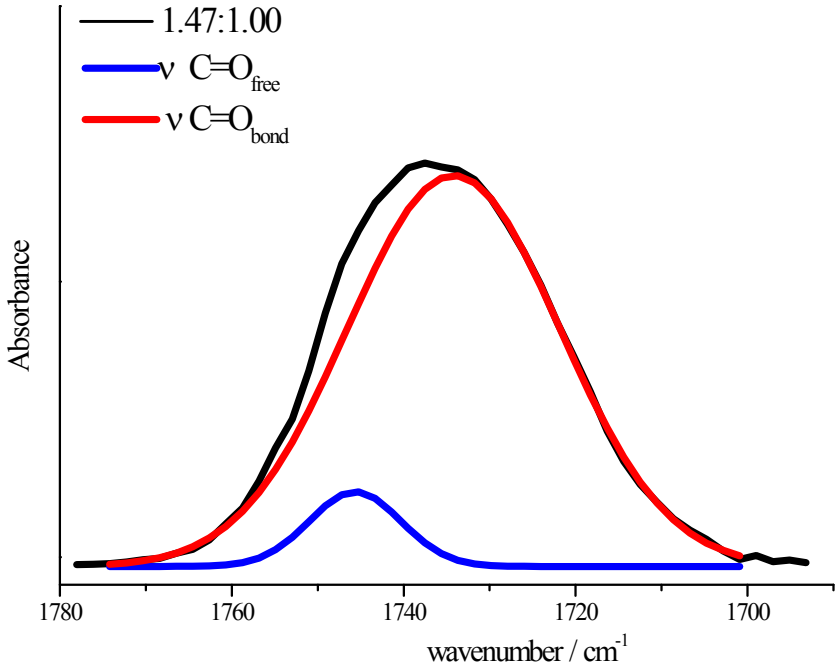

1,0
0,8
0,6
0,4
0,2
0,0
Absorbance
1.47:1.00
ν C=O free
ν C=O bond
ν C=O NAC
1800
1780
1760
1740
1720
1700
1680
Wavenumber / cm-1


2.93:1.00
ν C=O free
ν C=O bond
ν C=O NAC
Absorbance
1,0
0,8
0,6
0,4
0,2
0,0
1800
1780
1760
1740
1720
1700
1680
Wavenumber / cm-1

B (pH 7)


0.37:1.00
ν C=O free
ν C=O bond
Absorbance
1780
1760
1740
1720
1700
wavenumber / cm-1


1.10:1.00
ν C=O free
ν C=O bond
Absorbance
1780
1760
1740
1720
1700
wavenumber / cm-1


1.47:1.00
ν C=O free
ν C=O bond
Absorbance
1780
1760
1740
1720
1700
wavenumber / cm-1

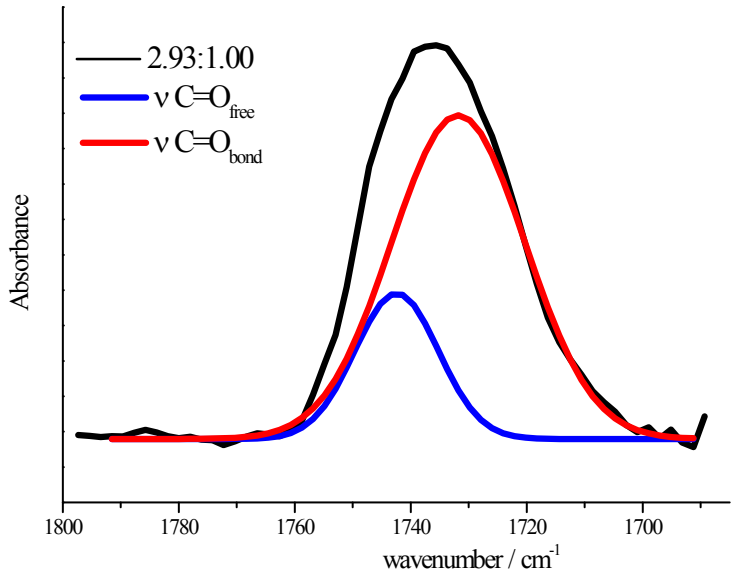
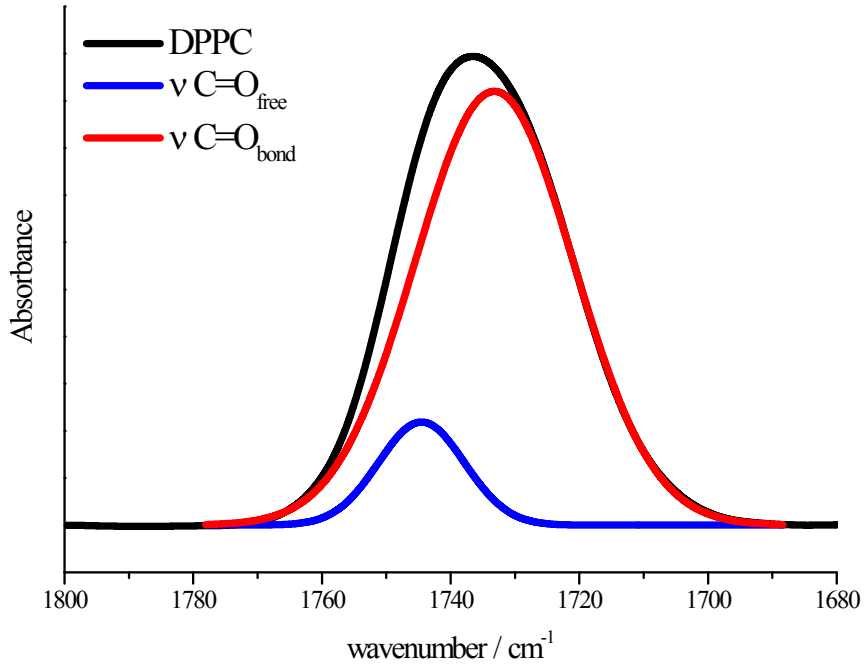
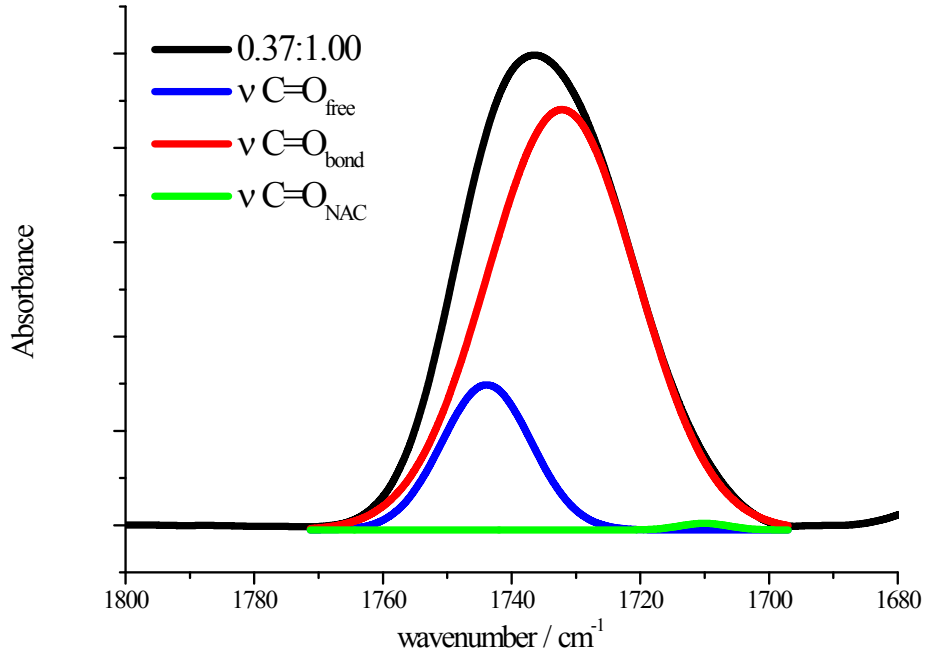
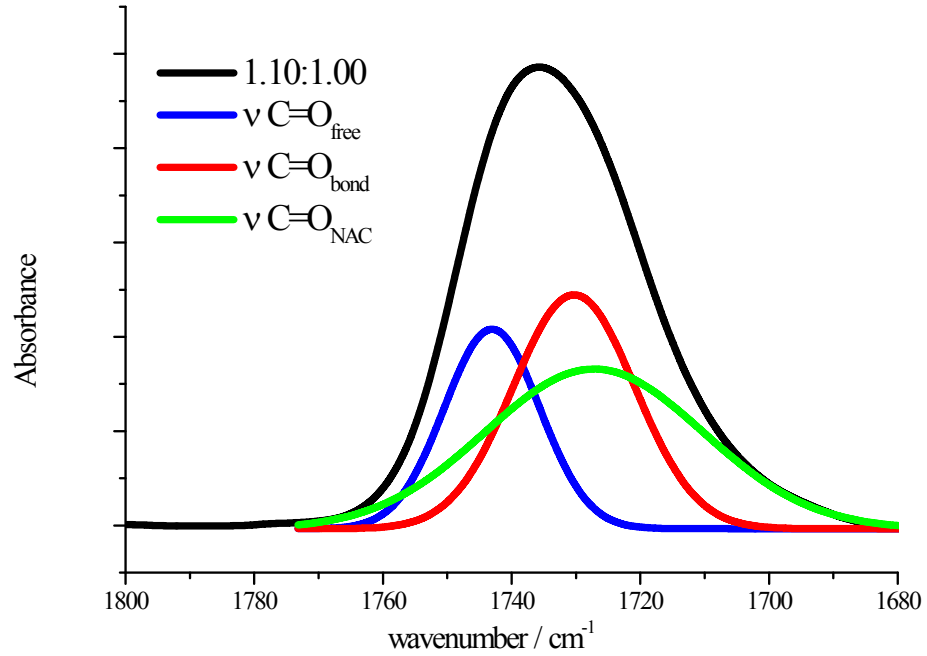


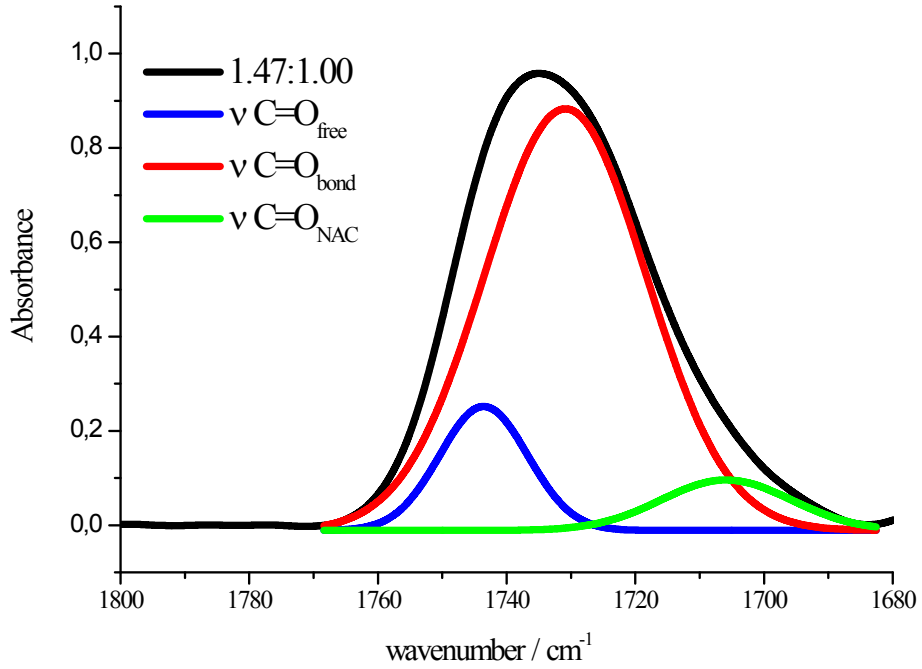
Figure S3. FTIR spectral bands of carbonyl groups of NAC: DPPC in gel state (30ºC) at pH 2 (A) and pH 7 (B). The black line represents the contours of the spectra acquired and the blue and the red lines represent our estimates of the position and relative intensities of the component bands after deconvolution and fitting.

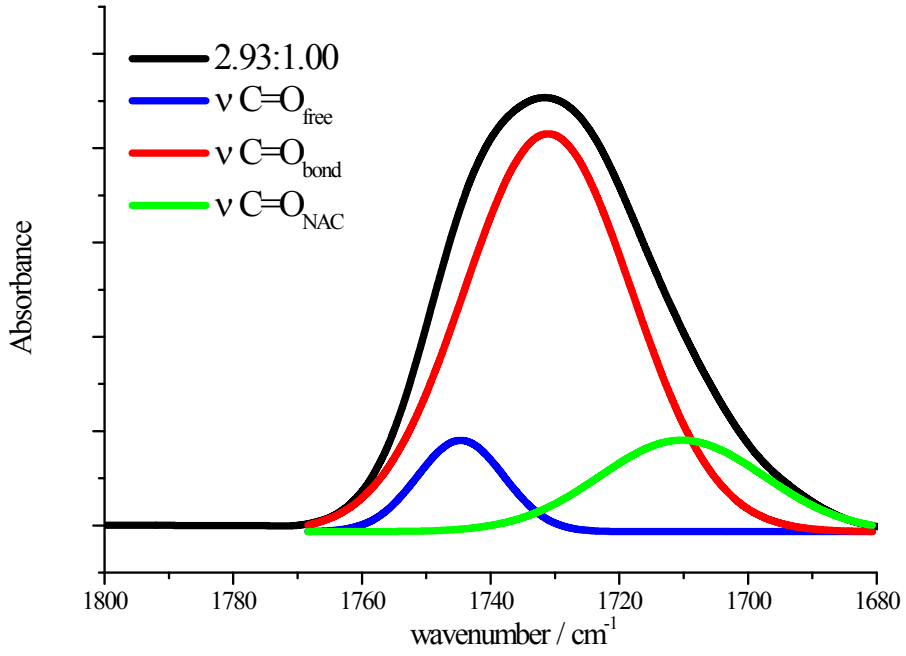

DPPC
ν C=O free
ν C=O bond
Absorbance
1800
1780
1760
1740
1720
1700
1680
wavenumber / cm-1

A (pH 2)

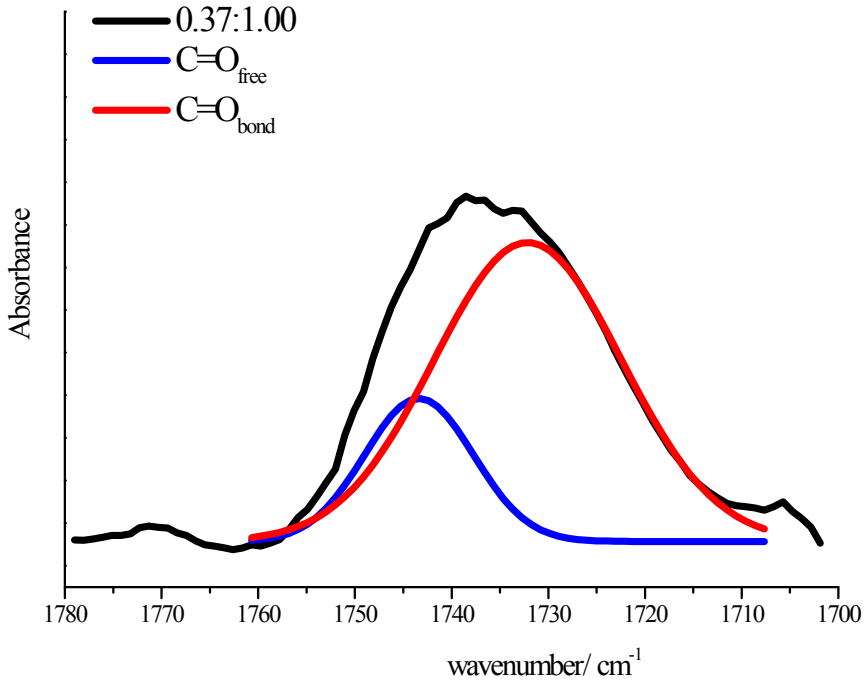

0.37:1.00
ν C=O free
ν C=O bond
ν C=O NAC
Absorbance
1800
1780
1760
1740
1720
1700
1680
wavenumber / cm-1

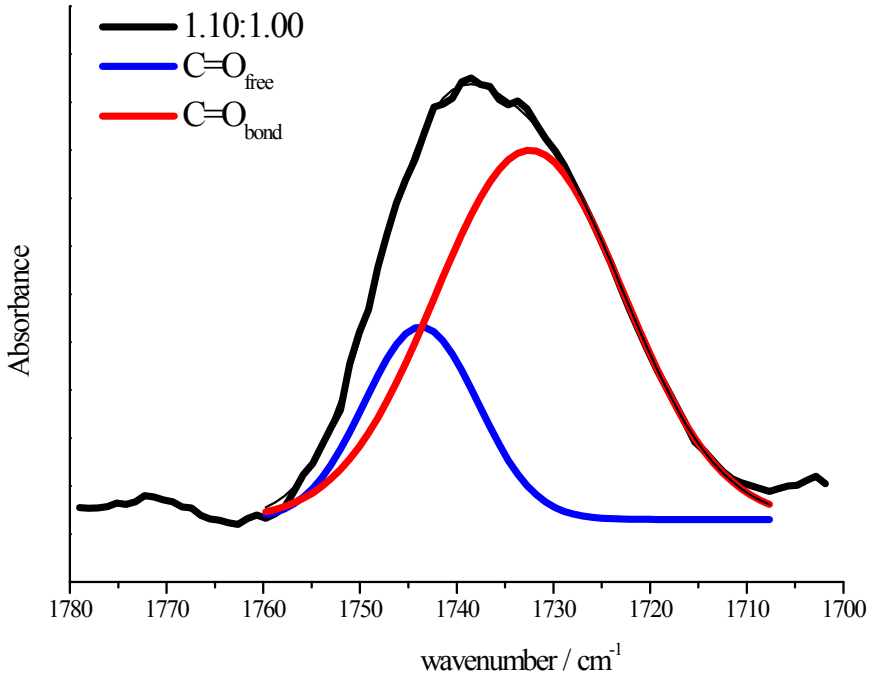

1.10:1.00
ν C=O free
ν C=O bond
ν C=O NAC
Absorbance
1800
1780
1760
1740
1720
1700
1680
wavenumber / cm-1

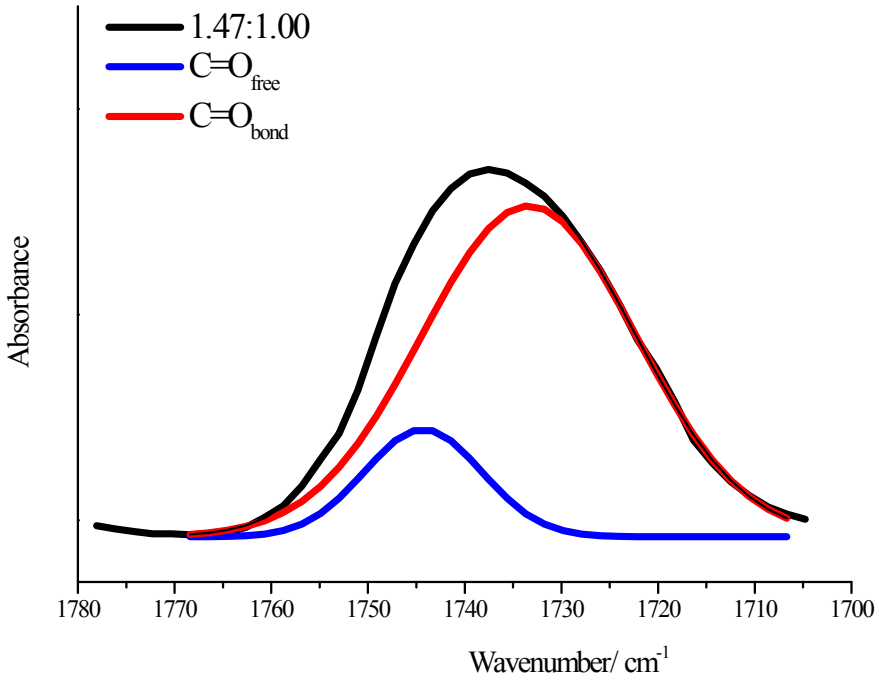

1,0
0,8
0,6
0,4
0,2
0,0
Absorbance
1.47:1.00
ν C=O free
ν C=O bond
ν C=O NAC
1800
1780
1760
1740
1720
1700
1680
wavenumber / cm-1


Absorbance
2.93:1.00
ν C=O free
ν C=O bond
ν C=O NAC
1800
1780
1760
1740
1720
1700
1680
wavenumber / cm-1

B (pH 7)


0.37:1.00
C=O free
C=O bond
Absorbance
1780
1770
1760
1750
1740
1730
1720
1710
1700
wavenumber/ cm-1


1.10:1.00
C=O free
C=O bond
Absorbance
1780
1770
1760
1750
1740
1730
1720
1710
1700
wavenumber / cm-1


1.47:1.00
C=O free
C=O bond
Absorbance
1780
1770
1760
1750
1740
1730
1720
1710
1700
Wavenumber/ cm-1

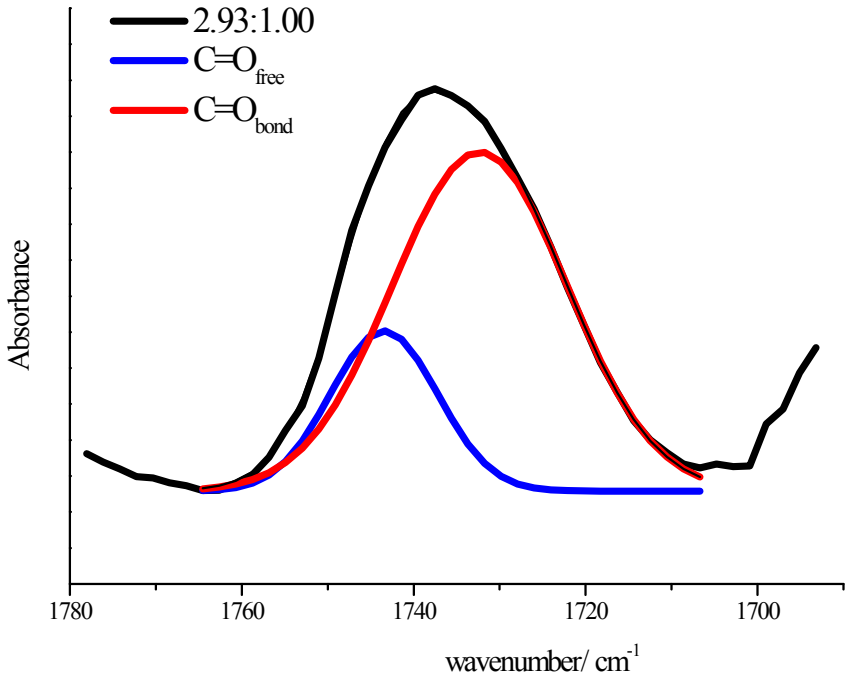


Figure S4. FTIR spectral bands of carbonyl groups of NAC: DPPC in liquid crystalline state (50ºC) at pH 2 (A) and pH 7 (B). The black line represents the contours of the spectra acquired and the blue and the red lines represent our estimates of the position and relative intensities of the component bands after deconvolution and fitting.

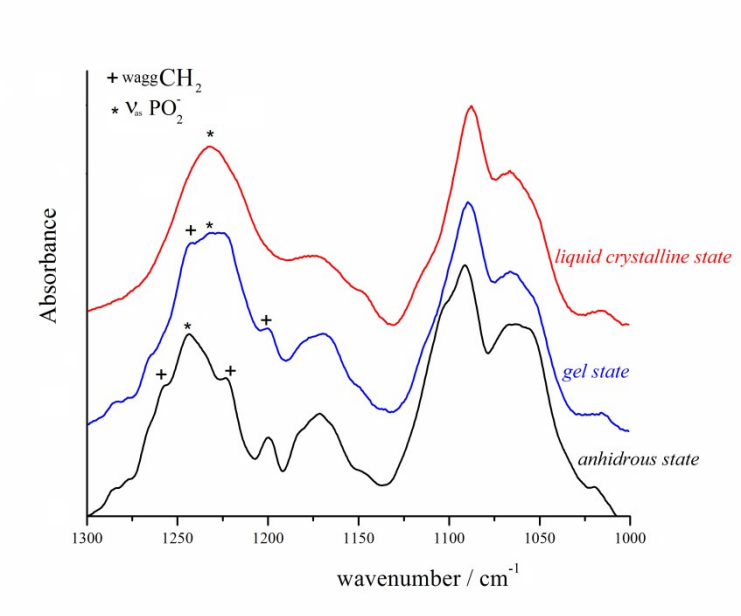


Figure S5. FTIR spectra corresponding DPPC liposomes lyophilized and hydrated in the region of the stretching mode of the phosphate group.

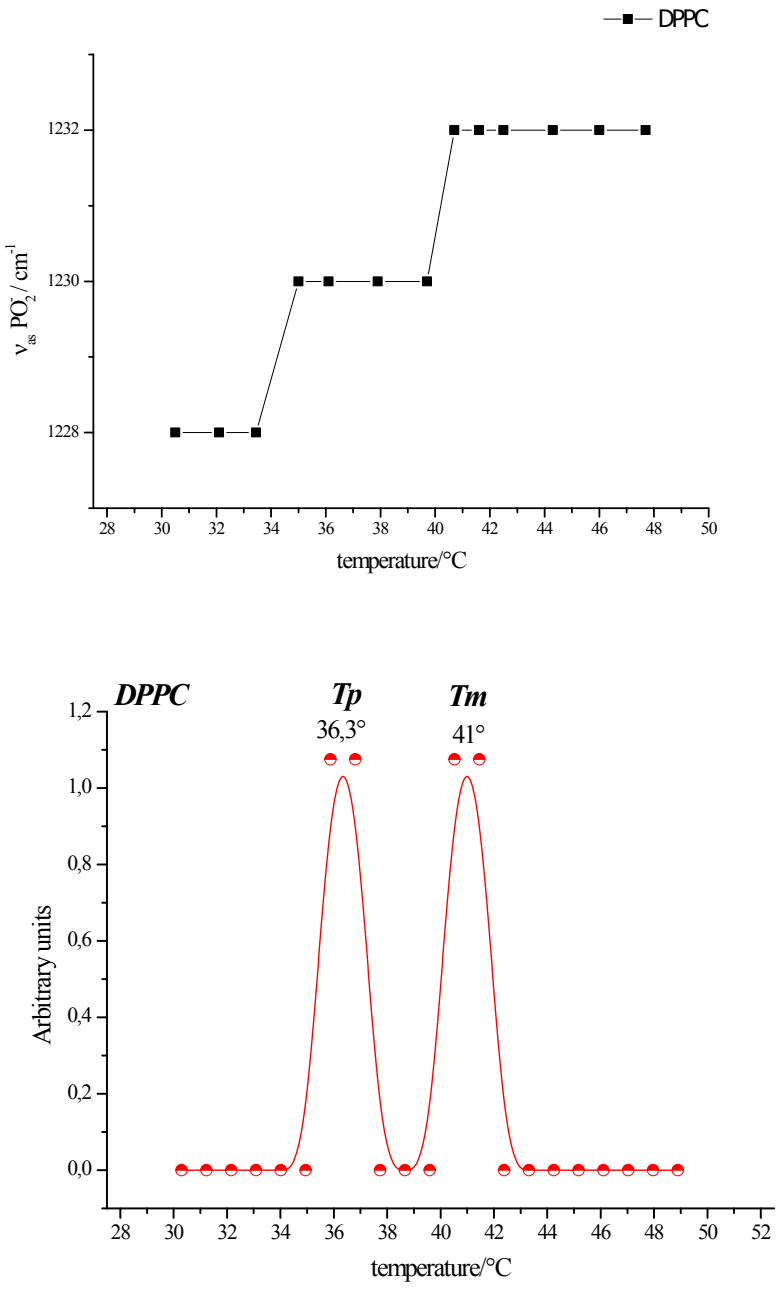


Figure S6. Wavenumbers vs temperature corresponding DPPC liposomes hydrated in the region of the asymmetric stretching mode of the phosphate group, from the FTIR spectra (upper). First derivative of the values of ν_{as} PO^-_2 versus temperature (down).