ESI-2

The relationship between the structure and functionality of the essential PUFA delivery systems based on sodium caseinate with phosphatidylcholine liposomes without and with a plant antioxidant: an in vitro and in vivo study

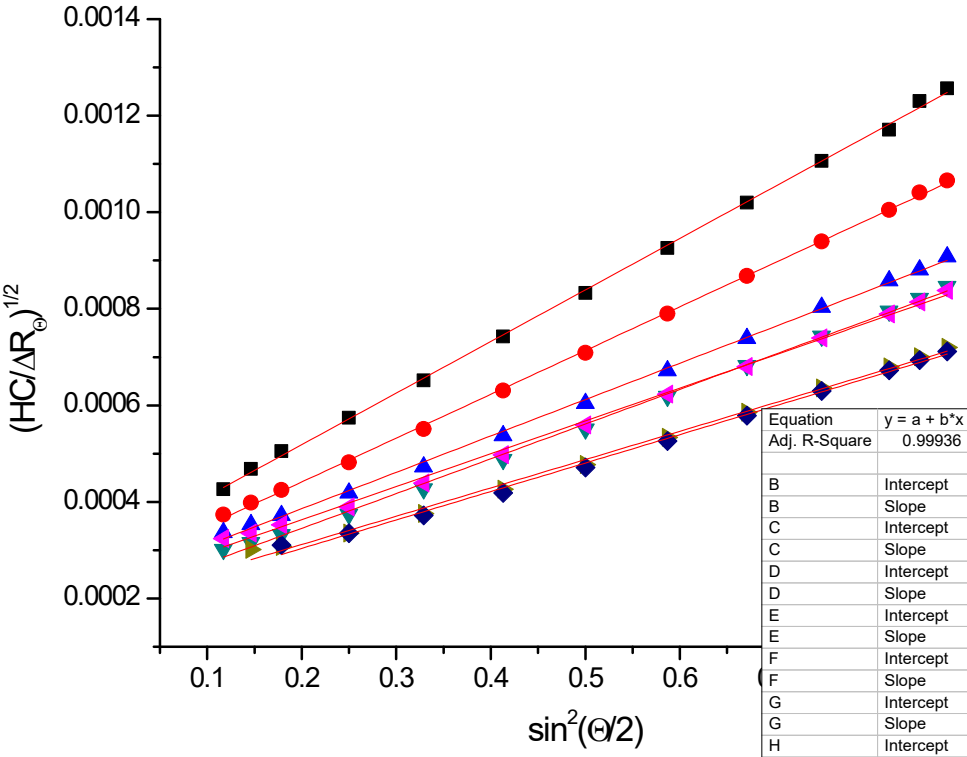
Maria G. Semenova,* Anna S. Antipova, Elena I. Martirosova, Sergey A.Chebotarev, Nadezhda P. Palmina, Natalya G. Bogdanova, Natalya I. Krikunova, Daria V. Zelikina, Maria S. Anokhina, Valery V. Kasparov

Emanuel Institute of Biochemical Physics of Russian Academy of Sciences, Russian Federation.

An example of graphical processing (a double extrapolation) of static light scattering measurements according to the Berry method[1]

The raw SLS data were used to plot the angular and concentration dependencies of the ratio $(HC/\Delta R_\Theta)^{1/2}$ according to the Berry method[2]. Here, C is the protein concentration (g/ml), ΔR_Θ is the excess light scattering over that of the solvent at angle Θ, and H is an instrumental optical constant equal to $4\pi^2 n^2 \nu^2 / N_A \lambda^4$, where N_A is Avogadro's number, λ is the wavelength of incident vertically polarized light (633 nm) in vacuo, n is the refractive index of the solvent, and ν = dn/dc is the refractive index increment of the biopolymer-based particles. Values of the weight-average molecular weight, M_w, were estimated **as** averages from the intercepts of both the concentration dependence of $(HC/\Delta R_\Theta)_{C=0}^{1/2}$ as $\Theta \rightarrow 0$ (the extrapolation was performed on 13 angles) and the angular dependence of $(HC/\Delta R_{\Theta=0})^{1/2}$ as $C \rightarrow 0$ (the extrapolation was performed on 5–8 concentrations). Values of the radius of gyration, R_G, were estimated from the slope of the angular dependence of $(HC/\Delta R_\Theta)_{C=0}^{1/2}$ as $\Theta \rightarrow 0$. Values of the second virial coefficient, A_2, were estimated from the slope of the concentration dependence of $(HC/\Delta R_{\Theta=0})^{1/2}$ as $C \rightarrow 0$.

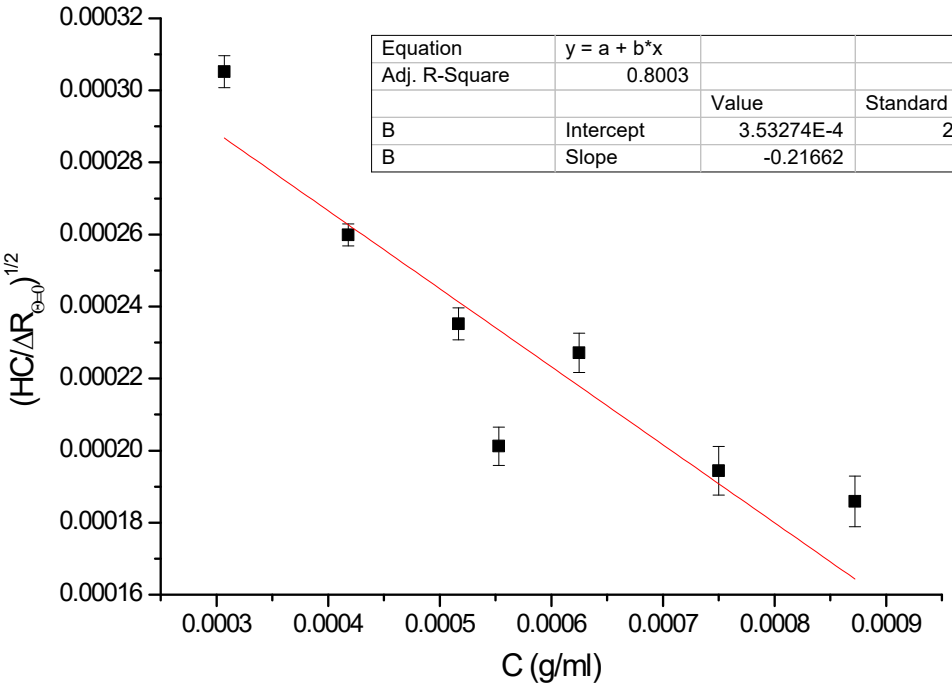
A



Equation	y = a + b*x		
Adj. R-Square	0.99936	0.99958	0.99872
		Value	Standard Error
B	Intercept	3.05235E-4	4.43779E-6
B	Slope	0.00107	7.80885E-6
C	Intercept	2.59876E-4	3.04232E-6
C	Slope	9.07301E-4	5.35334E-6
D	Intercept	2.35157E-4	4.42606E-6
D	Slope	7.53572E-4	7.78819E-6
E	Intercept	2.01212E-4	5.29933E-6
E	Slope	7.20048E-4	9.32483E-6
F	Intercept	2.2713E-4	5.425E-6
F	Slope	6.81253E-4	9.54595E-6
G	Intercept	1.94421E-4	6.74241E-6
G	Slope	5.86502E-4	1.14173E-5
H	Intercept	1.85878E-4	7.00745E-6
H	Slope	5.89205E-4	1.13901E-5

B

SC-PC-FO



Equation	y = a + b*x		
Adj. R-Square	0.8003		
		Value	Standard Error
B	Intercept	3.53274E-4	2.2805E-5
B	Slope	-0.21662	0.04329

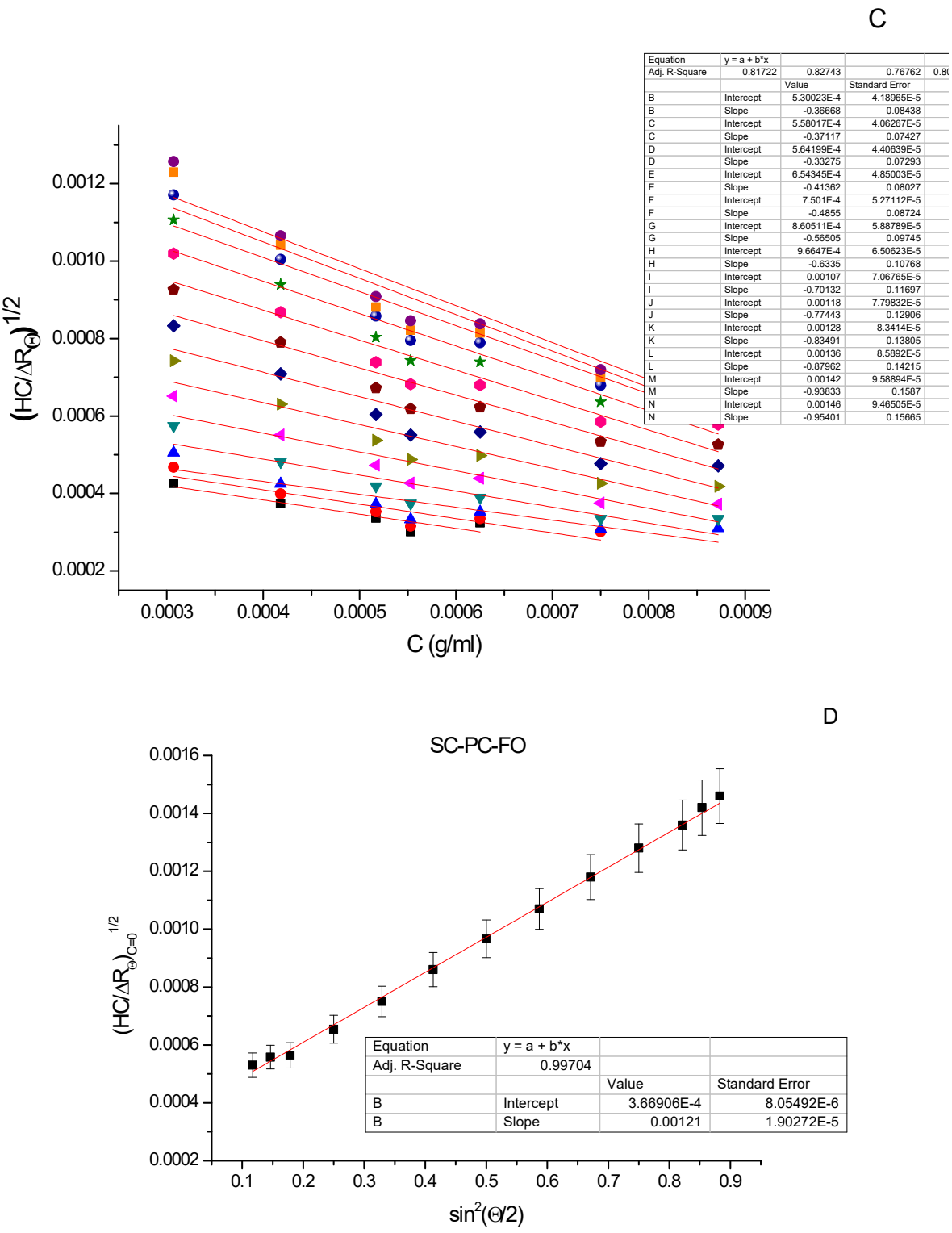


Fig. S1 An example of graphical processing (a double extrapolation) of static light scattering measurements according to the Berry method[2] in the case of the hydrolyzed SC–PC–FO complex particles in the simulated conditions of the small intestine (SIF, pH 7.0, 37 ^{0}C, ionic strength = 0.266 M): A – the values of the ratio $(HC/\Delta R_\Theta)^{1/2}$ are extrapolated to the $\Theta \rightarrow 0$; B – the values of the ratio $(HC/\Delta R_{\Theta=0})^{1/2}$ are extrapolated to the $C \rightarrow 0$; C – the values of the ratio $(HC/\Delta R_\Theta)^{1/2}$ are extrapolated to the $C \rightarrow 0$; D – the values of the ratio $(HC/\Delta R_\Theta)_{C=0}{}^{1/2}$ are extrapolated to the $\Theta \rightarrow 0$.

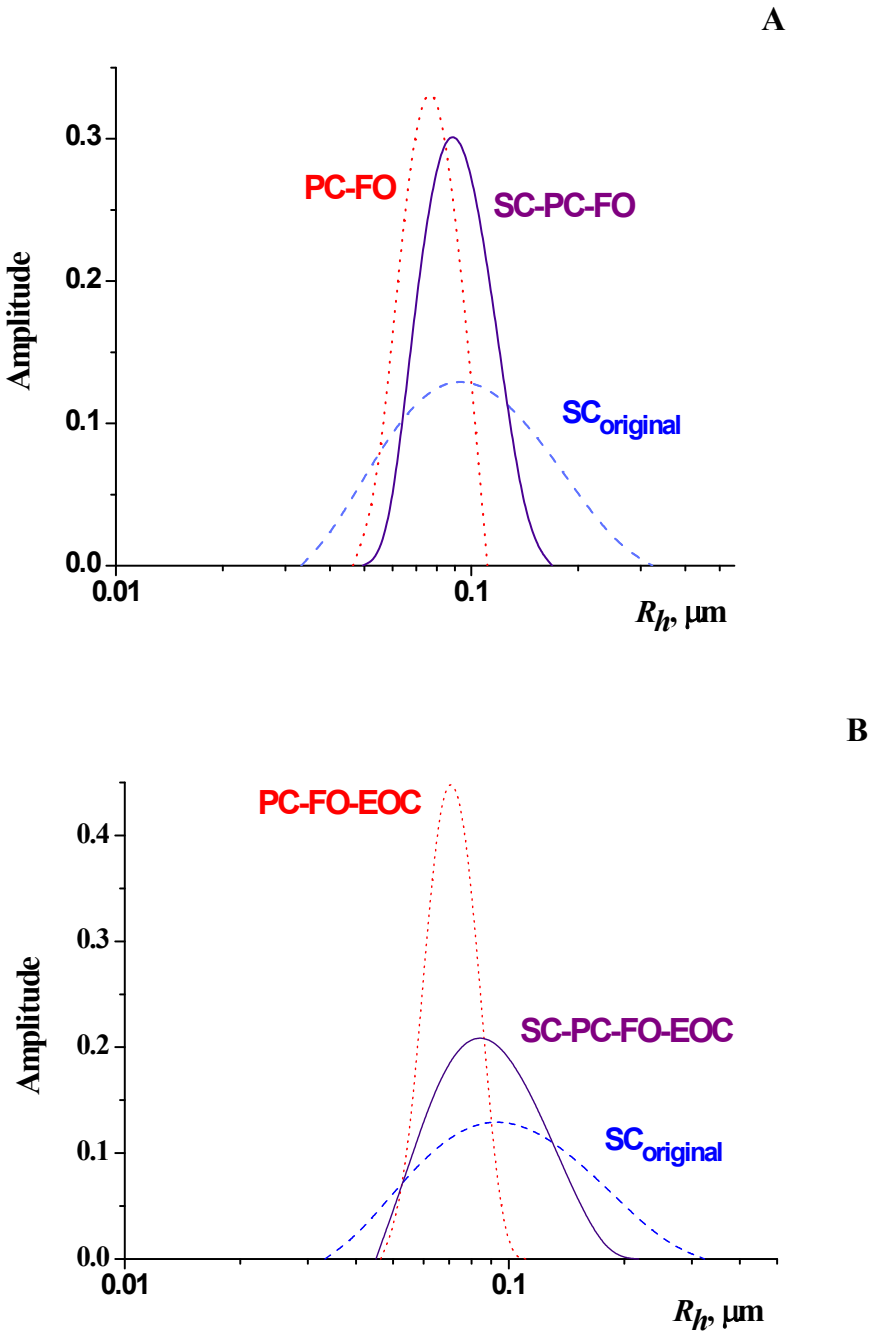


Figure S2. The DLS particle size distribution profiles of the following samples: A - original sodium caseinate (SC)(blue; dash), PC–FO liposomes (red; dot), the complex SC–PC–FO (purple; solid); B - original sodium caseinate (SC)(blue; dash), PC–FO–EOC liposomes (red; dot), the complex SC–PC–FO–EOC (purple; solid). The aqueous medium is a phosphate buffer (pH 7.0, I = 0.001M, 25°C).

Table S1 A detailed description of the DLS particle size distribution profiles presented in Fig. S2 for the PC–FO and PC–FO–EOC liposomes, original sodium caseinate (SC) particles, and the complexes of the SC with both PC–FO and PC–FO–EOC liposomes in an aqueous medium (phosphate buffer: pH 7.0, I = 0.001M) at 25 ^{0}C.

Sample	$R_h^{average}$ (nm)	R_h^{min} (nm)	R_h^{max} (nm)	A peak width, ΔR_h (nm)
PC–FO	74 ± 7	55 ± 6	94 ± 9	39 ± 4
PC–FO–EOC	78 ± 8	55 ± 6	94 ± 9	39 ± 4
SC	105 ± 11	40 ± 4	272 ± 27	232 ± 23
SC– PC–FO	101 ± 3	59 ± 6	142 ± 14	83 ± 8
SC–PC–FO–EOC	102 ± 4	54 ± 5	183 ± 18	129 ± 13

A comparison of the molecular parameters of SC with those of the complexes SC–PC– FO and SC–PC– FO– EOC

Table S2 presents the structural parameters of both the initial SC and its complexes with the PC-FO and PC-FO-EOC liposomes in an aqueous medium. The M_w of the complexes exceeded the initial molar mass of the protein by 6.6 and 5 times for the SC-PC-FO and SC-PC-FO-EOC, respectively. At the same time, the radii of gyration (R_G) of the complexes differed from that of the initial protein particles by only 1.2 times and 1.1 times for the SC-PC-FO and SC-PC-FO-EOC, respectively. The hydrodynamic radii (R_h) of the complexes did not differ from the R_h of the initial SC particles within the experimental errors. In addition, (Fig. S2) shows the DLS particle size distribution profiles of the initial SC, liposomes (PC-FO, PC-FO-EOC) and the complex (SC-PC-FO, SC-PC-FO-EOC) particles. Table S1 gives a detailed description of them.

All these data indicate a significant association of the initial SC particles, as if the liposomes acted as crosslinking agents. This crosslinking was accompanied by a tendency towards the shrinkage the initial rather porous and flexible structure of the SC within the complex particles.

In addition, Table S2 shows the thermodynamic parameters, namely the second virial coefficients (A_2*), in the virial series of chemical potentials by concentration (a molal scale) of both biopolymer-based particles (SC; SC-PC-FO; SC-PC-FO-EOC) and solvent molecules in a solution (Eq. 1S, 2S):[2,3,5]

Table S2 Molecular parameters of original sodium caseinate and its complexes with PC-FO liposomes without and with EOC in an aqueous medium (phosphate buffer: pH 7.0, I = 0.001M) at 25 ^{0}C. All data were analyzed by one-way ANOVA with Tukey's test ($p < 0.05$). Different simple letters in the same column indicate significant differences between samples. Capital letters (A, B) indicate significant differences between absolute values of A_2^{excl} and $A_2^{el}+A_2^{h/b}+A_2^{h/a}$ for the same sample.

Sample	$M_w \times 10^{-6}$ (Da)	R_G (nm)	R_h (нм)	$\rho = R_G/R_h$	$d \times 10^3$ (g/см^3)	A_2* (m^3/mol)	A_2^{excl} (m^3/mol)	$A_2^{el}+A_2^{h/b}+A_2^{h/a}$ (m^3/mol)	ζ-potential (mV)
SC	11.9 ± 1.1^{c}	159 ± 16^{c}	105 ± 11^{a}	1.51 ± 0.19^{b}	1.20 ± 0.11^{b}	12.4 ± 1.2^{c}	23.6 ± 2.4^{Aa}	-11.2 ± 1.1^{Bb}	-34 ± 1^{b}
SC-PC-FO	79 ± 12^{a}	194 ± 12^{a}	101 ± 3^{a}	1.93 ± 0.27^{a}	4.29 ± 0.11^{a}	247 ± 25^{a}	20.5 ± 2.1^{Ba}	226 ± 23^{Aa}	-38 ± 2^{a}
SC-PC-FO-EOC	63 ± 3^{b}	180 ± 1^{b}	102 ± 4^{a}	1.76 ± 0.22^{ab}	4.31 ± 0.23^{a}	18.0 ± 0.6^{c}	21.7 ± 2.2^{Aa}	-3.7 ± 0.4^{Bc}	-36 ± 2^{ab}

M_w is the weight-average molar mass; R_G is the radius of gyration; R_h is the hydrodynamic radius; ρ is the structure sensitive parameter; d is the density that is calculated using the equation: $d = M_w/(N_A V)$, where N_A is the Avogadro number and $V = 4/3\pi R_h^3$ for spherical particles; A_2* is the molal osmotic second virial coefficient in the virial series of chemical potentials by concentration (a molal scale) of both biopolymer-based particles (SC-PC-FO; SC-PC-FO-EOC) and solvent molecules in a solution; $A_2^{excl} = 10^{-3}\, 4\pi\, N_A/3(2R_h)^3$ (where N_A is Avogadro number) is the contribution to the value of A_2*from the excluded volume effects; $A_2^{el}+A_2^{h/b}+A_2^{h/a} = A_2^* - A_2^{excl}$ is the total contribution to the value of the A_2*from the pair interactions of different nature (A_2^{el} - electrostatic, $A_2^{h/b}$ – hydrogen bonding, $A_2^{h/a}$ -hydrophobic attraction).

$$\mu_1 = \mu_1^0 - (RT/m_1) \times (m_2 + ½\, A_2^* m_2^2), \qquad (1S)$$

$$\mu_2 = \mu_2^0 + RT\,[\ln(m_2/m^0) + A_2^* m_2], \qquad (2S)$$

where, μ_i^0 and m_i are the standard chemical potential and concentration (the molal scale) of the *i*-component (*i* = 1 for solvent molecules, *i* = 2 for the biopolymer-based particles); A_2^* is the osmotic second virial coefficient (in molal scale units of m^3/mol, i.e., taking the molar mass of the biopolymer-based particles into account); and m^0 is the standard-state molality for the biopolymer-based particles.

The sign and the magnitude of the A_2^* characterize the nature and intensity of pair interactions, respectively, between the biopolymer-based particles, as well as between the biopolymer-based particles and solvent molecules in solutions.[2,3]

A positive sign was found for the A_2^* of both the initial SC and the (SC-PC-FO, SC-PC-FO-EOC) complexes. On the one hand, the positive sign of the A_2^* indicates the thermodynamically unfavorable repulsive interactions between a pair of the biopolymer-based particles. Such interactions are mainly determined by the excluded volume effects and the electrostatic repulsive forces acting between the likely charged functional groups of the biopolymer-based particles[2,3,4], which are quantitatively characterized by the values of A_2^{excl} and A_2^{el}, respectively (Table S2).

The thermodynamically unfavorable interactions between the biopolymer-based particles cause an increase in the magnitude of the chemical potential of the biopolymer-based particles (Eq. 2S). On the other hand, the positive sign of the A_2^* indicates the thermodynamically favorable pair interactions between the biopolymer-based particles and the solvent molecules; i.e., such interactions lead to a decrease in the magnitude of the chemical potential of the solvent molecules in the presence of the biopolymer-based particles (Eq. 1S). Thus, the positive sign of the A_2^* means the thermodynamically good quality of aqueous medium for the biopolymer-based particles,[2,3,4] which, therefore, underlies their good solubility in an aqueous medium.

At the same time, the magnitude of the positive A_2^* indicates that both complex particles (SC-PC-FO; SC-PC-FO-EOC) have a higher thermodynamic affinity for an aqueous medium than that of the initial SC. This result can be attributable to the detected association of the initial SC as a result of its interactions with the liposomes during the formation of the complexes. It can be assumed that this association may hide a hydrophobic surface, that is initially available for the

contact with an aqueous medium, inside the complex particles, thereby increasing their hydrophilic surface, containing polar and charged functional groups. This was most pronounced for the SC-PC-FO particles, for which an order of magnitude greater positive A_2* value was found compared to that for the SC-PC-FO-EOC particles, despite the found equal contributions of the excluded volume effects (A_2^{excl}) to the magnitude of the A_2*. This result can be attributable to the greatest increase in the M_w (i.e., the highest degree of the association of the initial SC) during the complexation of the SC with the liposomes (PC-FO) (Table S2).

In addition to this, both initial SC and the complex particles (SC-PC-FO; SC-PC-FO-EOC) have a negative ζ-potential > 30 mV (Table 3). This result shows that these particles are highly stable and less prone to aggregation in their aqueous solutions.[6] It is also important to note here that there are lower values of the experimentally measured ζ-potentials of the complexes (Table S2) compared to those hypothetically calculated as the sum of the absolute values of the ζ-potentials of the components of the complexes: SC (-34.0 ± 1.1)+ PC-FO (-22.0 ± 1.7) = -56 ± 2.8 and SC (-34.0 ± 1.1)+ PC-FO-EOC (-21.0 ± 1.3) = -55 ± 2.3. This result indicates the participation of electrostatic attraction forces between oppositely charged functional groups of SC and the (PC-FO; PC-FO-EOC) liposomes in the formation of their complexes.

References

1. W. Burchard, in Light scattering, ed. S. B. Ross-Murphy, Blackie, Glasgow, 1994, Ch. Physical techniques for the study of food biopolymers, 151–214.
2. M. G. Semenova and E. Dickinson, Biopolymers in food colloids: Thermodynamics and molecular interactions, Brill Leiden, 2010.
3. C. Tanford, Physical chemistry of macromolecules, Wiley, New York, 1961.
4. M. Nagasawa and A. Takahashi, In, Light scattering from polymer solutions, ed. M. B. Huglin, Academic Press, London, 1972, Ch. 16, Light scattering from polyelectrolyte solutions, 671–723.
5. A. G. Ogston, Some thermodynamic relationships in ternary systems, with special reference to the properties of systems containing hyaluronic acid and protein. Arch. Biochem. Biophys, 1962, 38, 39–51.
6. E. Dickinson, Hdrocolloids as emulsifiers and emulsion stabilizers, Food Hydrocoll., 2009, 23(6), 1473-1482.