Analysis of structure of the PTNS vesicles based on small-angle neutron scattering data obtained at the “Yellow Submarine” spectrometer

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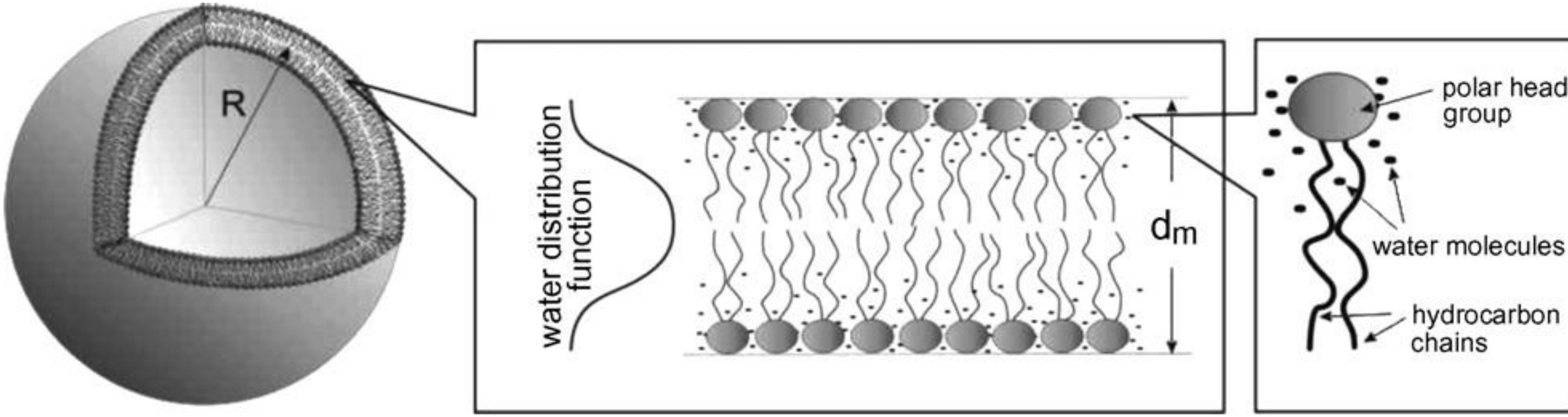
LXXV International Conference «NUCLEUS–2025»
1–6 July 2025, St. Petersburg State University

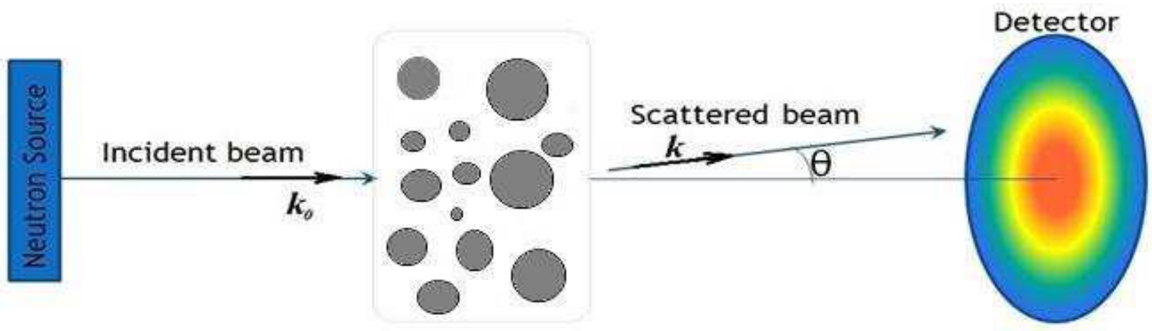
Introduction: Vesicles



- Unilamellar vesicles (ULVs) of phospholipids are nanosized objects with a nearly spherical shape. The vesicle shell (bilayer) consists of phospholipid molecules that form a special structure.
- Interest in the study of vesicular systems is due to the need to obtain fundamental knowledge about the structure and properties of biological membranes, as well as the prospects for practical applications in medicine and cosmetology.
- The phospholipid transport nanosystem (PTNS), developed at the Research Institute of Biomedical Chemistry (Moscow), increases the effectiveness of drugs “built into” the PTNS. Experiments show that the suspension obtained by dissolving the lyophilized PTNS powder in water has a vesicular structure consisting of small-diameter ULVs.

Introduction: Method

- Small angle scattering technique is known as powerful tool for investigation of structure of nanosystems.
- Small-angle scattering of neutrons (SANS) and X-rays (SAXS) is an experimental technique that uses elastic neutron (X-ray) scattering at small scattering angles to investigate the structure of various substances at a mesoscopic scale of about 1-100 nm; the direction of the scattered rays is only slightly (at small angles) deviated from the direction of the incident beam.
- Small-angle neutron scattering (SANS) and X-ray scattering (SAXS) are widely used experimental methods for studying the structure of ULV systems.



Aim of Study

- We study a structure of ULVs of PTNS and PTNS-based medical drug “Indolip” by means of analysis of SANS data obtained on the “Yellow Submarine” small angle spectrometer (Budapest).
- SANS measurements were performed on polydispersed populations of PTNS ULVs in heavy water with different PTNS concentrations and with different purity of soybean phospholipids in the PTNS samples.
- Results of the analysis are compared with the characteristics of PTNS vesicular systems obtained earlier in the analysis of other small-angle scattering data from PTNS samples.
- One of purposes of our analysis is to manifest an influence of purity of soybean phospholipids in the PTNS samples on basic parameters of PTNS ULVs: radius, polydispersity of radius, thickness of bilayer etc.
- Experiment: “Yellow Submarine”, BNC, Budapest, Hungary. Sample-detector distances: 120 and 540 cm (neutron wavelengths: 4.1 and 12.3 Å, respectively).
- Samples: PTNS powder was dissolved in heavy water with concentrations 5%,10%, and 25%. Sample temperature 22°C. Two variants of samples were prepared using the PTNS products of different purity: S80 (74%) and S100 (95%).

Data analysis: Formulae (1/3)

Macroscopic coherent scattering of a monodisperse population of ULVs:

$$\frac{d\Sigma}{d\Omega_{mon}}(q) = n \cdot A^2(q) \cdot S(q)$$

n – the number of vesicles per unit volume, *A(q)* – the ULV scattering amplitude, *S(q)* – the structure factor, *q* – the scattering vector modulus $q = 4\pi \sin(\theta/2)/\lambda)$, θ is the scattering angle, λ is the neutron wavelength.

Scattering amplitude in the centrally symmetric case:

$$A(q) = 4\pi \cdot \int \rho(r) \cdot \frac{\sin(qr)}{qr} \cdot r^2 \cdot dr$$

$\rho = \rho_C - \rho_{D2O}$ the neutron contrast between the scattering length density of the lipid bilayer ρ_C and the density of D_2O or solution (e.g. $\rho_{D2O} = 6.4 \cdot 10^{10}$ cm^{-2}).

or:

$$A(q) = 4\pi \cdot \int_{-d/2}^{d/2} \rho(x) \cdot \frac{\sin[(R+x) \cdot q]}{(R+x) \cdot q} \cdot (R+x)^2 \cdot dx$$

where *R* is radius of ULV and *d* – thickness of bilayer, *x* – distance from the center of the bilayer in the direction perpendicular to the plane of the membrane.

Data analysis: Formulae (2/3)

Integrating in assumption R>>d/2, R+x≈R yeilds:

$$A_{sff}(q) = 4\pi \cdot \frac{R^2}{qR} \cdot \sin(qR) \cdot \int_{-d/2}^{d/2} \rho(x) \cdot \cos(qx) \cdot dx$$

$$\frac{d\Sigma}{d\Omega}_{mon}(q) = n \cdot F_s(q,R) \cdot F_b(q,d) \cdot S(q)$$

$F_s(q,R)$ – form factor of sphere with infinitely thin shell and radius R

$$F_s(q,R) = \left(4\pi \cdot \frac{R^2}{qR} \cdot \sin(qR) \right)^2$$

$F_b(q,d)$ – form factor of a symmetric lipid bilayer:

$$F_b(q,d) = \left(\int_{-d/2}^{d/2} \rho(x) \cdot \cos(qx) \cdot dx \right)^2$$

Data analysis: Formulae (3/3)

Structural factor :

$$S(qR) = 1 - 8V_v n \cdot \left(\frac{\sin(2qR)}{qR} \right)$$

where $V_v = 4/3\pi R^3$ – vesicle volume, n – number of vesicles per unit volume.

Polydispersity of radius is accounted based the Schultz distribution

$$G(R) = \frac{R^m}{m!} \cdot \left(\frac{m+1}{\overline{R}} \right)^{m+1} \cdot \exp\left[-\frac{(m+1)\cdot R}{\overline{R}} \right]$$

Root-mean-square deviation of vesicle radius (polydispersity) from the average radius <R>

$$\sigma = \sqrt{(1+m)^{-1}}$$

Finally, accounting for the spectrometer resolution and incoherent background IB, we have:

$$I_m(q) = \frac{\int_{R\min}^{R\max} \frac{d\Sigma}{d\Omega}_{mon}(q,R)\cdot G(R,\overline{R})\cdot dR}{\int_{R\min}^{R\max} G(R,\overline{R})\cdot dR}$$

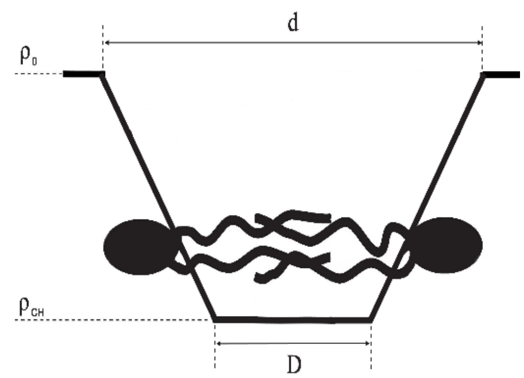
$$I(q) = I_m(q) + \frac{1}{2}\cdot\Delta^2\cdot\frac{d^2 I_m(q)}{dq^2} + IB$$

Δ^2 – second moment of the resolution function

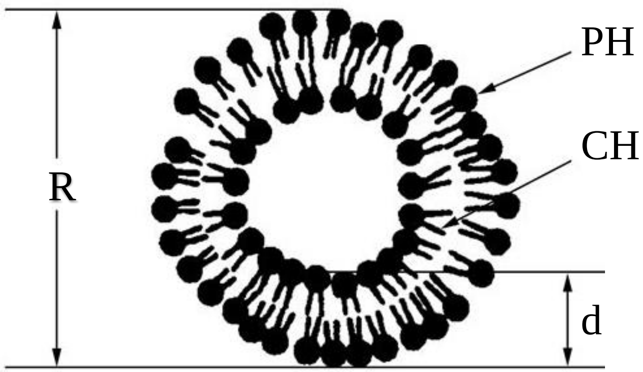
Software

HH model of bilayer

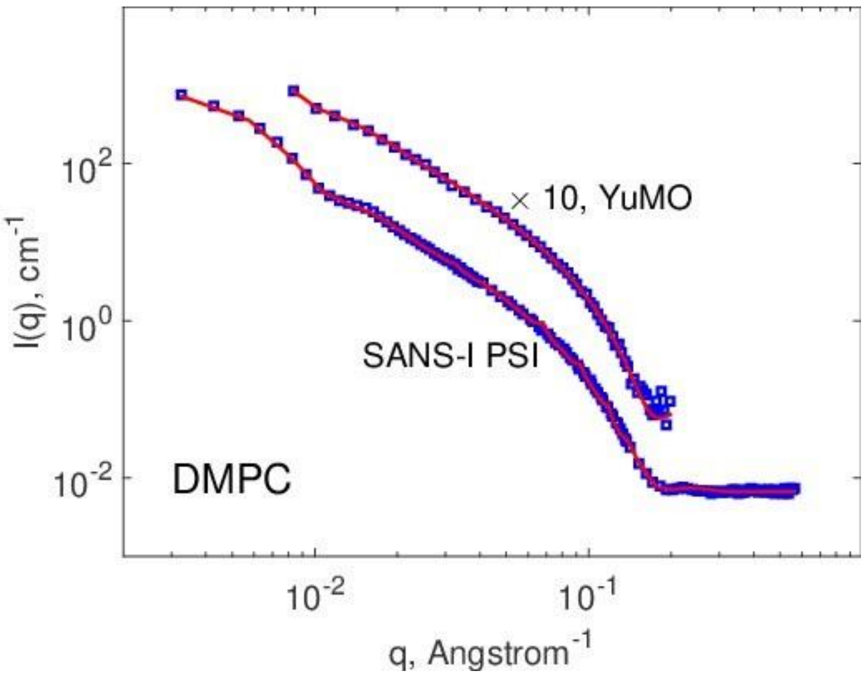
$$\chi^2 = \frac{1}{N} \cdot \sum_{i=1}^{N} \left(\frac{\left|\frac{d\Sigma}{d\Omega}(q_i)\right| - \left|\frac{d\Sigma}{d\Omega}_{exp}(q_i)\right|}{\left|\frac{d\Sigma}{d\Omega}_{exp}(q_i)\right|} \right)^2 \times 100\%$$

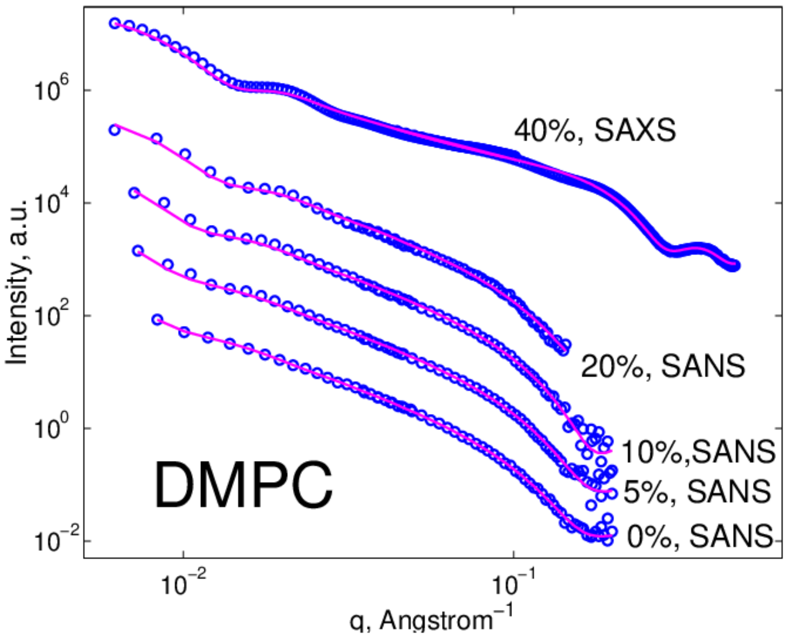


- Fitting procedure: FUMILI
- HH-model of bilayer
- The main fitting parameters are:
  - average radius of vesicles,
  - polydispersity parameter,
  - number of vesicles per unit volume,
  - incoherent background,
  - bilayer thickness,
  - thickness of the hydrophobic region in the central region of the bilayer,
  - etc.



Representative Case of "Standard" ULVs: DMPC



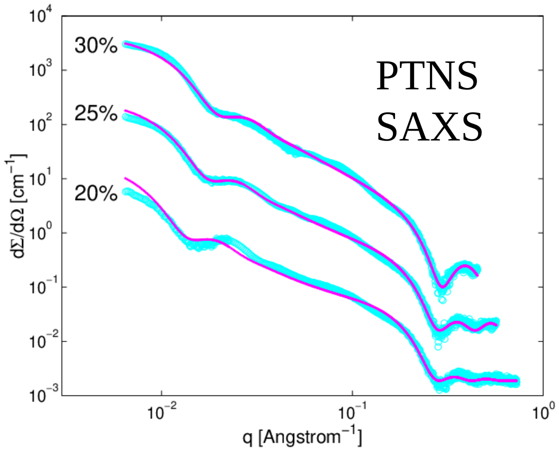


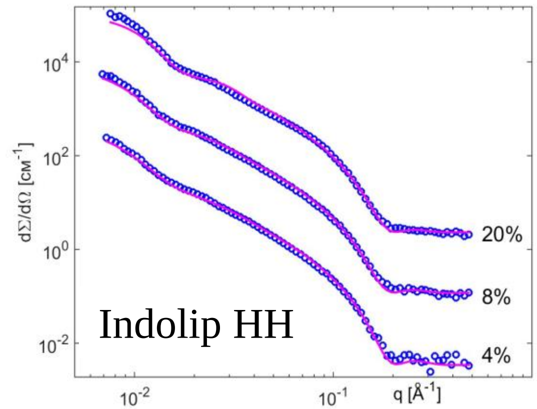
DMPC ULVs (PSI & YuMO),
EuroBioPhys J 35 6 (2006) 477-493

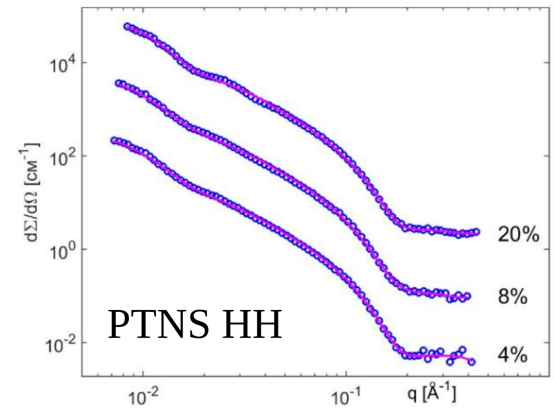
DMPC ULVs (SAXS & SANS)
Crystallography Rep 60 1 (2015) 143-147

- Results of analysis of within different approaches are in reasonable agreement (~10%)
- Relatively low polydispersity (about 20%)
- Explicit dependence of average radius on the disaccharide concentration in water solution

Previous Studies of PTNS & Indolip ULVs

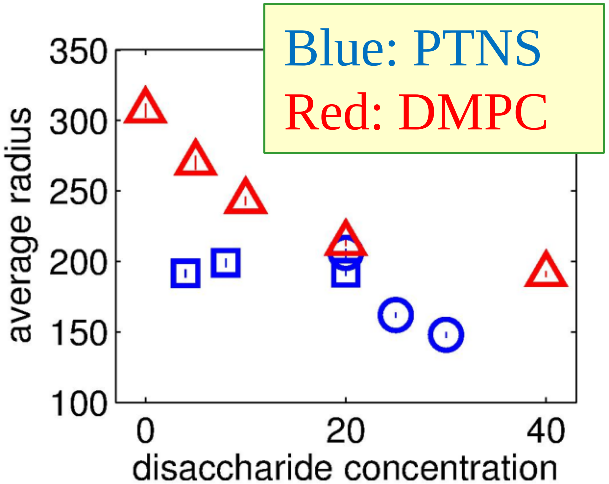


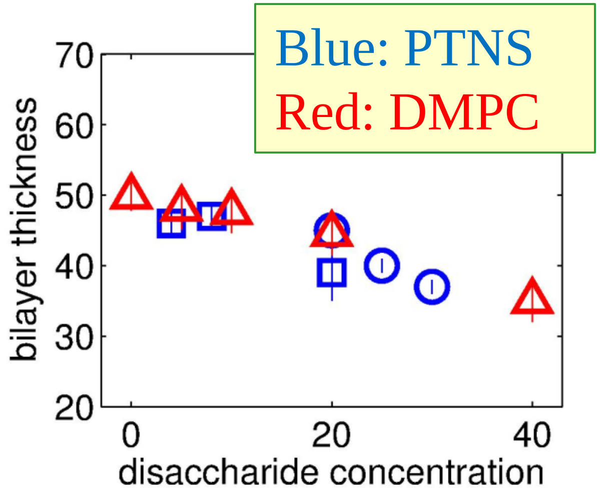




PTNS SAXS
J. Surface Investigation
13 1 (2019) 111–116

PTNS & Indolip – SANS YuMO
J Surface Investigation 17 1 (2023) 1–6

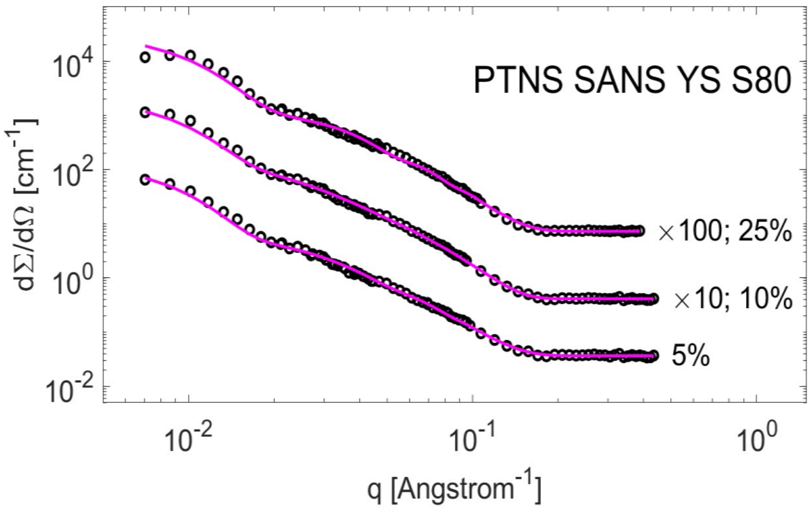


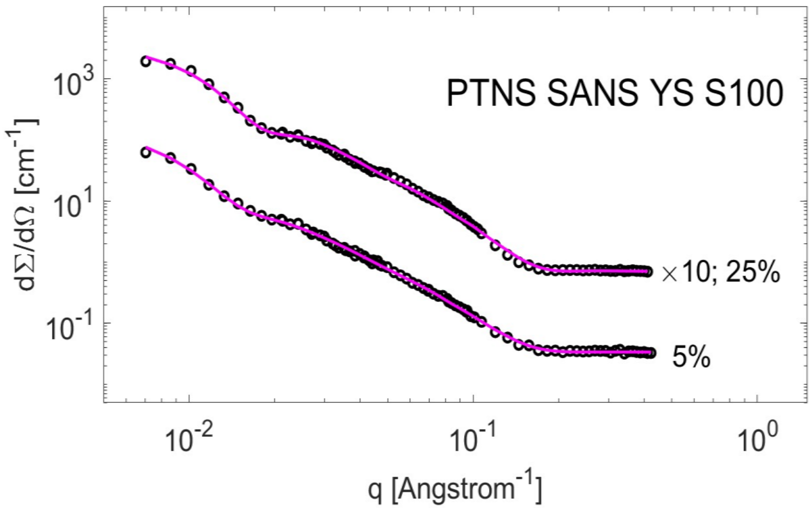


J Phys CS 1023
(2018) 012017

The PTNS ULV radius in case of the 20% disaccharide concentration is estimated
from 14.3 to 20.6 nm

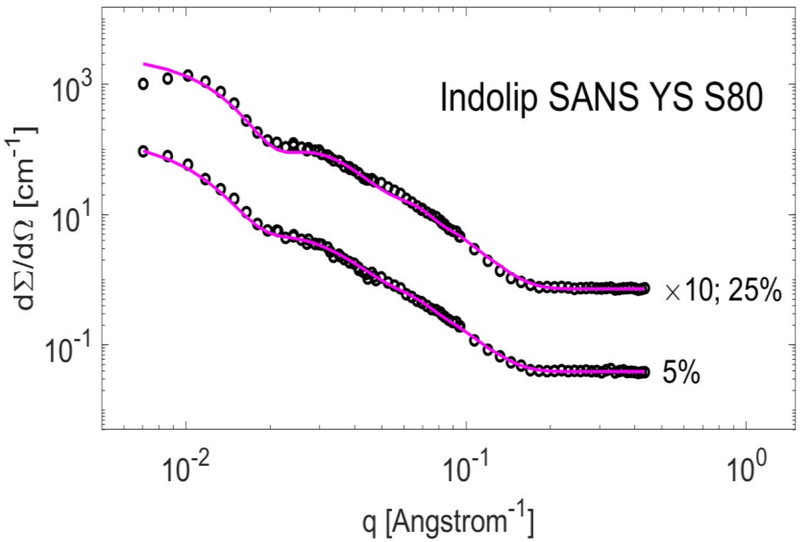
Results of Analysis of the YS Data): PTNS

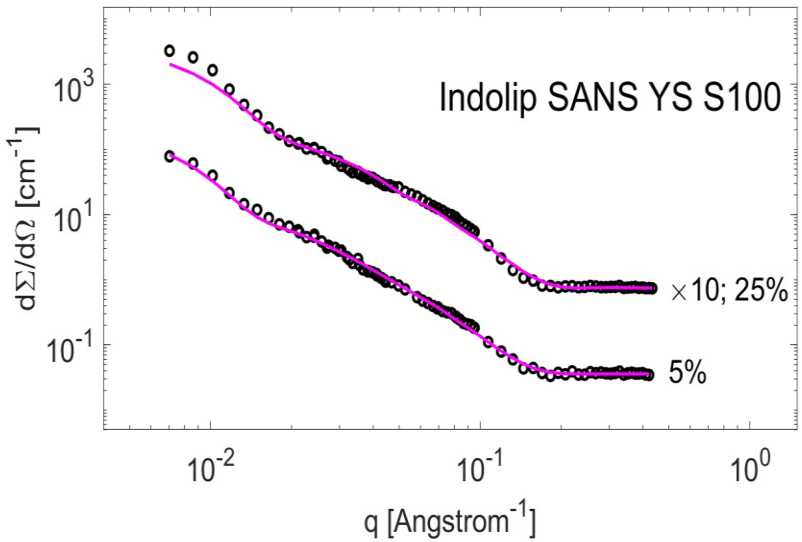




PTNS Concentration	Disaccharide Concentration	Experiment	R, Å	σ_R %	d, Å	χ^2
5%	4%	YS (S100) YS (S80)	163±4 142±2	31% 33%	40.7±0.5 46.9±1.3	1.3 3.5
10%	8%	YS (S80)	140±1	33%	47.1±0.4	1.7
25%	20%	YS (S100) YS (S80)	146±3 139±2	28% 36%	41.8±1.3 47.1±2.0	4.1 6.5

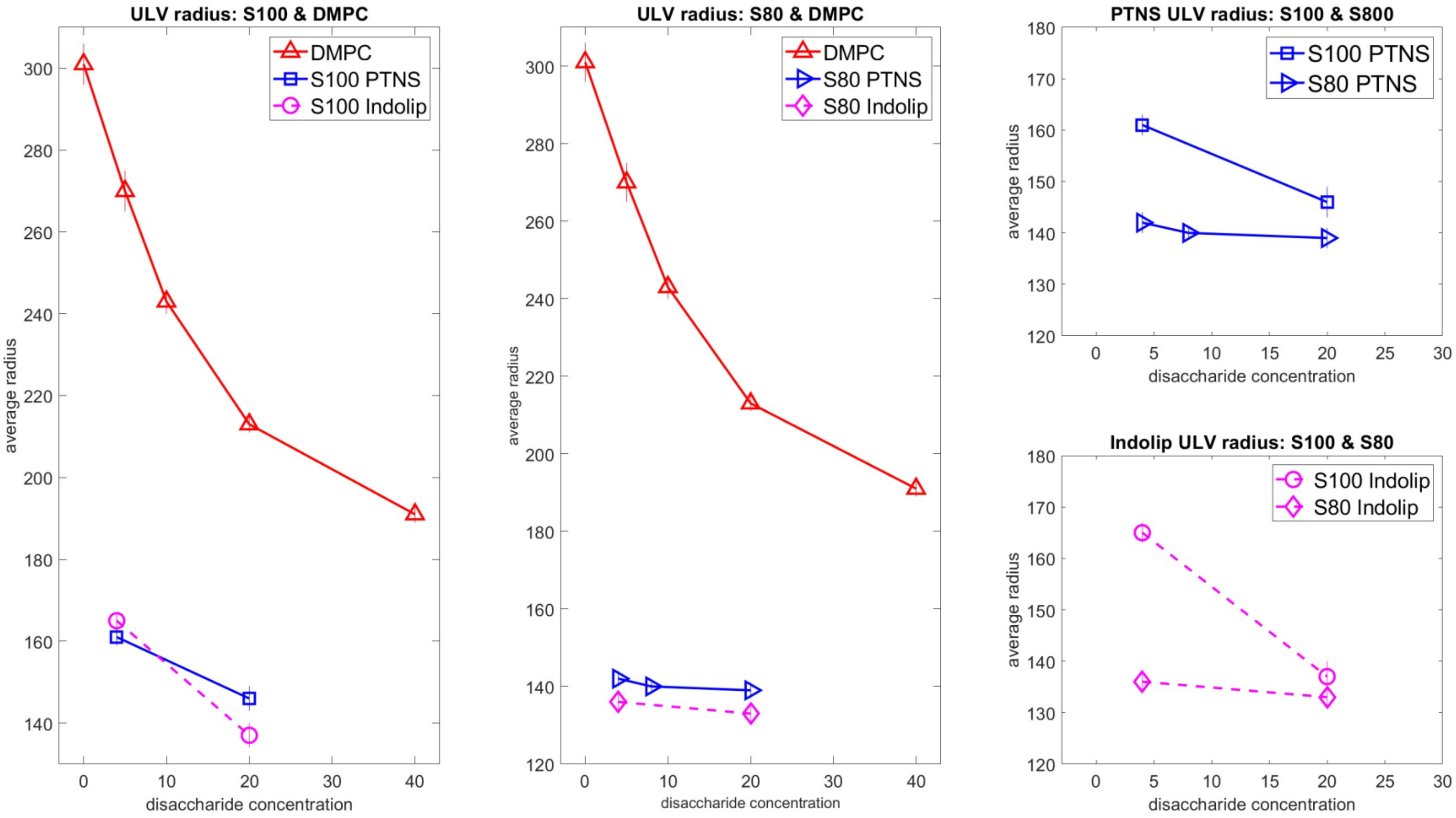
Results of Analysis of the YS Data): Indolip





Indolip Concentration	Disaccharide Concentration	Experiment	R, Å	σ_R %	d, Å	χ^2
5%	4%	YS (S100)	165±2	33%	40.9±0.5	3.4
		YS (S80)	136±2	30%	47.1±0.3	3.3
25%	20%	YS (S100)	137±3	35%	40.8±1.5	6.2
		YS (S80)	133±2	27%	44.9±2.2	7.1

ULVs of PTNS & Indolip in comparison with DMPC: radius dependence on the disaccharide concentration in heavy water



SUMMARY

- YS SANS data from PTNS and Indolip populations of ULVs have been analyzed depending on the concentration in heavy water (5%, 10%, 25%) and on purity of soybean phospholipids in the sample (74% and 95%).
- Basic parameters of the PTNS and Indolip ULVs have been determined.
- Calculations for the S100 samples with the higher phospholipid content demonstrate a noticeable decrease in the ULV radius with increasing concentrations of PTNS and Indolip in heavy water. This dependence is consistent with the results of the analysis of DMPC ULVs.
- As for the S80 samples, the dependence of radius on the concentration is rather weak.
- The difference between radiuses of S80 and S100 samples becomes smaller as the concentration of PTNS and Indolip in heavy water is growing.
- Such properties of PTNS and PTNS-based ULVs have to be accounted in practical applications.

Thank you for your attention!