Возможности малоуглового нейтронного и синхротронного рассеяния для исследования везикулярных переносчиков лекарств

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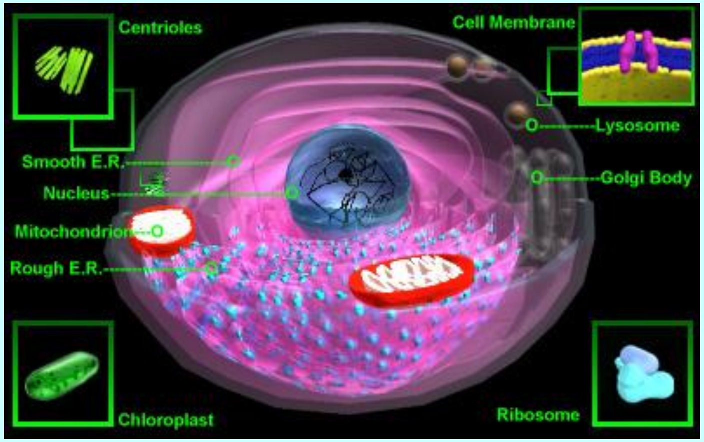
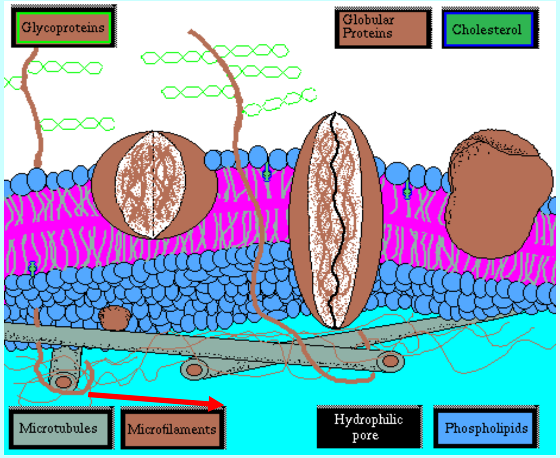
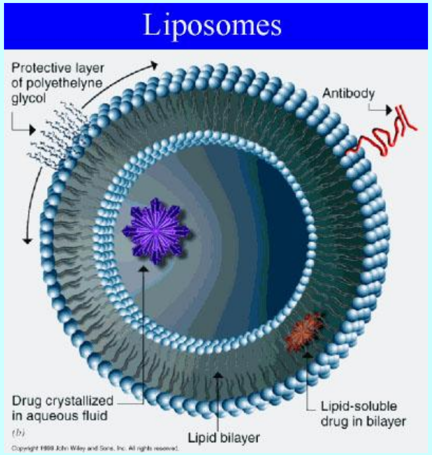
Совещание по использованию рассеяния нейтронов и синхротронного излучения в конденсированных средах (РНСИ-КС-2014), Старый Петергоф, 27-31 октября 2014

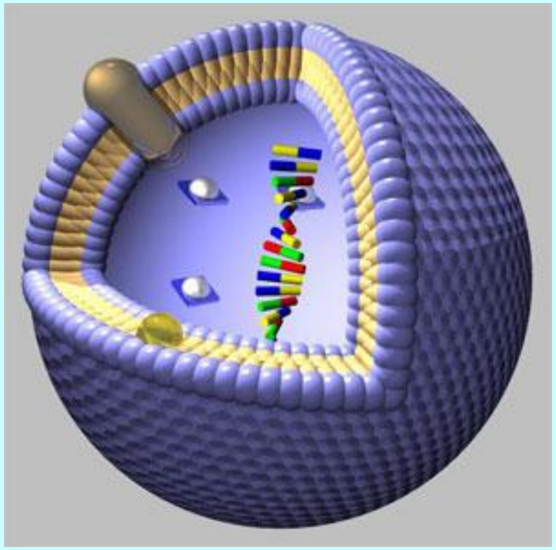
Phospholipids – major component of cell membranes and important materials for drug and cosmetic industry.







Practical applications



Phospholipid transport nanosystem (PTNS) - nanoparticles based on the phospholipids

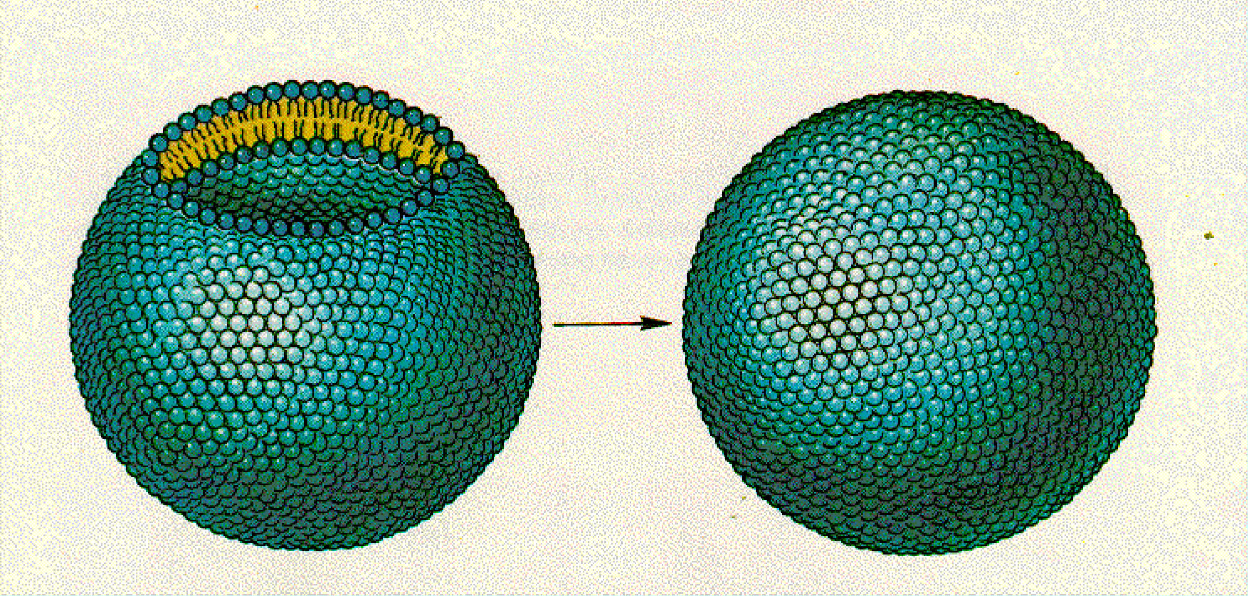
80% products in the bionanotechnology are drug delivery systems !!!

Turnover - 6 billion USD/year

Unilamellar vesicles from phospholipid molecules

From point of physics
it is nanospheres
with liquid crystal surface

R=250Å
n≅2 ·10^{14} vesicles/cm^3 for 1% DMPC (w/w)
6500 DMPC molecules in one layer for 30^{o}C



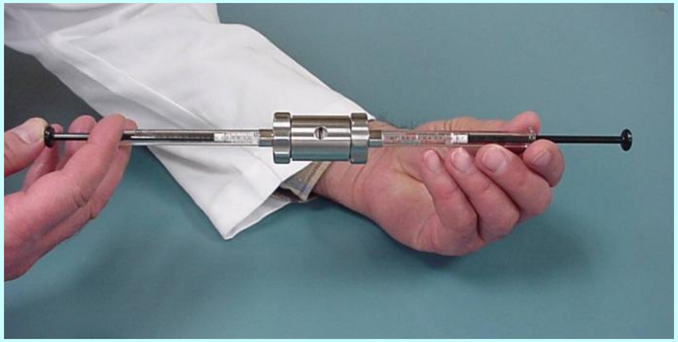
Neutron small-angle scattering in D_2O

X-ray small-angle scattering in sucrose solutions

M.A. Kiselev, P. Lesieur, A.M. Kisselev, D. Lombardo, M. Killany, S. Lesieur. Sucrose solutions as prospective medium to study the vesicle structure: SAXS and SANS study. J. Alloys and Compounds 328 (2001) 71-76.

Vesicle preparation

Micrometer
100-1000 layers



50 – 100 nanometer

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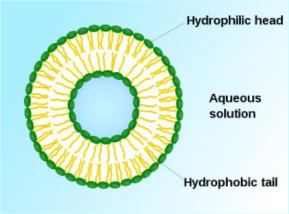
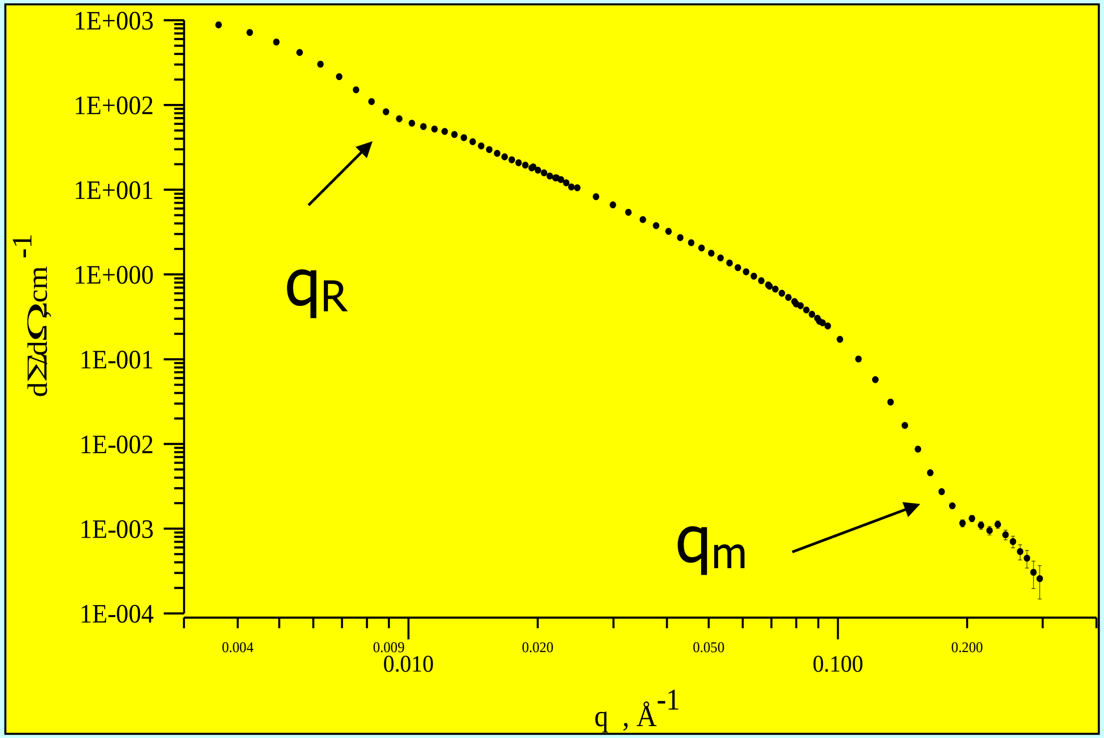
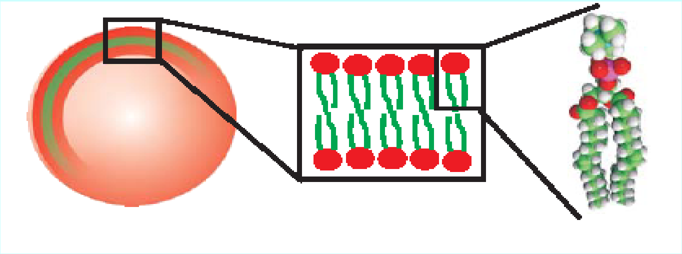
BENSC

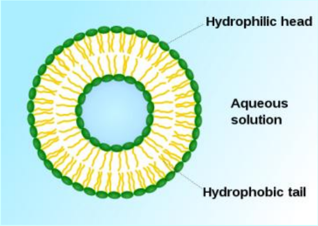
SANS pattern at 30 °C of unilamellar DMPC vesicles prepared by extrusion through 500 Å pores at DMPC concentration of 1% (w/w).

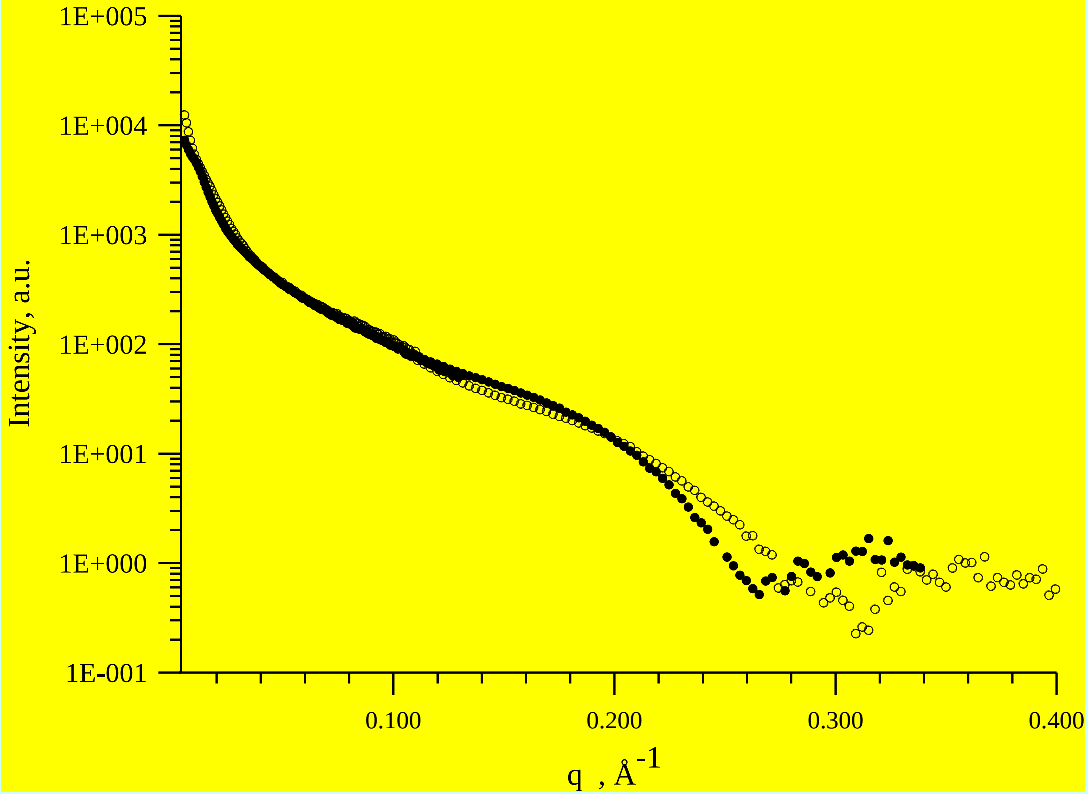


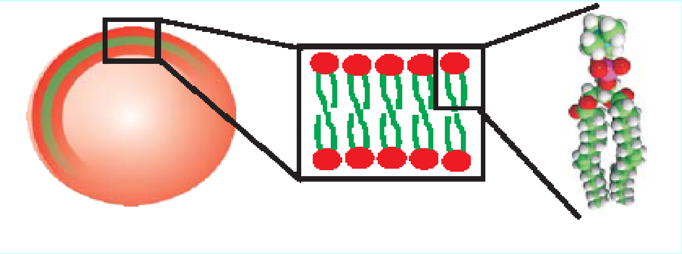


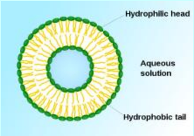


SAXS patterns at 10 °C (circles) and 30 °C (open circles) of unilamellar DMPC vesicles in 40% aqueous trehalose solution, DMPC concentration 3%(w/w)









Method of Separated Form Factors (SFF)

$$\frac{d\Sigma}{d\Omega}_{mon}(q) = n \cdot A^2(q) \cdot S(q) + IB$$

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

$\rho_c(x) = \rho(x) - \rho_{D2O}$ is contrast, the difference between scattering length densities of the bilayer $\rho(x)$ and the heavy water ρ_{D2O}.

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

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

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

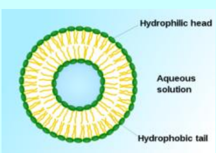
For the case of $\rho(x)$=Const

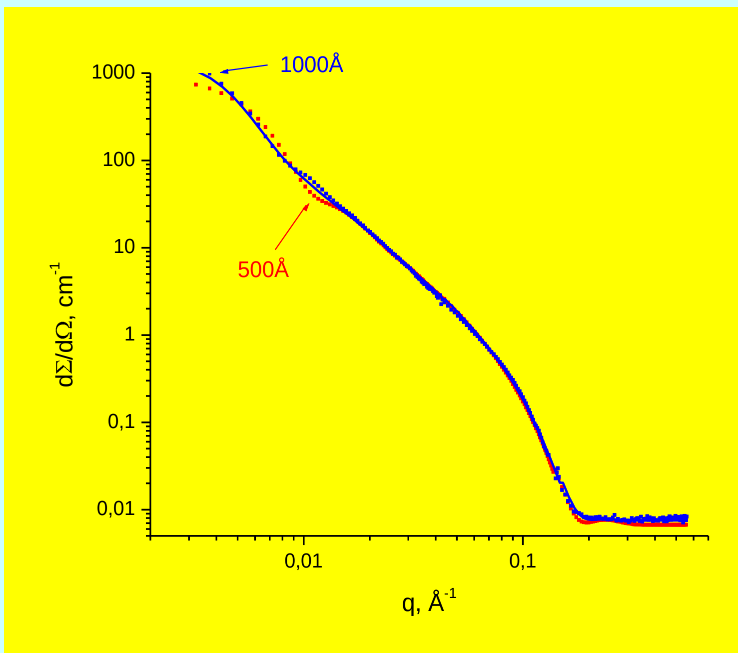
$$F_b(q,d) = \left(\frac{2\Delta\rho}{q} \cdot Sin\left(\frac{qd}{2}\right)\right)^2$$

M.A. Kiselev, P. Lesieur, A.M. Kisselev, D. Lombardo, V.L. Aksenov. Model of separated form factors for unilamellar vesicles. J. Applied Physics A 74 (2002) S1654-S1656

Results for liquid phase at 30^{o}C, HH approximation of $\rho(x)$

Model	D_F, Å	<R>, Å	σ, %	d, Å	D, Å	N_w	A, Å^2	IB, cm^{-1}
SFF	500	275.6 0.5	27	47.8 0.2	20.5 0.3	11.9±0.3	61.0±0.4	0.007
Full	500	275.7 0.4	27	47.8 0.2	20.5 0.3	11.9±0.3	61.0±0.4	0.007
SFF	1000	500*	48	45.5 0.6	20.8 0.4	10.8 0.4	62.6 1.0	0.007





Multilamellar (small curved)
vesicles
d = 44.2 Å
A=59.6 Å^2
J.Nagle, S. Tristram-Nagle.
Structure of lipid bilayers.
BBA 1469 (2000) 159-195

M.A. Kiselev, E.V. Zemlyanaya, V.K. Aswal, R.H.H. Neubert.
What can we learn about the lipid vesicle structure from the small angle neutron scattering experiment? *European Biophysics J.* 35 (2006) 477-493.

Structure of DMPC vesicles in liquid, ripple and gel phases. SF approximation of $\rho(x)$

T=30°C (liquid L_α phase)

D_F, Å	<R>, Å	σ, %	d_m, Å	ρ_{PH}, 10^{10} cm^{-2}	$N_{w,\ PH}$	A, $Å^2$
500	275.1 0.5	27	45.5 0.7	3.7 0.2	6.8±0.6	57±1
1000	450*	48	45.7 0.7	4.0 0.2	8.0±0.6	59±1

d = 44.2 Å, A=59.6 $Å^2$, $N_{w,\ PH}$ = 7.2 for multilamellar vesicles

T=20°C (ripple $P_{\beta'}$ phase)

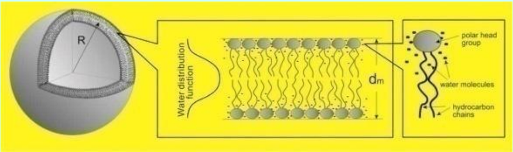
D_F, Å	<R>, <a>, Å	ε	σ, %	d_m, Å	ρ_{PH}, 10^{10} cm^{-2}	$N_{w,\ PH}$	A, $Å^2$
500	187 1	1.63 0.02	22	47.9 0.7	3.6 0.3	5.3±0.5	50±1
1000	450*	1	35	48.3 0.6	3.8 0.2	5.9 0.4	50.4 0.8

T=10°C (gel $L_{\beta'}$ phase)

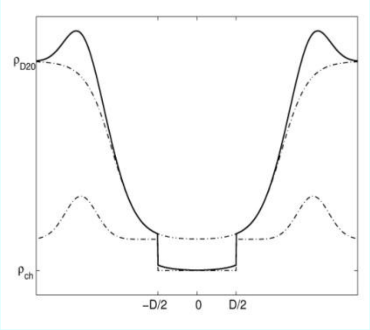
D_F, Å	<R>, <a>, Å	ε	σ, %	d_m, Å	ρ_{PH}, 10^{10} cm^{-2}	$N_{w,\ PH}$	A, $Å^2$
500	185 1	1.62 0.02	21	49.4 0.7	3.7 0.2	5.2±0.5	48.8±0.9
1000	450*	1	37	49.6 0.5	3.6 0.2	5.9 0.5	49.2 0.9

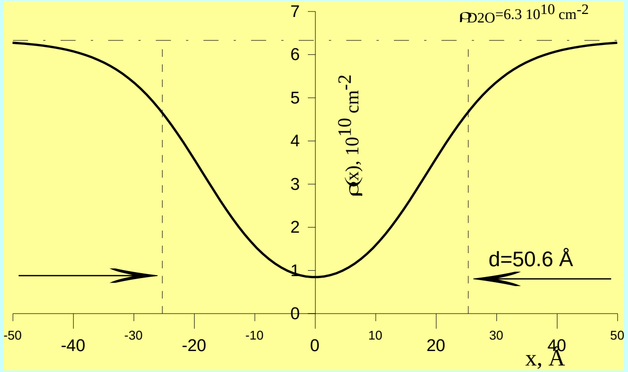
d=48.2 Å, A=47$Å^2$ for multilamellar vesicles

* - from DLS



Water distribution function across the lipid bilayer of the unilamellar vesicles demonstrates penetration of water through the bilayer in the L_α phase.





$$\rho(x) = \rho_{ph}(x) + \rho_{ch}(x) + \rho_w(x)$$

$$\rho_{ph}(x) = \frac{l_{ph}}{A_{dr}} \cdot \frac{1}{\sqrt{2\pi} \cdot \Sigma} \cdot \exp\left(-\frac{(x - x_o)^2}{2\Sigma^2} \right)$$

$$\rho_{ch}(x) = \begin{cases} l_{ch} \cdot 2/(D \cdot A), & -D/2 < x < D/2 \\ 0, & D/2 \le |x| \le d_m/2 \end{cases}$$

$$\rho_w(x) = \frac{\rho_{D2O}}{1 + \exp\left(\dfrac{x_W - x}{\sigma_W} \right)} + \frac{\rho_{D2O}}{1 + \exp\left(\dfrac{x_W + x}{\sigma_W} \right)}$$

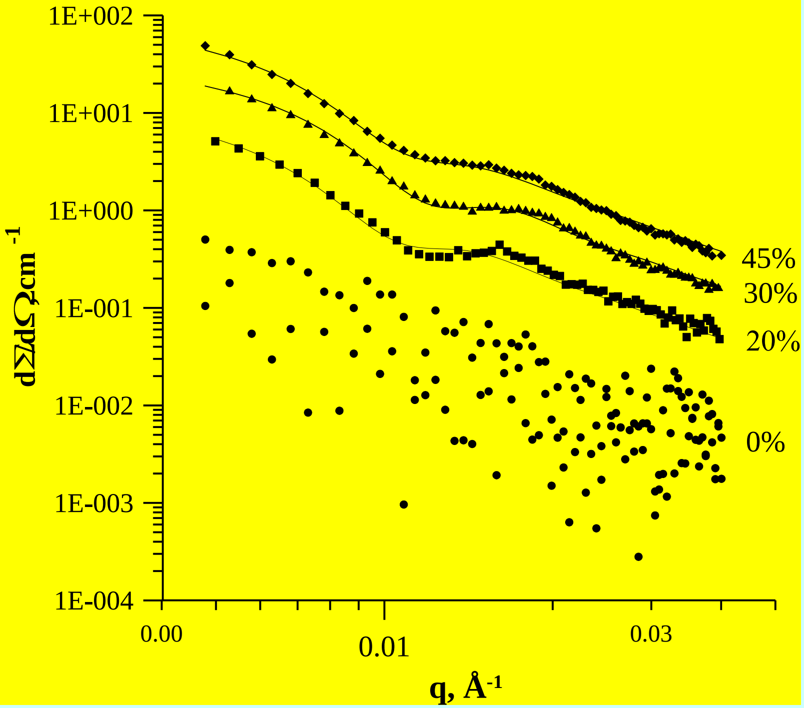
For drugs preparation we used H_2O, but not a D_2O

Disaccharide possess:

- **Bioprotection properties relative to vesicles from phospholipid**
- **Decrease the polidispersity of the vesicular population**
- **Decrease the vesicle aggregation from the unilamellar vesicles to the multilamellar vesicles**

M.A. Kiselev, P. Lesieur, A.M. Kisselev, D. Lombardo, M. Killany, S. Lesieur, M. Ollivon. A sucrose solutions application to the study of model biological membranes. *Nuclr. Inst&Method A* 470 (2001) 409-416.

Contrast variation of theX-ray scattering length density ρ (x) by disaccharide solutions in the water

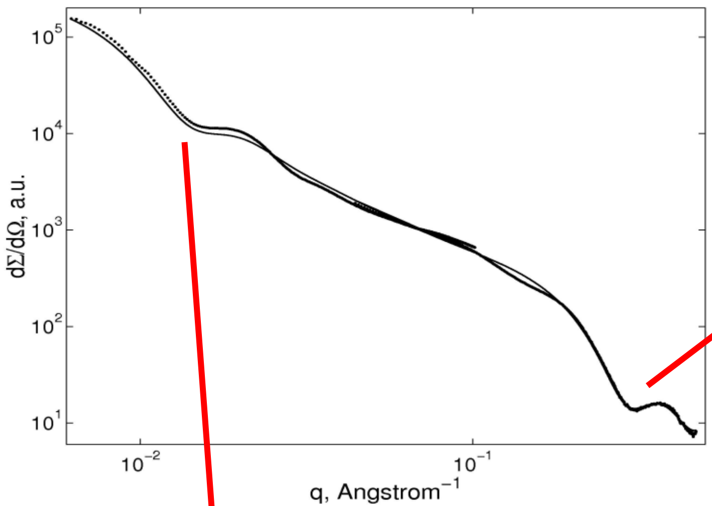


Dependence of scattering intensity on the sucrose concentration in water

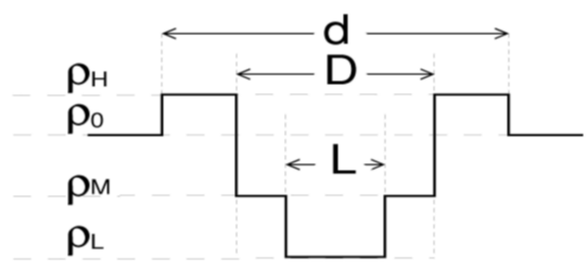
M.A. Kiselev, S. Wartewig, M. Janich, P. Lesieur, A.M. Kiselev, M. Ollivon, R. Neubert. Does sucrose influence the properties of DMPC vesicles? *Chemistry and Physics of Lipids* 123 (2003) 31-44.

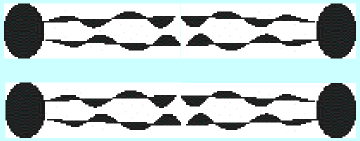
DMPC vesicle structure in sucrose solution

SAXS curve can be described only based on the fluctuation of the bilayer structure



d=35±1 Å
D=27±1 Å
L=3.9±0.2 Å
$\sigma_{d,\ D,\ L}$=32%

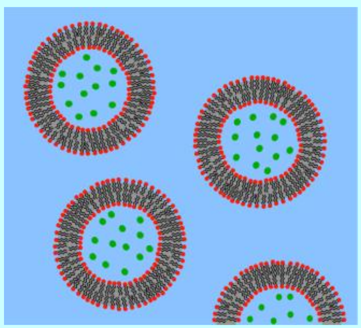




<R>=190±2 Å σ_R=26%

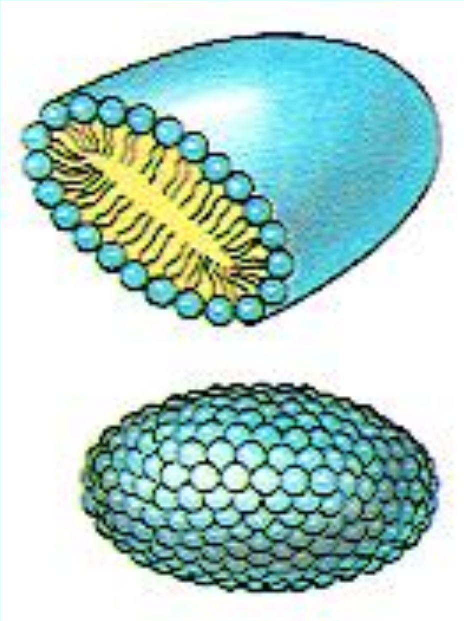
М.А. Киселев, Е.В. Земляная, Е.И. Жабицкая, В.Л. Аксенов. Исследование однослойных везикул ДМФХ в водных растворах сахарозы методами малоуглового рассеяния нейтронов и рентгеновских лучей. *Кристаллография*, т. 60, №1 (2015) 140-150.

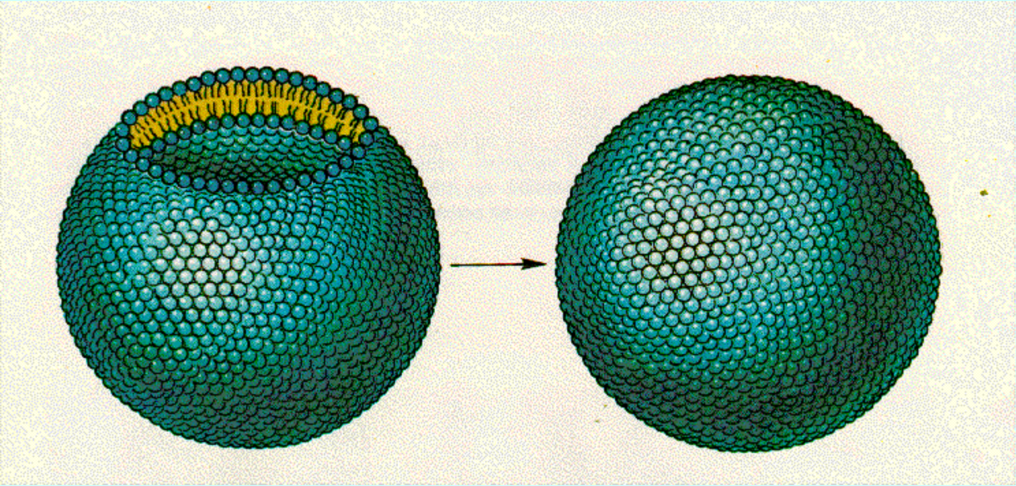
Nanodrugs based on the phospholipids



Composition	drug incorporation, %	Size, nm	pharmacological action
Phospholipid transport nanosystem (PTNS)		19 2	Acute toxic exposure, Precoma
Arbidol + PTNS	98	17 2	Antiviral action
Doxorubicin+PTNS	96	21 3	Antineoplastic action
Chlorin E6 + PTNS	90	22 5	Photodynamic therapy and diagnostics
Budesonite + PTNS	99	35 5	Anti-inflammatory action
Indometacin+ PTNS	98	35 3	Nonsteroidal, anti-inflammatory drug
Lipoic acid + PTNS	99		Anti-oxidant action

What is a morphology?
Micelles or vesicles?



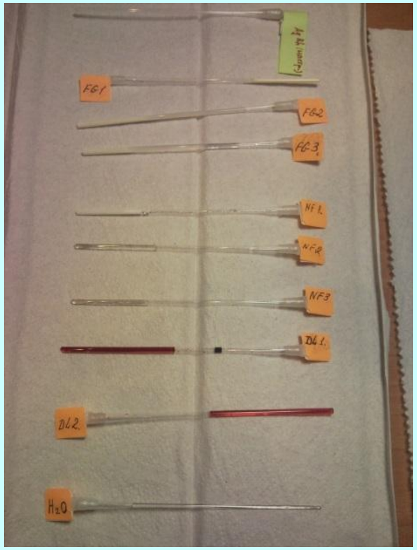


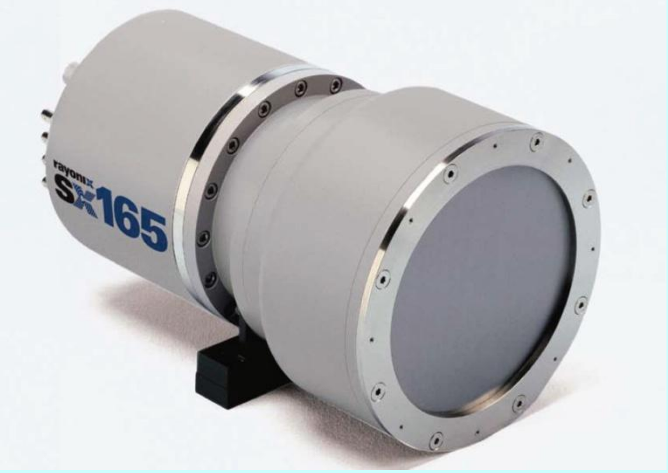
Nanodrugs are created in water solution of maltose

Liophylisation

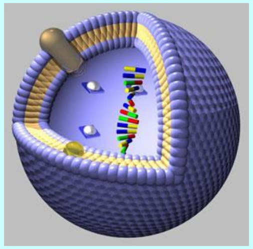
Nanodrugs are stored in the maltose matrix for a long time

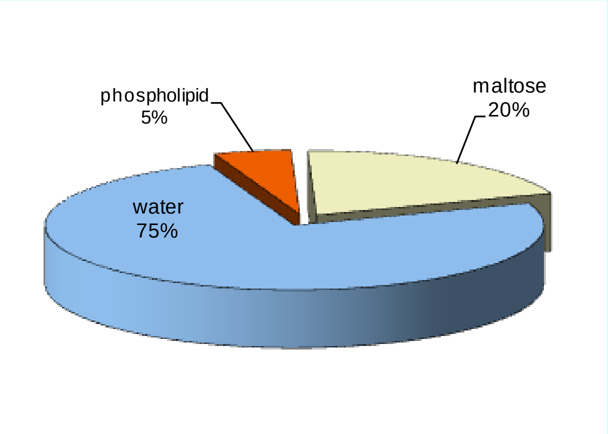
Spectrometer DICSI, synchrotron Siberia-2, Moscow

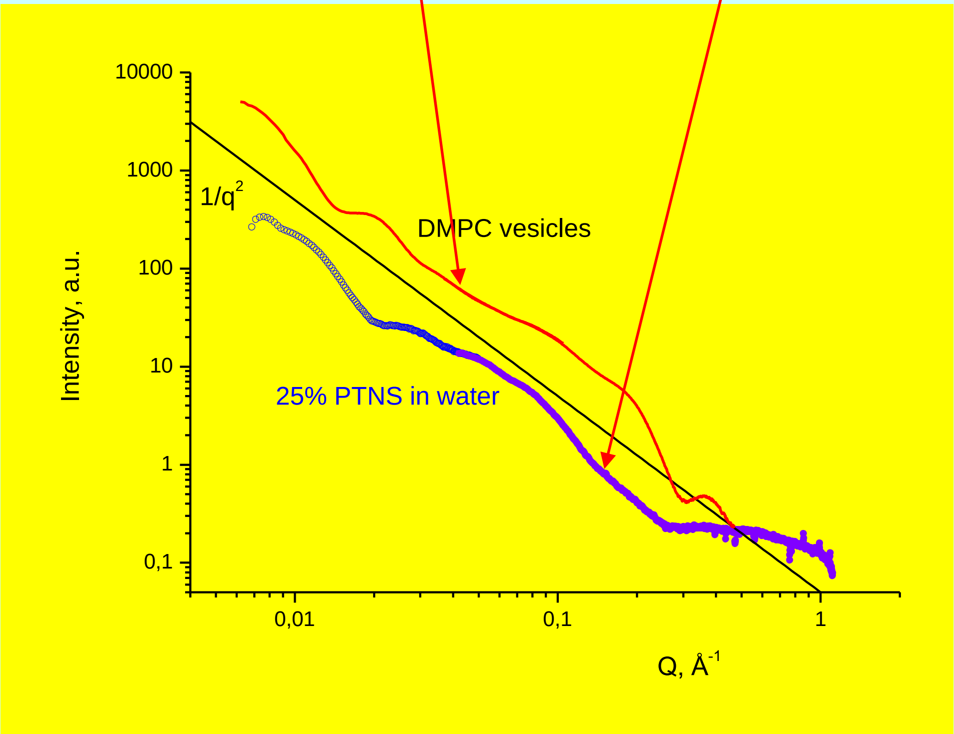




Phospholipid transport nanosystem (PTNS)







SAXS patterns. DMPC vesicles in 40% sucrose buffer – red line (LURE, France). 25% solution of FTNS in water – blue line (KSSR, Moscow). Black line - 1/q^2 law.

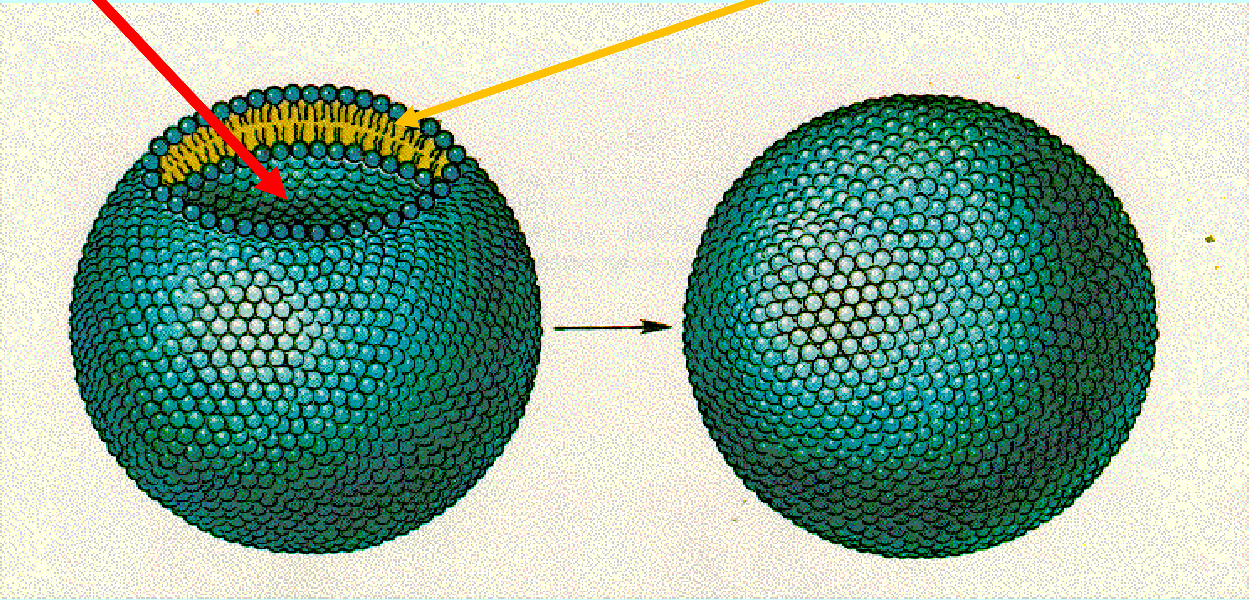
Average diameter PTNS - 380Å.
Average diameter DMPC - 420Å

Volumes for drugs

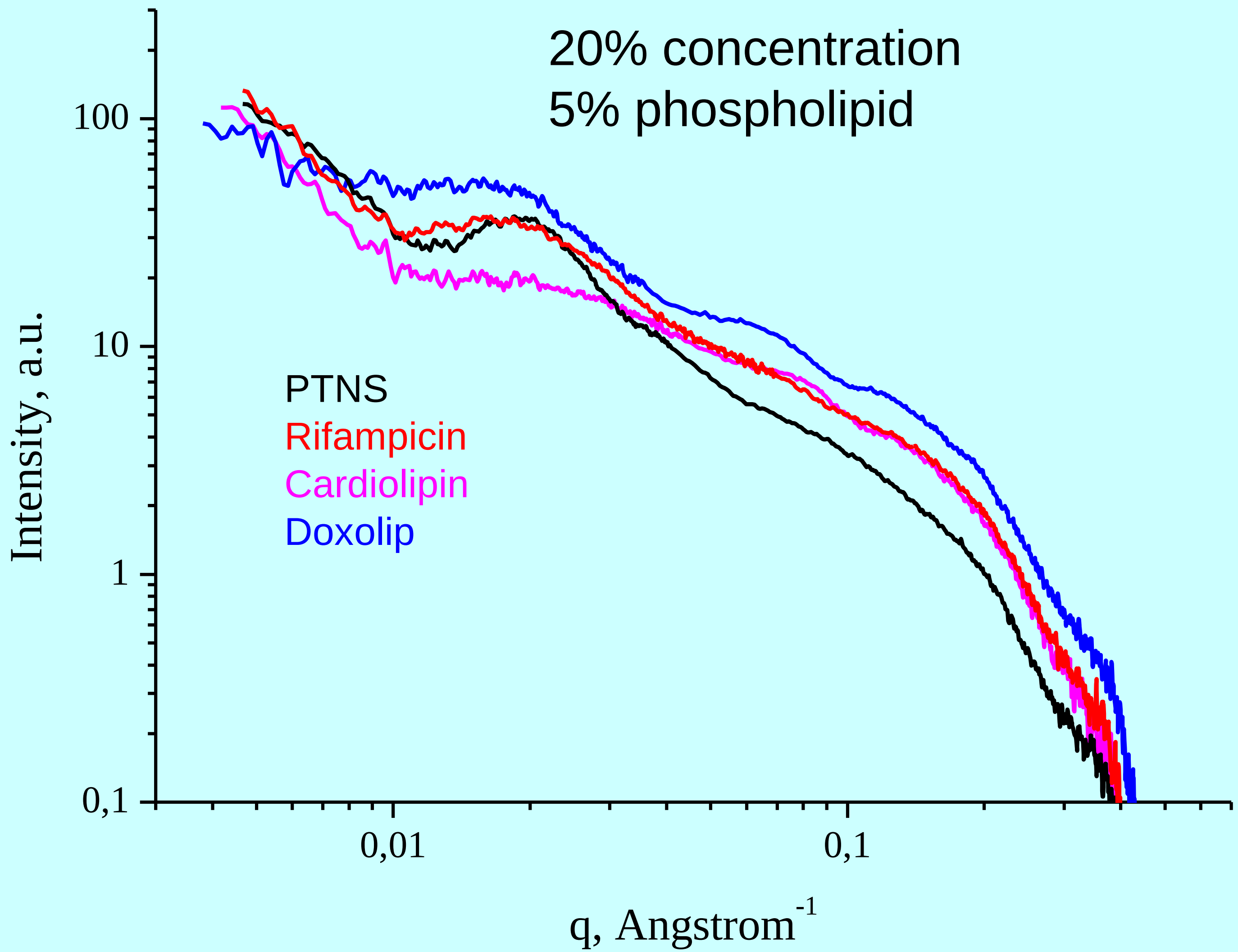
R=160Å, d=27,2Å

V hydrophilic=17 150 nm^3

V lypophilic=8 745 nm^3



SAXS curves from different nanodrugs



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