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Concentration-dependent effect of DMSO on lipid vesicle structure under physiological conditions

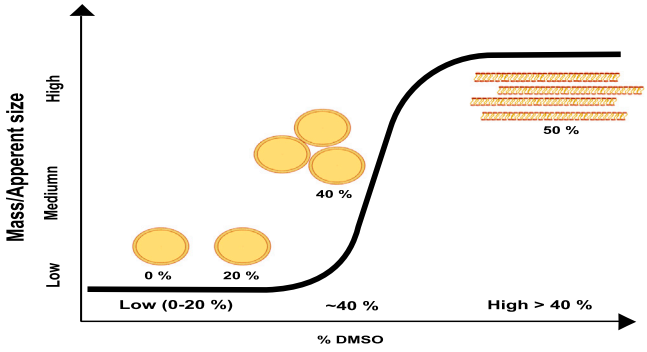
Alice Piccinini [a,b,c,1], Anastasiia Murmuliuk [c,2], Guillaume Gilliard [c,3], Gouranga Manna [d], Valeria Rondelli [c], Anja Winter [b,*], Sylvain Prévost [a,*]

[a] Institut Laue-Langevin – The European Neutron Source, 38042 Grenoble, France
[b] School of Life Sciences, Keele University, ST55BG Staffordshire, UK
[c] Dept. of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, 20054, Segrate, Italy
[d] ESRF – The European Synchrotron, 38043 Grenoble, France

HIGHLIGHTS

- A threshold DMSO concentration for utilization of lipid vesicles as drug delivery systems is proposed.
- Low concentrations of DMSO, up to 20 %, do not have a measurable effect on vesicle structure.
- The main effect of DMSO on vesicles is a reduction in inter-membrane distance, which leads to an increase of stacking.
- DMSO's effects on lipid vesicles are both concentration- and lipid-composition-dependent.

GRAPHICAL ABSTRACT



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ABSTRACT

Dimethyl Sulfoxide (DMSO) is widely used for drug delivery and in biology as a cryoprotectant because of its ability to dissolve drugs and biomolecules and due to its membrane permeating properties. Despite its extensive use, it is still unclear how this solvent affects the membrane structure and whether its effect is strongly dependent on membrane lipid composition. We employed vesicles of different complexity, from the simplest membrane model (DOPC) up to more complex ones (polar lipid extract of yeast and *E. coli*) in physiological conditions and under osmotic stress. A wide range of DMSO content was studied, from 0 % to ≥40 %, using dynamic light scattering, small-angle neutron and X-ray scattering techniques. Our results show that vesicles in PBS maintain their integrity and structure up to 20 % DMSO, but only at higher DMSO concentrations (≥40 %) a drastic alteration of vesicle structure will occur. The main effect of DMSO appears to be a reduction in inter-membrane distances, linked to dehydration, and therefore an increase in stacking of membranes. We also demonstrated that DMSO's effects on lipid vesicles generated in PBS are dependent on lipid composition, with more complex multi-component membranes, such as lipid polar extracts, demonstrating a higher resilience towards dehydration. Our findings highlight the importance of defining a DMSO threshold concentration that would allow stable and defined vesicle formation for any given lipid composition.

* Corresponding authors.
E-mail addresses: a.winter@keele.ac.uk (A. Winter), prevost@ill.fr (S. Prévost).
[1] Current affiliation: ChIP, UNICAM, 62032 Camerino MC, Italy.
[2] Current affiliation: UR Molecular Systems, Department of Chemistry, University of Liège, 4000 Liège, Belgium.
[3] Current affiliation: Laboratoire de génie enzymatique et cellulaire, Université de Picardie Jules Vernes, 80039 Amiens, France.

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1. Introduction

Dimethyl Sulfoxide (DMSO) is an organic polar solvent widely used due to its ability to solubilize both polar and apolar compounds for applications such as the delivery of hydrophobic drugs or biomolecules (e.g. hydrophobic peptides) [1], as a solvent for fluorescent dyes [2], as a cellular cryoprotectant and as an enhancer of membrane permeability [3–5]. Its ability to enhance membrane permeability for the delivery of transmembrane and topical drugs is widely exploited in therapeutics [6], however, an in-depth understanding of how this solvent can affect the structure of a biological membrane at the molecular level is still lacking. In particular, it is still unclear whether DMSO disturbs the membrane structure and to what extent this depends on its concentration, on the membrane lipid composition and perhaps on any osmotic stress across the membrane. Increasing our understanding of the effect of DMSO on the membrane is particularly interesting for applications such as drug delivery systems or drug screening in cell systems, as this may affect efficacy of drugs and biological outcomes of assays.

Several factors influence vesicle structure in a binary DMSO/H_2O solution. Firstly, DMSO has been shown to compete with surface water to effectively dehydrate the lipids [7,8], which leads to an expulsion of water from the interstitial space in multilamellar vesicles (MLVs), decreasing the intermembrane distance [9]. Secondly, the mechanical properties of membranes are altered, leading to a DMSO-concentration-dependent increase in membrane permeability for smaller molecules, water and ions [5,6], but also larger molecules such as Yo-Pro-I iodide (a green fluorescent dye) [5]. Thirdly, the vesicle's lipid composition not only influences the chain melting and therefore their thermodynamic state [6,10], but also how DMSO influences this thermodynamic state, where a more complex membrane is more resistant to an increasing DMSO concentration, potentially due to their increased bending rigidity [6]. Notably, different DMSO concentrations can have different or opposing effects on membranes [6,7], potentially due to competing thermodynamic, chemical and physical forces, which necessitate the careful examination of the DMSO effect at a defined concentration on specific vesicles.

Membrane composition is an important factor in vesicle structure and behaviour [10,11], therefore, choosing a model system that represents biological membranes closely is of utmost importance. In biophysical studies, often very simple membrane models are used, such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), which is not physiologically relevant due to the short acyl chains and absence of unsaturation. In contrast, 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) with its unsaturated C_{18} acyl chains is a common lipid found in mammalian membranes, and therefore represents a more biologically relevant membrane model. We compared the effect of DMSO on the simplest membrane model (DOPC) to more complex systems, which are biological extract (polar lipid extracts of *E. coli* an yeast). Although complex membrane models present analytical challenges, they provide indispensable insights into the true biological effects of DMSO.

In this study, we investigated changes in lipid vesicle structures caused by addition of DMSO in the surrounding solvent, and examined how different membrane compositions may alter this behaviour. We conducted our experiments to mimic physiological conditions, such as temperature, salt concentration and osmotic shock, which are important factors that are very often underappreciated by other studies. We employed dynamic light scattering (DLS), small-angle neutron and X-ray scattering (SANS and SAXS) to determine structural effects on simple and complex lipid vesicles while systematically increasing the DMSO concentration. Our findings show that up to 20 % DMSO vesicle structure is largely maintained, which is an important threshold for utilization of lipid vesicles as drug delivery systems. Additionally, more complex lipid systems, such as lipid polar extracts, are more tolerant of DMSO as part of their solvent. Our data also show that the main effect of DMSO on vesicles is a reduction in inter-membrane distance, which leads to an increase in stacking.

2. Experimental

2.1. Materials

DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine, Cat. no. 4235-95-4) and polar lipid extract from yeast (Cat no. 190001P) and *E. coli* (Cat no. 1240502-50-4) were purchased from Sigma-Aldrich and stored at −20 °C until use. *E. coli* polar lipid extract is composed of PE :PG: CL (67 %:23 %:10 % by weight), while yeast polar lipid extracts is composited of PC: PI: PS: PE: LysoPC: LysoPE (30 %: 26 %: 8 %: 8 %: 4 %: 1.4 % by weight). For the SANS experiment, heavy water (D_2O) (Eurisotop, 99.9 % D) was used to prepare solutions to increase contrast and reduce the incoherent scattering background; for all other experiments, ultrapure water was used. Organic solvents were purchased from Sigma-Aldrich. DMSO, chloroform, and methanol were used without further purification. Syringe filters 0.22 μm were purchased from Merck (Millex™, PVDF, Cat. no SLGVR33RS).

2.2. Methods

2.2.1. Vesicle preparation

Lipids were dissolved in a mixture of $CHCl_3$:MeOH 2:1. Once the lipids were dissolved, the solvent was first evaporated under a nitrogen flow and subsequently under vacuum overnight. Lipidic solutions were hydrated in D_2O, H_2O or Phosphate buffer saline (PBS) as specified for each experiment to reach a lipid concentration of 5 mg mL^{-1}. PBS is composed of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 (here called "full PBS"). Seven freeze-thaw cycles were performed to increase the monodispersity of the samples. The extrusions were carried out at least 11 times across two polycarbonate membranes (Whatman Cat no. WHA10417304) separated by support membranes PE drain discs (Whatman, Cat. no. 230 300). Extrusion was performed with an Avanti Polar extruder (Cat. no. 610 000) on a hot plate at approximately 50 °C, with 50 and 80 nm pore sizes, no more than 24 h before measurements.

2.2.2. Vesicle preparation by quenching of solvent

Lipids (DOPC, *E. coli* and yeast polar lipid extract) were dissolved directly in DMSO at a concentration of 20 mg mL^{-1}. In the case of *E. coli* and yeast, samples were bath-sonicated for 30 min and then tip sonicated while placed in an ice bath (BANDELIN SONOPLUS ultrasonic processor) for 10 min using 10 s pulse sonication with 10 s waiting time, operating at a fixed frequency of 20 kHz, with the amplitude set to 20 %. The lipidic solutions were then diluted to 1 mg mL^{-1} by a unique and fast addition into water (fast addition), or by adding drop-wise an aqueous solution to the concentrated lipidic stock solutions in DMSO while vortexing, here called slow addition.

2.2.3. Dynamic light scattering

Dynamic Light Scattering (DLS), also known as Photon Correlation Spectroscopy (PCS), is a quasi-elastic light scattering technique that analyses temporal fluctuations in scattered light intensity. The rate at which the resulting intensity autocorrelation function decays reflects the Brownian motion of the particles, which is related to their diffusion coefficient (D). From this, the hydrodynamic radius (R_h) can be determined using the Stokes–Einstein equation:

$$\frac{k_B T}{6\pi\eta R_h}$$

where k_B is the Boltzmann constant, T is the temperature, η is the dynamic viscosity of the solvent and R_h the particles' radius.

All solutions used for the DLS analyses were degassed in the ultrasound bath and filtered with 0.2 μm pore size syringe filters (Millex™, PVDF, Cat. no SLGVR33RS). Lipids–aqueous samples at 0.4 mg mL^{-1} were extruded through a 50 nm membrane using an Avanti Polar extruder (Cat. no 610000). DMSO-PBS solutions were measured as a

blank. DLS was measured on an ALV CGS-3 Compact Goniometer System instrument (ALV GmbH, Langen, FRG) in the PSCM at ILL. The angles tested were from 30° to 150°, every 10° or 30°, with 5 runs at 10 s per run, using a wavelength of 632.8 nm. Measurements were performed at 37 °C in glass test tubes (75 × 10 × 1.0 Boro 3,3, gIRd, rdBdn Lot. 90106007). R_h were determined for the monodisperse and polydisperse case (PDI), with a Schulz–Zimm distribution. Details on data fitting are provided in the supplementary information (SI results).

2.2.4. Small-angle neutron scattering

For SANS experiments, lipid vesicles were not extruded prior the experiments for two reasons: (i) extrusion might change the overall structure and/or shape of the vesicles, which is especially relevant in the presence of salts; (ii) the Q-range of our interest in this study is not strongly affected by the monodispersity of the size distribution of the vesicles and therefore does not affect the data analysis. Although local membrane curvature may influence molecular interactions and supramolecular organization, these curvature-dependent effects were not considered in the present SANS analysis. Instead, we addressed them using SAXS on extruded samples, enabling a detailed examination of bilayer organization. SANS data were collected at SANS-I (PSI) during beam time 20212436 using a two-dimensional ^{3}He detector with 128 × 128 elements of 7.5 × 7.5 mm^2 using Quartz cells (Hellma, type 100-QS) of 2 mm path length. Only one configuration was used to cover the mid-Q-range between 1×10^{-2} and 1×10^{-1} $Å^{-1}$ with a sample to detector distance of 6 m. Data were reduced with BerSANS [12].

DOPC, *E. coli* and yeast polar lipid extract at 5 mg mL^{-1} were hydrated in "light PBS" having the same composition as the "full PBS" but containing NaCl at 13.7 mM instead of 137 mM, to induce osmotic stress. Immediately before the measurements, NaCl was added to reach a final concentration of 137 mM ("full" PBS). Then, DMSO in a volume fraction of 0, 5, 10, 20, 40 and 50 % was added to the DOPC vesicles, while for *E. coli* and yeast 20, 30 and 40 % were used. Aqueous solution containing 5, 10, 20, 30, 40 and 50 % of DMSO was used as a blank. Measurements were performed at 37 °C. Data analyses and treatments are outlined in the supplementary material. Data remain available at DOI: 10.13140/RG.2.2.35231.62888.

2.2.5. Small-angle X-ray scattering

SAXS data were collected at ESRF - The European Synchrotron, Grenoble, France, on SAXS beamline ID02 (Proposal number SC-5603, https://doi.org/10.15151/ESRF-ES-1837447272) [13,14], which is equipped with an Eiger2 4M detector using an energy of 12.23 keV corresponding to a wavelength of 1.0137 Å to cover a Q-range of 5.7×10^{-2} to 5.7 nm^{-1}, where Q is the magnitude of the wave vector: $Q = \frac{4\pi}{\lambda} \sin\left(\frac{\theta}{2}\right)$ with λ the wavelength and θ the scattering angle. The sample-to-detector distance was set at 1.5 m. To reduce radiation damage, solutions were pushed through a 2 mm thick flow-through quartz capillary while being measured. The azimuthally averaged 1D intensity profiles were obtained after applying standard detector corrections and normalization to absolute intensity. Further data processing was performed using the SAXSutilities package. 10 frames were acquired systematically for all samples and buffers, and buffers were always measured between each sample measurement. After visual inspection for radiation damage, the frames were averaged, creating uncertainties due to the standard deviation between intensity values. The average of the frames was subtracted from the buffers measured before and after the sample. Reduced data can be obtained on demand, and remain available at DOI: 10.13140/RG.2.2.35231.62888. Data analyses were performed with SASfit [15] employing a form factor of discs with 3 homogeneous layers (a single inner layer for the acyl chains and two outer layers for the solvated headgroups exposed to solvent) and a structure factor to account for multi-lamellarity (paracrystalline model with polydispersity implemented on the discrete number of layers) [11,16].

DOPC, *E. coli*, and yeast polar lipid extract were measured at 5 mg mL^{-1} after hydration in H_2O under the following conditions: (i) addition of 5, 10, 20, 30, 40, 50 and 75 % by volume of DMSO which were performed just before measurements; (ii) addition of DMSO at 5, 10, 20, 30, 40 and 50 % to DOPC vesicles (with an overnight incubation) with subsequent induction of osmotic stress using PBS solution for 3 h. A mixture of H_2O containing 5, 10, 20, 30, 40, 50 and 75 % DMSO was used as a blank. Measurements were performed at 37 °C.

3. Results and discussions

3.1. Effect of DMSO content on the vesicle's hydrodynamic radius

The effect of different volume fractions of DMSO (ranging from 0 % to 40 %) on the size distribution of DOPC vesicles was investigated by DLS using DOPC lipid vesicles hydrated in "light PBS" in order to facilitate extrusion at 50 nm [10]. Osmotic shock was induced to the vesicles before measurement via addition of concentrated salt (NaCl) to reach "full PBS" content. Thereafter, DMSO at the appropriate amount was added. The R_h was extrapolated at zero angle (see SI results for more details). Data were fitted with Γ (decay rate) and β (coherence factor) assuming a monodisperse population, and consequently, the mean hydrodynamic radius was derived. More fitting details are given in the SI Results. DLS data show that R_h remains unchanged up to 20 % DMSO, suggesting that the overall size and/or integrity of the vesicles is maintained. On the other hand, when the amount of DMSO is ≥ than 40 %, R_h increases up three-fold, suggesting aggregation of vesicles. The fitting results up to 20 % show good fit with a $\chi^2_{red} \leq 1$, with values of R_h between 58 nm and 64 nm (Table S2 and Fig. 1) with an overall standard deviation of 1.6 nm, without the need to include additional polydispersity, suggesting a narrow range of size distribution. On the other hand, for 40 % DMSO, a standard deviation (SD) of 39 nm is shown with a χ^2_{red} of 8.4, suggesting a poorer fitting, which could be due to the presence of bigger objects ($x \approx 400$ nm diameter) that could mask the detection of smaller ones.

Our results suggest that up to 20 % DMSO does not significantly alter the hydrodynamic radius of the vesicles (Fig. 1). This is in line with other studies, where up to 10 % of DMSO was demonstrated to cause membrane loosening, permeation, and deformability in fibroblasts [6], but generally the membrane remained intact. In contrast, a more dramatic effect is observed when DMSO is employed in an amount greater than 40 % (approximately 6 water molecules per DMSO molecule) where pore formation occurs, leading to rupture of the bilayer, according to MD simulations [5]. Our DLS results provide a systematic investigation of the effect of DMSO on the hydrodynamic radius (R_h) of DOPC vesicles, and therefore important information on vesicle swelling or contraction as well as aggregation, all effects that may be detrimental to therapeutic applications as drug delivery systems or on a research setting. The use of DOPC represents a simplified yet reliable model for biological membranes, owing to its C_{18} carbon chains and unsaturation, and the use of PBS to induce osmotic shock. This offers new and valuable insight into the careful use of DMSO under physiological conditions (PBS, 37 °C) in a vesicle that is osmotically shocked, and constitutes a novel contribution to the current literature describing vesicle morphology and stability when DMSO is added to their environment.

3.2. Concentration-dependent effect of DMSO on vesicle structure

Next, we took advantage of SAXS and SANS as techniques that probes different and complementary features of the vesicles. Using SANS we studied the morphology and lamellarity of vesicles represented in the mid-Q- range (≈ 6 nm^{-1}), which reflects the vesicles' structure on a larger scale, sacrificing details on the bilayer structure such as thickness, headgroups and tails, which can be better investigated with X-ray scattering. We employed three different membrane

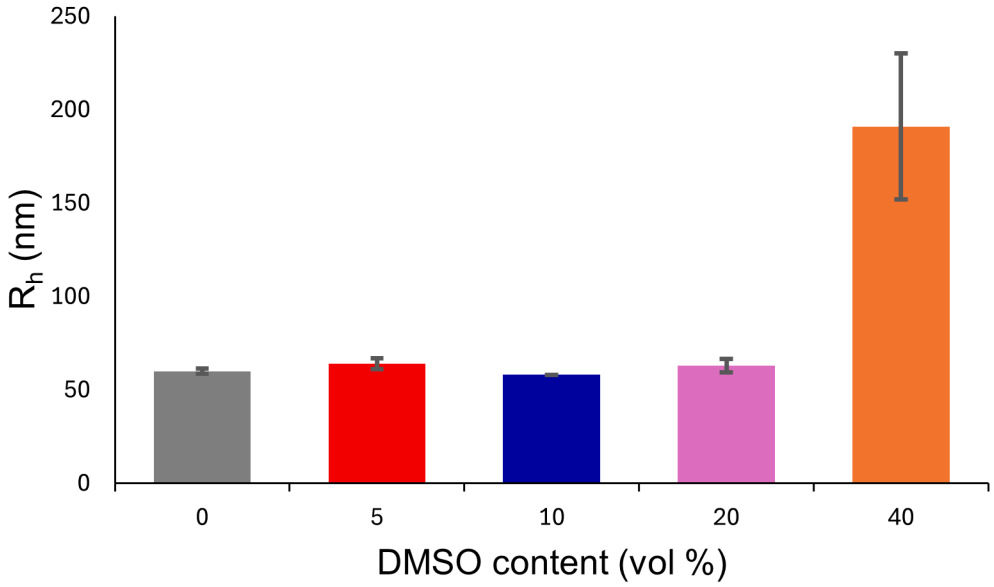

Fig. 1. R_h extrapolated at 0° by DLS investigation of DOPC vesicles hydrated in PBS with addition of DMSO from 0 % to 40 %. On the *X*-axis is represented the DMSO content as volume fraction, while in y-axes the hydrodynamic radius in nm. Colour code is applied along the full manuscript, 0 % in grey, 5 % in red, 10 % in blue, 20 % in light purple, 40 % in orange. The vertical error bars represent the SD of each single DMSO content: 0 % 1.3 nm, 5 % 3.0 nm, 10 % 0.1 nm, 20 % 3.6 nm, 40 % 39 nm.

models of varying complexity, namely (i) DOPC and polar lipid extracts from (ii) *E. coli* and (iii) yeast. Lipid vesicles probed with neutrons present the great advantage of isotope sensitivity that can be leveraged to enhance the visibility of vesicles in the bulk medium. This can be achieved by using deuterated water (D_2O) instead of H_2O, as D_2O has a significantly higher scattering length density (SLD = 6.36×10^{-4} nm^{-2}) compared to H_2O (SLD = -0.56×10^{-4} nm^{-2}); SLDs of DOPC, acyl chains (twice $C_{17}H_{33}$) and headgroup ($C_{10}H_{18}O_8NP$) are respectively 0.30, −0.21 and 1.85×10^{-4} nm^{-2}; DMSO has an SLD of -0.04×10^{-4} nm^{-2}. On the other hand, X-rays offer higher contrast between the head groups and tails of the lipids, and by probing a much shorter *Q*-range (≈ 0.1 nm to 2 nm), we will be able to obtain significantly more detailed insights into the features of the bilayer [17]. However, it must be considered that in X-ray scattering, the bulk medium tends to mask the lipid overall contrast (SLD of H_2O 9.38×10^{-4} nm^{-2}, SLDs of DOPC, acyl chains and headgroup 9.36, 7.74 and 14.25×10^{-4} nm^{-2}, SLD DMSO 9.94×10^{-4} nm^{-2}) SI Table S1.

The effect of different DMSO content (from 0 % to 50 %) on different lipid vesicles (i) DOPC, (ii) *E. coli*, and (iii) yeast polar lipid extracts was first evaluated with SANS. Lipid vesicles were hydrated in PBS and osmotically shocked to mimic physiological conditions. DOPC vesicles were extruded to increase the size homogeneity [18]. However, forcing the extrusion of complex lipid mixtures in the presence of salts can lead to the formation of highly elongated vesicles [10], caused by the passage of the vesicles through the extruder itself. Therefore, extrusions of vesicles generated from polar lipid extracts were avoided. Aqueous solutions were measured, then DMSO was progressively added to them with measurements at every step; thus, samples are getting progressively diluted.

We note that a qualitative interpretation of the data can be misleading because of the different incoherent backgrounds along the samples caused by the different amounts of hydrogenated DMSO added to a lipid solution in D_2O. To overcome this problem, the incoherent background was subtracted according to the hydrogen content added (h-DMSO) to D_2O. Furthermore, the intensity was normalized by the volume fraction of lipids as well as the square of the contrast between the lipids and the binary solvent, which assumes that the DMSO contributes exclusively to the solvent (Fig. 2) (non-treated data are shown in SI Figure S4).

The scattering profiles of DOPC with increasing DMSO content up to 50 % remained largely unchanged, resulting in superimposed SANS profiles with a slope of Q^{-3} at low-*Q*, characteristic of the presence of several bilayers (Fig. 2, green dashed line). This is in line with results presented previously [10], where the hydration of PC vesicles in PBS leads to MLVs or elongation of vesicles, which cannot be avoided even with extrusion. The superimposition of the DOPC scattering profiles for all DMSO amounts indicates no change in vesicle structure, and therefore also seems to indicate that DMSO does cross the membrane of DOPC vesicles.

On the other hand, when using a more complex model membrane, such as yeast and *E. coli* vesicles, at up to 20 % DMSO content, a Q^{-2} slope is observed, suggesting the formation of non-stacked membranes (Fig. 2, grey dashed line). When the DMSO content is increased ≥ 30 %, an increase of the slope at low-*Q* up to Q^{-3} was induced, suggesting a stacking of layers or the presence of an extended shape such as an elongated vesicle, causing the presence of a correlated membrane due to the proximity of their bilayers [11]. To fully understand our SANS data, we formulated different hypotheses. The simulations for each hypothesis can be found in Figure S3.

(i) DMSO is homogeneously distributed, both outside and inside the vesicles, i.e. the solvent is the binary D_2O-DMSO, leading to a similar scattering profile, but with dilution and additionally a progressively reduced contrast. This is the hypothesis used to normalize the scattering data (Fig. 2), and is valid for DOPC at all DMSO additions where the data completely overlap. For polar lipid extracts of *E. coli* and yeast this hypothesis is only valid up to 20 %, while it is not confirmed at concentrations ≥30 %, where we observe a change of the slope from Q^{-2} to Q^{-3}.

(ii) DMSO contributes to the solvent but exclusively on the outside of the vesicles (it does not go through the membrane), and as a result, the scattering contrast will increase between the D_2O core and the DMSO-D_2O solvent outside the vesicles, leading to a new scattering contribution. This will result in a progressive increase of the slope at low-*Q* towards Q^{-4}. However, we can completely exclude this hypothesis, due to the absence of this slope in the lower *Q* range of our data. Moreover, an osmotic pressure would be created between the pure water core and the DMSO-D_2O solvent, resulting, for example, in a flattening out of the vesicles, the evidence of which is absent in our data. A flattened

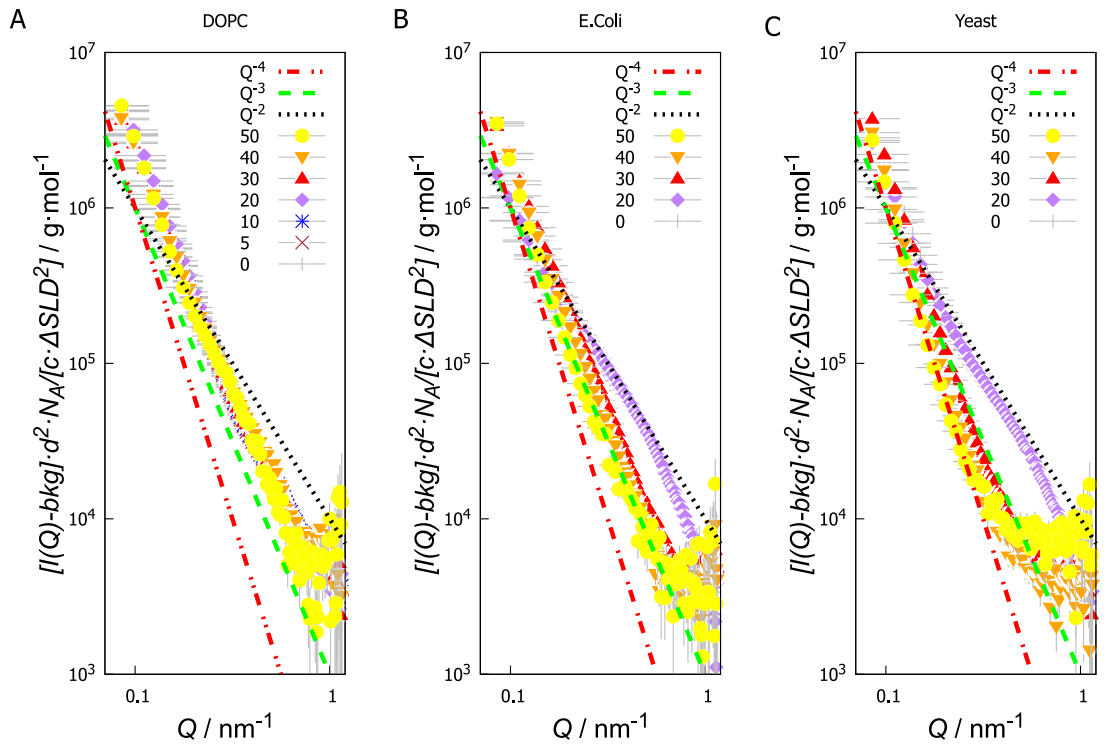

Fig. 2. SANS profiles of MLV of DOPC (A), *E. coli* (B) and yeast (C) polar lipid extract with the addition of different DMSO content by volume: 0 % (grey), 5 % (brown cross), 10 % (purple star), 20 % (purple rhombus), 30 % (red triangles), 40 % (orange triangles), 50 % (yellow). Data were subtracted from their incoherent background given by the respective hDMSO content, and normalized by the volume fraction of lipids and the square of the contrast between the lipids and the binary solvent. Lines with slope at Q^{-2} are represented in black, at Q^{-3} in green, at Q^{-4} in red.

out vesicle would result in an elongated, squeezed, or even bilamellar vesicle. For comparison, a similar effect was described in our previous work, where the addition of PBS exerts an osmotic pressure on vesicle membranes [10].

(iii) DMSO preferentially locates in the membrane, but the majority is mixed with water. This is compounded by the fact that DMSO is a good solvent for lipids, and this would become especially apparent at high DMSO content. The outcome would be an increase in the membrane volume fraction and, therefore, in the scattering intensity, which we can readily exclude based on our data.

To summarize, in DOPC membranes, DMSO permeates the bilayer without inducing structural changes. The absence of detectable structural alterations may also be influenced by layer stacking resulting from osmotic shock caused by salts, as we established previously [10]. In contrast, in more complex membranes, such as polar lipid extracts from *E. coli* and yeast, the addition of DMSO at concentrations ≥ 30 % induces the formation of stacked membranes. In these systems, the presence of cardiolipin (CL) in both *E. coli* and yeast membranes, which increases the likelihood of elongated membrane morphologies [11], may facilitate the interaction of DMSO with the membrane, altering its structure. Once again, the present study, together with our previously reported work [10,11] and others [6], highlights the critical importance of lipid composition and the methodology employed to form the vesicles (i.e. hydration in absence or presence of salts, temperature, extrusions etc.) in what type and shape of vesicle would be present in the sample.

To further explore details on the bilayer structure such as thickness, headgroups and tails of vesicles in solution with different DMSO content, we employed SAXS and used membrane lipid compositions of different complexity: DOPC or yeast polar lipids extract with a general composition of PC:PI:PS:PE:LysoPC:LysoPE (30 %:26 %:8 %:8 %:4 %:1.4 % by weight).

We first studied the addition of DMSO (from 0 % to 75 % by volume fraction) to DOPC vesicles hydrated in H_2O and extruded at 80 nm. Visual inspection of our SAXS data indicated membrane correlation in all samples, even without DMSO, as evidenced by the presence of correlation peaks (humps) showing at the Q-vector values of 1 nm^{-1} and 2 nm^{-1} (Fig. 3A). This membrane correlation becomes more pronounced, as evidenced by the increase in intensity of the first correlation peak at 1 nm^{-1} at 30 and 40 % DMSO (Fig. 3, red and orange curves), until reaching a completely stacked membrane at 50 % with a very sharp Bragg peak. This membrane correlation further increased at 75 % DMSO, suggesting an almost crystalline structure (Fig. 3, yellow and brown curves).

Our results indicate that the binary mixture of DMSO-H_2O above 40 % is not a good solvent for vesicles, because it changes the structure of the vesicles. The membrane correlation bump around 1 nm^{-1} remains initially at this position until 20 % DMSO, then shifts to higher Q, i.e. shorter distances. In the presence of 50 % DMSO, membranes in solution are completely stacked and become an almost crystalline structure in 75 % of DMSO.

With regards to the DOPC vesicles without DMSO, it is unclear whether the membrane correlation is caused by the presence of spherical bilamellar vesicles (BLVs) (which should be present only as a small fraction when hydration occurs with water [19]) or by the flattening of the vesicles passing through the extruder, which may induce formation of cylindrical vesicles [10]. This distinction cannot be made using our data because we chose a shorter Q-range, which allows us to focus on the bilayer details, but loses information on the overall shape of the vesicles at low-Q. However, the shift towards a higher Q of the Bragg peak at ≥ 50 % of DMSO is undeniable. This sharp Bragg peak may be caused by infinite stacking as a result of dehydration of the headgroup caused by DMSO, which led to the vesicle's structural collapse and a decrease in inter-membrane distance. Indeed, from 0 % to 50 % DMSO, the first distance of the Bragg peak suggests that the distance from centre to centre of the layers is ≈ 4.9 nm, while at 75 % the distance decreases to ≈ 4.3 nm, confirming an increase in membrane stacking.

Data fitting was performed with SASfit [15] employing a form factor of discs with 3 homogeneous layers (a single layer for the hydrophobic

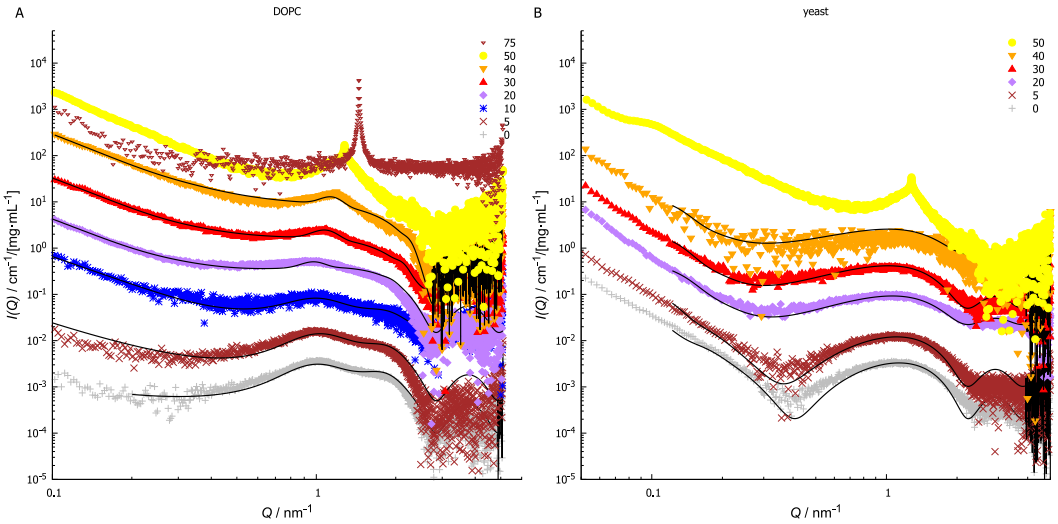

Fig. 3. SAXS profiles of DOPC vesicles (A) and vesicles from yeast polar lipid extract (B). Vesicles were hydrated in H_2O and DMSO added subsequently: 0 % (grey), 5 % (brown cross), 10 % (purple star), 20 % (purple rhombus,) 30 % (red triangles), 40 % (orange triangles), 50 % (yellow). Data fitting using discs with 3 shells (A, black lines) or using or ULV model (B, black lines). An incremental multiplication factor along $I(Q)$ was applied for clarity.

part of the bilayer and two outer layers for the head groups) and a structure factor to account for multi-lamellarity (paracrystalline model with polydispersity implemented on the discrete number of layers) [16]. Details of the fit parameters and model employed are in SI results. Fitting of the data does not highlight any difference in the thickness of the bilayer upon DMSO addition, with all membrane thicknesses reported as between 3.5 nm to 3.7 nm ±1. On the other hand, above 20 % DMSO, a slight increase in the number of layers from 2 to 3 is observed, together with a slight decrease in the distance between layers. This suggests an increase in the stacking of layers due to the excess DMSO (Table S3). This supports our observation regarding the position of the first Bragg peak. Our experimental results are in line with previous studies [20–24] where a near-constant thickness of the bilayer upon DMSO addition was reported. However, it is in contrast with MD studies where an increased DMSO content lead to less packed chains, causing a progressive thinning of the membrane until pore formation [25].

Next, we performed measurements on a more complex membrane model, yeast polar lipid extract. No evident changes in the SAXS profile up to 40 % of DMSO were present, showing a typical scattering profile of a unilamellar vesicle (ULVs) (Fig. 3B). We suggest that the relative complexity of this lipid mixture, which contains all unsaturated lipids compared to DOPC-only vesicles, together with the presence of cardiolipin, led to the formation of ULVs in the vesicle preparation process rather than BLV [11]. However, the SAXS profile of the vesicles changes completely when using 50 % of DMSO, with the appearance of a distinct Bragg peak at 1.1 nm^{-1}, indicative of a stacked membrane with an almost crystalline structure, similar to the peak observed for DOPC vesicles (Fig. 3A). Data analyses were performed with SASfit, with a core–shell model with a three-slabs model, to represent two layers for the internal and external head-groups, and the tail as a unique layer. Fitting parameters confirmed the qualitative data analysis, showing no differences except for vesicles in 50 % DMSO, where the vesicle structure is completely lost (Table S3). Fitting also revealed that the overall membrane thickness remains unchanged up to 40 % of DMSO at 4.1 nm, values comparable with literature (Fig. 3).

Our results not only confirm the lipid-composition-dependent effect of DMSO on altering the membrane structure but also highlight the importance of how high the DMSO content in the solution is. In particular, high concentrations of DMSO (≥ 40 %) have a pronounced impact on vesicle structure, whereas less DMSO content is well tolerated. Secondly, we confirm that the effect of DMSO is dependent on the lipid composition, as was also observed by a previous study [6]. The authors demonstrate that DMSO had a greater effect on the two-membrane system than when cholesterol was present as a third component. This effect has been attributed to the ability of DMSO to reduce the free volume on the bilayer of the two components, oppositely to the three-lipidic classes one, which was attributed to the lipid specificity and their thermodynamic properties. However, our data show a similar effect for the simplest (DOPC) or more complex membrane model employed (yeast). This discrepancy can likely be attributed to the use of different amounts of DMSO in both studies as well as membrane models with different complexities, highlighting the intricate interplay between DMSO content in the solvent and lipid composition of the vesicle membrane.

Our data show that the relationship between DMSO content in the solvent and lipid composition is more convoluted than to simply look at a one-lipid system or a mixture. When examining our SANS data, we observe a clear difference between DOPC and yeast and *E. coli* membranes when adding DMSO to the surrounding solvent, suggesting changes at the vesicle structure. In contrast, when examining shorter distances (range towards larger Q as observed with SAXS), we can observe that in the presence of the more complex membrane model (yeast), more DMSO is needed to induce the stacking of membranes (50 %), while for DOPC changes in the membrane structure occur more gradually. This indicates that the effect of DMSO is most prominent at high concentrations and affects shape, morphology and lamellarity.

Importantly, our study highlights that one effect of DMSO on vesicles is the reduction of membrane distances, and therefore an increase in stacking, which is likely caused by the dehydration of the lipid membranes, specifically the head groups, as was suggested before [7,26]. In the frequently studied DMPC vesicle membranes, several SAXS and SANS studies revealed that the multilamellar repeat distance decreases sharply with increasing DMSO concentration up to 13 % DMSO (see e.g. [7]), although the thickness of the DMPC bilayer remains unchanged up to 30 % DMSO. This was also observed in our data using DOPC vesicles and may point to a general phenomenon. Crucially, our comparison with more complex membrane models such as yeast and *E. coli* polar lipid extracts, which we examined with scattering techniques under a long and a short Q range, allowed us to obtain a threshold of DMSO concentration at which an effect on the membrane becomes apparent, demonstrating once again the criticality in choice of the membrane model.

The main question remaining is whether possible membrane thinning and pore formation occur in the presence of high DMSO content,

which is not observed in experimental data but is shown in MD simulations [25]. Pore formation would ultimately lead to an increase in permeability for larger molecules and ions, but it is unclear what the molecular determinants are that would govern this behaviour. Therefore, we set out to verify whether DMSO could facilitate the diffusion of PBS to the inside of the vesicles by examining the vesicle structure using SAXS. We employed DOPC vesicles, hydrated in H_2O and incubated them for 9 h overnight with increasing amounts of DMSO to induce permeability, followed by the addition of PBS. We expected that, should DMSO increase the membrane permeability, the vesicles would be more susceptible to osmotic stress caused by the PBS, which would manifest in a change in vesicle morphology, e.g. elongation or flattening. However, our SAXS data show that from the outset, highly correlated membranes were formed as evidenced by two Bragg peaks at 1.1 and 2.2 nm^{-1}, which remain unchanged up to 20 % of DMSO. However, a small shift to the right is highlighted with 40 % and 50 % DMSO, where the Bragg peaks move to 1.2 and 2.3 nm^{-1}. This suggests that when DMSO is added, the membrane correlation only slightly increases (Figure S5). We conclude that the presence of MLVs, from the start, did not allow for better visualization of whether DMSO could further change the vesicle structure. This could be due to the added rigidity and therefore resistance to osmotic stress afforded by MLV formation, or that DMSO was unable to penetrate the multiple layers in the MLV sufficiently to allow PBS to reach the core of the vesicle and thus facilitate osmotic stress. This is compounded by the fact that the effect of DMSO on membrane curvature remains poorly characterized [27].

Lastly, we verified the possibility of forming lipid vesicles by exchange of the solvent, employing a mixture DMSO-H_2O, where the DMSO content was 5 %. Lipid vesicles of yeast polar lipids, *E. coli* polar lipid extract and DOPC were dissolved in DMSO. Then, two mixing methods were used: a "slow" addition consisted in adding drop-wise an aqueous buffer to the lipidic stock-solution, while the "fast" addition consisted in quickly injecting the lipidic solution into an aqueous buffer.

Resulting vesicles were analysed with SAXS due to the sensitivity to the eventual formation of the structure factor characteristic of BLVs or MLVs (Figure S6). For both methods, fast and slow additions, the formation of ULVs can be observed for the yeast polar lipid extract only, without any correlation peak. This could be due to a higher content of lipids with large polar head groups in the yeast polar lipid extract, such as PI, which could promote vesicle formation. In contrast, no ULVs were formed with DOPC and *E. coli* polar lipid extract, and we obtained a very low scattering signal. We interpret this to be due to fast aggregation and precipitation in these samples. A more thorough and systematic study may provide additional parameters and thresholds as to which lipid composition and preparation method may result in the best and most reliable ULV formation. We conclude that the lipid composition has a profound effect on whether spontaneous vesicles (ULVs) can be formed with rapid solvent exchange. This has an important application in the food industry [28], and for pharmaceutical applications [29], where lipid preparation in organic solvents and rapid solvent exchange ("solvent shifting" or "solvent quenching") facilitates an increased sample homogeneity [30].

4. Conclusions

Dimethyl Sulfoxide (DMSO) is ubiquitous in pharmacology and cell biology, yet molecular insights into its membrane interactions have historically relied on simplified models (e.g., DMPC in pure water). In this work, we demonstrate that the structural perturbations caused by DMSO are highly dependent on the interplay between solvent concentration, lipid complexity, and physiological environmental factors (salts/osmotic stress).

By comparing DOPC with complex polar lipid extracts (yeast, *E. coli*) under physiological saline conditions, we provide three key insights that extend beyond previous literature:

1. *Robustness of the* 20 % *Threshold:* We confirmed that vesicle integrity is maintained up to 20 % DMSO even in the presence of osmotic shock and salts.
2. *Composition defines the structural transition:* We observed distinct behaviours depending on lipid complexity. Simple DOPC vesicles are prone to immediate stacking under osmotic stress, showing a gradual flattening effect with increasing DMSO. In contrast, complex biological extracts (yeast, *E. coli*) exhibit higher initial resilience, maintaining unilamellar morphology in PBS. However, these complex membranes display a sharp stability threshold: once critical DMSO concentrations are reached, they undergo a dramatic structural collapse into stacked multilayers.
3. *Mechanism of Collapse:* We corroborate that the primary failure mode at high concentrations ($\geq$ 40 %) of DMSO is a reduction in inter-membrane distance leading to stacking. Crucially, we show this mechanism dominates even in high-ionic-strength environments.

In summary, our study emphasizes the importance of preserving physiologically relevant conditions in membrane models. We conducted a mapping of the DMSO concentration thresholds at which effects become apparent. Our findings highlight that while the specific collapse threshold depends on lipid composition and initial structure, 20 % DMSO represents a safe operating limit for stabilizing vesicles of varying complexity.

Finally, comparing these findings with our previous work on salt-induced osmotic stress [10,11] highlights the critical role of membrane permeability in vesicle stability. We previously observed that physiological salts act as impermeable osmolytes, creating a strong osmotic gradient across the bilayer that efficiently depletes the vesicle core of water, leading to flattening or bilamellar structures even at relatively low molar concentrations ($\approx$ 0.15 M). In contrast, DMSO is membrane-permeable and equilibrates across the bilayer, preventing the formation of a transmembrane osmotic gradient. Consequently, DMSO does not mechanically flatten the vesicle; instead, it acts by directly competing with lipid headgroups for the hydration shell. This bulk dehydration mechanism is far less efficient than transmembrane osmotic stress, explaining why significantly higher molar concentrations of DMSO ($\geq$ 2.5 M to 3.0 M) are required to overcome hydration repulsion and induce membrane stacking.

Moving forward, understanding the dynamic prerequisites to the structural collapse of vesicles in high DMSO content is essential. We propose that future studies employ Neutron Spin Echo (NSE) and Quasi-Elastic Neutron Scattering (QENS) to investigate how additives such as DMSO can alter membrane bending rigidity and lipid lateral diffusion *before* structural failure occurs, providing a complete mechanical picture of complex solvent–membrane interactions under physiological stress.

CRediT authorship contribution statement

Alice Piccinini: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data acquisition and experimental design Formal analysis, Data curation. **Anastasiia Murmuliuk:** Writing – review & editing, Data acquisition, Resources. **Guillaume Gilliard:** Writing – review & editing, Data acquisition. **Gouranga Manna:** Writing – review & editing, Resources. **Valeria Rondelli:** Writing – review & editing, Resources, Funding acquisition. **Anja Winter:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Sylvain Prévost:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Data acquisition and experimental design, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used Gemini in order to improve readability and English grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sylvain Prevost reports equipment, drugs, or supplies was provided by Paul Scherrer Institute PSI. Anastasiia Murmuliuk reports equipment, drugs, or supplies and travel were provided by ESRF. Alice Piccinini reports financial support was provided by Italian Ministry of University and Research. Anastasiia Murmuliuk reports financial support was provided by Next Generation EU. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.colsurfa.2026.140014.

Data availability

Data will be made available on request.

References

[1] Nuno C. Santos, J. Figueira-Coelho, J. Martins-Silva, Carlota Saldanha, Multidisciplinary utilization of dimethyl sulfoxide: Pharmacological, cellular, and molecular aspects, Biochem. Pharmacol. (ISSN: 00062952) 65 (7) (2003) 1035–1041, http://dx.doi.org/10.1016/S0006-2952(03)00002-9,

[2] Koen R. Storm, Caroline Körösy, Enrico Skoruppa, Stefanie D. Pritzl, Pauline J. Kolbeck, Willem Vanderlinden, Helmut Schiessel, Jan Lipfert, The effects of DMSO on DNA conformations and mechanics, Biophys. J. (ISSN: 15420086) (2025) 2639–2654, http://dx.doi.org/10.1016/j.bpj.2025.06.041.

[3] Adrian C. Williams, Brian W. Barry, Penetration enhancers, Adv. Drug Deliv. Rev. (ISSN: 0169409X) 56 (5) (2004) 603–618, http://dx.doi.org/10.1016/j.addr.2003.10.025.

[4] William Wolkers, Harriette Oldenhof, Cryopreservation and freeze-drying, in: Methods in Molecular Biology, Humana Press, Totowa, NJ, ISBN: 978-1-4939-2192-8, 2021, p. 203.

[5] Marie-Amélie de Ménorval, Lluis M. Mir, M. Laura Fernández, Ramon Reigada, Effects of dimethyl sulfoxide in cholesterol-containing lipid membranes: A comparative study of experiments in silico and with cells, PLoS One 7 (7) (2012) http://dx.doi.org/10.1371/journal.pone.0041733.

[6] Beatrice Gironi, Zehra Kahveci, Beth McGill, Bob Dan Lechner, Stefano Pagliara, Jeremy Metz, Assunta Morresi, Francesca Palombo, Paola Sassi, Peter G. Petrov, Effect of DMSO on the mechanical and structural properties of model and biological membranes, Biophys. J. (ISSN: 15420086) 119 (2) (2020) 274–286, http://dx.doi.org/10.1016/j.bpj.2020.05.037.

[7] Chi Yuan Cheng, Jinsuk Song, Jolien Pas, Lenny H.H. Meijer, Songi Han, DMSO induces dehydration near lipid membrane surfaces, Biophys. J. (ISSN: 15420086) 109 (2) (2015) 330–339, http://dx.doi.org/10.1016/j.bpj.2015.06.011.

[8] Alex M. Schrader, Chi-Yuan Cheng, Jacob N. Israelachvili, Songi Han, Communication: Contrasting effects of glycerol and DMSO on lipid membrane surface hydration dynamics and forces, J. Chem. Phys. 145 (2016) 41101, http://dx.doi.org/10.1063/1.4959904.

[9] M.A. Kiselev, Combined application of neutron and synchrotron radiation for investigation of the influence of dimethyl sulfoxide on the structure and properties of the dipalmitoylphosphatidylcholine membrane, Biological Syst. 52 (3) (2007) 554–559, http://dx.doi.org/10.1134/S1063774507030352.

[10] Alice Piccinini, Andrew E. Whitten, Anja Winter, Sylvain Prévost, The effect of phosphate buffered saline and osmotic stress on phosphatidylcholine vesicles, J. Colloid Interface Sci. (ISSN: 10957103) 691 (December 2024) (2025) http://dx.doi.org/10.1016/j.jcis.2025.137363.

[11] Alice Piccinini, Joachim Kohlbrecher, Dihia Moussaoui, Anja Winter, Sylvain Prévost, Effect of cardiolipin on the lamellarity and elongation of liposomes hydrated in PBS, J. Colloid Interface Sci. (ISSN: 10957103) 669 (April) (2024) 844–855, http://dx.doi.org/10.1016/j.jcis.2024.04.211.

[12] U. Keiderling, The new 'BerSANS-PS' software for reduction and treatment of small angle neutron scattering data, 2001.

[13] Theyencheri Narayanan, Michael Sztucki, Pierre Van Vaerenbergh, Joachim Léonardon, Jacques Gorini, Laurent Claustre, Franc Sever, John Morse, Peter Boesecke, A multipurpose instrument for time-resolved ultra-small-angle and coherent X-ray scattering, J. Appl. Crystallogr. 51 (6) (2018) 1511–1524, http://dx.doi.org/10.1107/S1600576718012748.

[14] Theyencheri Narayanan, Michael Sztucki, Thomas Zinn, Jérôme Kieffer, Alejandro Homs-Puron, Jacques Gorini, Pierre Van Vaerenbergh, Peter Boesecke, Performance of the time-resolved ultra-small-angle X-ray scattering beamline with the extremely brilliant source, (ISSN: 16005767) 2022.

[15] Ingo Breßler, Joachim Kohlbrecher, Andreas F. Thünemann, SASfit: A tool for small-angle scattering data analysis using a library of analytical expressions, J. Appl. Crystallogr. (ISSN: 16005767) 48 (2015) 1587–1598, http://dx.doi.org/10.1107/S1600576715016544.

[16] Georg Pabst, Richard Koschuch, Beatriz Pozo-Navas, Michael Rappolt, Karl Lohner, Peter Laggner, Structural analysis of weakly ordered membrane stacks, J. Appl. Crystallogr. (ISSN: 00218898) 36 (6) (2003) 1378–1388, http://dx.doi.org/10.1107/S0021889803017527.

[17] Mária Klacsová, Peter Westh, Pavol Balgavý, Molecular and component volumes of saturated n-alkanols in DOPC + DOPS bilayers, Chem. Phys. Lipids (ISSN: 00093084) 163 (6) (2010) 498–505, http://dx.doi.org/10.1016/j.chemphyslip.2010.04.004.

[18] Sandy Gim, Ming Ong, Mallikarjun Chitneni, Kah Seng Lee, Long Chiau Ming, Kah Hay Yuen, Evaluation of extrusion technique for nanosizing liposomes, Pharmaceutics 8 (36) (2016) http://dx.doi.org/10.3390/pharmaceutics8040036.

[19] Haden L. Scott, Allison Skinkle, Elizabeth G. Kelley, M. Neal Waxham, Ilya Levental, Frederick A. Heberle, On the mechanism of bilayer separation by extrusion, or why your LUVs are not really unilamellar, Biophys. J. (ISSN: 15420086) 117 (8) (2019) 1381–1386, http://dx.doi.org/10.1016/j.bpj.2019.09.006.

[20] Luísa M.P.F. Amaral, Maria Rangel, Margarida Bastos, Effect of DMSO on structural properties of DMPC and DPPC liposome suspensions, J. Funct. Biomater. (ISSN: 20794983) 15 (3) (2024) http://dx.doi.org/10.3390/jfb15030067.

[21] Stephanie Tristram-Nagle, Tisha Moore, Horia I. Petrache, John F. Nagle, DMSO produces a new subgel phase in DPPC: DSC and X-ray diffraction study, Biochim. Biophys. Acta (BBA) - Biomembr. (ISSN: 0005-2736) 1369 (1) (1998) 19–33, http://dx.doi.org/10.1016/S0005-2736(97)00197-1.

[22] Z.W. Yu, P.J. Quinn, Phase stability of phosphatidylcholines in dimethylsulfoxide solutions, (ISSN: 00063495) 1995.

[23] M.A. Kiselev, P. Lesieur, A.M. Kisselev, C. Grabielle-Madelmond, M. Ollivon, DMSO-induced dehydration of DPPC membranes studied by X-ray diffraction, small-angle neutron scattering, and calorimetry, J. Alloys Compd. (ISSN: 09258388) 286 (1–2) (1999) 195–202, http://dx.doi.org/10.1016/S0925-8388(98)01006-8.

[24] J.E. Gorshkova, V.I. Gordeliy, Investigation of the interaction of dimethyl sulfoxide with lipid membranes by small-angle neutron scattering, Crystallogr. Rep. (ISSN: 10637745) 52 (3) (2007) 535–539, http://dx.doi.org/10.1134/S1063774507030364.

[25] Andrey A. Gurtovenko, Jamshed Anwar, Modulating the structure and properties of cell membranes: The molecular mechanism of action of dimethyl sulfoxide, J. Phys. Chem. B (ISSN: 15206106) 111 (35) (2007) 10453–10460, http://dx.doi.org/10.1021/jp073113e.

[26] V.I. Gordeliy, M.A. Kiselev, P. Lesieur, A.V. Pole, J. Teixeira, Lipid membrane structure and interactions in dimethyl sulfoxide/water mixtures, Biophys. J. (ISSN: 00063495) 75 (5) (1998) 2343–2351, http://dx.doi.org/10.1016/S0006-3495(98)77678-7.

[27] Sangita Kundu, Sheeba Malik, Meghna Ghosh, Sourav Nandi, Arghajit Pyne, Ananya Debnath, Nilmoni Sarkar, A comparative study on DMSO-induced modulation of the structural and dynamical properties of model bilayer membranes, Langmuir 37 (2021) 1078–2065, http://dx.doi.org/10.1021/acs.langmuir.0c03037.

[28] Nikita S. Bhatkar Adhikari, Vimal Challana, Shivanand S. Shirkole, Benu, Solvent exchange method in the food industry, Sustain. Food Technol. 2 (2025) 382–391, http://dx.doi.org/10.1039/d5fb00396b.

[29] Emmanouil Papadakis, Anjan K. Tula, Rafiqul Gani, Solvent selection methodology for pharmaceutical processes: Solvent swap, Chem. Eng. Res. Des. (ISSN: 0263-8762) 115 (2016) 443–461, http://dx.doi.org/10.1016/J.CHERD.2016.09.004.

[30] Jeffrey T. Buboltz, Gerald W. Feigenson, A novel strategy for the preparation of liposomes: rapid solvent exchange, Biochim. Biophys. Acta (BBA) - Biomembr. (ISSN: 0005-2736) 1417 (2) (1999) 232–245, http://dx.doi.org/10.1016/S0005-2736(99)00006-1.