Analysis of purification of charged giant vesicles in a buffer using their size distribution

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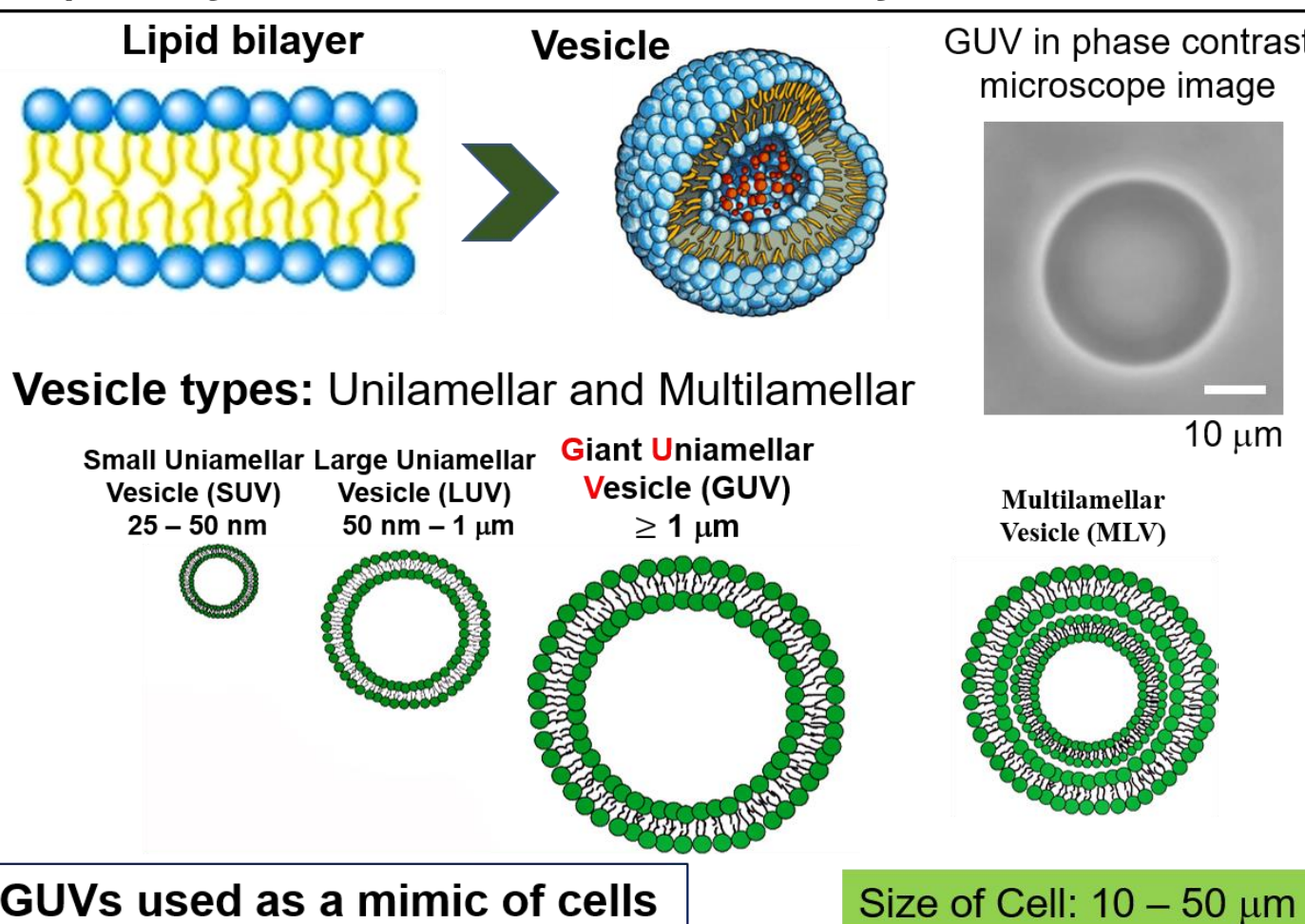Marzuk Ahmed and Mohammad Abu Sayem Karal

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Abstracts: We have analyzed the purification of charged giant unilamellar vesicles (GUVs) prepared in a buffer containing various concentrations of salt using their size distribution. The membranes of GUVs were synthesized by a mixture of dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylglycerol (DOPG) lipids with various DOPG mole fractions (X) and salt concentrations (C) in buffer. The size distribution histograms of experimentally investigated unpurified and purified GUVs were fitted with the lognormal distribution and obtained the multiplication factor γ for mean (μ) and η for standard deviation (σ) of the lognormal distribution. The key parameters γ and η were responsible for changing the average size and size distribution of unpurified GUVs to purified ones. The theoretically fitting equation of experimentally obtained X and C dependent values of γ and η provided the calibration equation for estimating the average size of purified GUVs theoretically for any values of X and C. These investigations might be helpful in the field of cell/chemical biology for understanding the process of purification of vesicles/cells investigated by any other techniques.

Vesicle and it's classification

A vesicles is an artificially prepared spherical compartment composed of a lipid bilayer. Vesicles have been extensively used as a model cells.

Lipid bilayer → **Vesicle**

GUV in phase contrast microscope image

10 µm

Vesicle types: Unilamellar and Multilamellar

- Small Uniamellar Vesicle (SUV) 25 – 50 nm
- Large Uniamellar Vesicle (LUV) 50 nm – 1 µm
- **Giant Uniamellar Vesicle (GUV)** ≥ 1 µm
- Multilamellar Vesicle (MLV)

GUVs used as a mimic of cells

Size of Cell: 10 – 50 µm

Preparation and purification of vesicles

Natural Swelling Method

(1) Lipid dried by N₂ and keep into vacuum desiccator (12 h)
→ (2) Pre-hydration 20 µL MilliQ
→ (3) Incubated with 0.1 M sucrose in buffer
→ (4) GUVs and smaller vesicles

200 µL Lipid | at 45°C for 8 min | at 37°C for 3.5 hours

Purification of GUVs

10 µm pore filter paper

Purified GUVs

50 µm | 50 µm

Results and Discussion

Unpurified GUVs

Purified GUVs

30 µm

Fig. 1: Effects of filtering on the phase contrast image and size distribution of 40% DOPG/60% DOPC-GUVs.

Size distribution and Average size

$$f_{\text{pur}}(D) = \frac{1}{D}\,\frac{1}{\sigma\sqrt{2\pi}}\exp\left[-\frac{\{\ln(D)-\gamma\mu\}^2}{2(\eta\sigma)^2}\right]$$

$$D_{\text{ave}}^{\text{pur}} = \int_0^\infty D f_{\text{pur}}(D)\,dD = \exp\left[\gamma\mu + \frac{1}{2}(\eta\sigma)^2\right]$$

$$D_{\text{ave}}^{\text{pur}} = D_{\text{ave}}^{\text{unp}}\exp\left[\mu(\gamma-1) + \frac{1}{2}\sigma^2(\eta^2-1)\right]$$
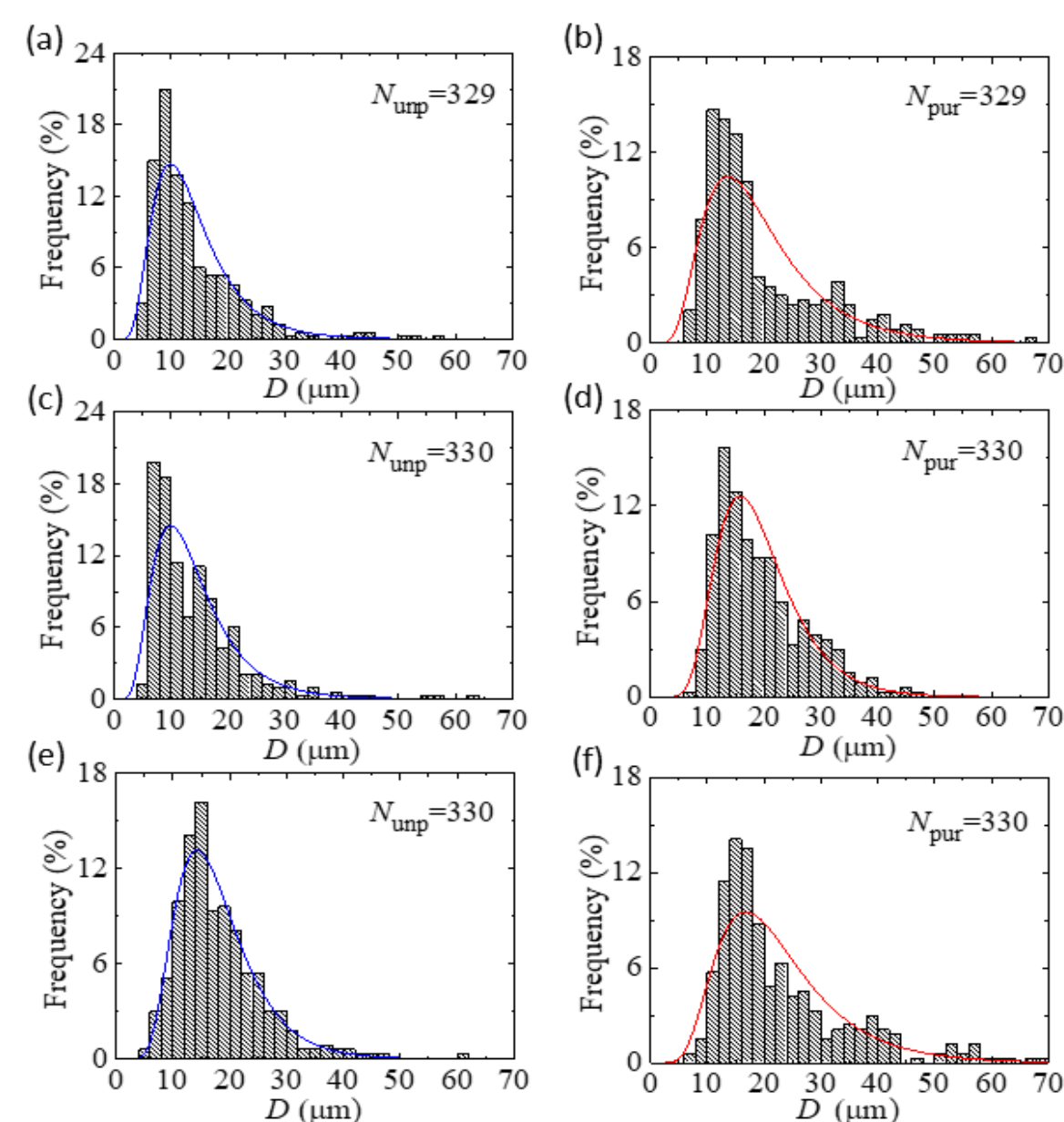

Fig. 2: Effects of filtering on the size distribution of unpurified GUVs (a, c, e) and purified GUVs (b, d, f) containing various X at $C = 162$ mM. (a, b) $X = 0.25$ (c, d) $X = 0.55$ and (e, f) $X = 0.90$.
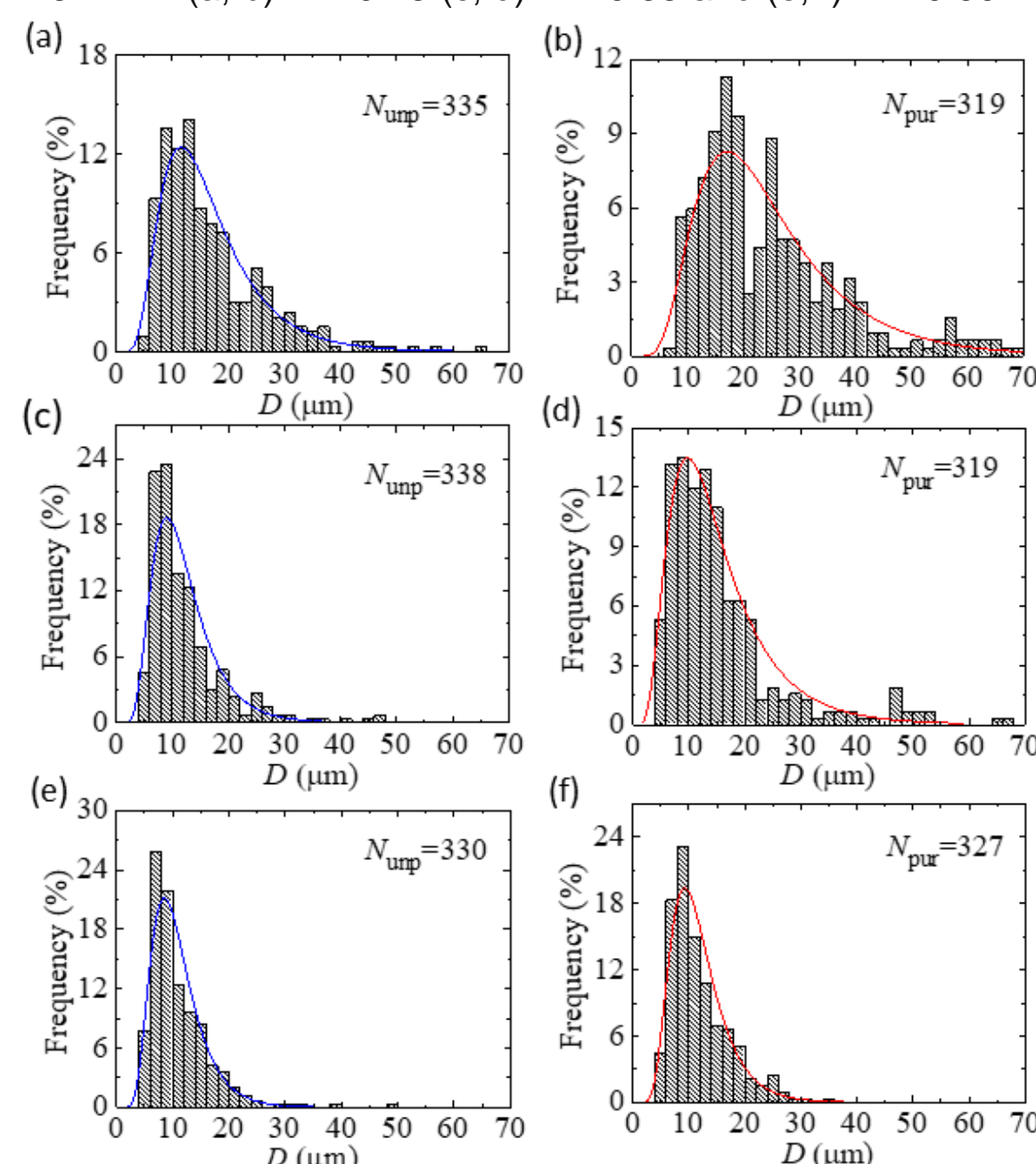

Fig. 3: Effects of filtering on the size distribution of unpurified GUVs (a, c, e) and purified GUVs (b, d, f) containing various C at $X = 0.40$. (a, b) $C = 62$ mM (c, d) $C = 212$ mM and (e, f) $C = 362$ mM.
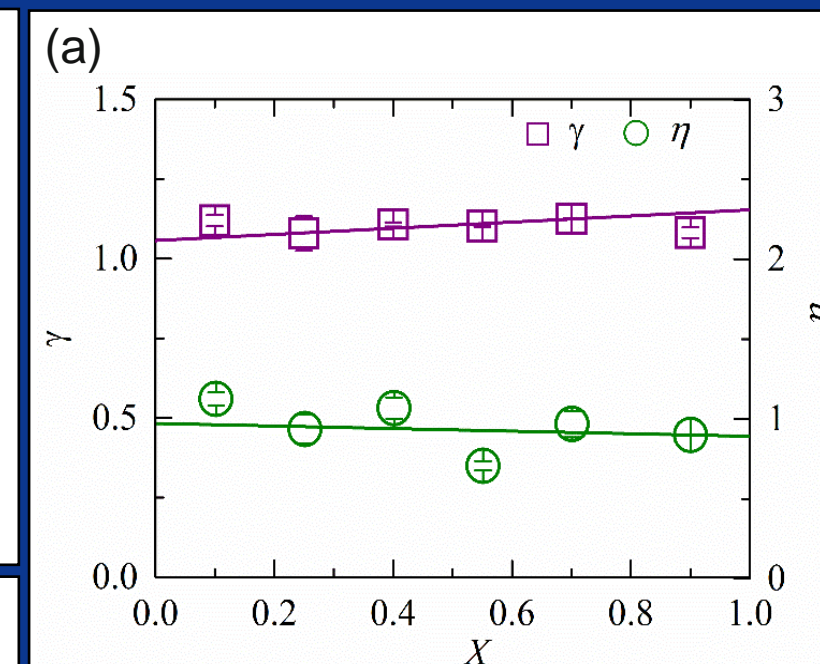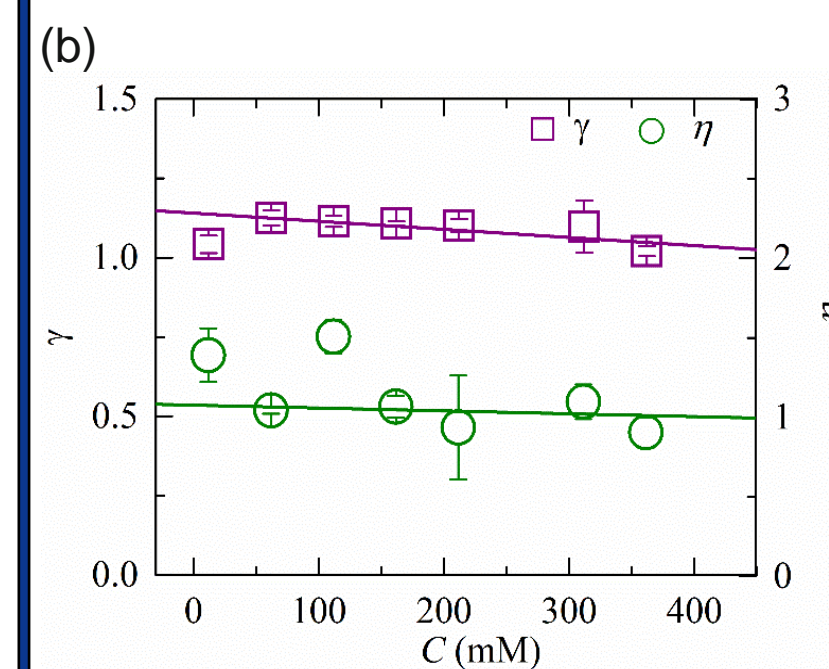

Fig. 4: Surface charge dependent average values of γ and η.

Fig. 5: Salt concentration dependent average values of γ and η.

Calibration equations

For any surface charge

$$\eta(X) = -0.081X + 0.971$$
$$\gamma(X) = 0.097X + 1.057$$

For any salt concentration

$$\eta(C) = 0.0001C + 1.076$$
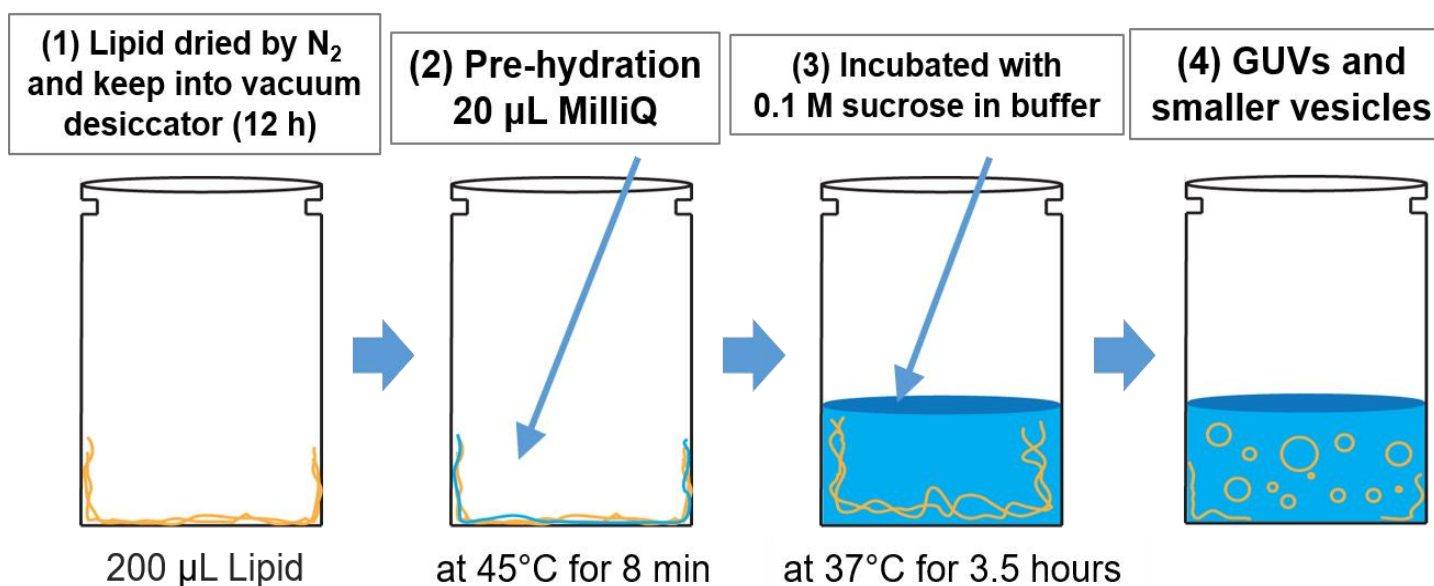$$\gamma(C) = -0.0003C + 1.141$$

Electrostatic bending modulus

$$f^{\text{el}} = 4k_B TX\left[\frac{1-q}{p} + \ln(p+q)\right]$$

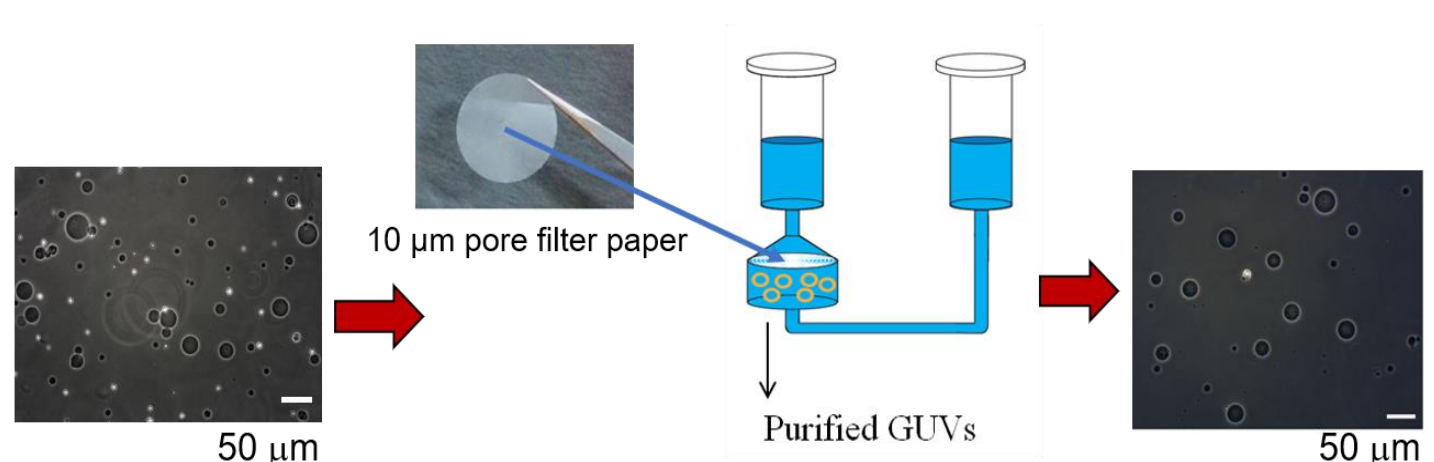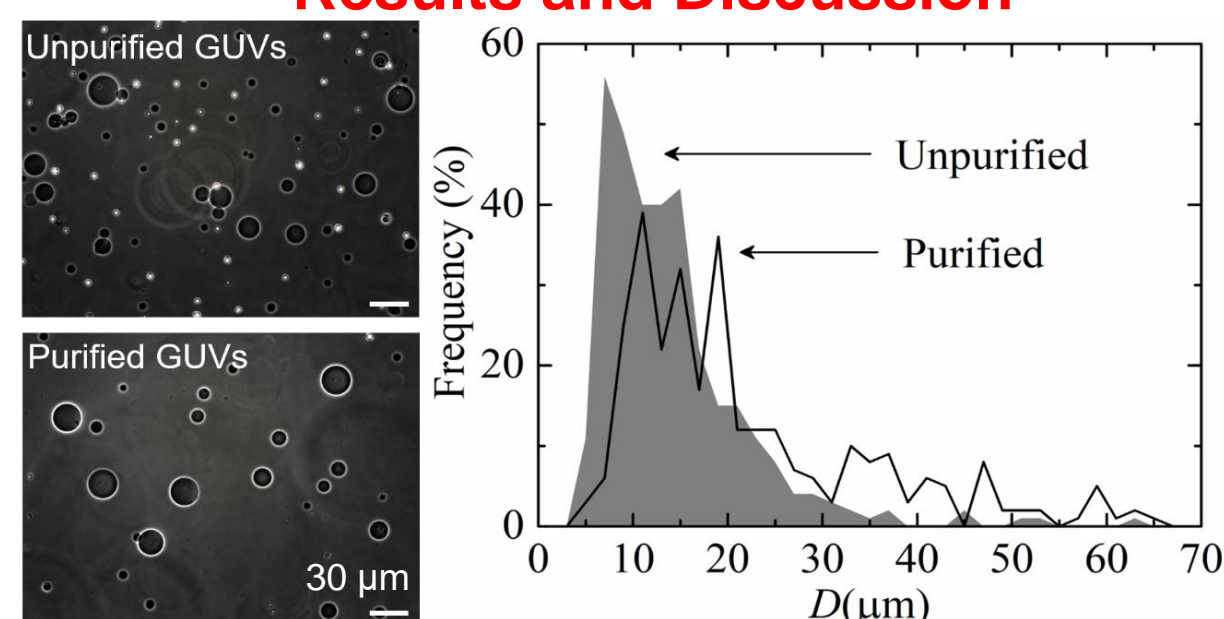$$D_{\text{ave}}^{\text{pur}} = bD_{\text{freq0}}\sqrt{1 + \frac{K_{\text{ben}}^{\text{el}}}{K_{\text{ben0}}}}$$

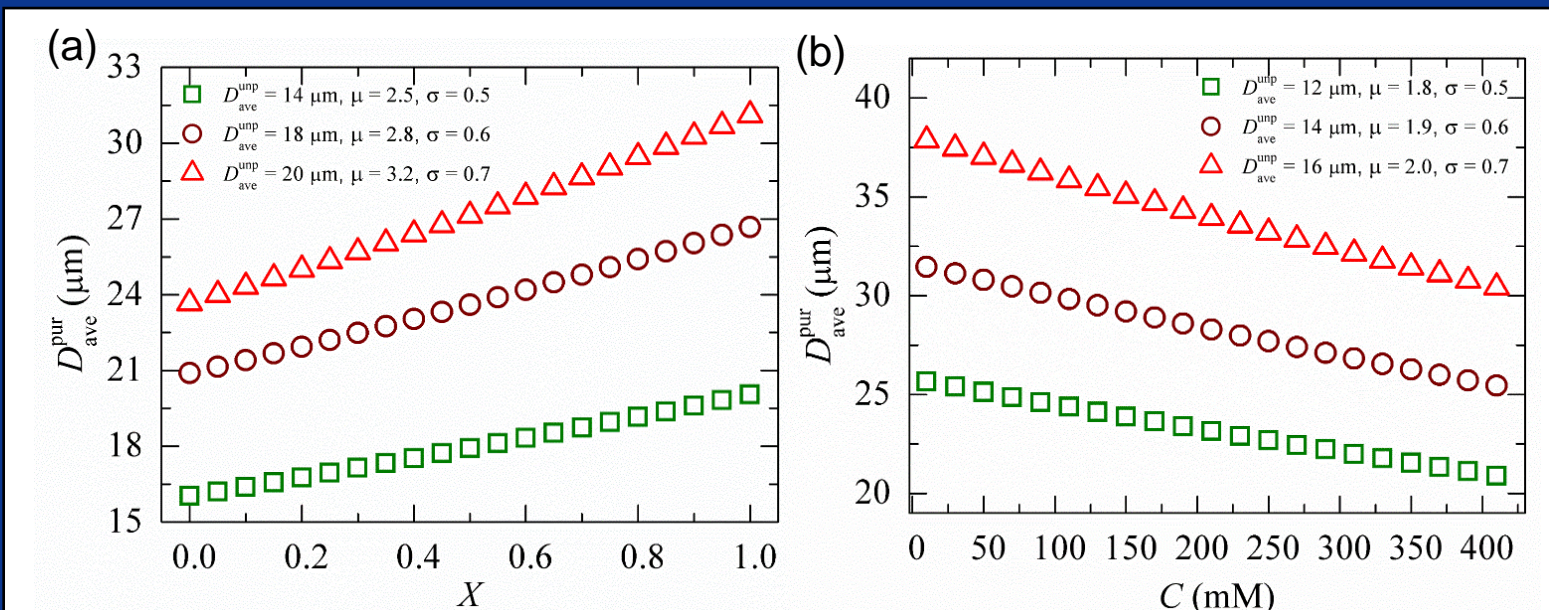$$K_{\text{ben}}^{\text{el}} = \chi\frac{X^2}{\sqrt{C^3}}$$

Fig. 6: Theoretical estimation of the average size for (a) different values of X at $C = 162$ mM and for (b) different values of C at $X = 0.40$.

Table : The experimentally determined values of γ and η for different X and different C

Salt concentration, C = 162 mM			DOPG mole fraction, X = 0.40		
X	$\gamma(X)$	$\eta(X)$	C (mM)	$\gamma(C)$	$\eta(C)$
0.10	1.12 ± 0.02	1.12 ± 0.04	12	1.04 ± 0.03	1.40 ± 0.17
0.25	1.08 ± 0.05	0.94 ± 0.09	62	1.13 ± 0.02	1.04 ± 0.02
0.40	1.11 ± 0.01	1.07 ± 0.07	112	1.11 ± 0.02	1.51 ± 0.10
0.55	1.10 ± 0.01	0.71 ± 0.03	162	1.11 ± 0.01	1.07 ± 0.07
0.70	1.13 ± 0.01	0.97 ± 0.08	212	1.10 ± 0.02	0.94 ± 0.33
0.90	1.08 ± 0.02	0.90 ± 0.01	312	1.10 ± 0.08	1.10 ± 0.11
-	-	-	362	1.02 ± 0.02	0.91 ± 0.10

Conclusion: With the help of γ & η, the average size and size distribution of purified GUVs are obtained theoretically from the experimentally observed unpurified vesicles for any values of X & C.