EFFECT OF VARIATION OF LIPID COMPOSITION ON PROPERTIES OF A LIPOSOMAL DELIVERY SYSTEM

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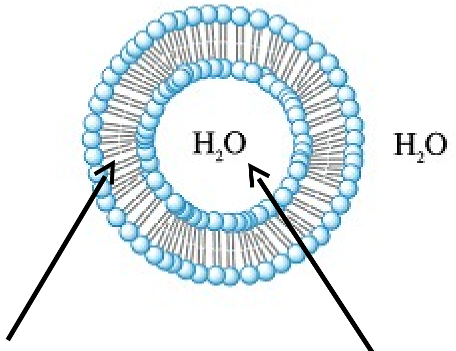
What are liposomes?

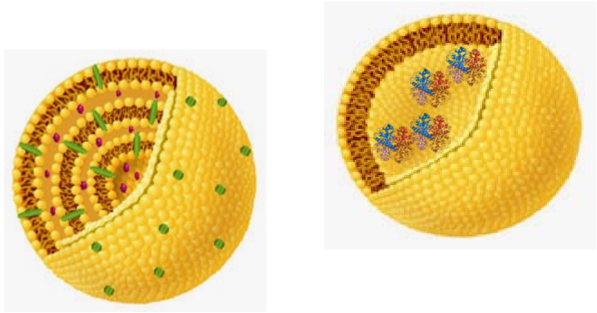
- Vesicular structures

Components
- Polar lipids
- Aqueous component



Liposome

- Carriers of various structures



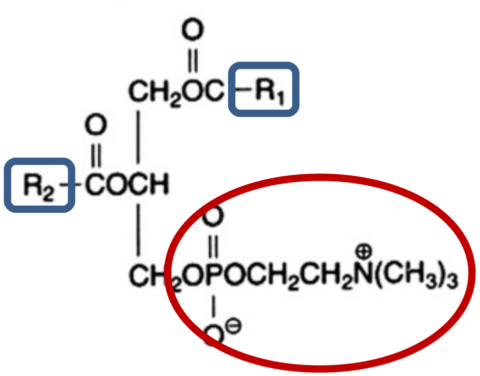
Liposomes encapsulating various structures

Polar lipids – major lipid component

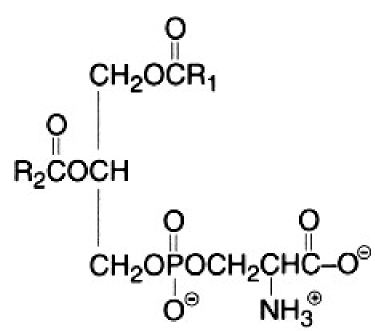
Polar lipids

- **Neutral**
- **Negatively charged**
- **Positively charged**

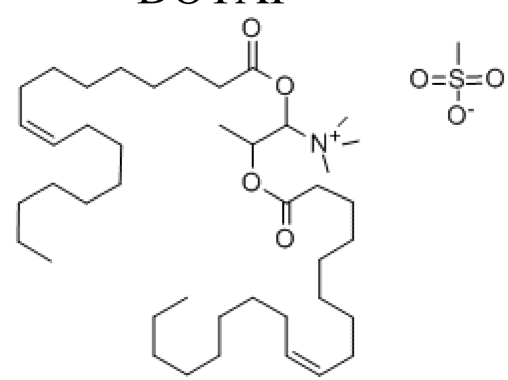
Phosphatidylcholine



Phosphatidylserine



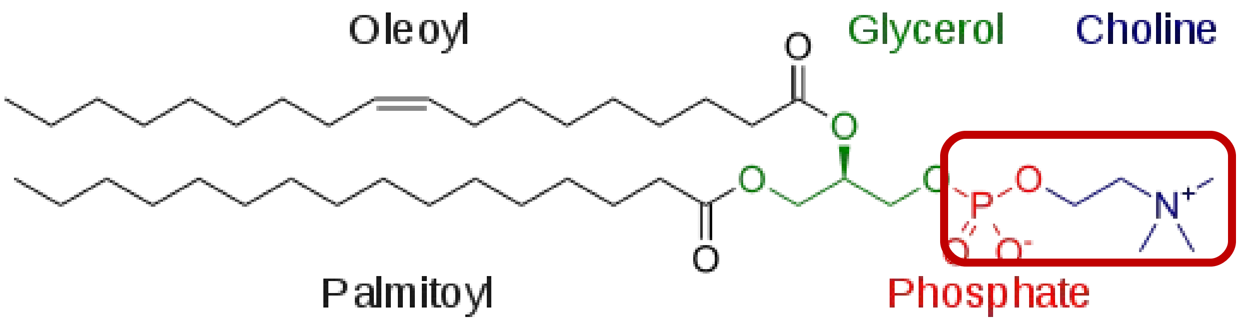
DOTAP



Phosphatidylcholine (PC)

- Neutral polar lipids

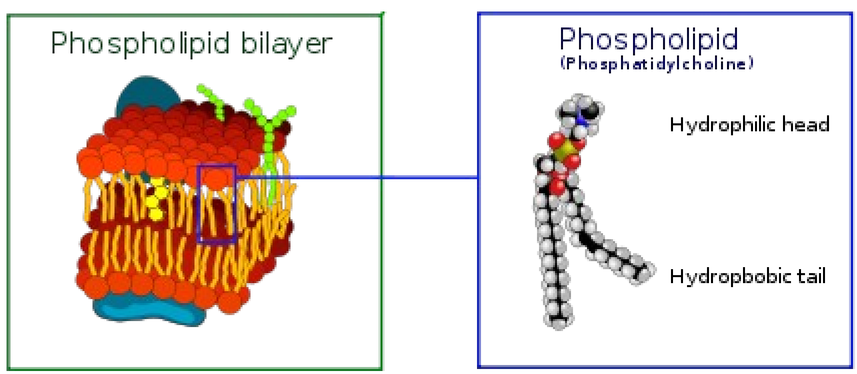
Backbone



Head group

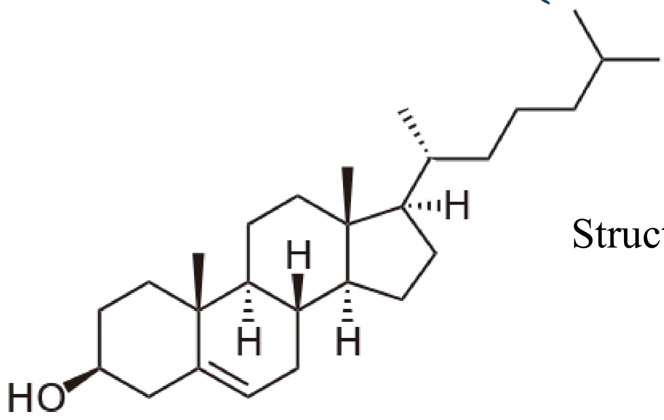
Structure of phosphatidylcholine

- Major component of cell membranes



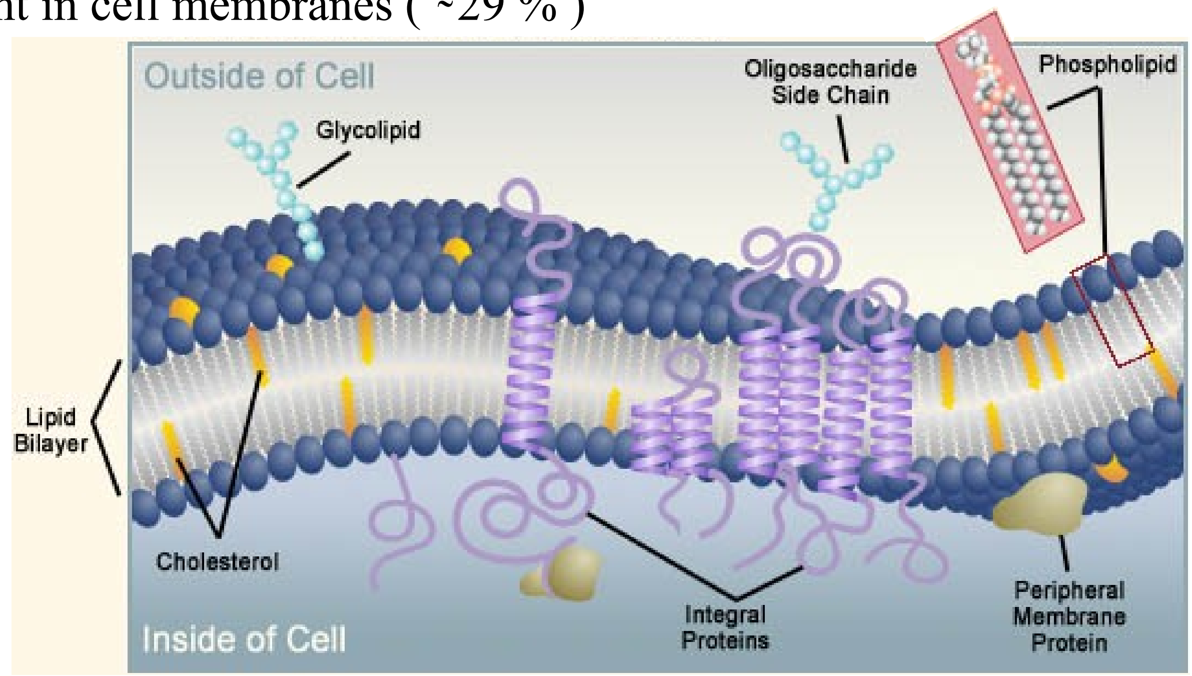
Cholesterol (CH)

•Neutral lipid



Structure of cholesterol

•Present in cell membranes (~29 %)

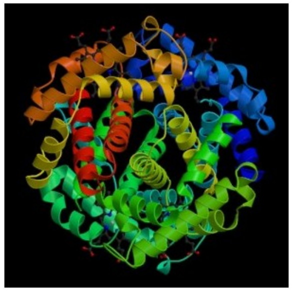


Fluid Mosaic Model of Cell Membranes

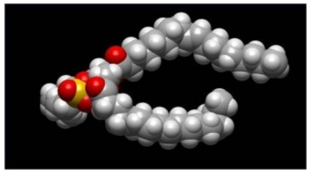
Antioxidants

- **What?** Molecules that prevent oxidation of other molecules
- **Role?** Protect the body from oxidative stress
    - Biological molecules protected:

DNA



Protein



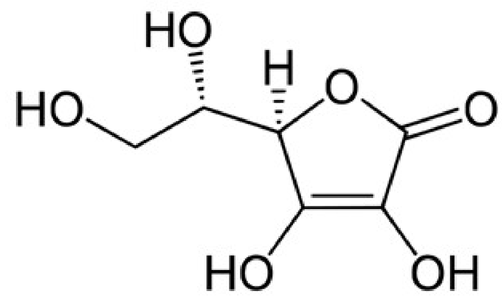
Lipid

- **How?** Neutralize reactive oxygen species

$^{\cdot}OH$	O_2^-	H_2O_2	$HClO$
Hydroxyl radical	Superoxide anion	Hydrogen peroxide	Hypochlorous acid

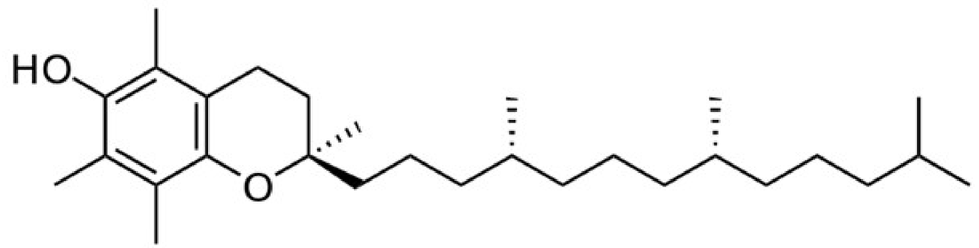
Antioxidants encapsulated in liposomes

Water soluble:
Glutathione
Lipoic acid
Water-soluble flavonoids (e.g. pycnogenol)
Ascorbic acid (vitamin C)



ascorbic acid

Lipid soluble:
ubiquinones (e.g CoQ10)
Carotenoids
lipid soluble flavanoids (e.g. naringenin),
vitamin E (tocopherols and tocotrienols



α-tocopherol

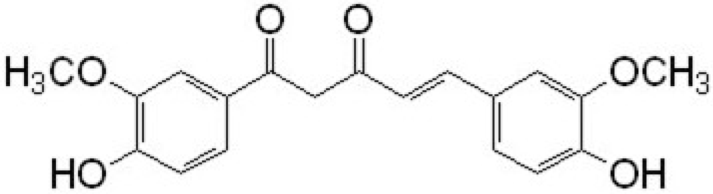
Enzymatic:
superoxide dismutase

superoxide dismutase

Plant extracts used in cosmetic formulations

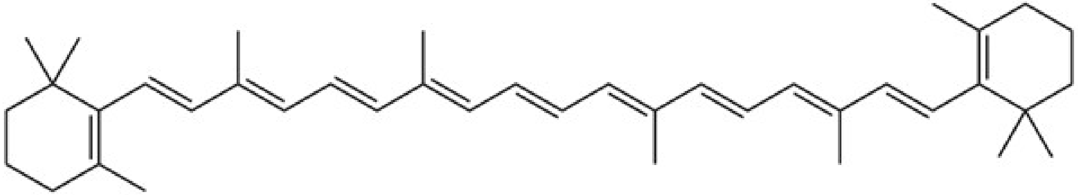
Turmeric

Curcumin – antioxidant activity



Carrot extract

Carotene – antioxidant activity



Papaya extract

Carotene – antioxidant activity

Schumacheria castaneifolia

Family - Dilleniaceae

Tropical trees, shrubs, climbers, herbs

Genus - *Schumacheria* :

Schumacheria castaneifolia
Schumacheria alnifolia
Schumacheria angestifolia

Endemic to Sri Lanka

Schumacheria castaneifolia

Gurni, A.A. and Kubitzki, K. (1981). Flavonoid Chemistry and Systematics of the Dilleniaceae. *Biochemical Systematics and Ecology* 9 (2/3): 109-114

Methanol extract of stem-bark of *Schumacheria castaneifolia*

High antioxidant activity; IC_{50} = 11.0 ppm (DPPH assay)

α-tocopherol – IC_{50} = 11.4 ppm (DPPH assay)

Very low cytotoxicity; (Brine shrimp assay)

May be utilized in pharmaceutical, cosmetic or food industry

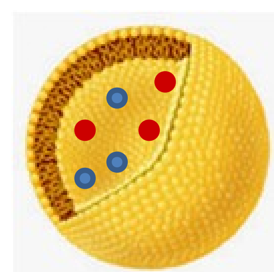
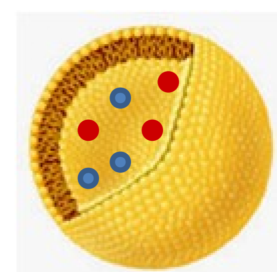
Bandara, R.M.C.J., Abeykoon, D.M.B., Bandara, B.M.R., Wickramasinghe, A., Wijesundara, D.S.A., Karunaratne, D.N. and Karunaratne, V. (2011). Antioxidant, Cytotoxic and Phytotoxic Activities of *Schumacheria castaneifolia*, a Plant Endemic to Sri Lanka. *Proceedings of the Peradeniya University Research Sessions (PURSE)* 16: 156

Aim

Can we prepare plant extract-encapsulated liposomes with improved properties by varying the lipid composition?

- To investigate the effect of lipid composition (i.e. ratio of PC to CH) on the following properties of liposomes:
  - encapsulation efficiency
  - loading capacity
  - particle size
  - zeta-potential
  - release properties



Preparation of liposomes

Reverse-phase evaporation method

Dissolve lipids in an organic solvent

↓

Dissolve *Schumacheria castaneifolia* MeOH extract in water

↓

Add aqueous phase to organic phase & sonicate to form an emulsion

↓

Evaporate organic solvent to form liposomes in the aqueous component

↓

Add more water & continue drying

↓

Sonicate, keep refrigerated overnight

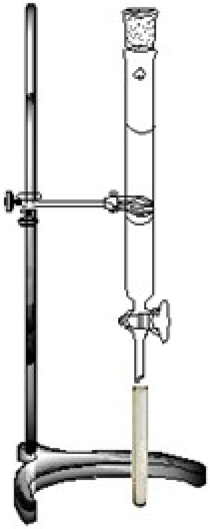
Removal of free extract from liposomes

- **Centrifugation**

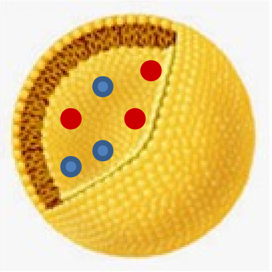
  Removal of supernatant

- **Sephadex G50**

  Collecting purified liposomes using an aqueous eluent



Liposomes prepared using phosphatidylcholine and cholesterol



	Liposomes			
	Plain		Extract encapsulated	
PC:CH	– sucrose	+ sucrose	– sucrose	+ sucrose
10:0	x	x	x	x
9:1	x	x	x	x
8:2	x	x	x	x
7:3	x	x	x	x
6:4	x	x	x	x

Encapsulation efficiency

Centrifuge

↓

Collect precipitated liposomes

↓

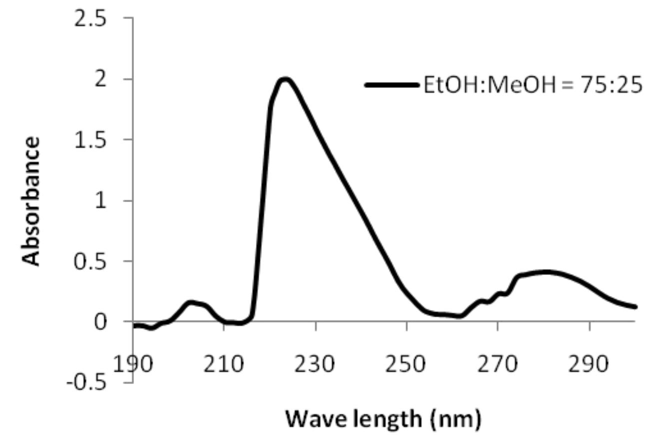
Freeze-dry liposomes

↓

Disrupt liposomes using Ethanol:Methanol 75:25 (v/v)

↓

Measure absorbance at 225 nm



$$EE\ (\%) = \frac{\text{amount of encapsulated extract}}{\text{total amount of extract introduced}} \times 100$$

Encapsulation efficiency (EE %)

Liposomal formulation PC:CH	Encapsulation efficiency (%)
10:0	66.81 ± 0.38
9:1	85.59 ± 0.26
8:2	86.41 ± 0.25
7:3	59.05 ± 0.32
6:4	44.55 ± 0.29

Most suitable lipid compositions; PC:CH = 9:1 and 8:2

Loading capacity

Centrifuge

↓

Collect precipitated liposomes

↓

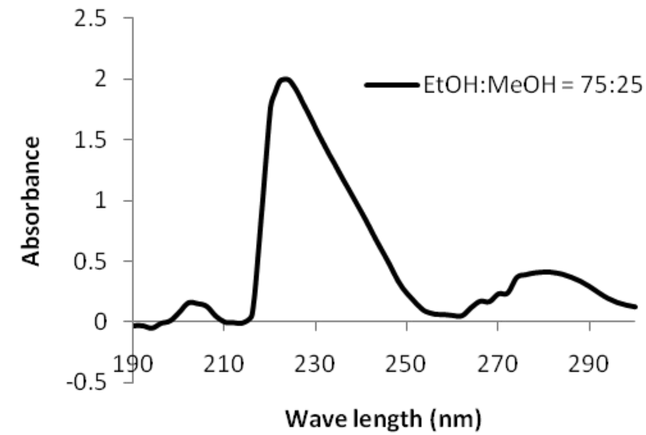
Freeze-dry liposomes

↓

Disrupt liposomes using Ethanol:Methanol 75:25 (v/v)

↓

Measure absorbance at 225 nm



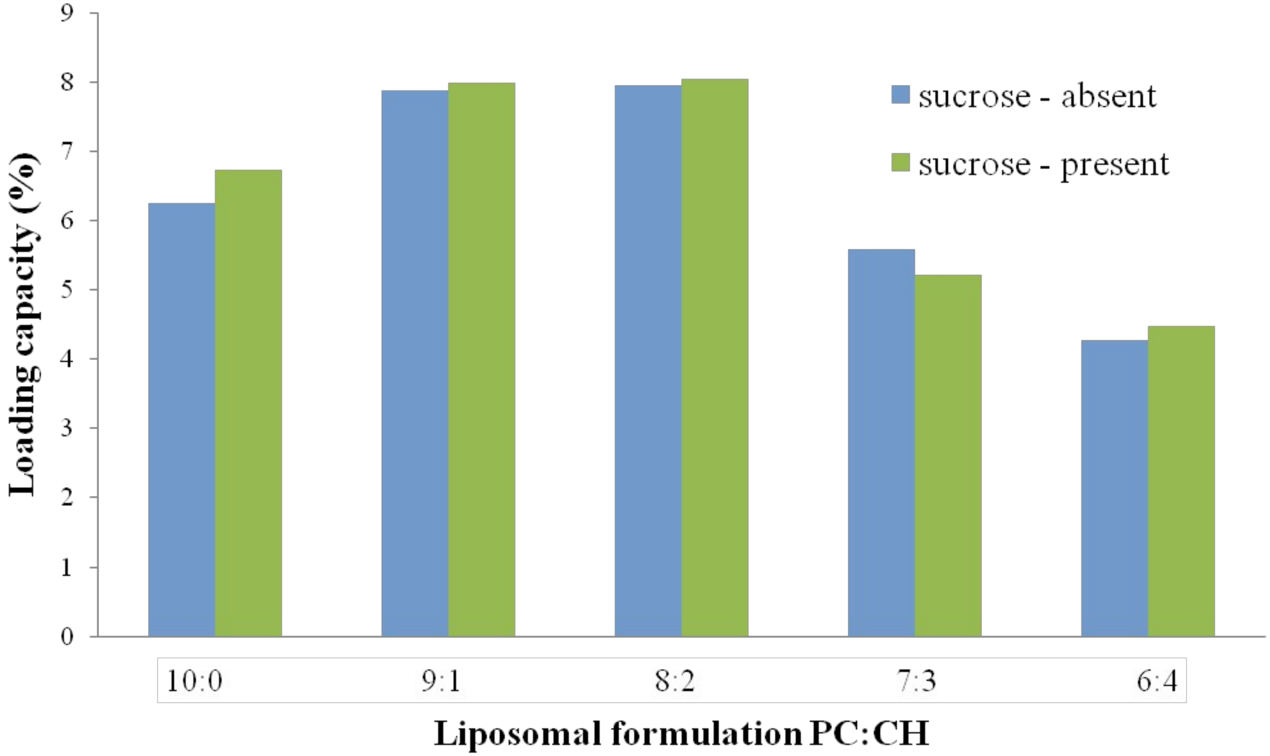
$$LC\,(\%) = \frac{\text{weight of encapsulated extract}}{\text{weight of liposomes}} \times 100$$

Loading capacity

Liposomal formulation PC:CH	Sucrose	LC (%)
10:0		6.26 ± 0.03
	✓	6.73 ± 0.07
9:1		7.88 ± 0.02
	✓	7.99 ± 0.02
8:2		7.95 ± 0.02
	✓	8.04 ± 0.02
7:3		5.58 ± 0.03
	✓	5.21 ± 0.03
6:4		4.27 ± 0.03
	✓	4.48 ± 0.04

Loading capacities are relatively high

Loading capacity



- **Loading capacities are similar in liposomes prepared in the presence or absence of sucrose.**

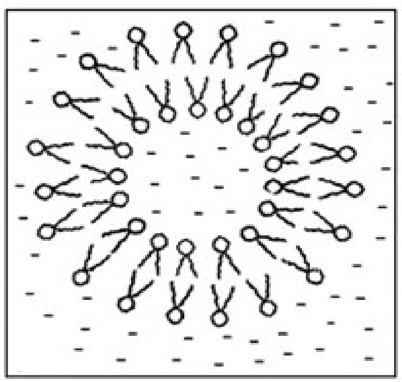
- **Optimum lipid composition; PC:CH – 9:1 and 8:2**

Lyoprotectants

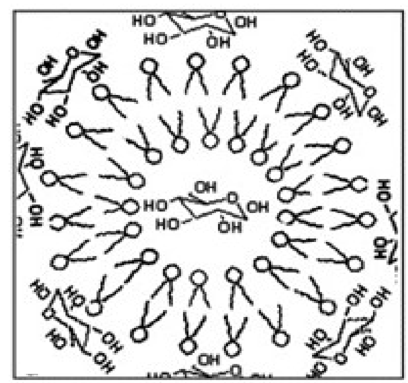
What? Compounds that protect freeze-dried material

Examples? Sugars, polyalcohols, their derivatives

Why in liposomes? Provide physical support for liposomes to maintain their structures upon freeze drying.



Liposome in aqueous medium

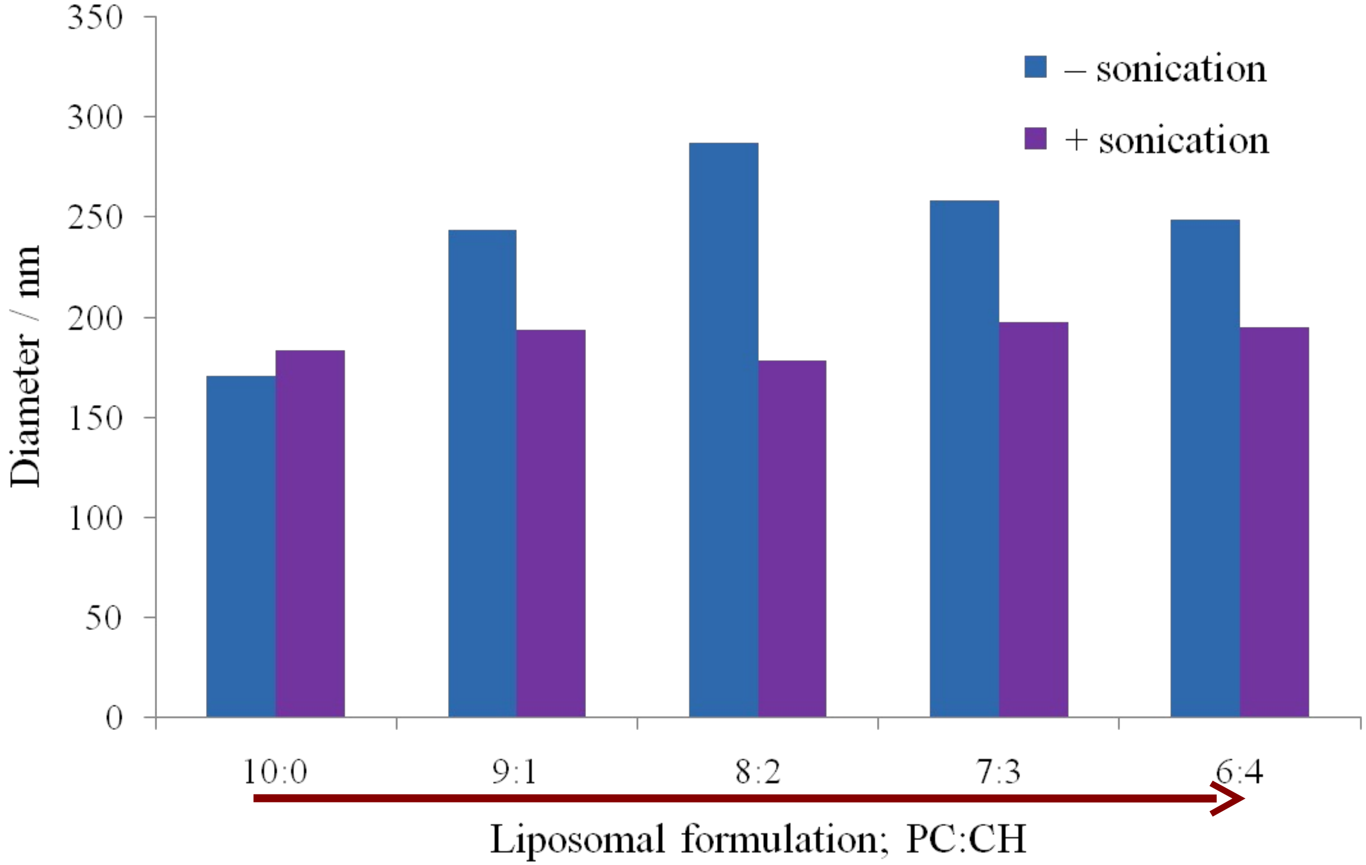


Liposome freeze dried with lyoprotectants

Advantage? Stability of liposomes

Wang, T., Deng, Y., Geng, Y., Gao, Z., Zou, J., Wang, Z. (2006). Preparation of submicron unilamellar liposomes by freeze-drying double emulsions. *Biochimica et Biophysica Acta* 1758: 222-231.

Particle sizes of plant extract-encapsulated liposomes



– sonication - Size increases with increasing cholesterol content

+ sonication - Sizes are comparable

Zeta – Potential of Plain Liposomes

Liposomal formulation PC:CH	Zeta-potential / mV
10:0	-35.0 ± 4.92
9:1	-45.0 ± 4.70
8:2	-44.5 ± 3.21
7:3	-51.9 ± 4.60
6:4	-50.5 ± 4.28

Zeta-potential becomes more negative with increasing cholesterol content

Zeta – Potential of Plain Liposomes (+ sucrose)

Liposomal formulation PC:CH	Zeta-potential / mV
10:0	-43.0 ± 5.3
9:1	-41.7 ± 4.0
8:2	-51.2 ± 4.7
7:3	-55.9 ± 5.4
6:4	-57.6 ± 14.2

Zeta-potential becomes more negative with increasing cholesterol content

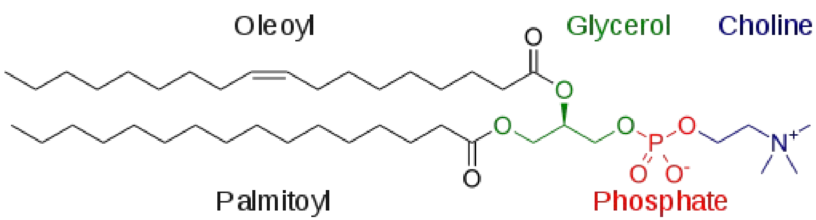
Zeta – Potential of Plant Extract-Encapsulated Liposomes (+ sucrose)

Liposomal formulation PC:CH	Zeta-potential / mV
10:0	-41.9 ± 7.0
9:1	-51.1 ± 4.3
8:2	-57.7 ± 4.9
7:3	-66.7 ± 4.9
6:4	-67.8 ± 4.3

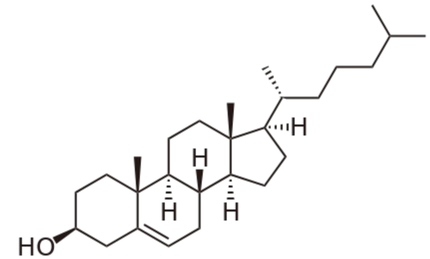
Zeta-potential becomes more negative with increasing cholesterol content

Negative zeta-potential?

- Lipids are neutral



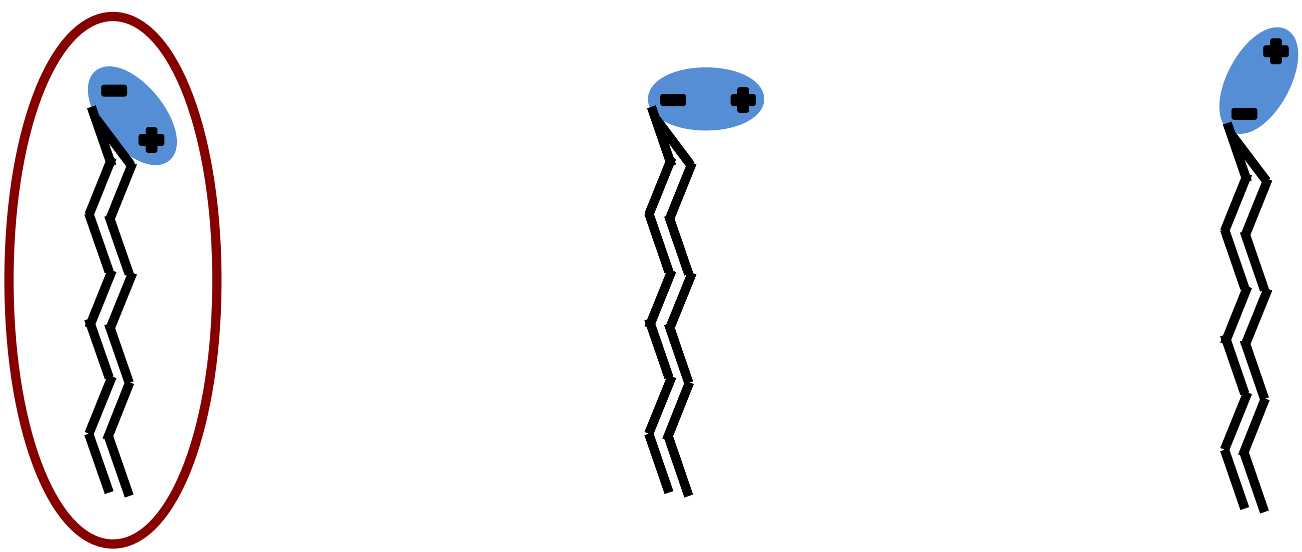
Phosphatidylcholine



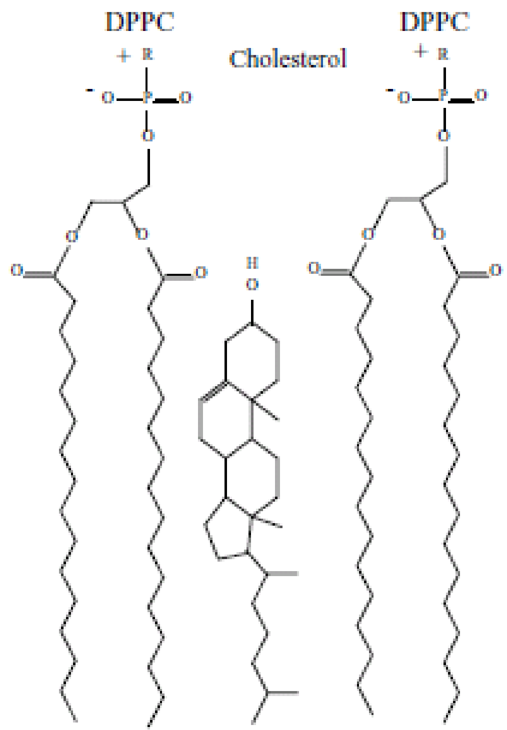
Cholesterol

- Why negative zeta-potentials?

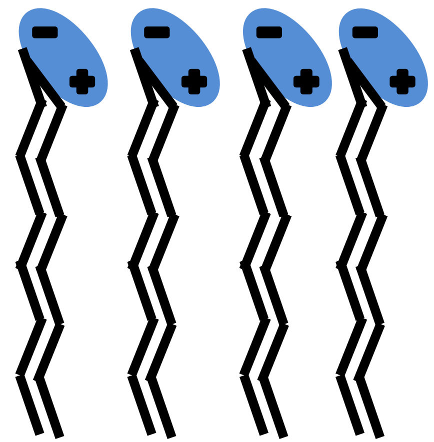
ORIENTATION of the HEAD GROUP



Increased negative Zeta-potential?



Orientation of cholesterol in phospholipid membrane



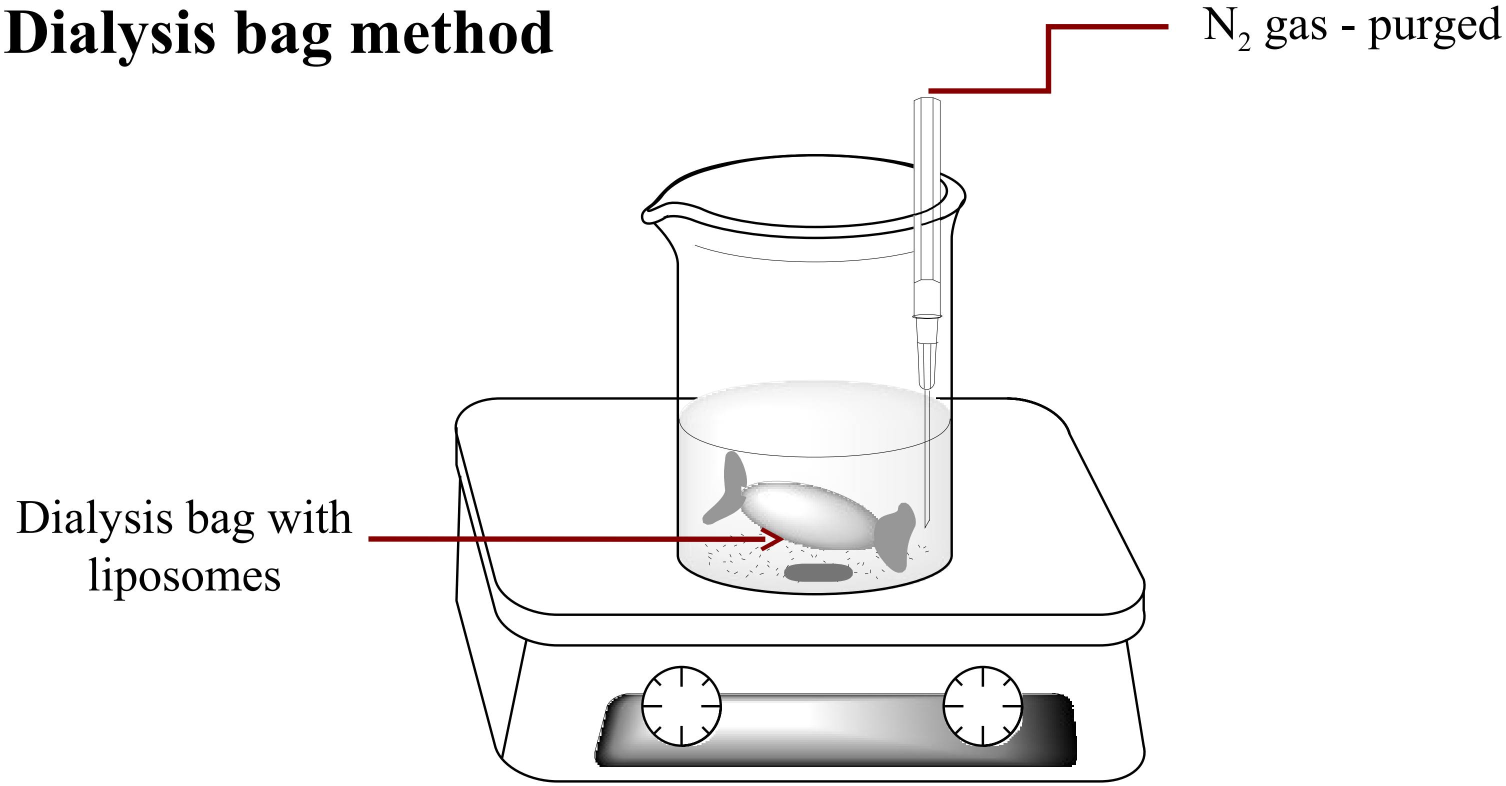
Orientation of PC zwitterionic head groups on membrane surfaces

- Cholesterol may stabilize the orientation of the head group via Hydrogen-bonding

El Maghraby, G.M.M., Williams, A.C. and Barry, B.W. (2004). Interactions of surfactants (edge activators) and skin penetration enhancers with liposomes. *International Journal of Pharmaceutics* 276: 143-161

In vitro release studies

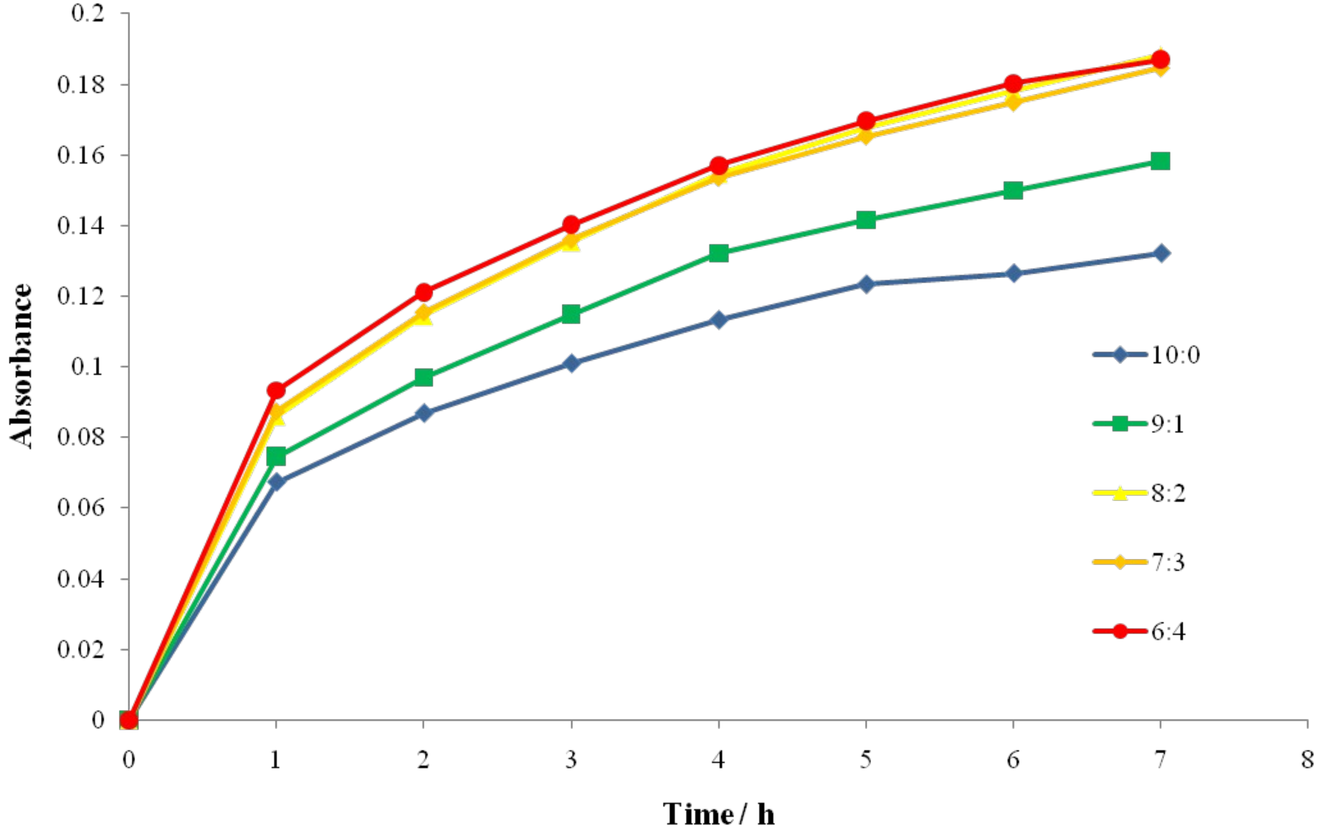
Dialysis bag method



Amount released – quantified using spectrometric method

Slow-release of encapsulated material

Medium – distilled water



Release of encapsulated material increases upon increasing the cholesterol content

Conclusions

•The methanol extract of stem-bark of *S.castaneifolia* can be encapsulated in liposomes with high encapsulation efficiencies and loading capacities.

- Liposomes may be used as delivery vehicles of plant extract

•The presence of lyoprotectants does not affect the loading capacity.

- Storage of liposomes for longer periods of time may be possible.

•The lipid composition has a significant effect on the properties of liposomes

- encapsulation efficiency and loading capacity
- size
- zeta-potential
- leakage of encapsulated material (according to liposomal formulations used in this study)

•Lipid composition may be varied to modulate properties of liposomes encapsulating the methanol extract of stem-bark of *S.castaneifolia*.

Acknowledgements

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Thank You