Investigating the Effects of Commercial Antimicrobial Agents on Human Corneal Epithelial Cell Membranes

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Abstract

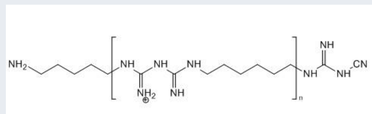
Constituents in multipurpose contact lens solution (MPS) have been suggested to cause corneal injury. To explore this issue we created an *in-vitro* liposome-based model of the corneal epithelial surface and we assessed the interactions of polyhexamethylene biguanide (PHMB) and polyquaternium-1 (PQ-1) on the biomembrane by using fluorescence spectroscopy, dynamic light scattering (DLS), and liquid chromatography/mass spectrometry (LC-MS). Fluorescence assessed the membrane surface polarity and stability through the temperature-dependent generalized polarization (GP) the gel-to-liquid transition temperature (T_m) and the associated van't Hoff enthalpy (ΔH_{VH}). DLS evaluated liposome-liposome aggregation. LC-MS determined the composition of any precipitates that formed. PHMB increased T_m, phospholipid cooperativity, and GP. In contrast, PQ-1 did not change T_m or phospholipid cooperativity, but it decreased GP. PQ-1 alone lead to liposome-liposome aggregation. The aggregates exhibited a liposome composition equivalent to the liposome prior to the addition of PQ-1.

Background

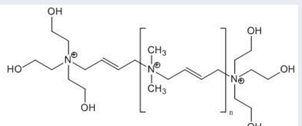
In the eye care industry, MPSs are used extensively for soft contact lens disinfection, cleaning and storage. Commercial MPSs are composed of a buffer system, at least one antimicrobial agent (AA), and other additives such as surfactants or humectants that are used to provide lens comfort and improve performance. PHMB and PQ-1 are among the most widely used AAs.[1]

After a contact lens is soaked, removed from a MPS and applied to the ocular surface, AAs begin to desorb from the contact lens at a rate that depends on the agent and the contact lens material.[2] Within the human pre-corneal tear film, PHMB and PQ-1 exist as polycations.[3] Thus, strong evidence for ion-ion interactions between PHMB and PQ-1 and oppositely charged functionalities present at cell surfaces is well established.[4]

Antimicrobial agent chemical structures:

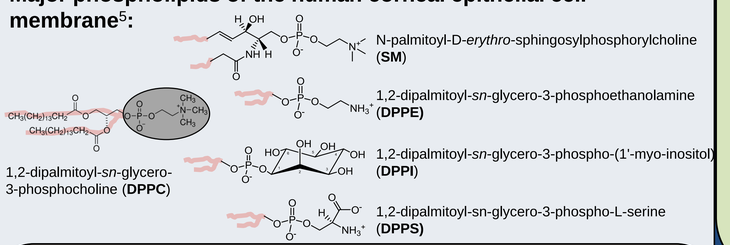


Polyhexamethylene biguanide (PHMB)



Polyquaternium-1 (PQ-1)

Major phospholipids of the human corneal epithelial cell membrane[5]:



N-palmitoyl-D-*erythro*-sphingosylphosphorylcholine (**SM**)

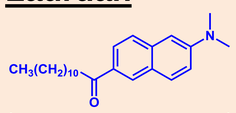
1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (**DPPE**)

1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-myo-inositol) (**DPPI**)

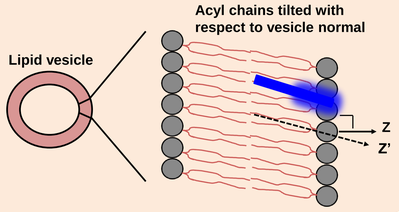
1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (**DPPC**)

1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine (**DPPS**)

Laurdan



24 Å



Theory

The fluorescent reporter, Laurdan, is sensitive to its local microenvironment. Laurdan displays a large emission red shift in polar solvents in comparison to non-polar solvents. To quantify the Laurdan spectral shifts we computed the generalized polarization (GP)[6]:

$$GP = (I_{440} - I_{490}) / (I_{440} + I_{490}) \qquad (1)$$

It is well-known that phospholipid bilayers undergo reversible temperature-dependent gel-to-liquid phase transitions. This transition is described by a characteristic T_m (defined by the mid-point in the GP vs. *T* profile[6]) and by an associated van't Hoff enthalpy (ΔH_{VH}) that reflects subunit (phospholipid) cooperativity during the gel-to-liquid phase transition.

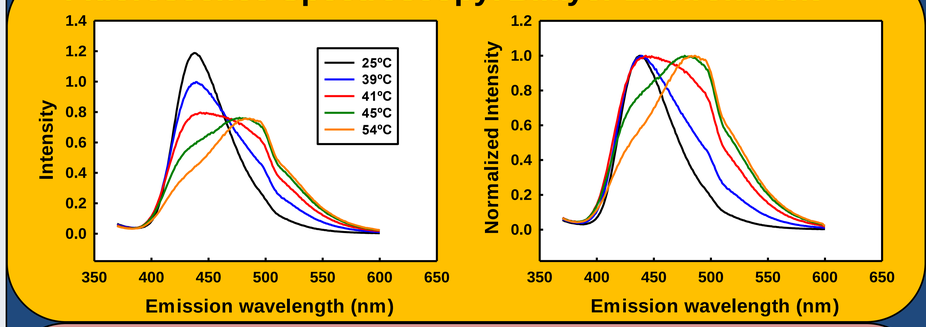
$$\Delta H_{VH} = 4RT_m^2(\delta\alpha/\delta T)_{Tm} \qquad (2)$$

where

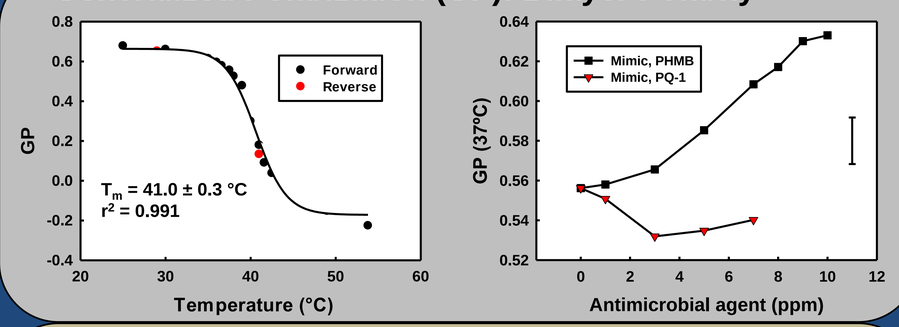
$$\alpha = [(GP)_{max}-(GP)_T] / (\Delta GP)_{\Delta T} \qquad (3)$$

Results

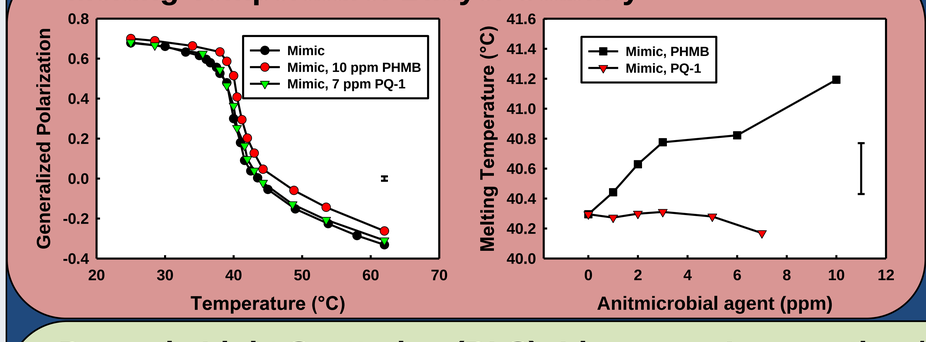
Fluorescence Spectroscopy: Bilayer Environment



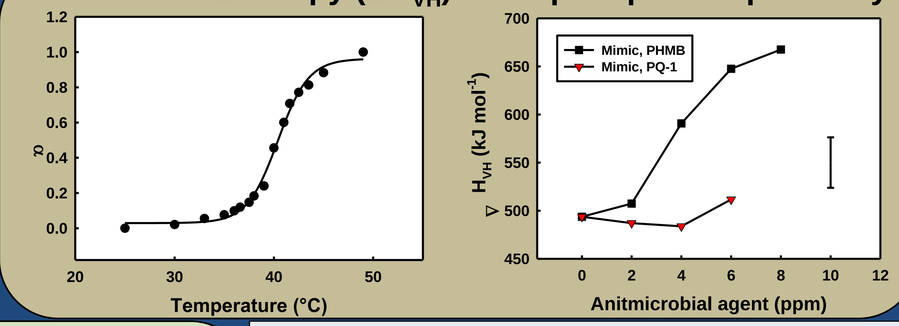
Generalized Polarization (GP): Bilayer Polarity



Melting Temperature: Bilayer Stability

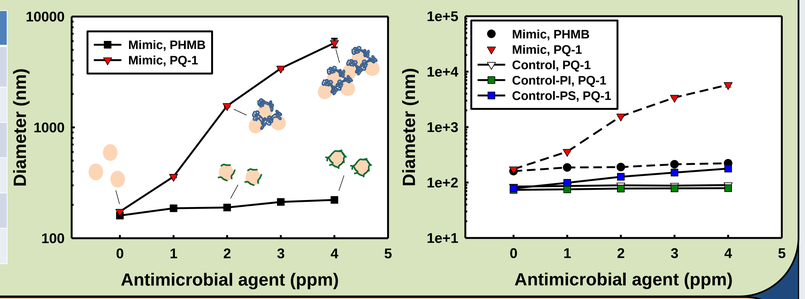


van't Hoff Enthalpy (ΔH_{VH}): Phospholipid Cooperativity

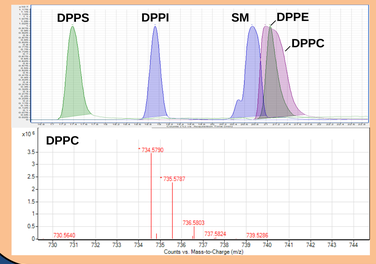


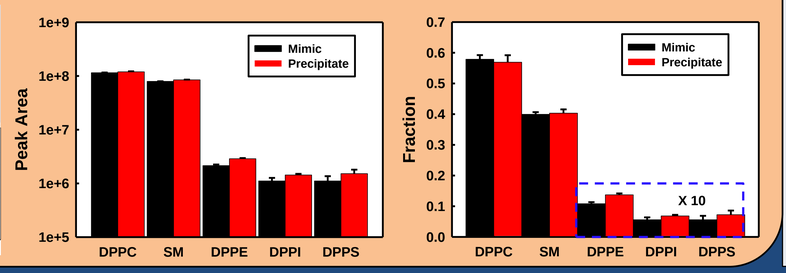
Dynamic Light Scattering (DLS): Liposome Aggregation / Size

Liposome Composition (%)				
	Mimic	Control	Control-PI	Control-PS
DPPC (±)	45	50	48	48
SM (±)	26	32	28	28
DPPE (±)	13	18	16	16
DPPI (-)	8	-	8	-
DPPS (-)	8	-	-	8



LC-MS: Liposome Composition





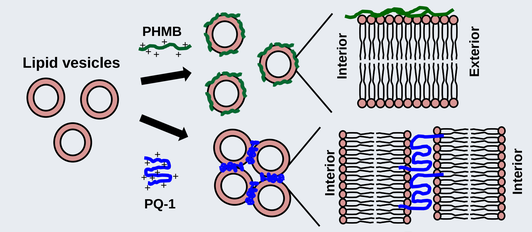
Conclusions

PHMB association resulted in:
- increase in bilayer T_m
- increase in phospholipid cooperativity (ΔH_{VH})
- decrease in surface polarity (GP)
- increase in particle size on a nanometer scale

This behavior is consistent with PHMB adsorbing ***onto*** the bilayer exterior (monolayer, multilayer) such that individual PHMB molecules interact with several phospholipids at the same time.

PQ-1 association resulted in:
- no change in bilayer T_m
- no change in phospholipid cooperativity (ΔH_{VH})
- increase in surface polarity (GP)
- increase in particle size on a micron scale

This behavior is consistent with PQ-1 intercalating ***into*** bilayers and facilitating liposome-liposome aggregation. The aggregates exhibited a liposome composition equivalent to the liposome prior to PQ-1 addition.



Acknowledgements

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