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Partition coefficients – what do we mean by lipid solubility and what it means for digestion

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Outline

- Effects of partitioning on emulsification properties of surfactants

- Bile salts – comparison with conventional surfactants

- Implications of above for digestion

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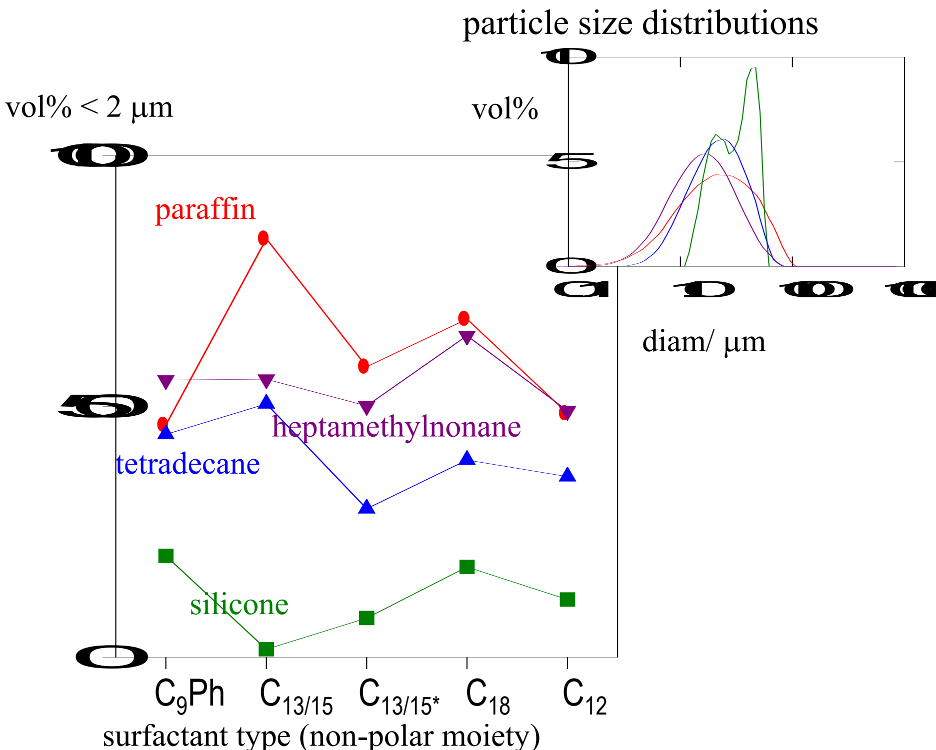
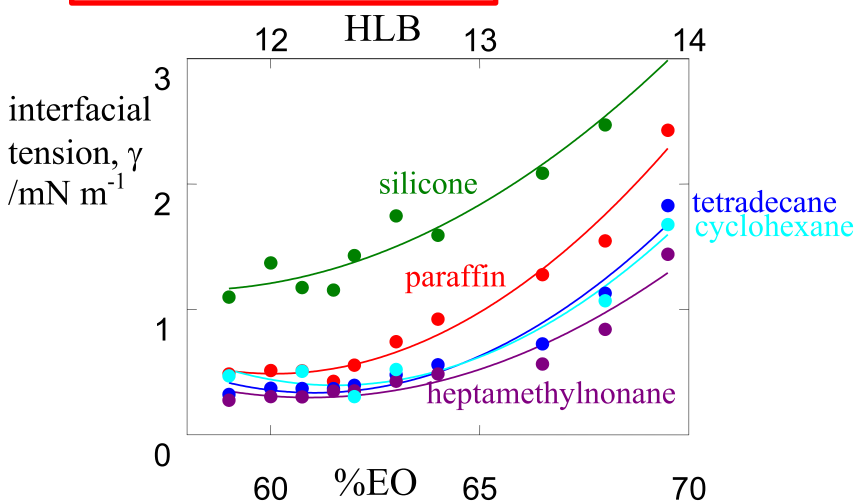


[all EO = 60% (HLB =12)]



The relative solubility of surfactants for polar or non-polar phases is traditionally given by the HLB number (Hydrophile-Lipophile Balance)

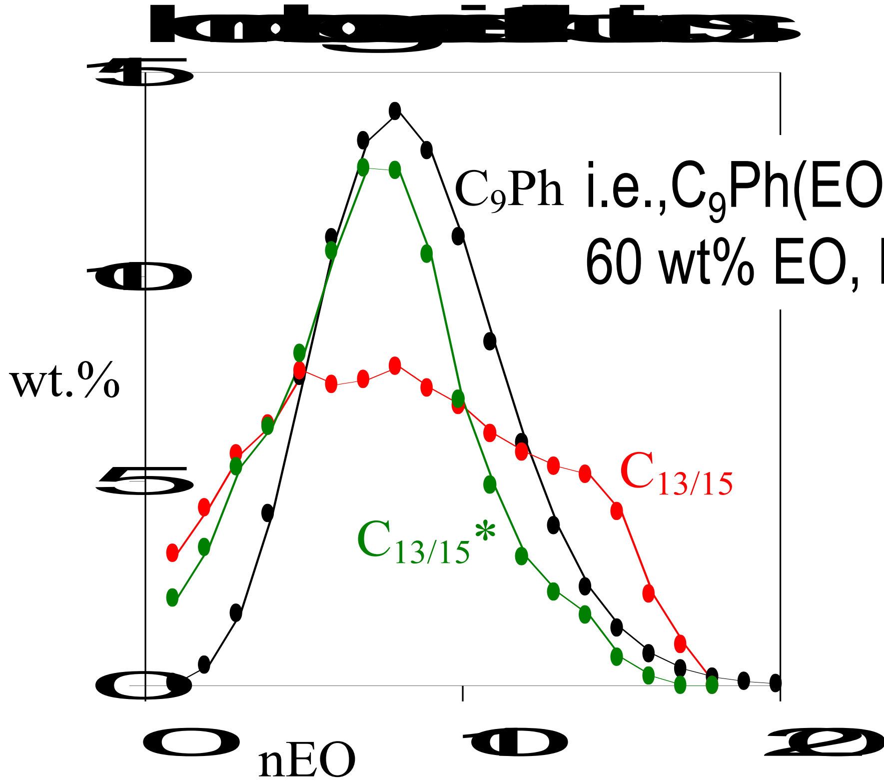
Originally defined for surfactants based on ethoxylated long chain alcohols, etc.

HLB = (wt. % ethylene oxide)/5

e.g.,$C_9Ph(EO)_7$, 60 wt% EO, HLB ≈ 12 "water-soluble"

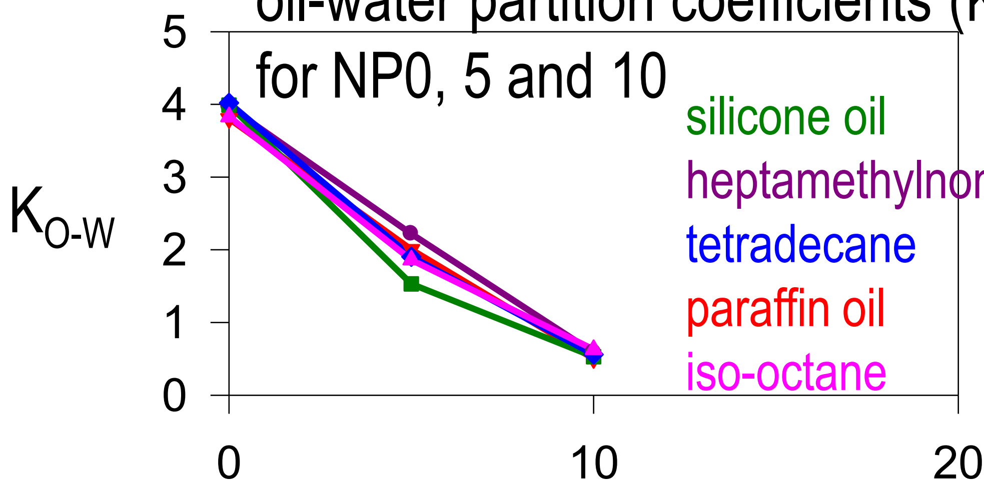
$C_9Ph(EO)_1$, 18 wt% EO, HLB ≈ 3.5 "oil-soluble"

'Dividing line' between oil- and water soluble ≈ HLB 7



i.e.,$C_9Ph(EO)_7$ (NP7)
60 wt% EO, HLB ≈ 12

oil-water partition coefficients (K_{O-W}) for NP0, 5 and 10



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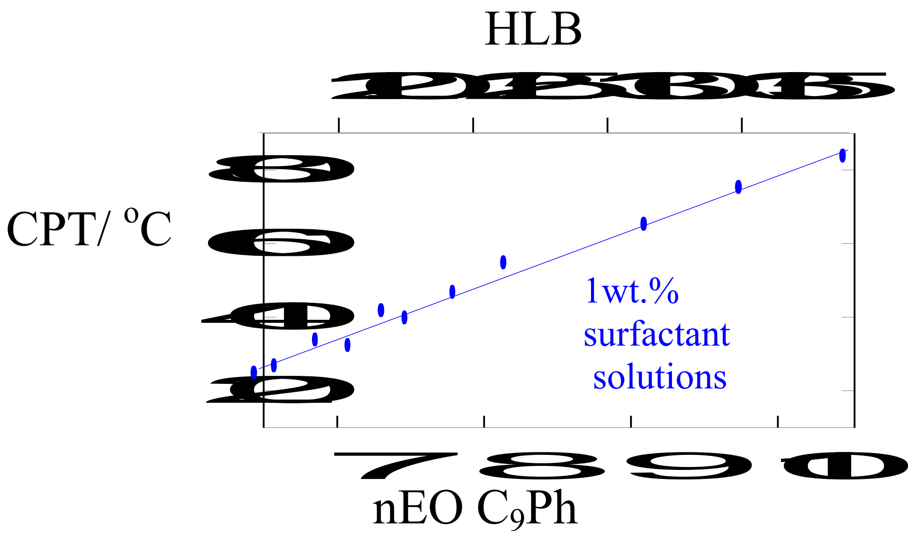
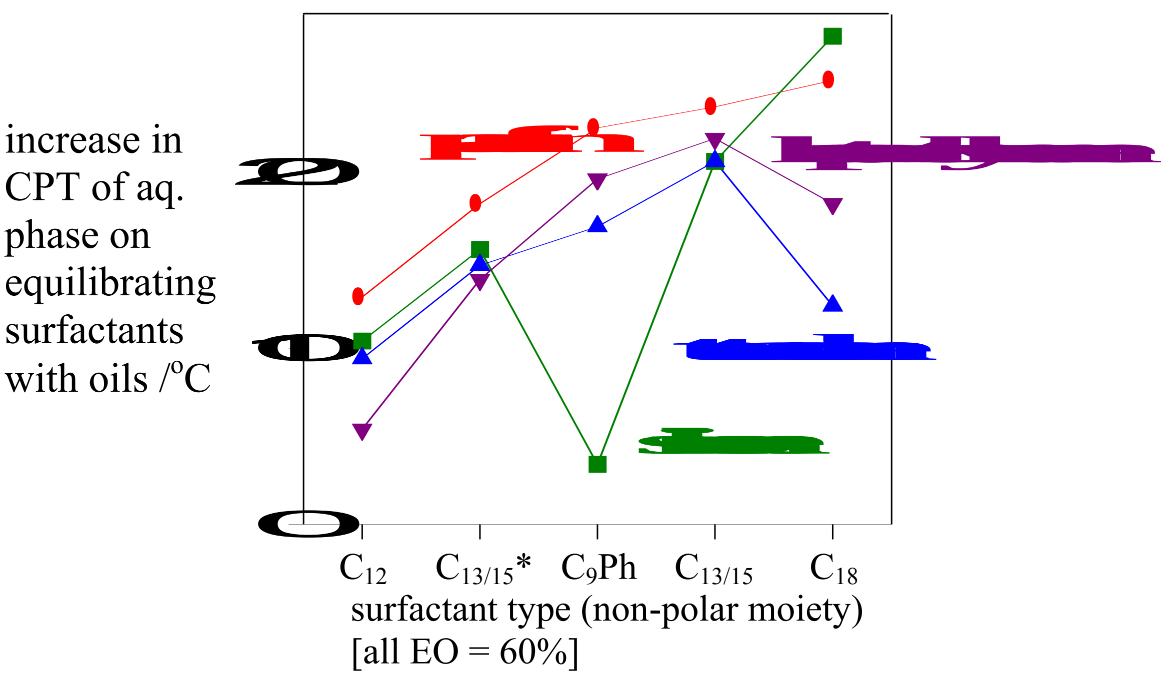
Measurement of partitioning from aq. phase – via cloud point temperature (CPT)





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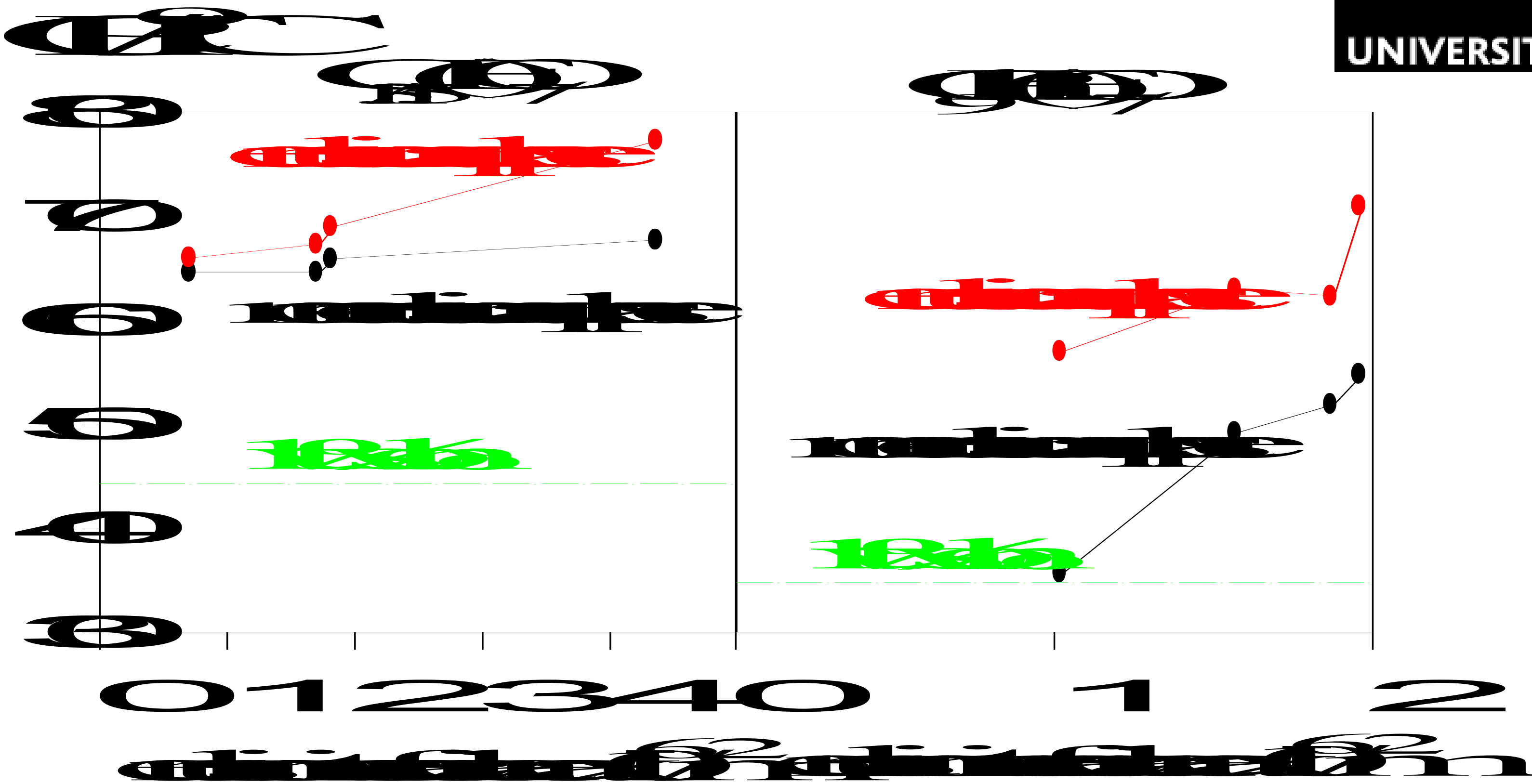

Increase in mean nEO of aqueous phase estimated from CPT

OIL	C_9Ph **not** emulsified	C_9Ph emulsified	$C_{13/15}$ **not** emulsified	$C_{13/15}$ emulsified
paraffin	1.5	3.6	1.0	2.5
heptamethyl nonane	1.4	3.1	1.1	1.4
tetradecane	1.1	3.6	0.8	1.3
silicone	0.2	1.9	0.8	0.8

Apparent oil-water partition coefficients $K_{O\text{-}W}$ of individual components of C_9PhEO_7 in emulsions (stabilized by 1 wt.% C_9PhEO_7)

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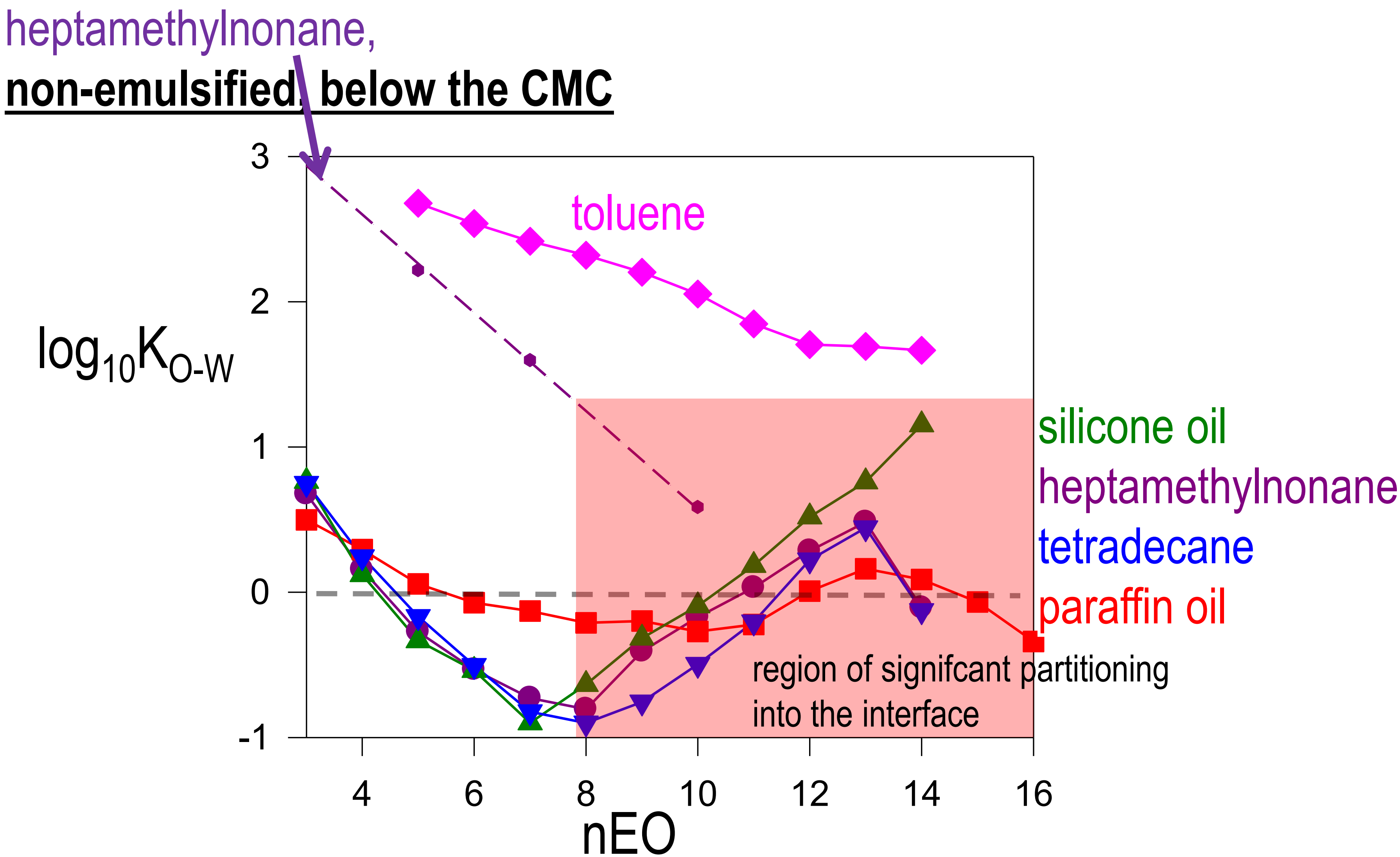
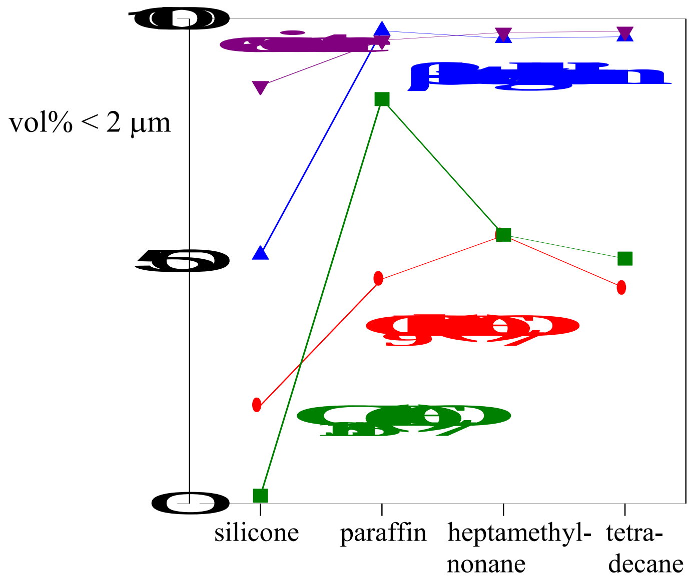
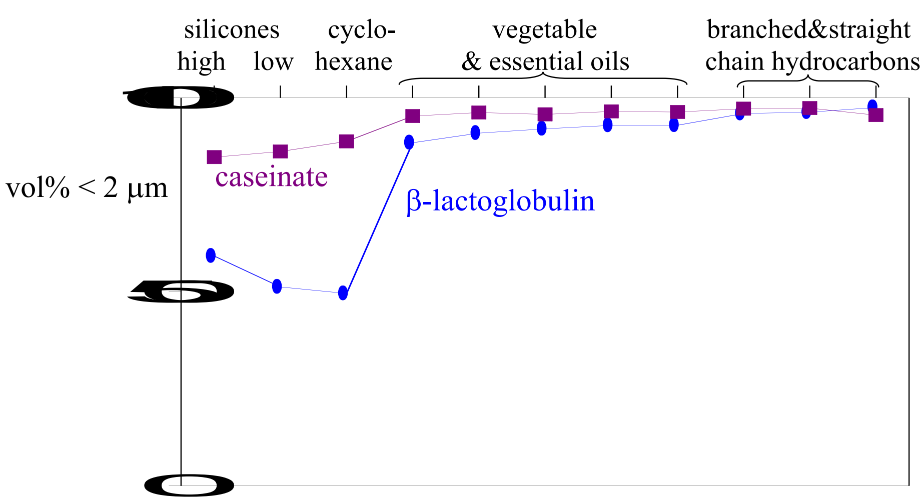


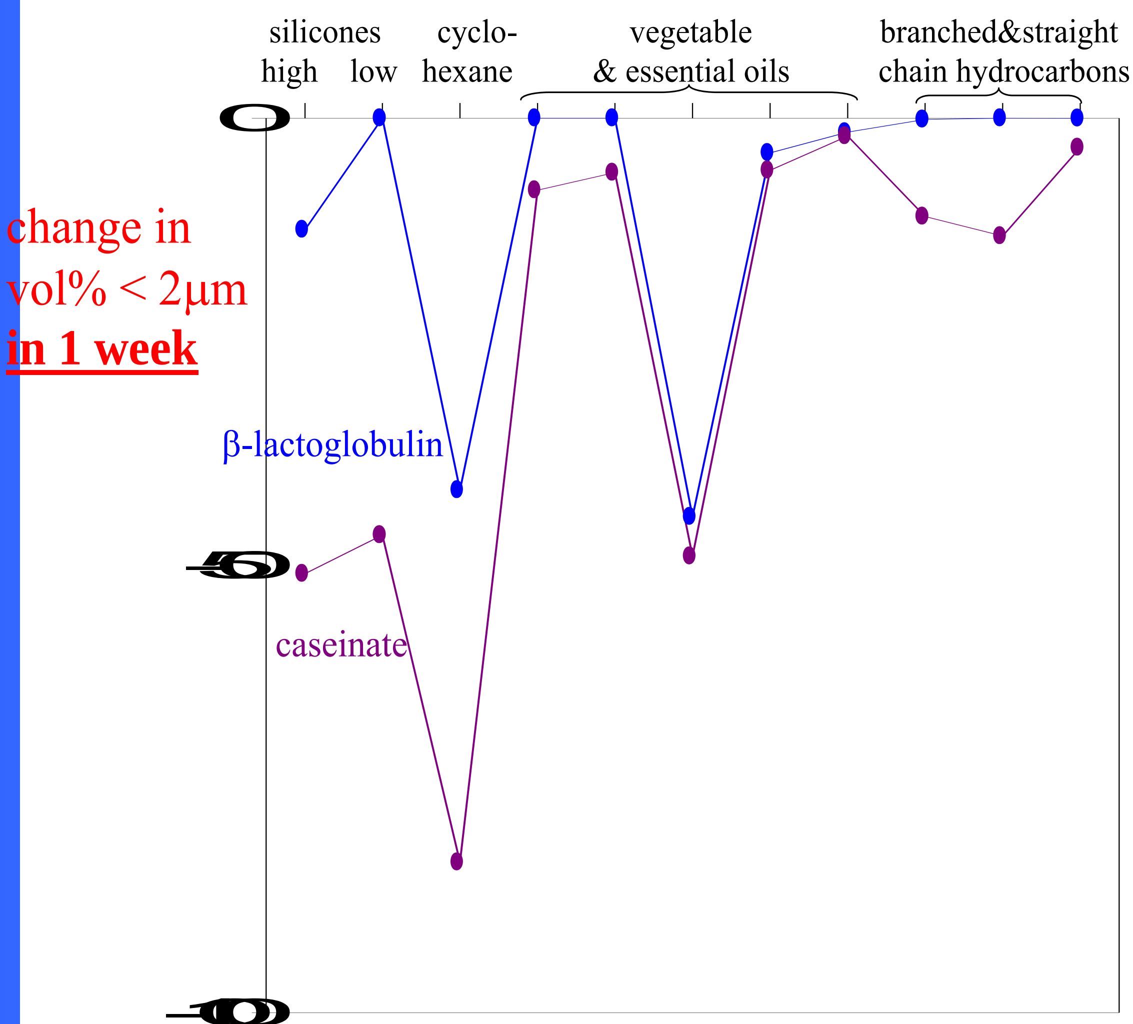
Comparison with protein as emulsifier



Oil	γ / mN m^{-1}	η x10^3 / N s m^{-2}	ρ / g cm^{-3}
tetradecane	53	2	0.76
heptamethylnonane	50	2	0.79
paraffin	49	25	0.85
silicone (high η)	33	350	0.92
(low η)	33	1	0.91
limonene	3	2	0.84
cyclohexane	50	2	0.78
impure veg oils	10	70 - 160	0.9
purified veg oils	32		0.9



"Long-term" stability of protein-stabilized emulsions



cf. low molecular weight $(EO)_n$ surfactants – protein stabilized emulsions of hydrocarbon and silicone oil emulsions show much greater stability over several months storage

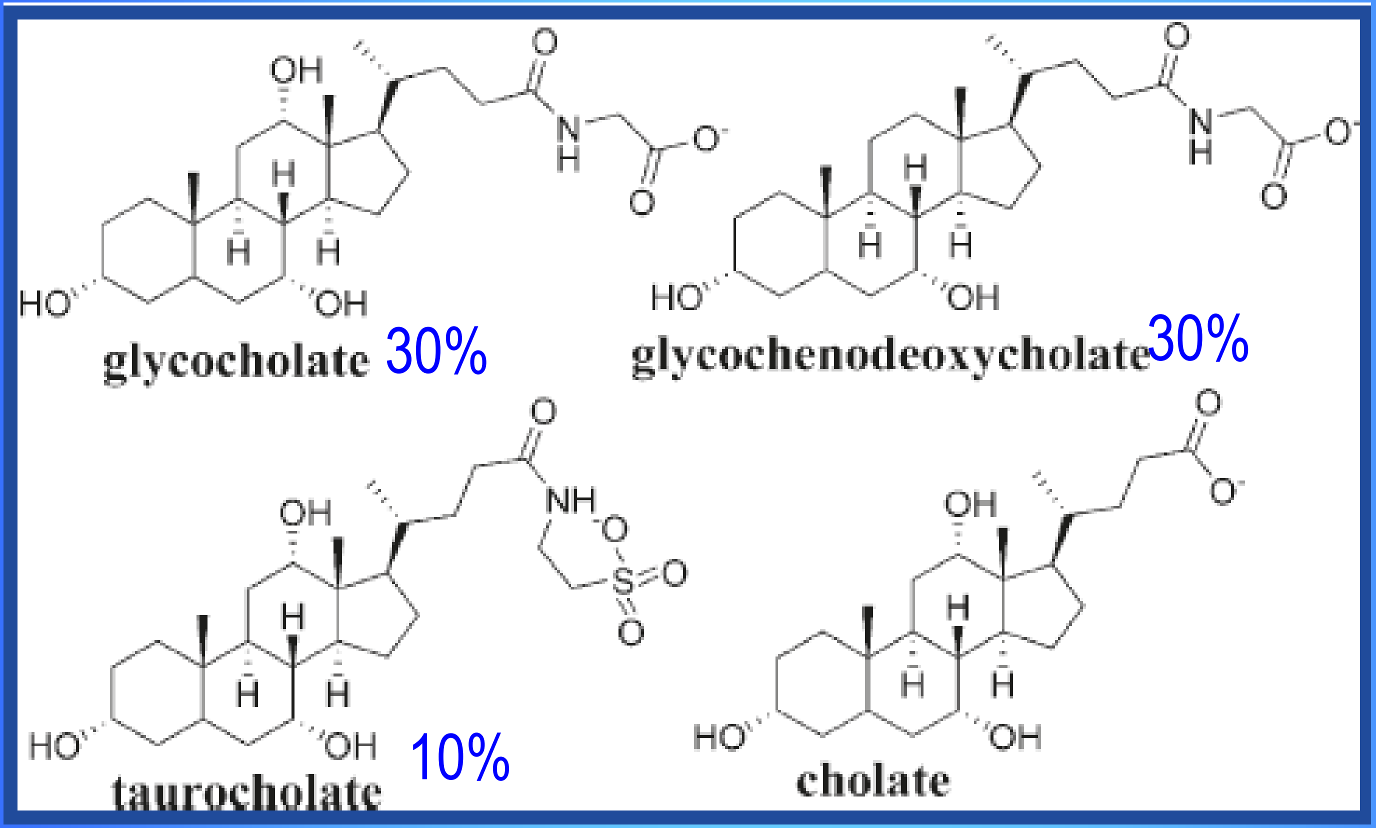
- Differences observed under quiescent conditions are often small, or slow to appear.

- Most surfactants give reasonable emulsion stability if adequate surface coverage achieved fast enough.

- But long-term instability can occur, reflecting partitioning, micellar solubilization & Ostwald ripening with LMWS; unfolding & cross-linking with proteins; chemical degradation (e.g., oxidation) with both.

Bile salts as surfactants in digestion

Chemical structures of the four common (primary) bile salts in human bile:



The gall bladder normally secretes into the duodenum at least 600 ml of bile per day

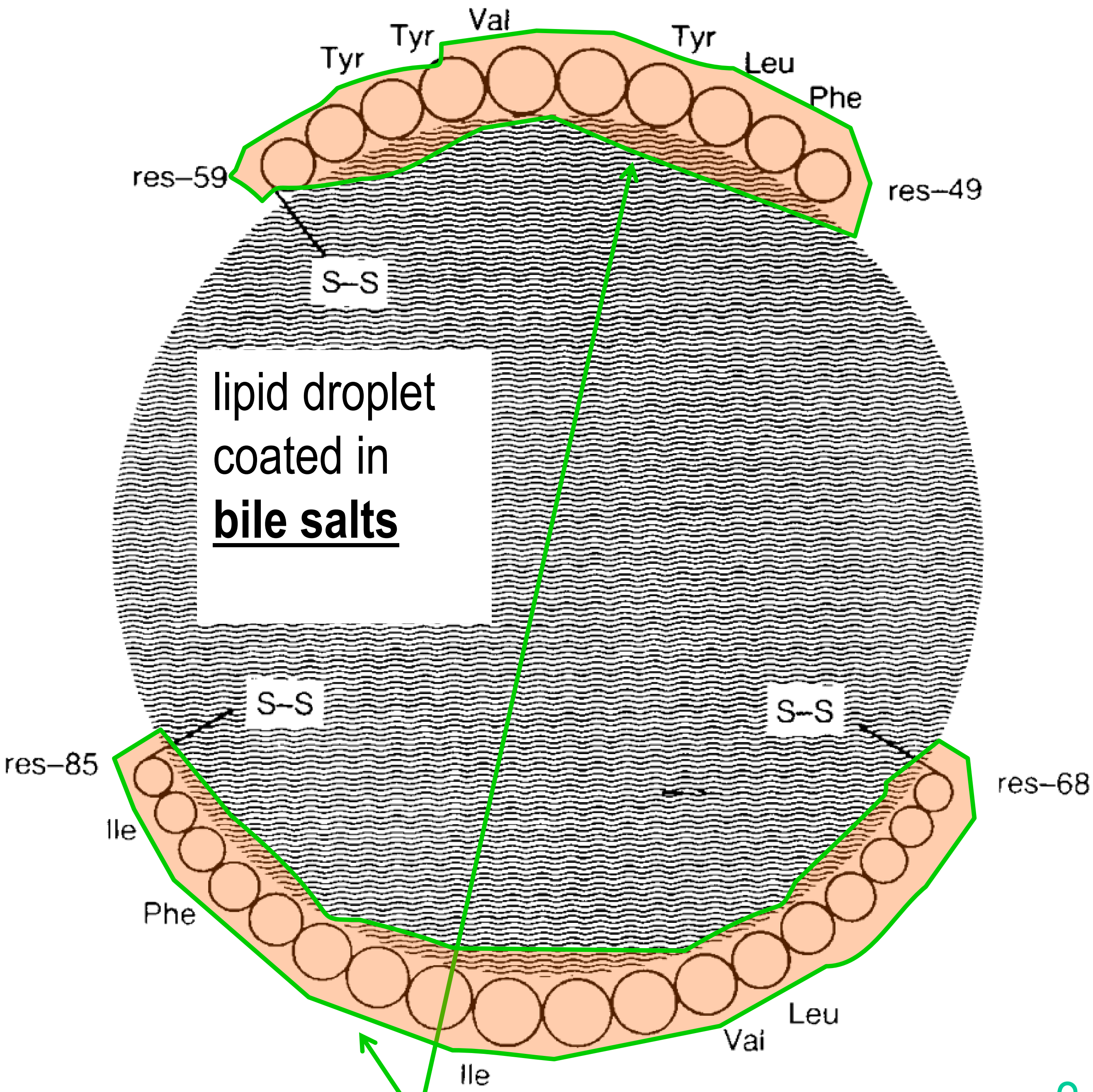
Bile salts and lipid digestion - the 'traditional' view

Bile salts 're-emulsify' the fat in the chyme, increasing the surface area of the fat and so increasing the efficiency of lipolysis, since lipases are soluble in water and therefore can only act at the surface of the fat particles/oil droplets.

Bile salts condition the surface of the fat so as to attract the polypeptide colipase to the surface, which in turn makes it easier for the lipase to adsorb.

Bile salt micelles solubilize into their structure the water-insoluble and soluble surface active products of lipase action

Bile salt micelles carry the products to the brush border where they are transported through or diffuse through membranes in the brush border epithelial cells, and thence into the blood stream.



The hydrophobic loops of colipase for binding of bile salts

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bile salt adsorption & desorption of other surface species aids co-lipase adsorption, thence lipase adsorption & action

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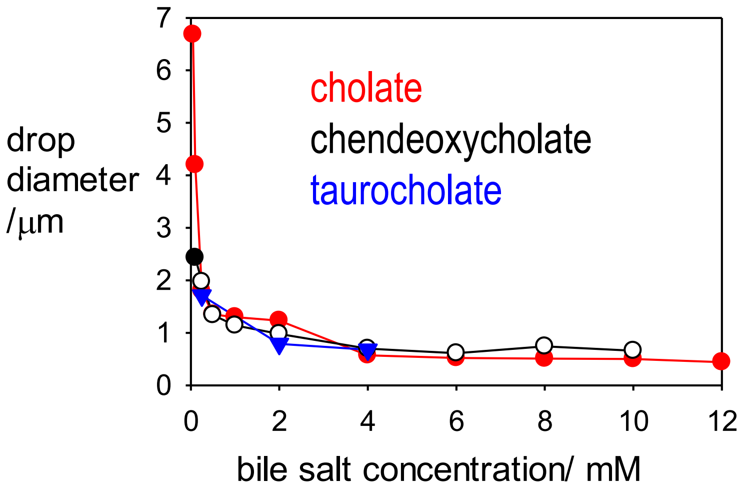
Problems with the traditional view of bile salts and lipid digestion

Bile salts are undoubtedly good emulsifiers:-

homogenization of 20 vol% hexadecane at pH 7



But...

- Although bile salts normally secreted into the duodenum at approx. 100 mM, the degree of dilution may vary widely
- The solubility of the glycocolates bile salts varies significantly across the range (pH 2 to 8) encountered during digestion
- Little evidence that there is enough shear for re-emulsification to takes place

Further problems with the traditional view of bile salts and lipid digestion

A) Bile salts do not have a typical surfactant structure, so cannot necessarily adsorb in the traditional head-tail fashion or form micelles with a simple hydrophobic core that easily accommodate/solubilize large quantities of lipid, or lipids beyond a certain size and shape.

B) 100 mM, is >> CMC values reported, but there is frequent disagreement on these values and although CMCs for glycholates will vary significantly with pH CMCs are rarely reported as a function of pH

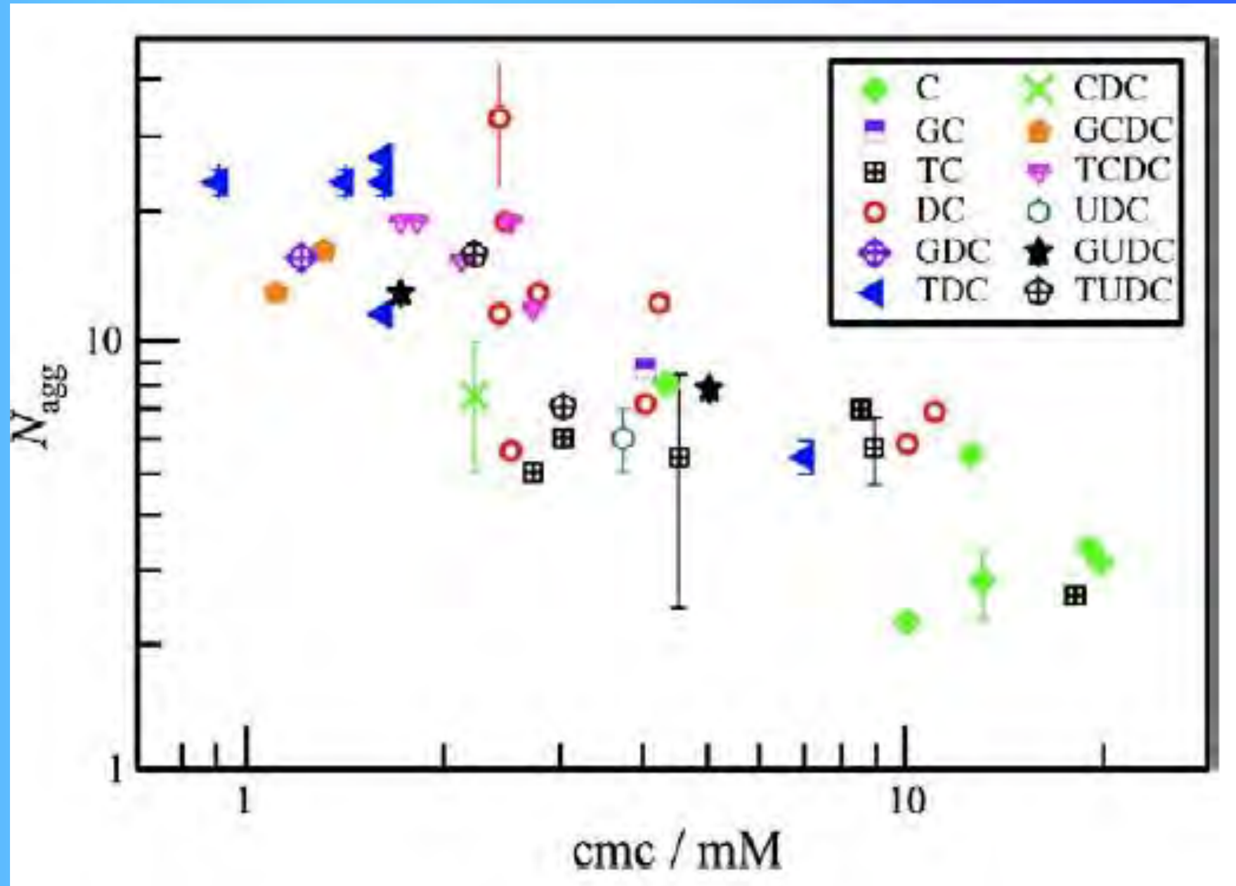
C) The efficiency of bile salt adsorption to pre-formed emulsions depends on what is already on the surface: proteins, protein-polysaccharide complexes and other surface active species present

D) Other species may complex with bile salts and restrict their availability.

E) As lipase degrades the surface layer of lipid - its composition changes and a range of LMWS may be produced that may displace lipase and bile salts

A) Bile salts do not have a typical surfactant structure.....

Bile salt	Mol %
Glycocholate	30.9
Taurocholate	12.0
Glycochenodeoxycholate	29.3
Taurochenodeoxycholate	11.2
Glycodeoxycholate	9.3
Taurodeoxycholate	2.2
Glycolithocholate	0.4
Taurolithocholate	0.3
Glycoursodeoxycholate	0.4
Tauroursodeoxycholate	1.6
Sulfoglycolithocholate	1.9
Sulfotaurolithocholate	0.5

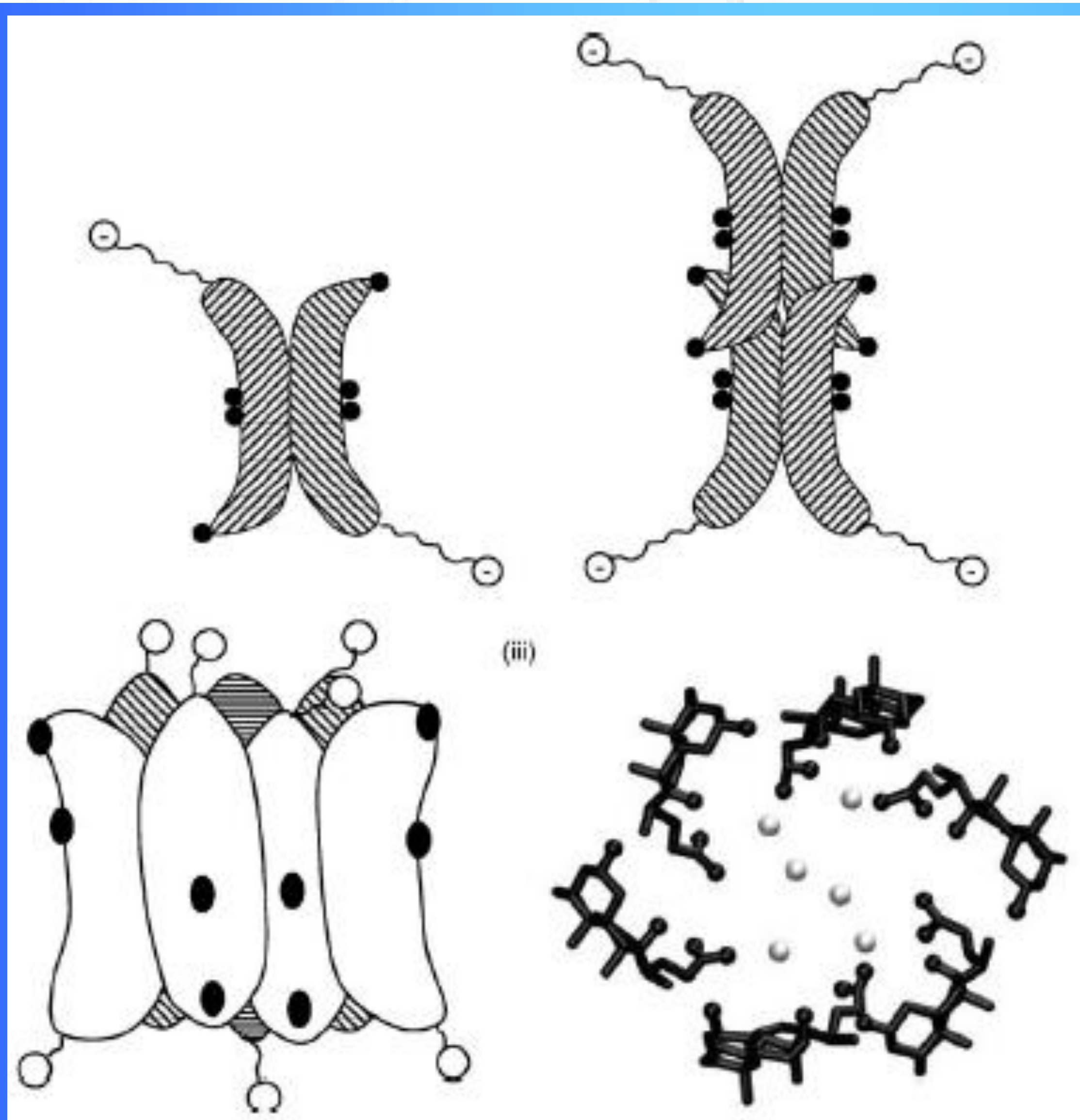


D. Madenci *, S.U. Egelhaaf [1]

Current Opinion in Colloid & Interface Science 15 (2010) 109–115

Dallas B. Warren [a], David K. Chalmers [b], Keith Hutchison [c], Wenbin Dang [c], Colin W. Pouton [a,*]

Colloids and Surfaces A: Physicochem. Eng. Aspects 280 (2006) 182–193



Dallas B. Warren [a], David K. Chalmers [b], Keith Hutchison [c], Wenbin Dang [c], Colin W. Pouton [a,*]

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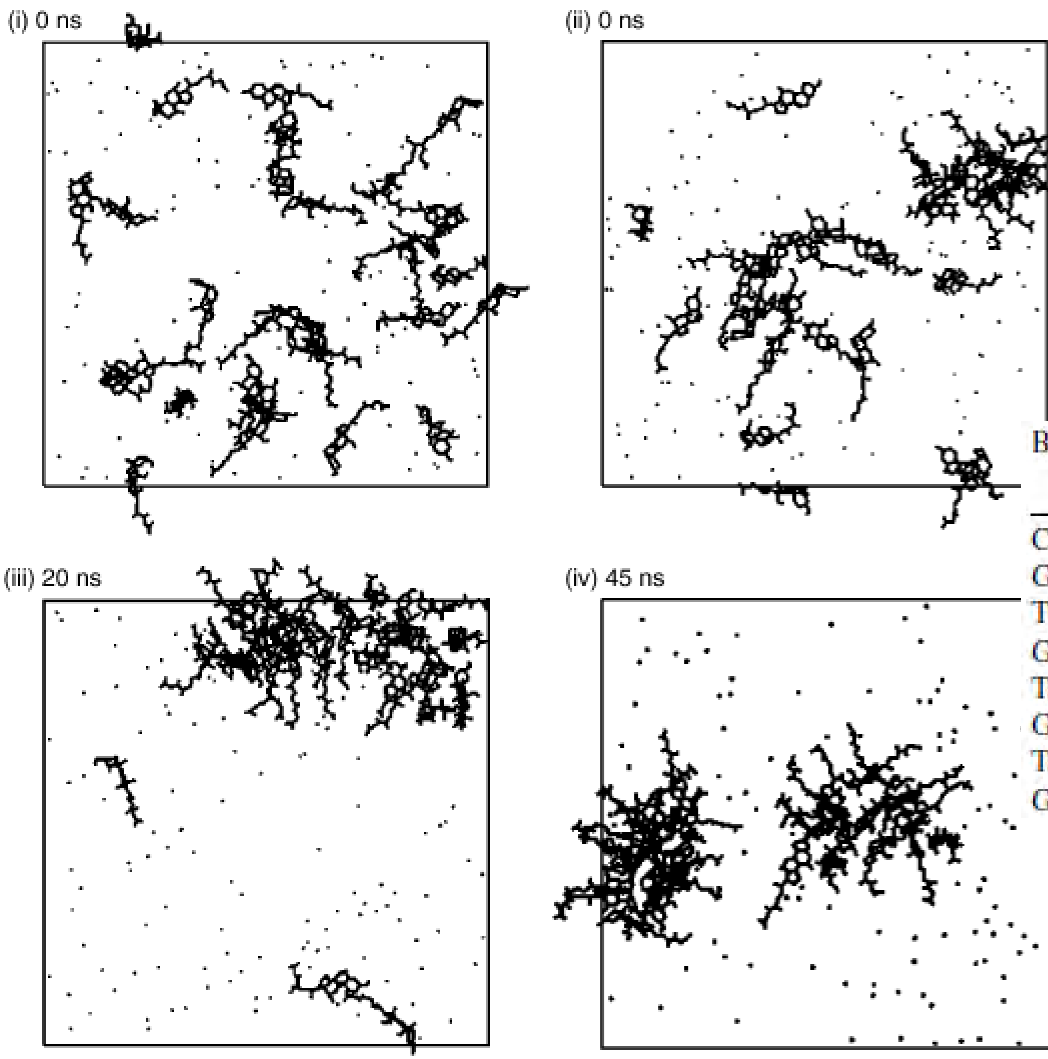
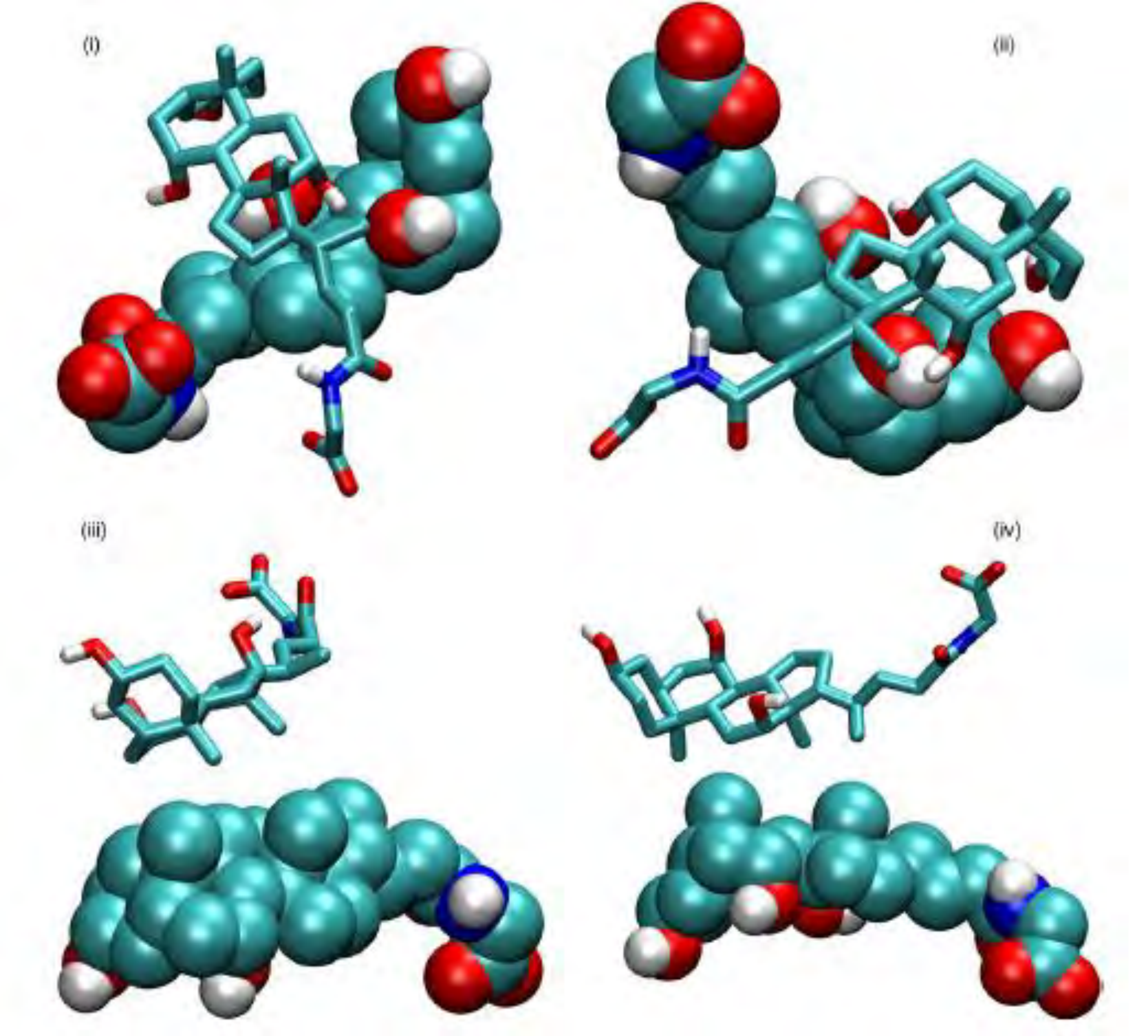


Bile salt	Simulation aggregate size	Literature aggregation
CHD	31	15–30 [50], 3.09–3.35
GCH	16, 15	–
TCH	11, 10, 10 × 1	3 [51], 2.59 [2]
GCD	16, 14, 1	–
Taurocheno-deoxycholate	–	20[51]
GDX	17, 11, 3 × 1	–
Taurodeoxy-cholate	–	24[51]
GLC	13, 10, 8	–

Example of evolution of ‘micelle: glycocholate (points = ions)



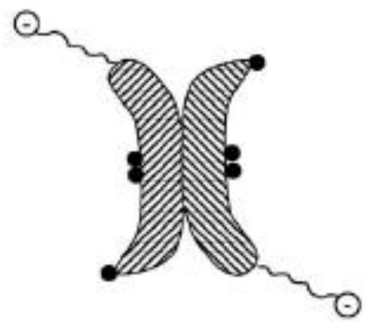
Cyan = C atom

Red = O atom

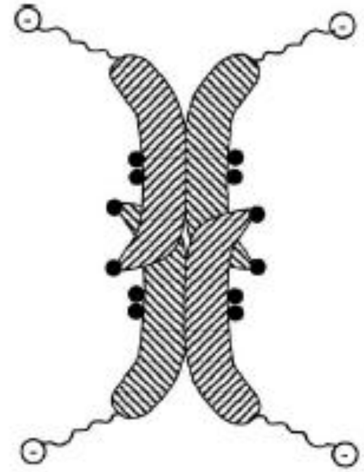
White = polar H atom, i.e., $H^{\delta+}$

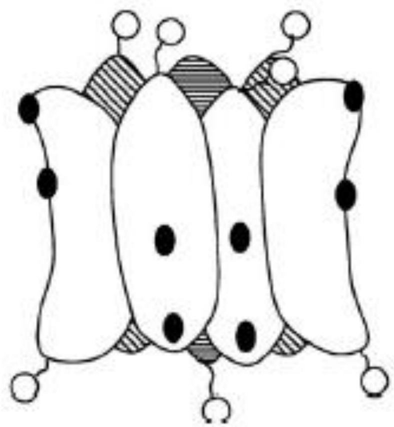
Blue = N atom

Probable



Possible





Only fleeting possibility

Very unlikely

Pouton et al.

In general:-
- Aggregates small & oblate
- Intermolecular H-bonding strong determinant of structure
- Structures very dynamic & disordered

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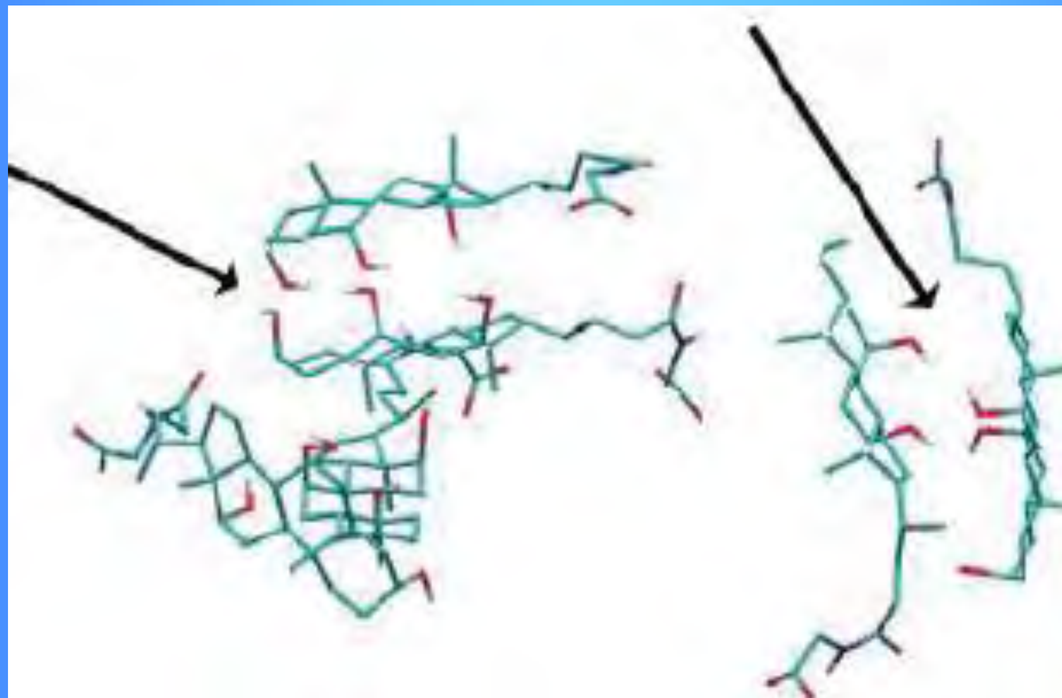
A) Bile salts do not have a typical surfactant structure,

B) so cannot necessarily adsorb in the traditional head-tail fashion

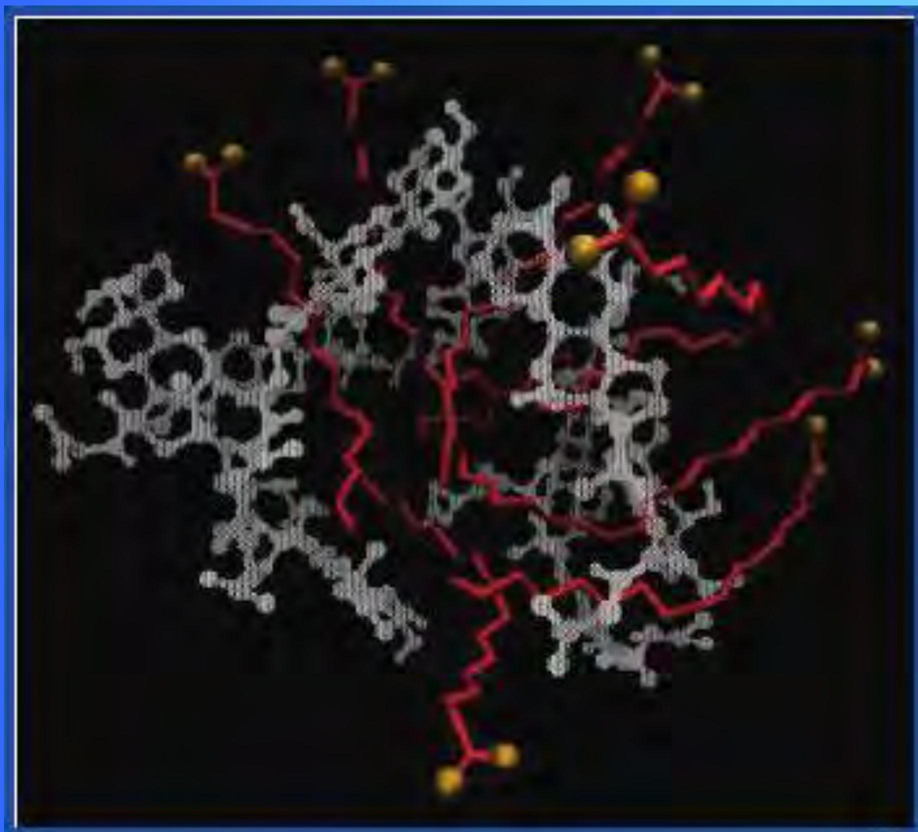


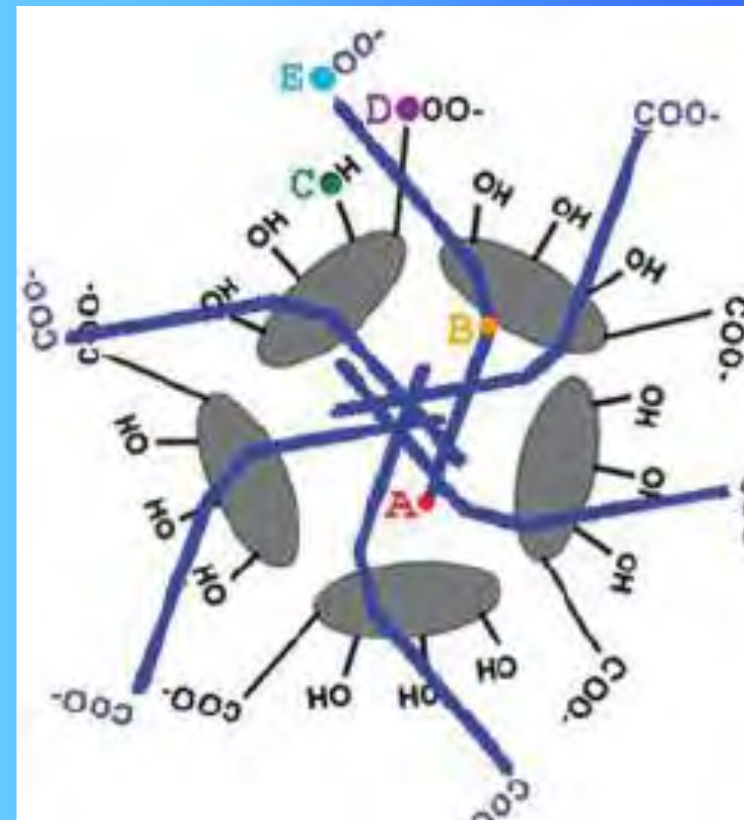
Premicellar dimers merging into a larger aggregate. Black arrows indicate glycocholate monomers maximizing hydrogen-bond interactions among R-hydroxyl groups.
The facial amphiphilic nature of bile salts is seen.

Cyan = C atom
Red = O atom
White = polar H atom, i.e., $H^{\delta+}$
Blue = N atom

Turner et al. Langmuir 2010, 26(7), 4687–4692

A) Bile salts ...cannot necessarily...form micelles with a simple hydrophobic core that easily accommodate/solubilize large quantities of lipid, or lipids beyond a certain size and shape.





Glycocholate-oleic acid mixed micelle.
[Glycocholate molecules = white, carbon chains of oleate = red
Gold spheres = carboxyl oxygens of the oleate anion]

Turner et al. Langmuir 2010, 26(7), 4687–4692

B) ...there is frequent disagreement on these CMC values....

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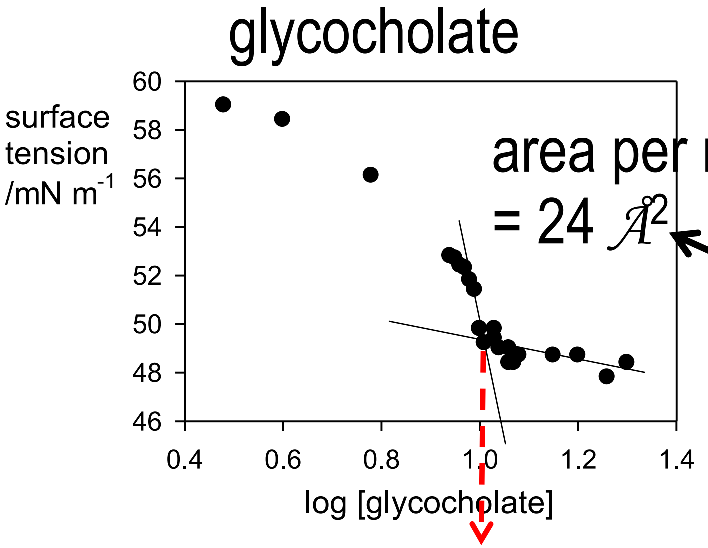
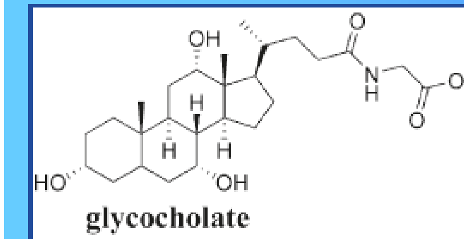


area per molecule = 24 Å^2

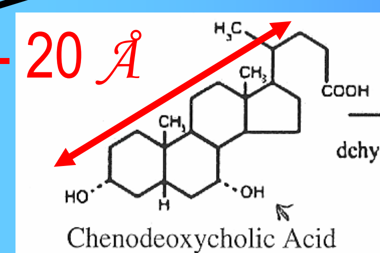


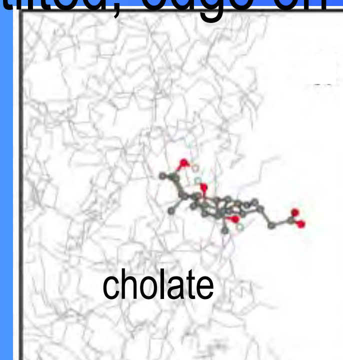
Adsorbing tilted, edge on ?

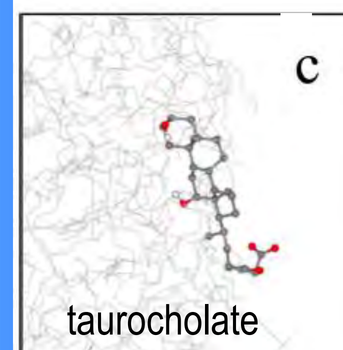
CMC = 11.8 mM [cf. lit. 12 mM]

chendeoxycholate
CMC = 3.6 mM
area per molecule
= 11 Å^2

15 - 20 Å



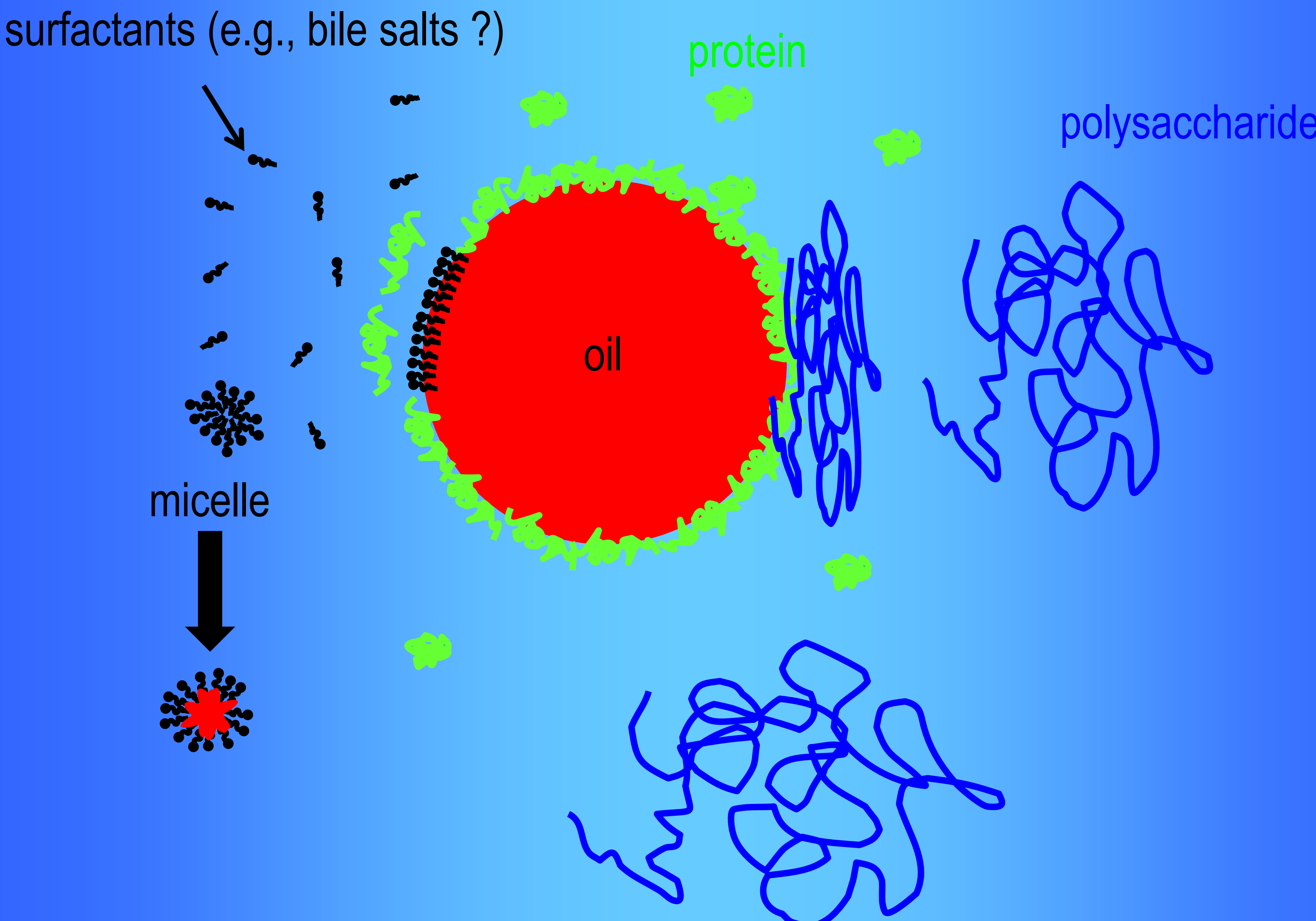




taurocholate
CMC = 8.8 mM [cf. lit. 10 mM]
area per molecule
= 160 Å^2

Adsorbing flat ?

C) The efficiency of bile salt adsorption to pre-formed emulsions depends on what is already on the surface.....



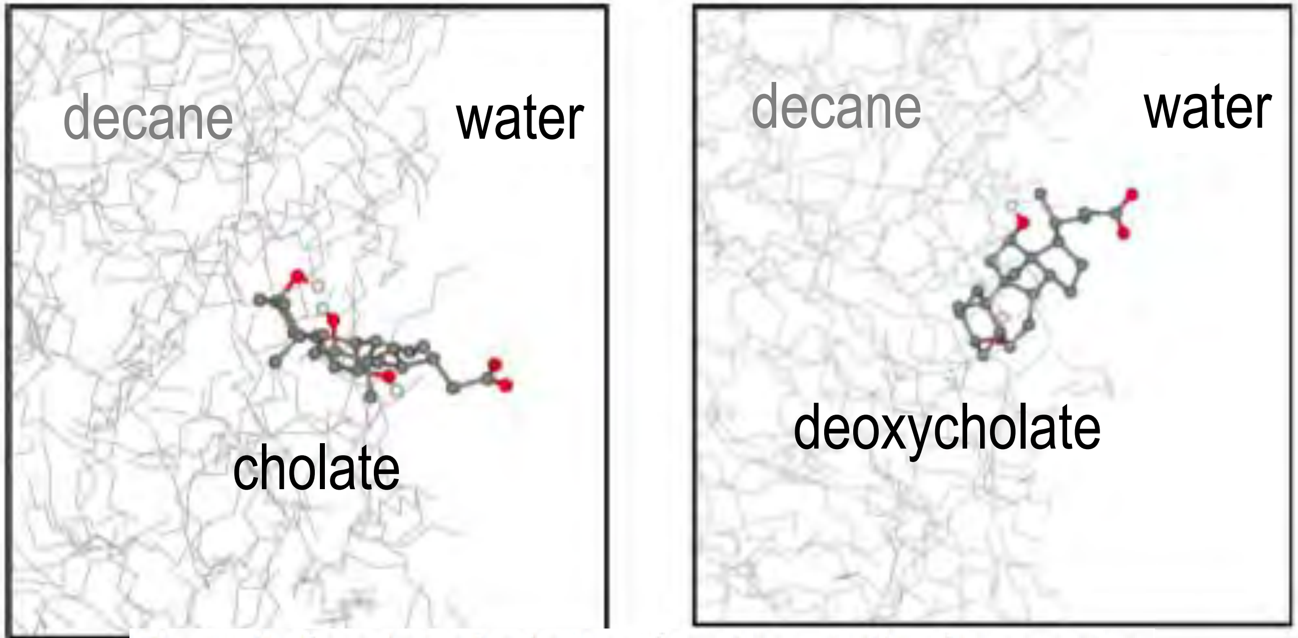
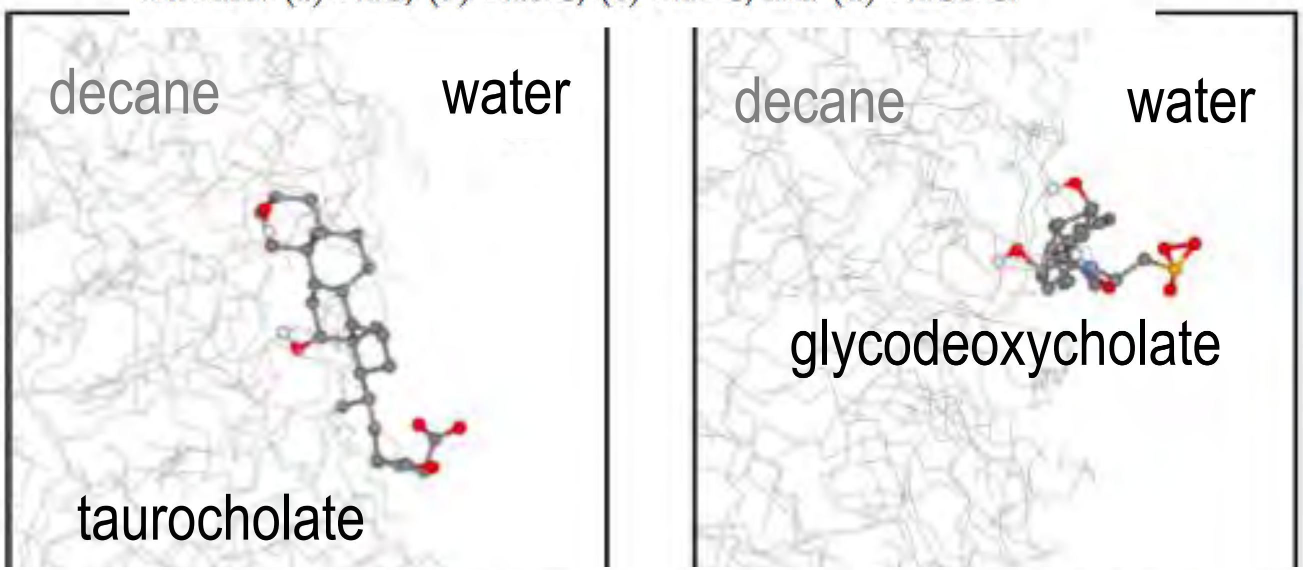


Figure 9. Snapshot adsorbed conformations at the decane−water interface: (a) NaC, (b) NaDC, (c) NaTC, and (d) NaGDC.



Removing one hydroxyl of cholate, i.e., as in deoxycholate, increases hydrophobicity and area per molecule at the interface that **increases** capacity to displace protein from the interface

Adding charge as glycine or taurine decreases hydrophobicity, causing molecule to sit further into aq. phase, which also **increases** its capacity to displace protein from the interface

Stephen R. Euston,* William G. Baird, Lydia Campbell, and Martin Kuhns†

Biomacromolecules 2013, 14, 1850−1858

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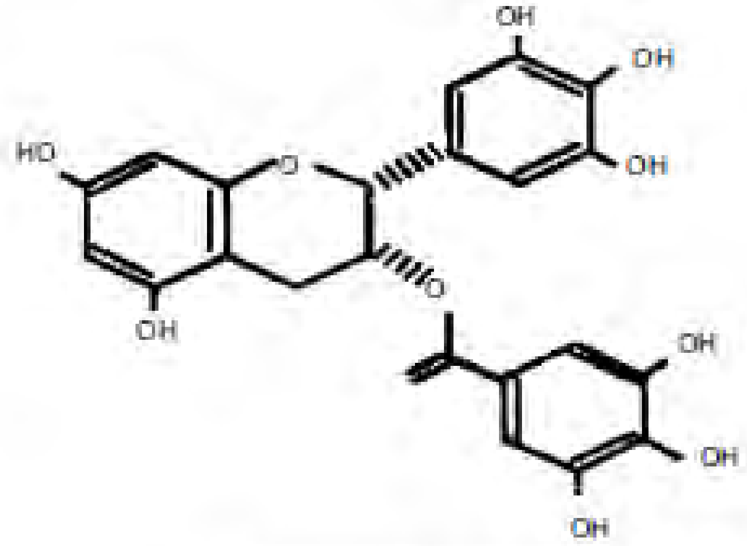
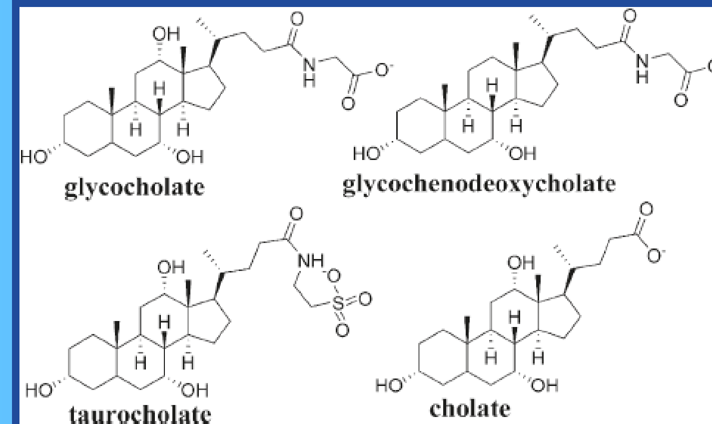
D) Other species may complex with bile salts and restrict their availability.

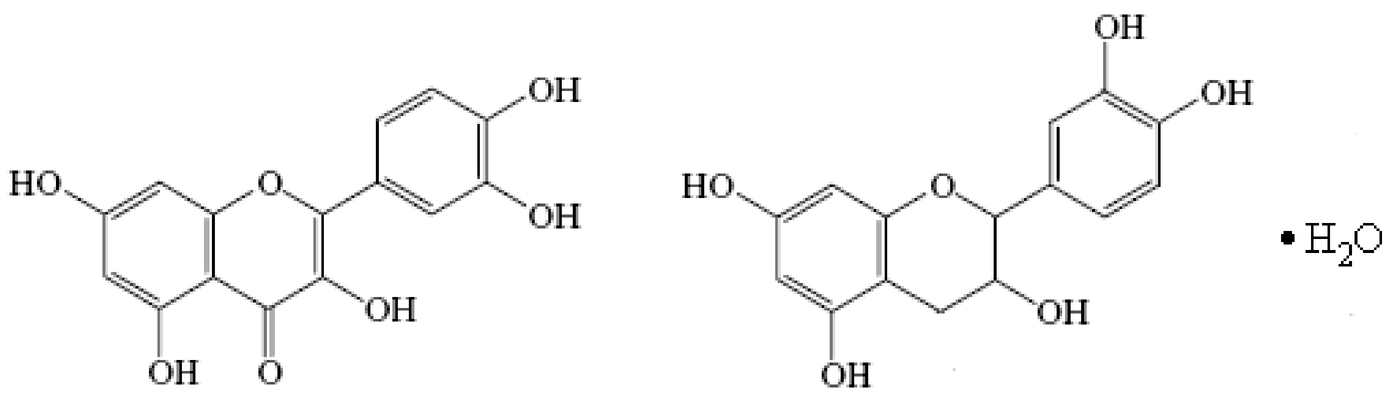
Polyphenols/flavonoids ??



(-)-Epigallocatechin gallate (EGCg)



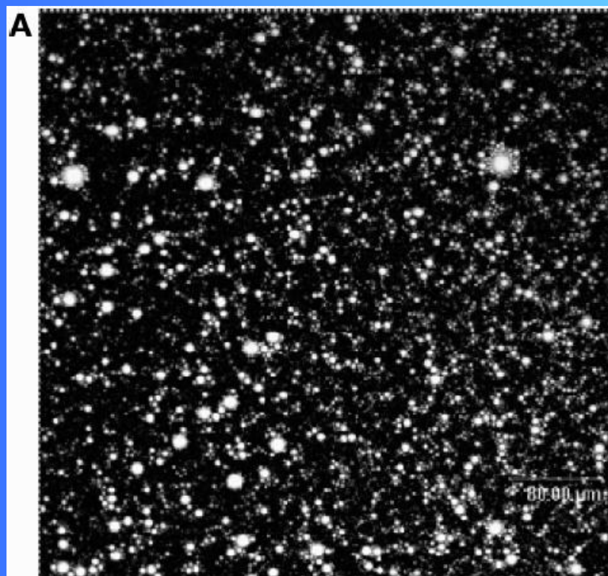
Effect efficiency of bile salt emulsification:
Shishikura, Khokhar & Murray
J Ag. Fd. Chem.(2006) **54**, 1906-1913.



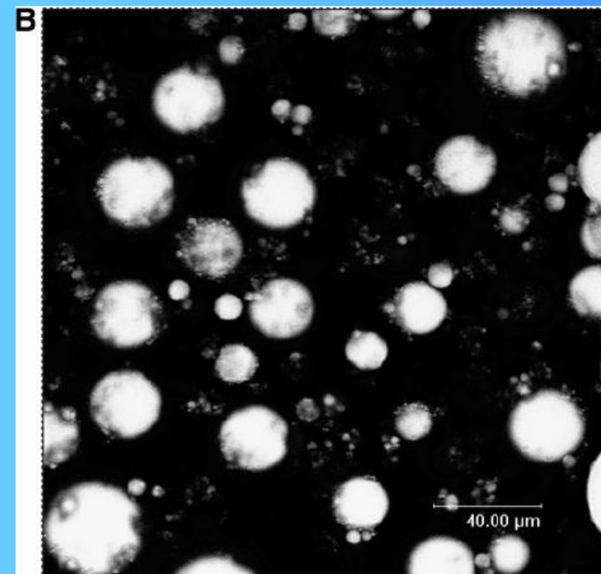
Quercetin

(+) - Catechin Hydrate

Bile salt stabilized vegetable O/W emulsions



In the absence of green tea catechins

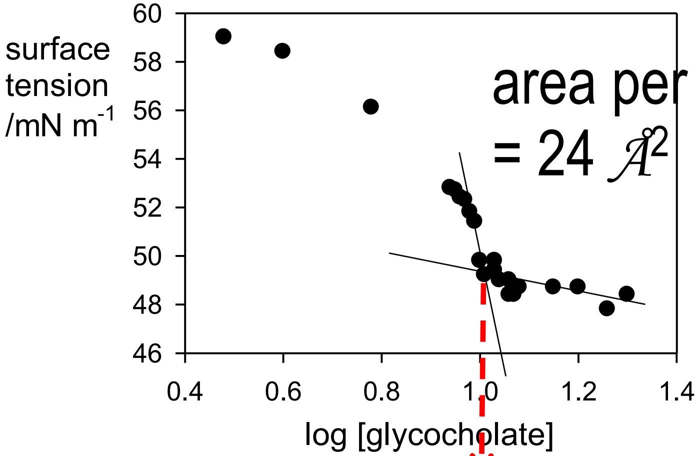


In the presence of 0.9 mg ml-1 total green tea catechins

Effect efficiency of bile salt emulsification:
Shishikura, Khokhar & Murray
J Ag. Fd. Chem.(2006) **54**, 1906-1913.

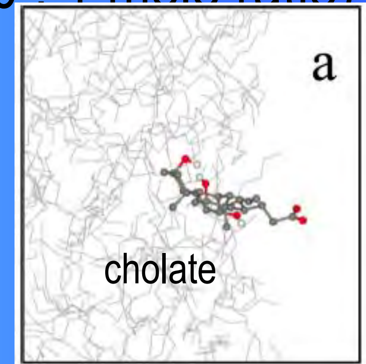
D) Other species may complex with bile salts and restrict their availability.

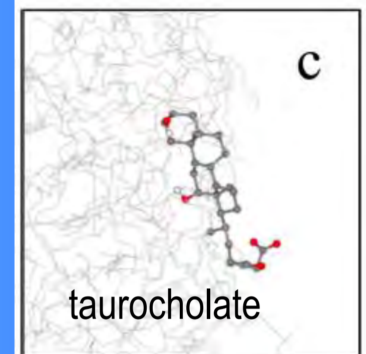
glycocholate



CMC = 11.8 mM

glycocholate + EGCG (20 : 1 mole ratio)
CMC = 9.5 mM
area per molecule = 21 Å^2





chendeoxycholate	chendeoxycholate + catechin (5 : 1)	
CMC = 3.6 mM	CMC = 5.9 mM	
area per molecule = 11 Å^2	area per molecule = 11 Å^2	

taurocholate	taurocholate + catechin (5 : 1)	+ quercetin (40 : 1)
CMC = 8.8 mM	CMC = 7.8 mM	CMC = 7.8 mM
area per molecule = 160 Å^2	area per molecule = 38 Å^2	area per molecule = 38 Å^2

D) Other species may complex with bile salts and restrict their availability.

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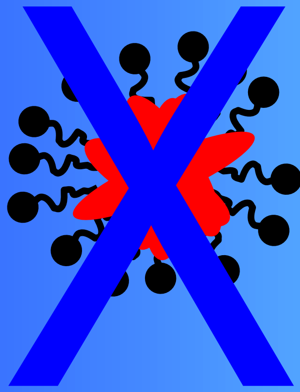
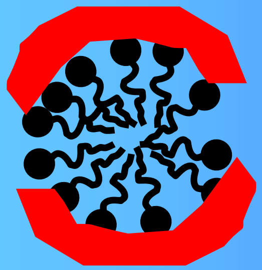
Solubilization of polyphenols or other species of similar size 'into' bile salt 'micelle's does not occur



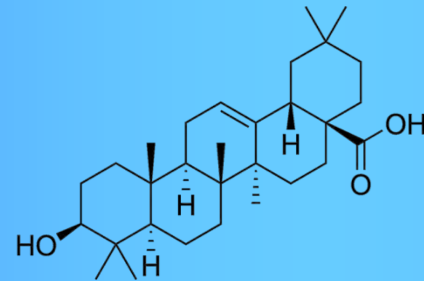
But complex formation on the surface of bile salt aggregates seems more likely



D) Other species may complex with bile salts and restrict their availability.

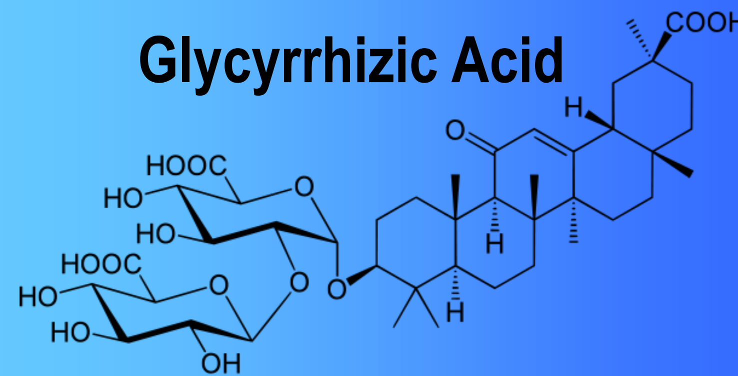
Saponins

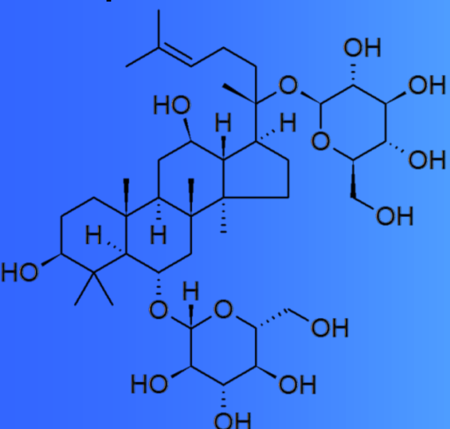
soybeans 6%
chick peas 4%
ginseng 0.5 – 3 %
herbal teas, Chinese teas, liquorice



oleanoic acid

Glycyrrhizic Acid

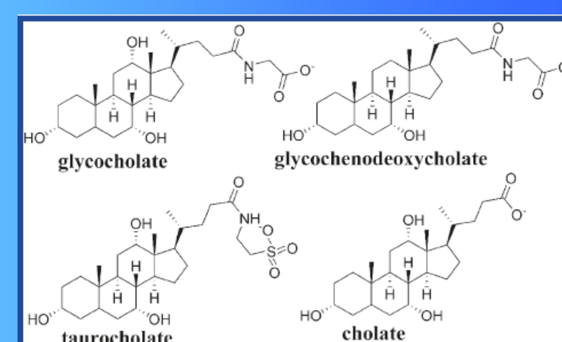




ginsenosides

Bile excretion is increased in the presence of saponins

Because bile salts are made from cholesterol, increased bile excretion can lower blood cholesterol



D) Other species may complex with bile salts and restrict their availability.

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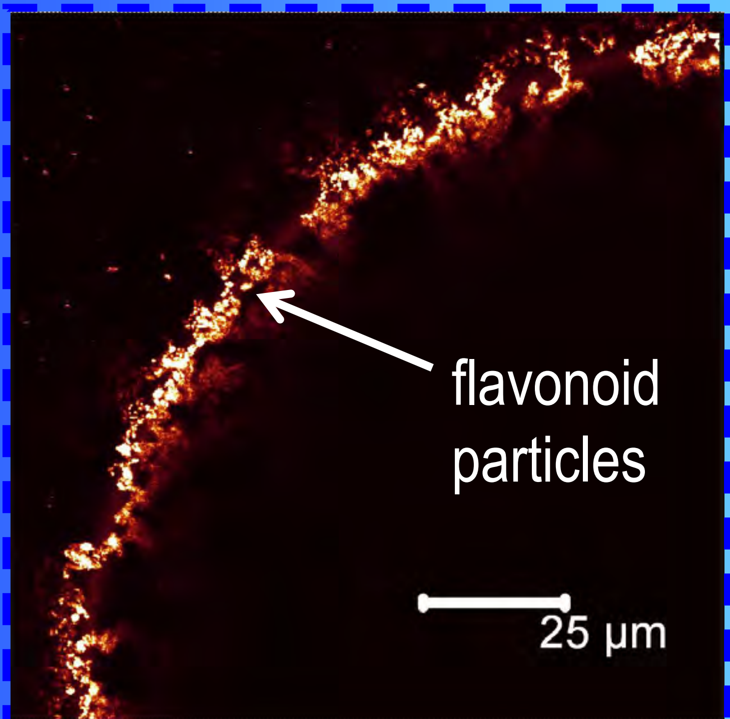
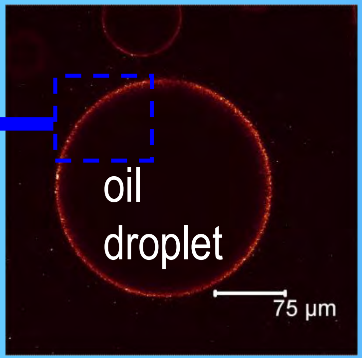
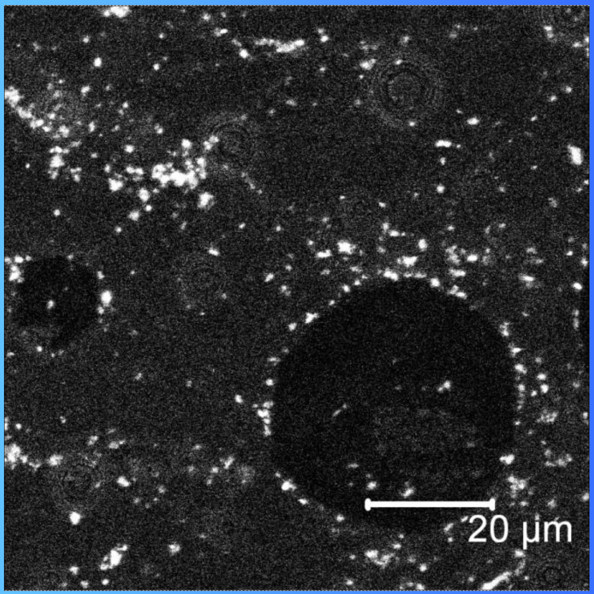
[flavonoid particles ?]







1.25 wt.% (≈ 25 mM) bile salts
+ 0.04 wt% (≈ 0.6 mM) tiliroside

(See next talk by
Andrea Day)

E) As lipase degrades the surface layer of lipid - its composition changes and a range of LMWS may be produced that may displace lipase and bile salts

P. Reis [a,*], H. Watzke [b], M. Leser [c], K. Holmberg [d], R. Miller [e]   Biophysical Chemistry 147 (2010) 93–103

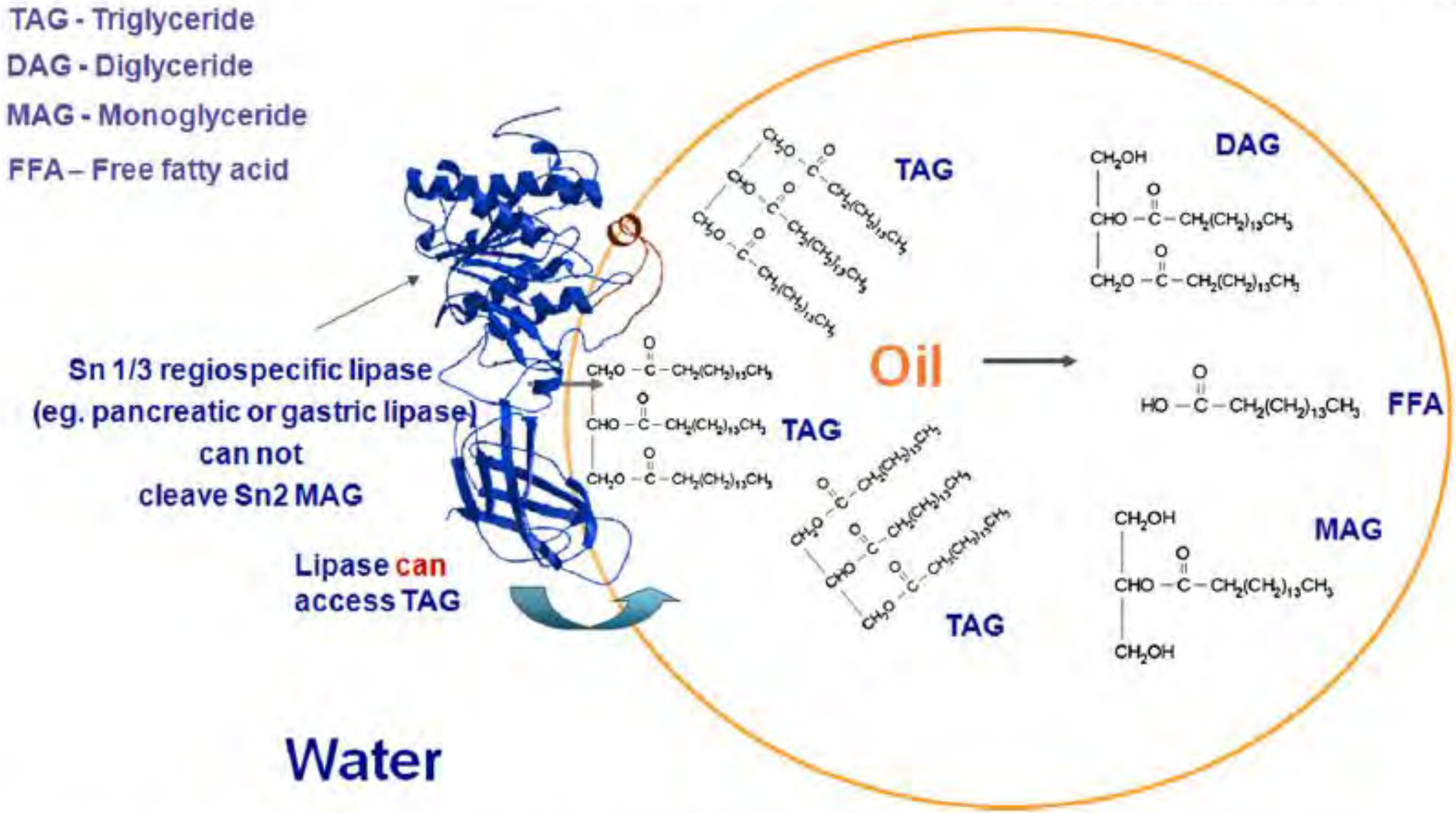


Fig. 3. Scheme of the enzymatic reaction of pancreatic lipase with triglycerides, leading to diglycerides (DAG), which are mainly oil-soluble, monoglycerides (MAG) and free fatty acids (FFA), which are mainly water-soluble.

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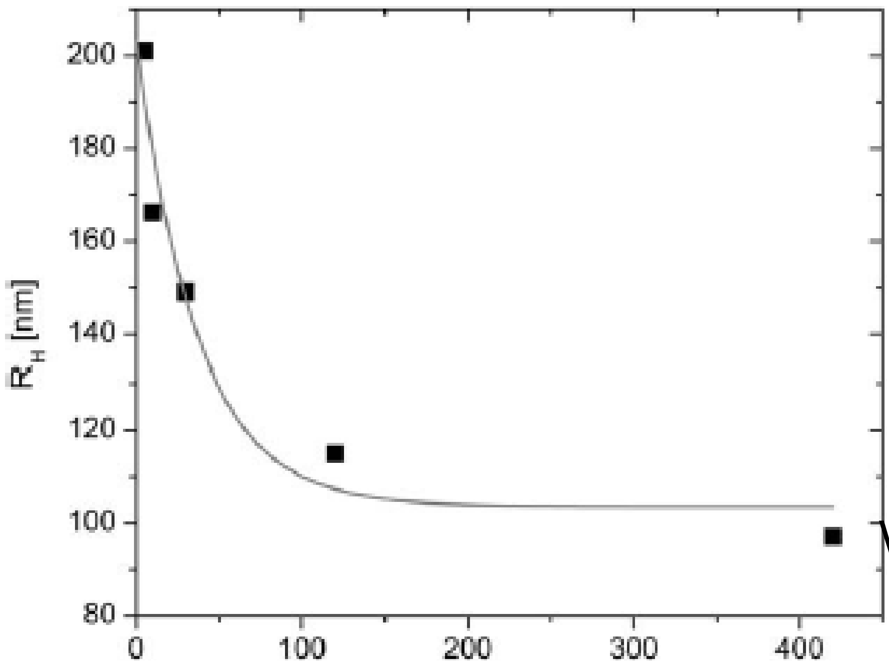
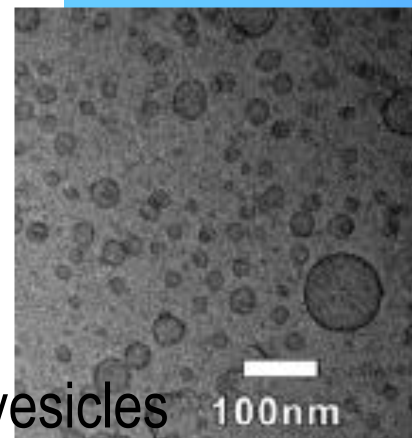
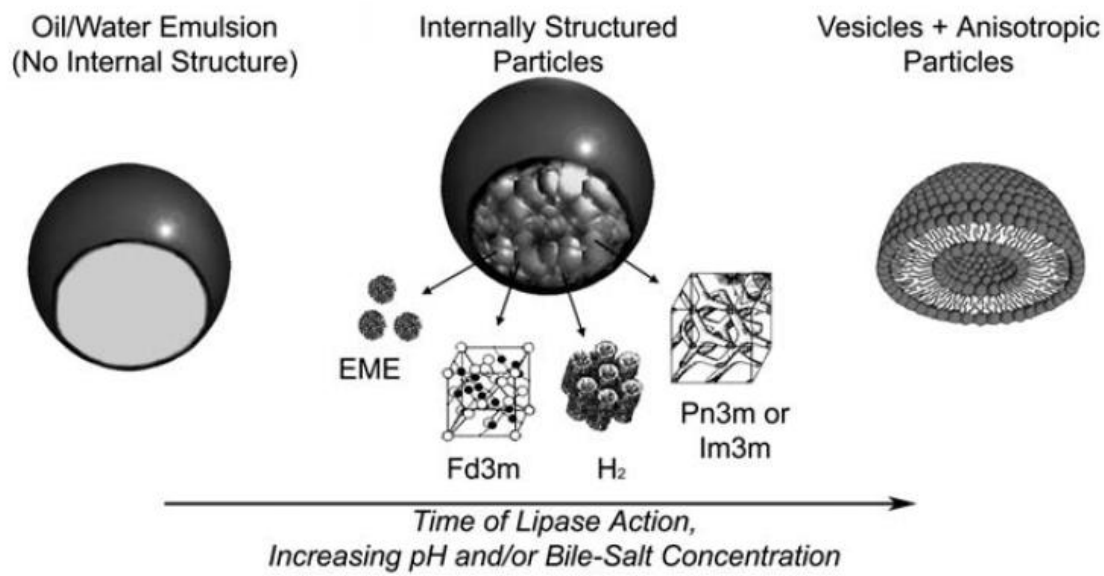


vesicles

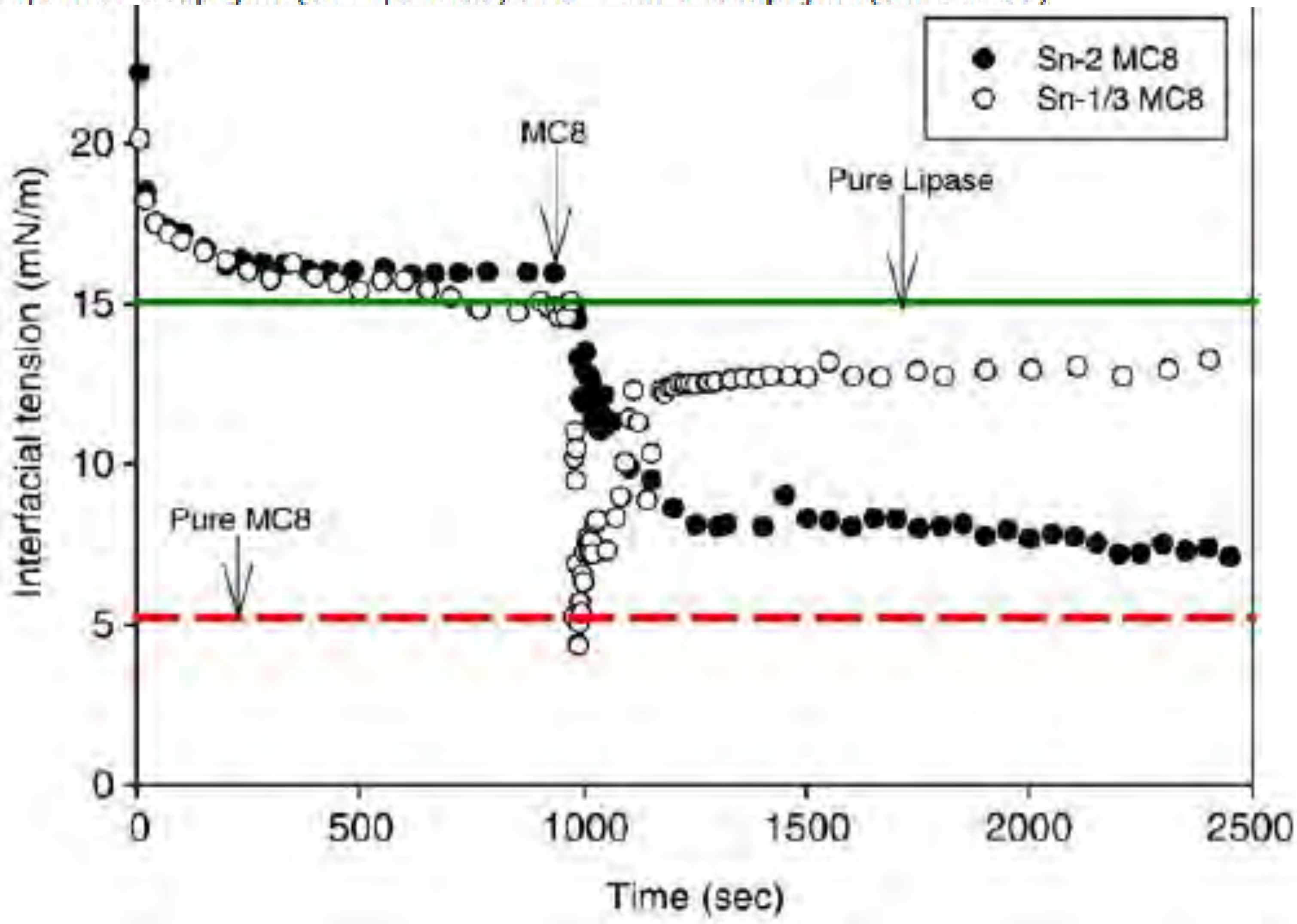




Salentinig, Sagalowitz, Leser, Tedeschi & Glatter
Soft Matter, 2011, **7**, 650-661.

E) As lipase degrades the surface layer of lipid - its composition changes and a range of LMWS may be produced that may displace lipase and bile salts

Fig. 16. Interfacial tension of a buffer/decane system with 3.3 E-5 M lipase from *Rhizomucor miehei* added followed by injection into the oil phase of (a): 5.0 E-3 M of either Sn-1/3 monocaprylin (Sn-1/3 MC8) or Sn-2 monocaprylin (Sn-2 MC8)



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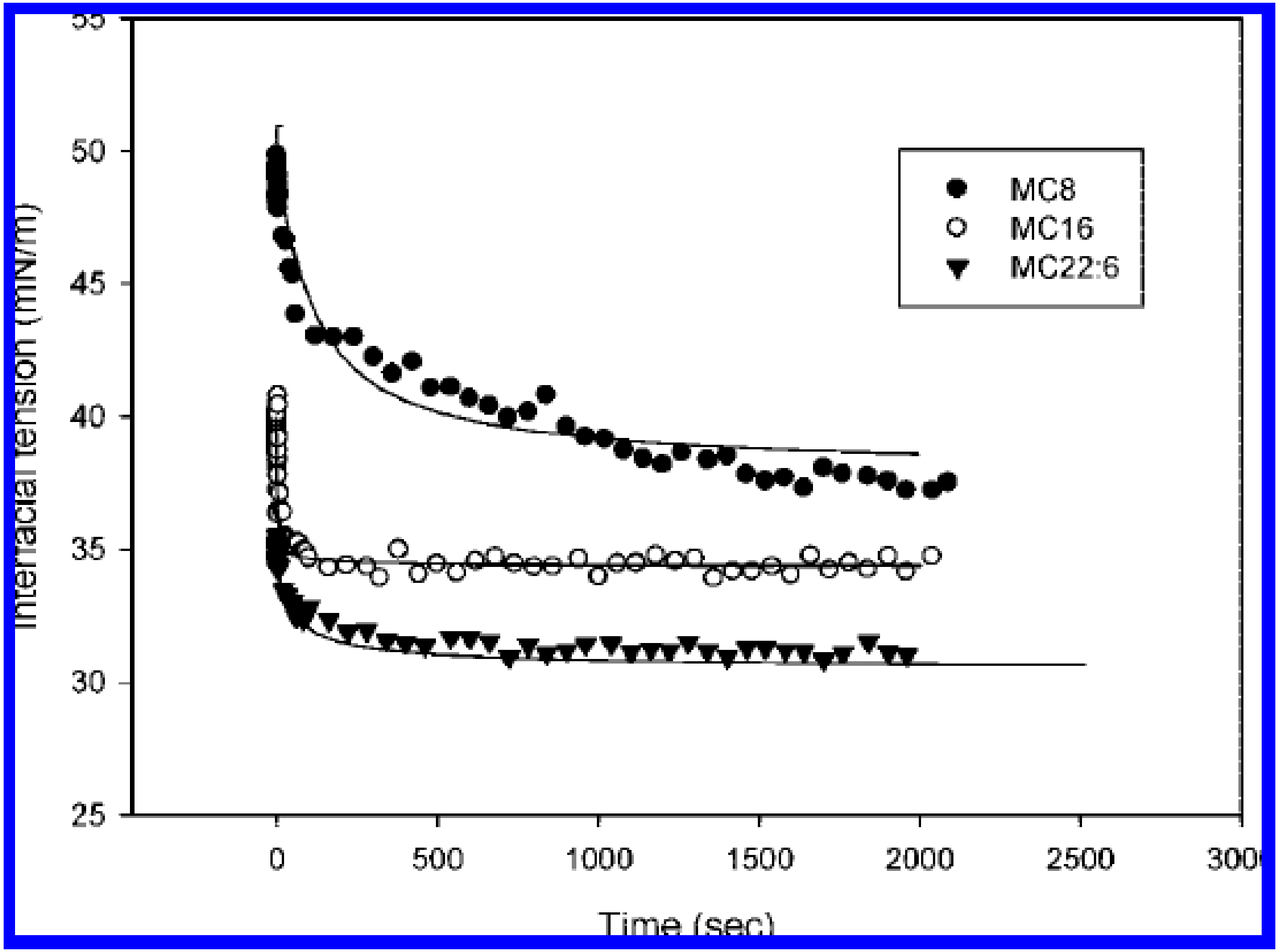
P. Reis,[†,‡] R. Miller,[§] M. Leser,[†] H. Watzke,[†] V. B. Fainerman,[||] and K. Holmberg*[,‡]

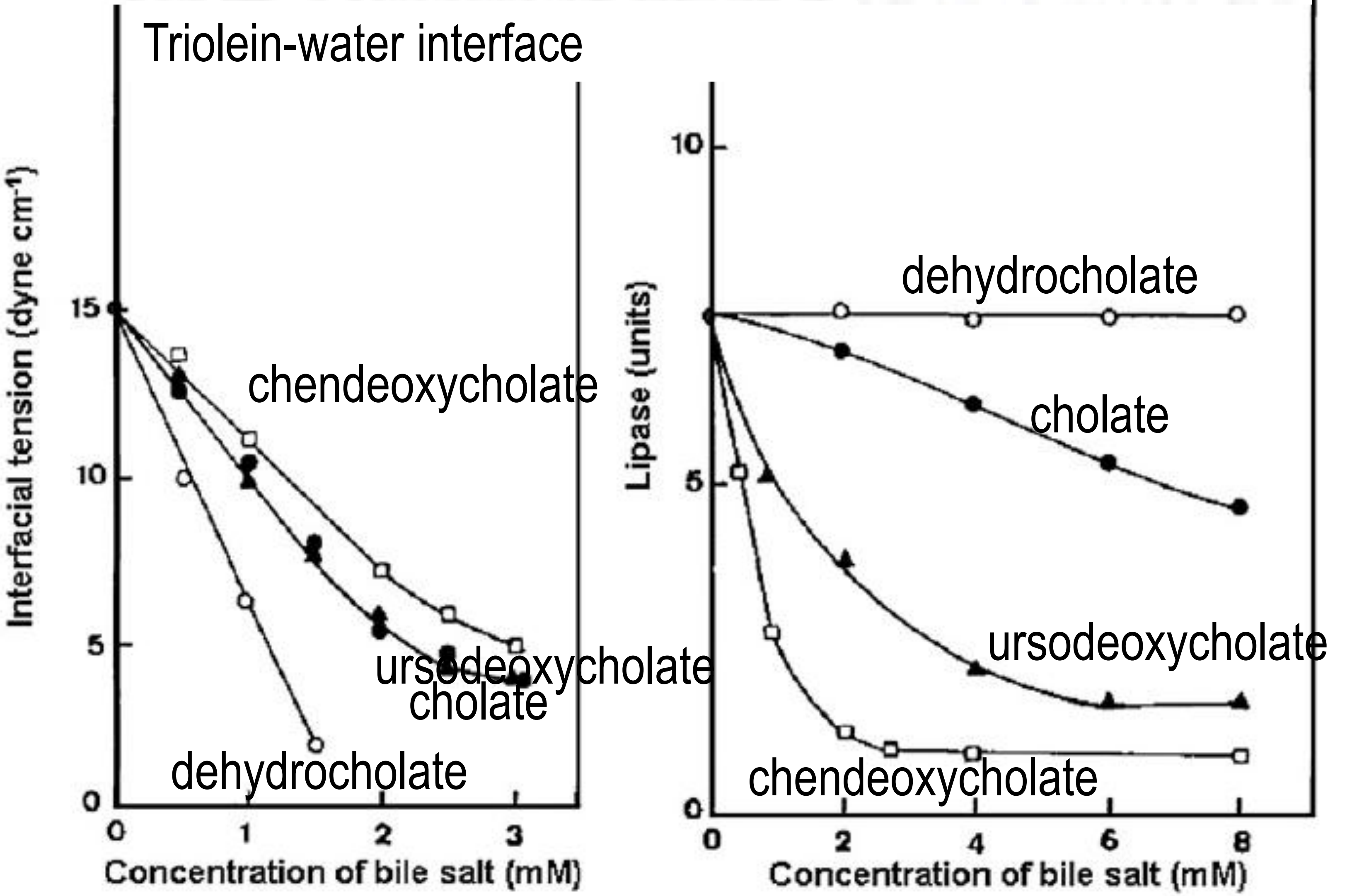
Langmuir **2008**, *24*, 5781−5786



E) As lipase degrades the surface layer of lipid - its composition changes and a range of LMWS may be produced that may displace lipase and bile salts

P. Reis [a,b], K. Holmberg [b], H. Watzke [a], M.E. Leser [a], R. Miller [c,*]

Advances in Colloid and Interface Science 147–148 (2009) 237–250



Other physiological issues with bile salts

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Gastro-oesphageal reflux disease (GORD), oesophageal adenocarcinoma OA), **Barrett's oesophagus**

Obese individuals – acid reflux – stomach content enter oesophagus
Most bile acid conjugates precipited by stomach acid, but treatment with anti-acids re-solubilizes them which therefore potentially membrane permeable.

Bile acids in oesophagus induces whole series of complex changes involving ROS leading to carninoma – **survaival rate < 10% !**

Conclusions

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• Partitioning of surfactants between oil phase, water phase and the interface can have large effects on the formation and stability of O/W emulsions

• The same effects apply to bile salts – although here the effects also influence lipase action and the product of lipase action

• The complex and as yet unresolved aspect sof bile salt 'micelle' formation mean that other components that interact with bile salts could have a strong influence on fat digestion & adsorption

Acknowledgements

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Aiman Al.Ghanem

Andrea Day
Laura Hardie

ICI Surfactants

But I leave you with a warning....

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"By the gods, you can swallow your own bile till it kills you"

Brutus to Cassius in *Julius Caesar*

The End

Thank you