The partitioning of emulsifiers in o/w emulsions: A comparative study of SANS, ultrafiltration and dialysis

Kathleen Oehlke[1], Vasyl Haramus[2], Anja Heins[1], Heiko Stöckmann[1], Karin Schwarz[1]

[1]Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany
[2]Helmholtz-Zentrum Geesthacht: Zentrum für Material- und Küstenforschung, Geesthacht, Germany

31.01.2012 / Delft

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

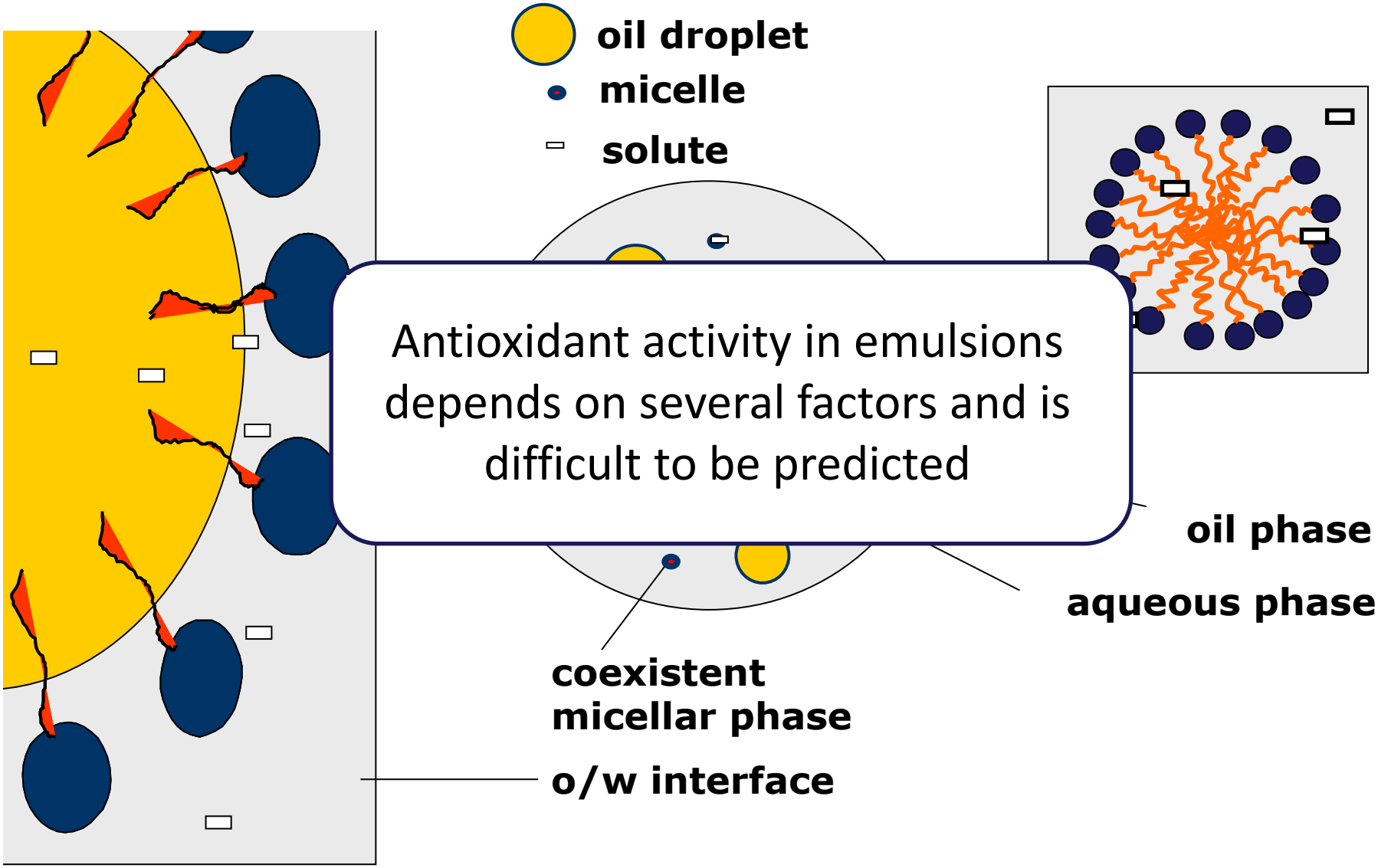
Introduction – why SANS

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

- Food is usually a complex matrix with different phases / constituents and hence different structures
- Phases or structures represent reaction sites for chemical reactions that are relevant e. g. for storage stability
- To understand reactions in food matrices it is important to know the structures

Solubilisation and reactions sites in o/w emulsions

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung



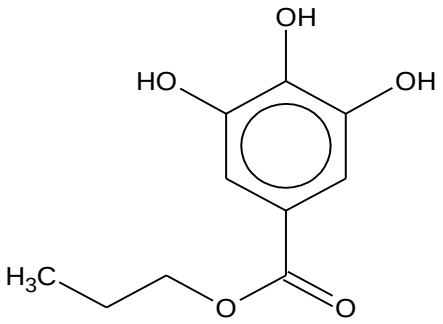
Lipid oxidation and antioxidant activity in emulsions

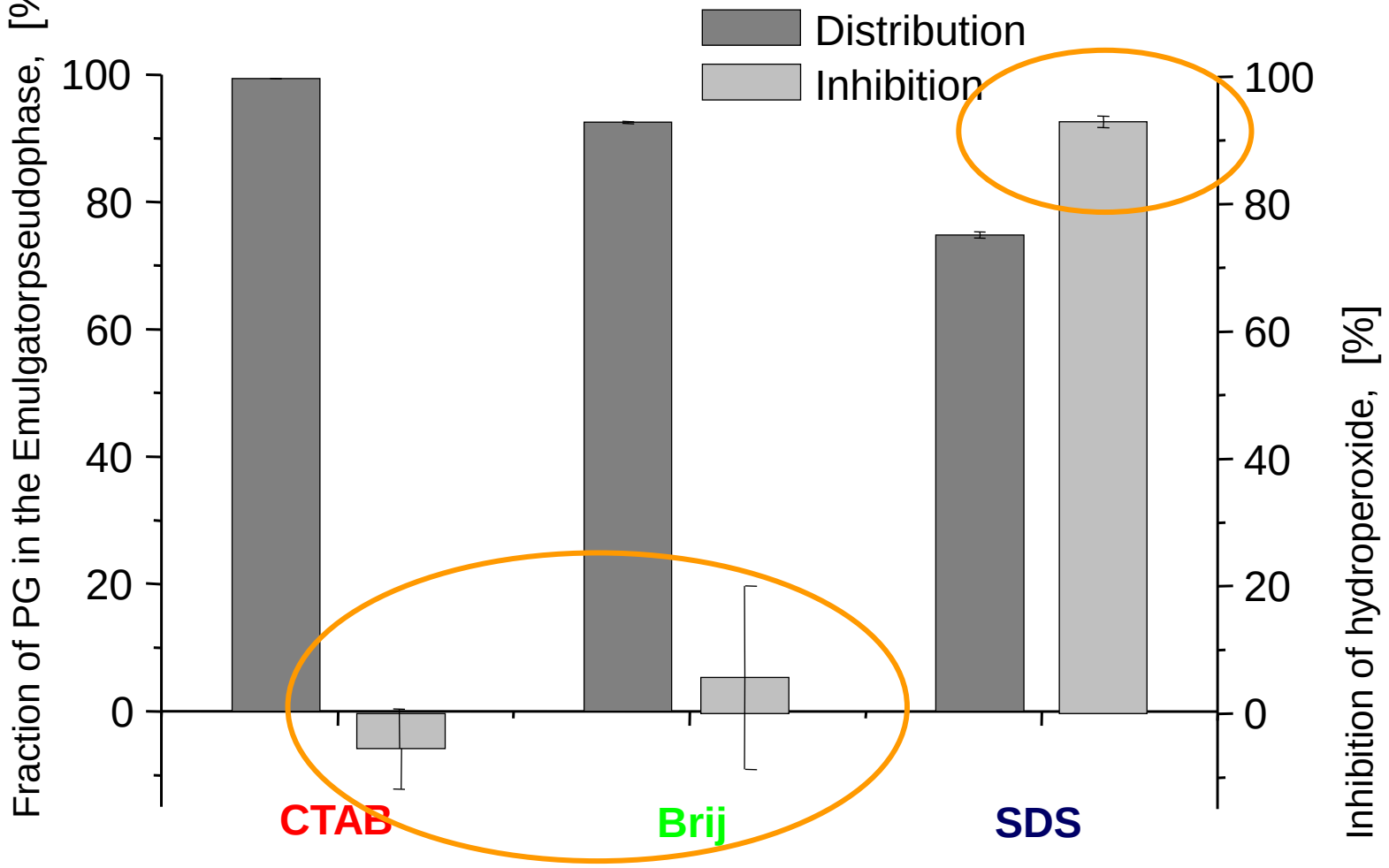
Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

- Progress of lipid oxidation in emulsions depends on the type and concentration of the emulsifier
- Activity of antioxidants in emulsions depends on type and concentration of the emulsifier
- Not always correlated to the partitioning of the antioxidants

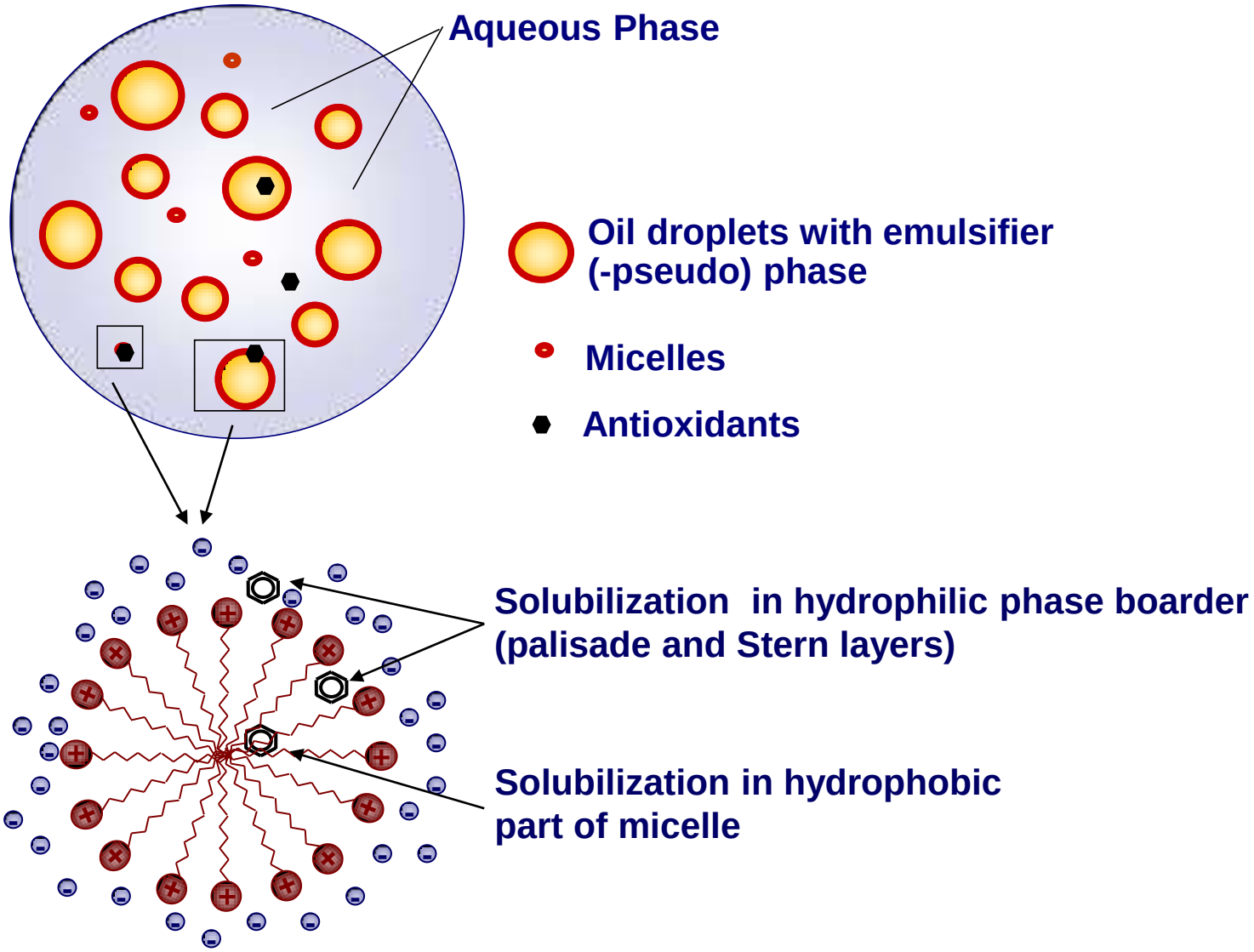
Activity vs. distribution of propyl gallate (PG) in different o / w emulsions

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung





Localization of Antioxidants in Dispersions



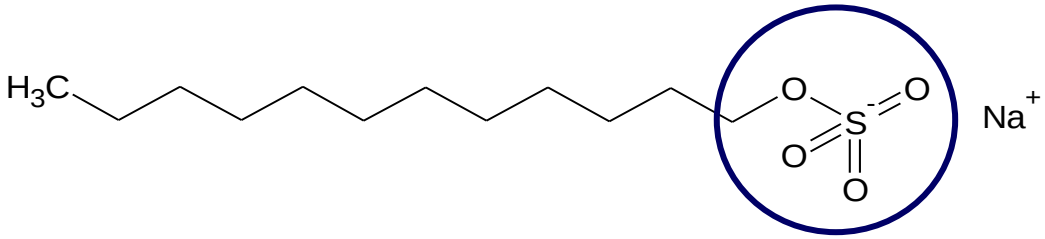
Different Electrical Charge of Emulsifiers

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

SDS

(Sodium dodecyl sulfate)

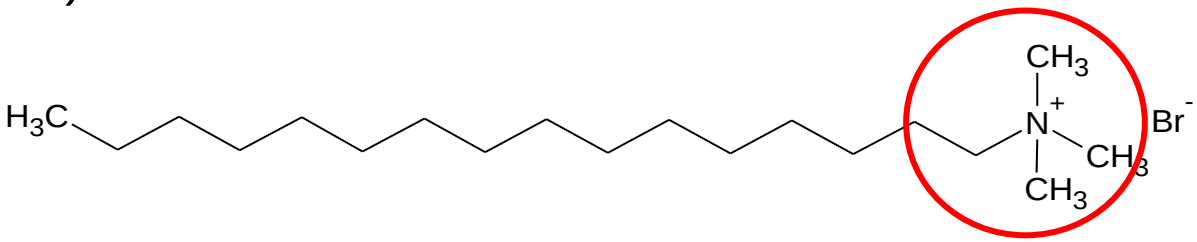
anionic head group



CTAB

(Cetyl trimethyl ammonium bromide)

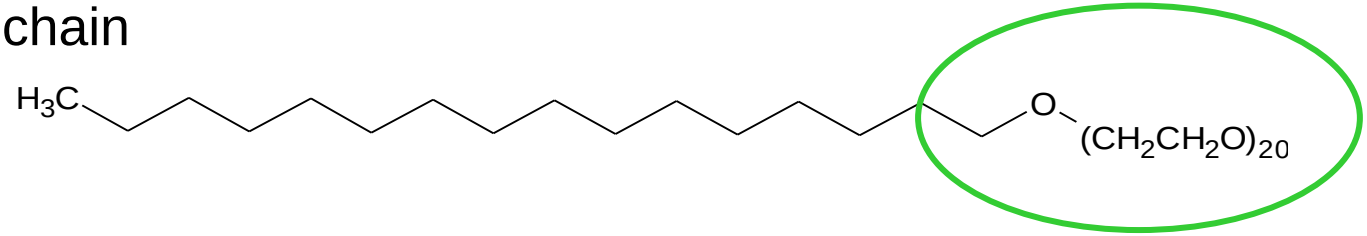
cationic head group



Brij 58

(Polyoxyethylene-20-cetyl ether)

nonionic Polyoxyethylene-chain



Antioxidants: Gallate Esters

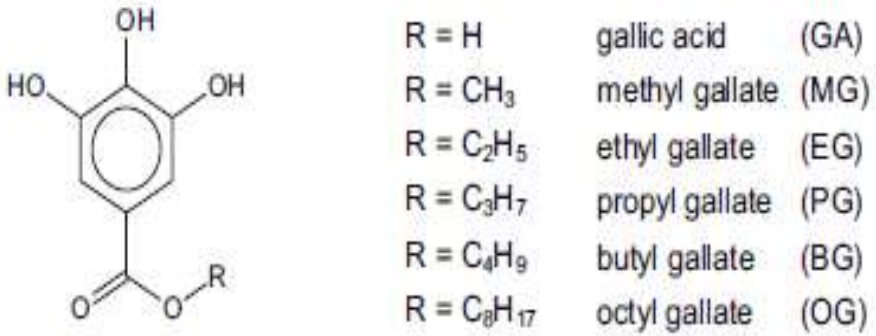
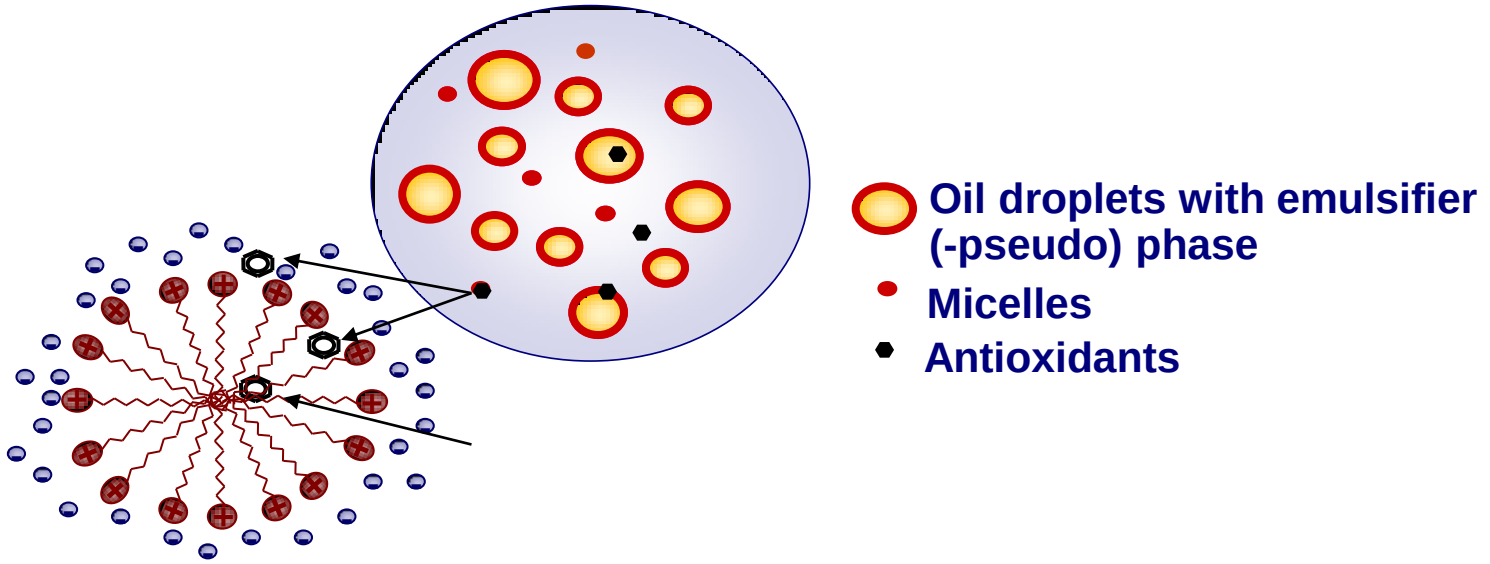


Fig. 1. Structures of gallates.

Sigma Aldrich, Seelze, Germany

TASK

Interaction between emulsifieir and antioxidant at micellar solution i.e., effect of antioxidant on micelle formation and micelle structure.



Methods

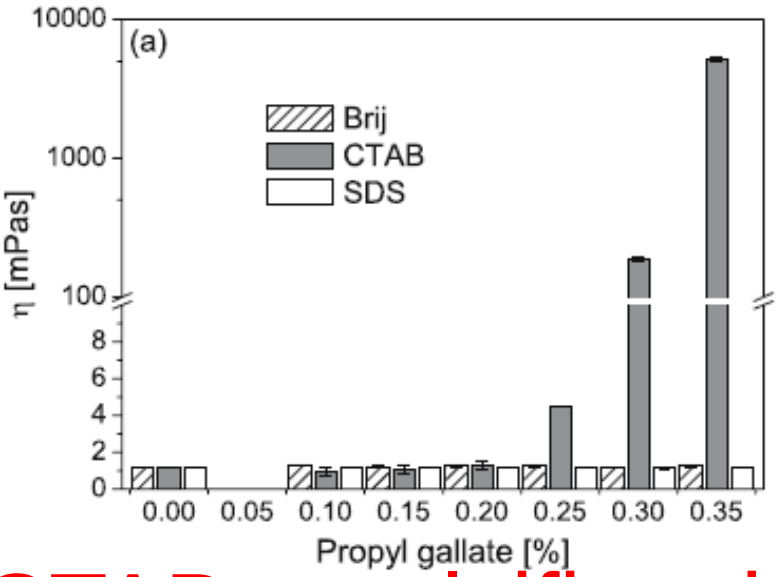
Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

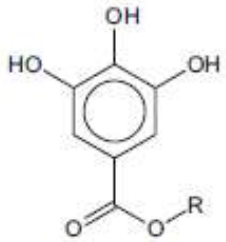
Viscosity: CVO 120. H_2O or D_2 easured at 25°C. Con osity η.

Critical m num plate method Kr M, 5.0 pH, σ = 69.7±0 ntration. T = 20 °C. **C**

SANS: SA o 0.25 Å^{-1}. T= 25.0± ct Fourier Transform stribution **function o $p_{CS}(r)$; radius of gyration R_g ($R_{CS,g}$); mass of aggregate M (M_L).**

Viscosity: Concentration and Type of Gallates

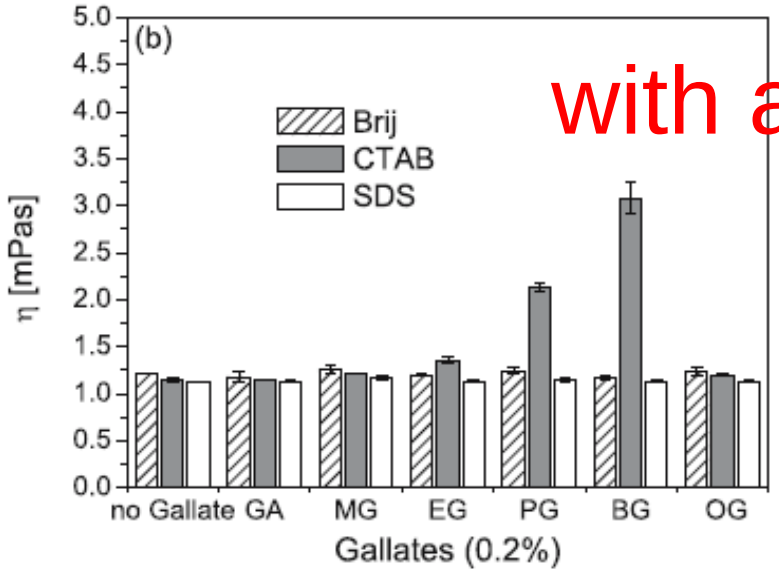


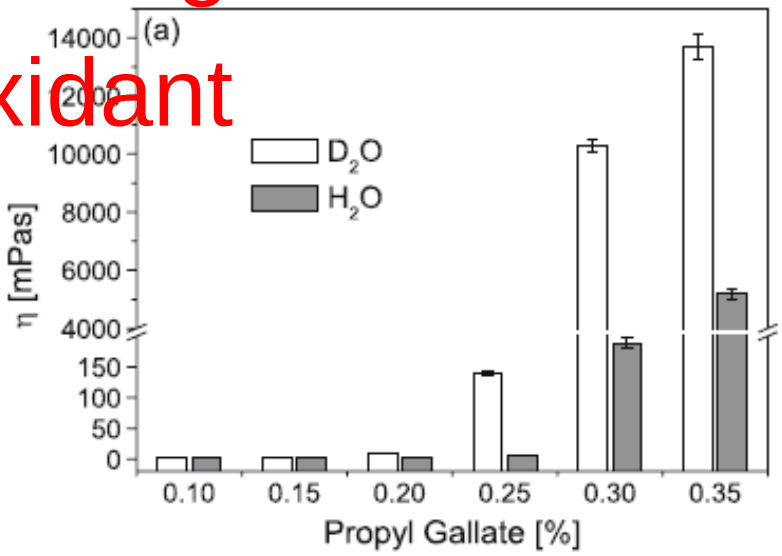


R = H	gallic acid	(GA)
R = CH_3	methyl gallate	(MG)
R = C_2H_5	ethyl gallate	(EG)
R = C_3H_7	propyl gallate	(PG)
R = C_4H_9	butyl gallate	(BG)
R = C_8H_{17}	octyl gallate	(OG)

Fig. 1. Structures of gallates.

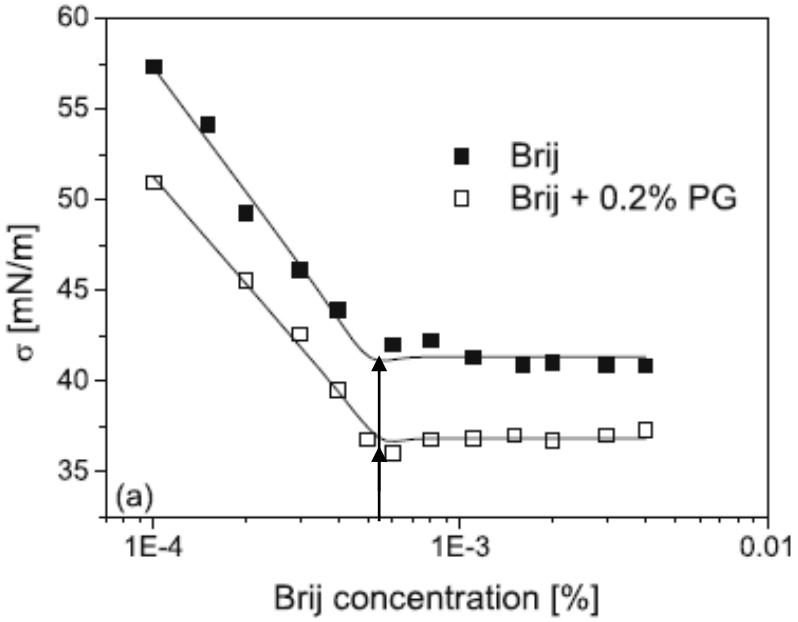
CTAB emulsifier shows strongest interaction with antioxidant





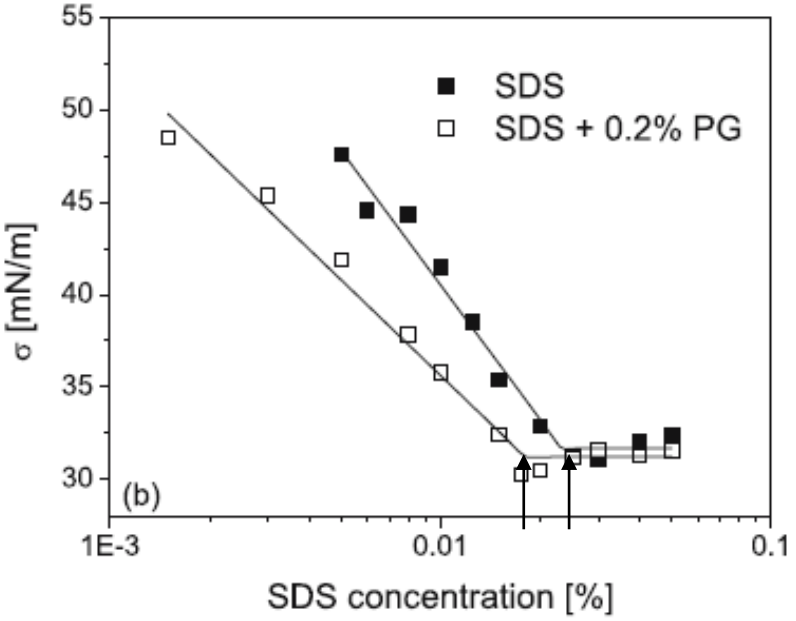
CMC and surface tension

Brij 58



Surface tension at CMC
is lower
CMC is constant

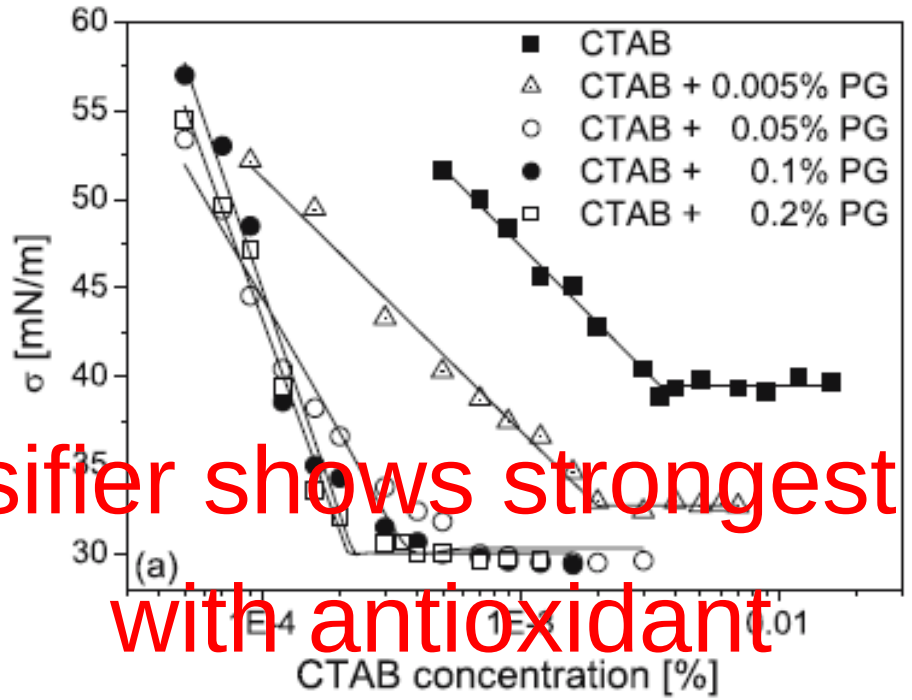
SDS



Surface tension at CMC
is constant
CMC is lower

CMC and surface tension

CTAB



CTAB emulsifier shows strongest interaction with antioxidant

Surface tension at CMC is lower

CMC is lower

Concentration and type of oxidant play role

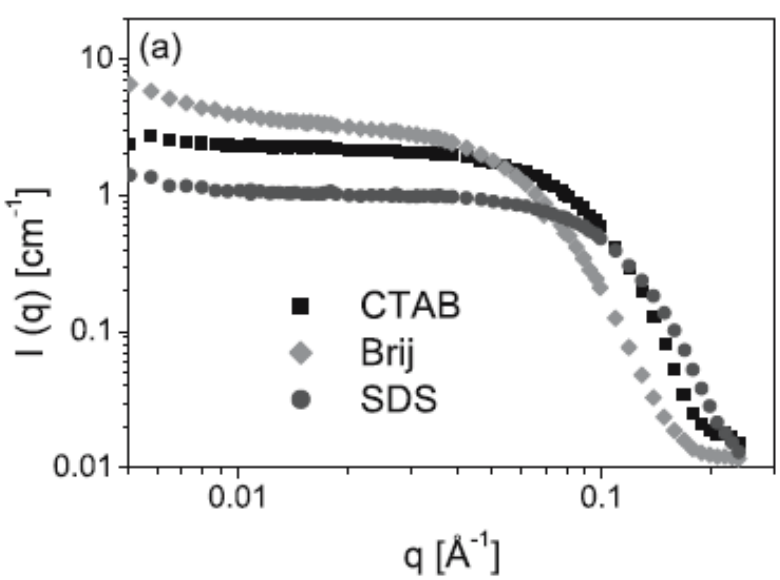
Antioxidants: CMC and surface area per molecule

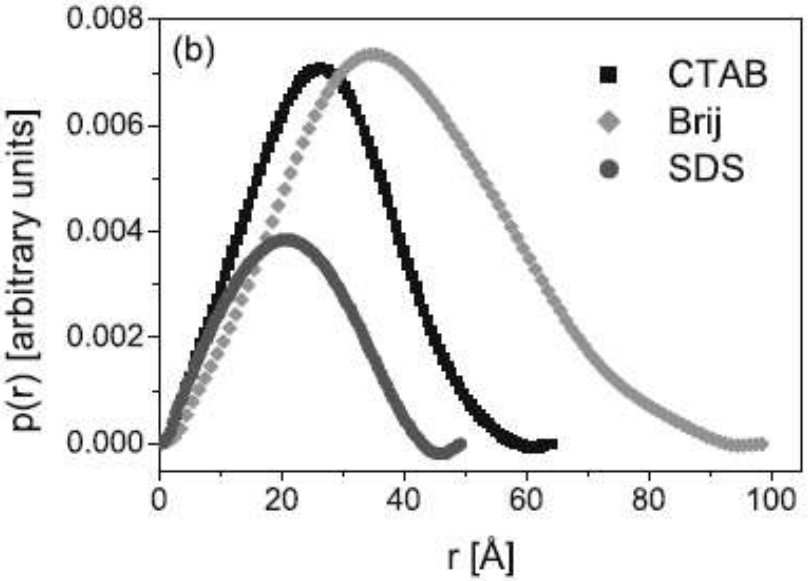
Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

Table 2 Determination of the cmc, specific excess surface concentration, and free energy of micellization of CTAB, Brij 58, and SDS in the absence and presence of selected antioxidants

	cmc[mg/L][c] mean ± s.d.[a]	σ_{cmc}[mN/m][cd] mean ± s.d.[a]	$\frac{\partial\sigma}{\partial \ln c_s}$[mN/m][ce] mean ± s.d.[a]	$\Gamma^* \cdot 100$[Å^{-2}][f] mean ± s.d.[b]	$\Delta G°_M$[kJ/mol][g] mean ± s.d.[b]
CTAB	$34.8 \pm 2.00^{\alpha}$	39.5 ± 0.17	6.3 ± 0.24	0.8 ± 0.03	-21.0 ± 0.13
+ 0.005%PG	$19.8 \pm 1.70^{\delta}$	32.8 ± 0.29	6.2 ± 0.25	0.8 ± 0.03	-22.3 ± 0.19
+ 0.05%PG	$3.7 \pm 0.40^{\eta}$	30.3 ± 0.50	10.8 ± 0.84	1.4 ± 0.11	-26.1 ± 0.25
+ 0.1%PG	$2.3 \pm 0.20^{\varphi}$	30.1 ± 0.59	18.3 ± 1.33	2.4 ± 0.18	-27.2 ± 0.20
+ 0.2%PG	$2.2 \pm 0.10^{\varphi}$	30.1 ± 0.38	17.3 ± 0.83	2.3 ± 0.11	-27.3 ± 0.10
+ 0.005%GA	$23.0 \pm 1.90^{\beta,\gamma}$	38.2 ± 0.28	6.0 ± 0.35	0.8 ± 0.09	-22.0 ± 0.19
+ 0.005%CA	$21.1 \pm 2.30^{\gamma}$	36.8 ± 0.33	5.1 ± 0.34	0.7 ± 0.09	-22.1 ± 0.25
+ 0.005%SA	$26.5 \pm 2.70^{\beta}$	39.1 ± 0.36	6.2 ± 0.41	0.8 ± 0.11	-21.6 ± 0.23
Brij	$5.0 \pm 0.30^{\varphi}$	41.4 ± 0.27	9.9 ± 0.61	2.6 ± 0.08	-28.0 ± 0.42
+ 0.2%PG	$5.5 \pm 0.20^{\varphi}$	36.9 ± 0.16	8.5 ± 0.33	2.2 ± 0.04	-27.8 ± 0.25
SDS	$232.1 \pm 19.1^{\alpha}$	31.7 ± 0.46	10.5 ± 0.76	1.4 ± 0.10	-16.2 ± 0.15
+ 0.2% PG	$180.3 \pm 14.4^{\beta}$	31.2 ± 0.36	7.5 ± 0.37	1.0 ± 0.05	-16.7 ± 0.14

Antioxidants: Micellar size and shape





→Micelles are spherical in binary solution (emulsifier/acetic buffer)

→Addition of antioxidant gives sphere-rod transion for CTAB solution

→Addition of antioxidant gives minor changes for SDS and Brij

Micellar size and shape

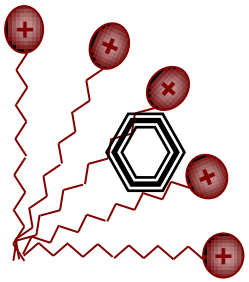
Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

Table 3 Calculations of the shape, size and aggregation number per micelle using to the fitted SANS data

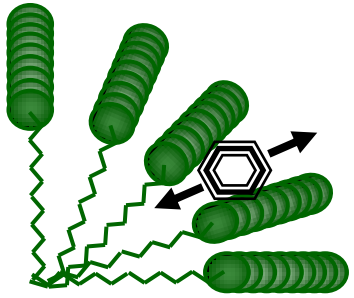
	shape	R[Å]^d mean ± s.d.^{a}	L_{app}[Å]^e mean ± s.d.^{b}	R_{cs}[Å]^f mean ± s.d.^{b}	$N_{agg}{}^g$ mean ± s.d.^{c}
1% SDS	sphere	$16.1 \pm 0.1^{\alpha}$			$80 \pm 1^{\alpha}$
+ 0.1% PG	sphere	$15.1 \pm 0.1^{\beta}$			$72 \pm 1^{\beta}$
+ 0.2% PG	sphere	$15.2 \pm 0.1^{\beta}$			$69 \pm 1^{\gamma}$
+ 0.3% PG	sphere	$15.0 \pm 0.1^{\beta}$			$68 \pm 1^{\gamma}$
1% Brij	sphere	$30.9 \pm 0.3^{\alpha}$			$73 \pm 1^{\alpha}$
+ 0.1% PG	sphere	$30.5 \pm 0.3^{\alpha}$			$74 \pm 1^{\alpha}$
+ 0.2% PG	sphere	$29.7 \pm 0.3^{\beta}$			$75 \pm 1^{\alpha}$
+ 0.3% PG	sphere	$29.2 \pm 0.3^{\beta}$			$75 \pm 1^{\alpha}$
1% CTAB	sphere	$20.5 \pm 0.1^{\alpha}$			$70 \pm 0^{\alpha}$
+ 0.1 % PG	sphere	$23.8 \pm 0.1^{\delta}$			$155 \pm 0^{\eta}$
+ 0.15% PG	rod-like		$421 \pm 12^{\lambda}$	21.4 ± 0.1	944 ± 17^{o}
+ 0.2 % PG	rod-like		$609 \pm 17^{\upsilon}$	21.6 ± 0.1	$1,414 \pm 10^{\theta}$
+ 0.25% PG	rod-like		$659 \pm 23^{\upsilon}$	22.1 ± 0.1	$1,610 \pm 8^{\tau}$
+ 0.3 % PG	rod-like		$712 \pm 19^{\pi}$	22.1 ± 0.1	$1,540 \pm 6^{o}$
+ 0.35% PG	rod-like		$635 \pm 21^{\upsilon,o}$	21.8 ± 0.1	$1,525 \pm 8^{\rho}$
+ 0.2% GA	sphere	$22.0 \pm 0.1^{\gamma}$			$118 \pm 0^{\delta}$
+ 0.2% MG	rod-like		$406 \pm 9^{\lambda}$	21.4 ± 0.1	$780 \pm 5^{\mu}$
+ 0.2% EG	rod-like		$674 \pm 25^{\pi,o}$	21.2 ± 0.2	$1,269 \pm 11^{\pi}$
+ 0.2% PG	rod-like		$609 \pm 17^{\upsilon}$	21.6 ± 0.1	$1,414 \pm 10^{\theta}$
+ 0.2% BG	rod-like		$718 \pm 23^{\pi}$	21.8 ± 0.2	$1,438 \pm 14^{\theta}$
+ 0.2% OG	rod-like		$812 \pm 32^{\rho}$	27.3 ± 0.2	$2,340 \pm 33^{\omega}$
+ 0.2% SA	sphere	$20.5 \pm 0.1^{\alpha}$			$100 \pm 1^{\beta}$
+ 0.2% FA	sphere	$20.9 \pm 0.1^{\beta}$			$105 \pm 0^{\gamma}$
+ 0.2% pC	rod-like		$287 \pm 8^{\eta}$	19.7 ± 0.2	$471 \pm 6^{\lambda}$
+ 0.2% CA	rod-like		$482 \pm 15^{\mu}$	20.6 ± 0.1	$829 \pm 6^{\upsilon}$

CTAB emulsifier shows strongest interaction with antioxidant

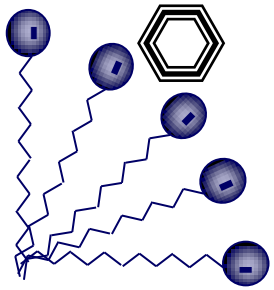
Location by Solubilization



⇨ **Solubilization in pallisade layer of CTAB micelles**



⇨ **Solubilization in head group area of Brij micelles**



⇨ **Solubilization in Stern layer of SDS micelles**

Antioxidant: action and location

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

Anionic surfactant - SDS. Antioxindants locate at Stern layer and act as cosolvent.
Smaller micelles from decreasing of of dielectric constant due to the cosolvent.
Weak H-acceptor properies and driving force is hydrophobic effects.

Nonionic surfactant – Brij 58. Antioxidants locate at polyoxyethylene chain and act as alcohol cosolvent.
Lose the surfactant structure, polyoxythylene chain is more closer to hydrophobic core and hydrophilic micellar shell is thinner . Decreases slightly aggregation number of micelles and number of molecules per surface.

Cationic surfactant – CTAB. Antioxindants locate at palisade layer and act as cosurfactants.
Antioxidants leads to sphere-to-rod transition, increases the aggregation number of micelles and makes tigher packing of CTAB molecules. It means that Columb forces are weaken. Confirmed by NMR. CTAB is strong H-bond acceptor.

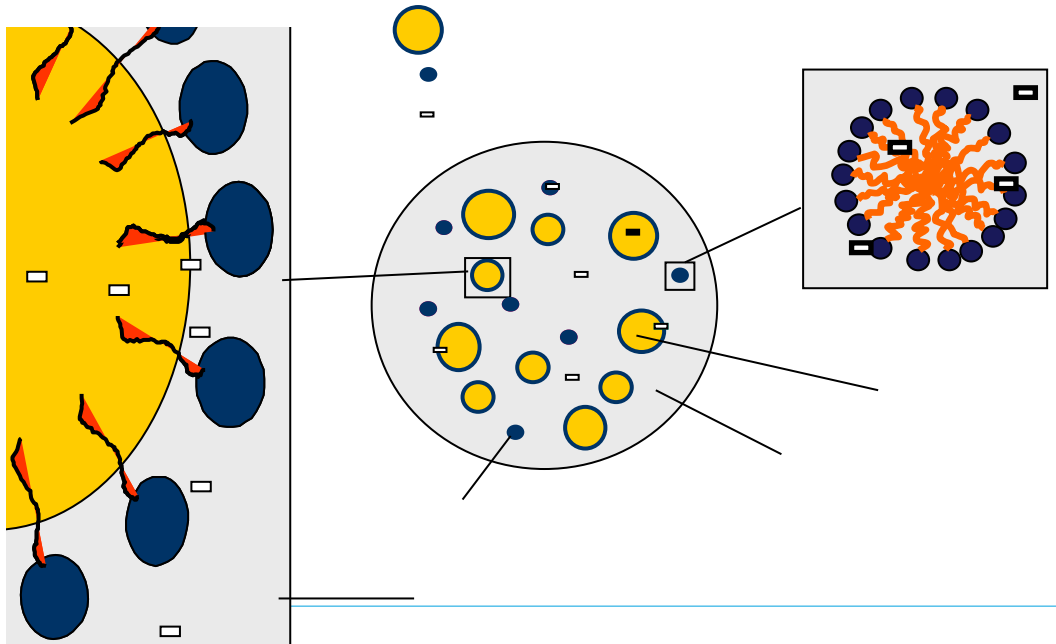
Heins A., Garamus V. M., Steffen B., Stöckmann H., Schwarz K.
“Impact of Phenolic Antioxidants on Structural Properties of Micellar Solutions”
Food Biophysics, 2006, v. 1, P. 189-201.

Interface Structure of Emulsifier

Activity of antioxidant is inverse proportional to strength of interaction between antioxidant and emulsifier.

Good antioxidant activity is strongly depended on proximity of antioxidant and radical which is controlled by emulsifier at interface – results of electron spin resonance
Heins et al. (2007) *Lipids*, 42: 561 - 572

Task is to get concentration (structure) of emulsifier at oil droplet interface with aqueous solution



Partioning of Emulsifiers: Methods

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

Droplet size: Laser diffraction – Helos Laser Diffraction instrument. Samples were diluted ~ 1500-fold. Dilution did not affect of droplet size. **Hydrodynamic radius and total interfacial area.**

Ultrafiltration: Centrifugal devices with 2 pore sizes. Regenerated cellulose membranes with nominal molecular weight cut-off (MWCO) of 3 kDa (Centricon YM3, Millipore) – **molecular aqueous solution**. MWCO of 100 kDa (Centricon YM100) - **SDS micelles + monomers**. Inorganic membranes with pore size 20 nm (VestaSpin micro, Whatman) – **CTAB micelles + monomers**. Equilibration steps. Room temperature.

Diallysis: Side-by-side diffusion cells. Cellulose ester membrane with MWCO of 3.5 kDa – **molecular aqueous solution** and 300 kDa (SpectraPor, Spectrum Laboratories, USA) - **SDS micelles + monomers**. 72 h at room temperature.

Quantification: SDS - 4 µL filtrate or permetate were added to 3 mL of 45 µM methyl orange and 35 cetyl pyridinium bromide. **CTAB** - 30 µl of filtrate or permeate were added to 3 ml of a 60 µM methyl orange solution in degassed deionised water/ethanol (80/20), absorbance at 465 nm.

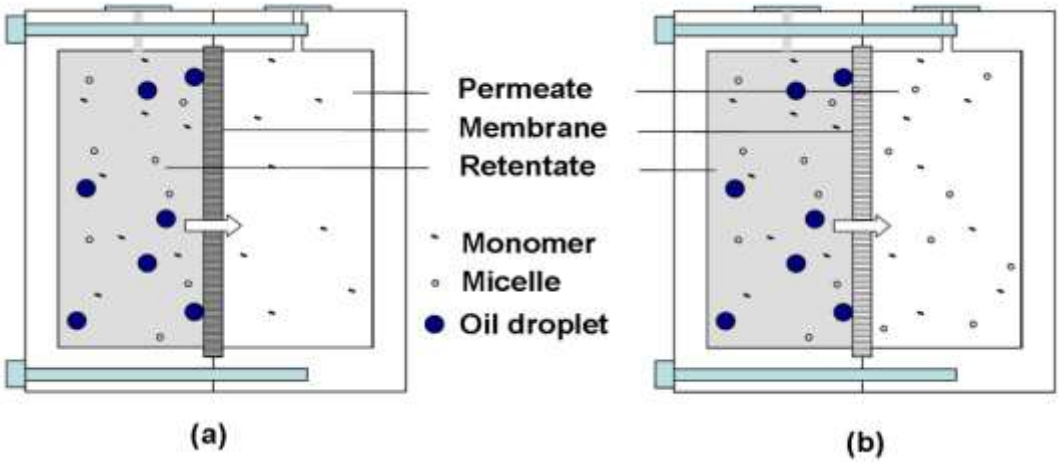
Partioning of Emulsifiers: Methods

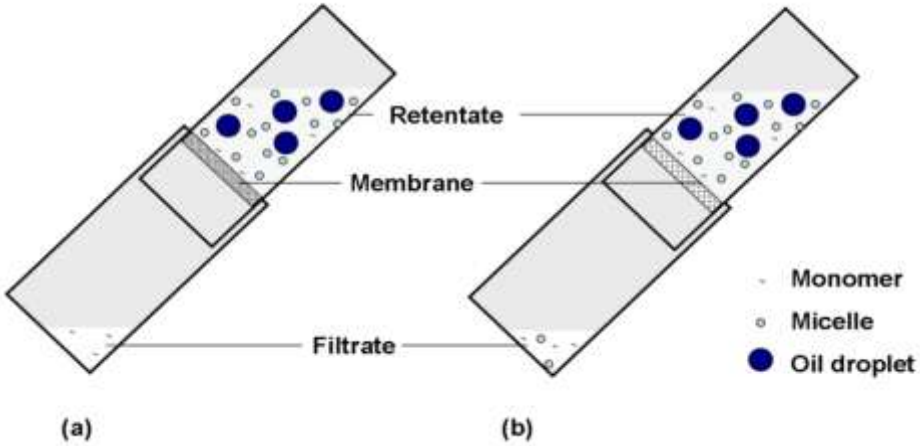
SANS as nondestructive (nonseparation) method. IFT analysis as results **p(r) - pair distance distribution function, radius of gyration R_g and scattering at "zero angle" I(0) .** (i) the trace scattering from large particles was approximated by the power law $q^{-\alpha}$ and (ii) the studied domains were spherical 3D objects. Best fits - when $\alpha = 4$, which correlates to a smooth, sharp interface of large emulsion aggregates.

$$c_{tot} = c_{int} + c_{mic} + c_{mon}$$

The total emulsifier concentration c_{tot} can be fractionated into the o/w interfacial concentration c_{int}, the micellar concentration c_{mic} and the monomer concentration c_{mon}.

Dialysis vs. Ultrafiltration of Emulsions

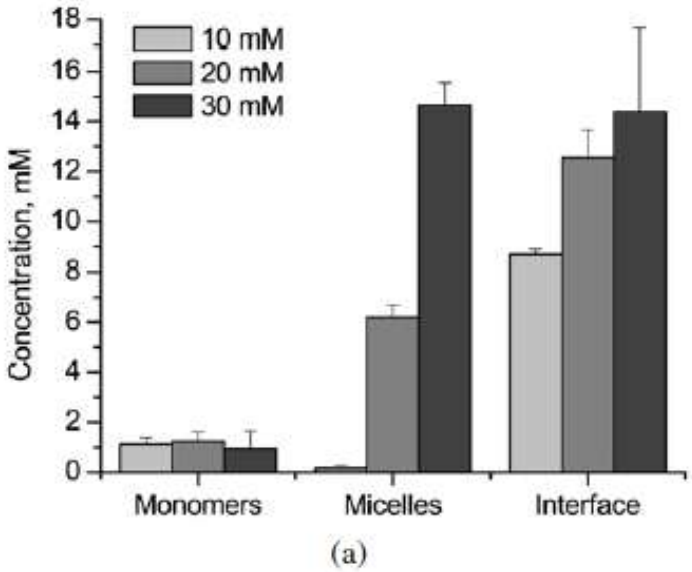




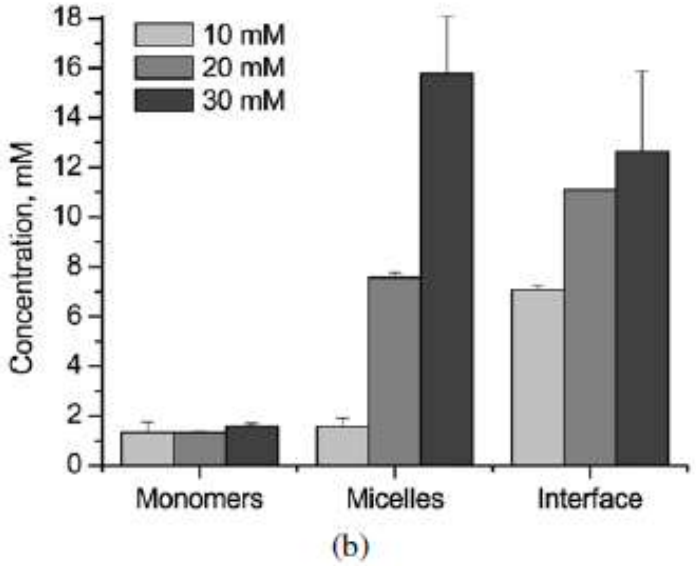
Partioning of Emulsifiers: SDS

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

Ultrafiltration



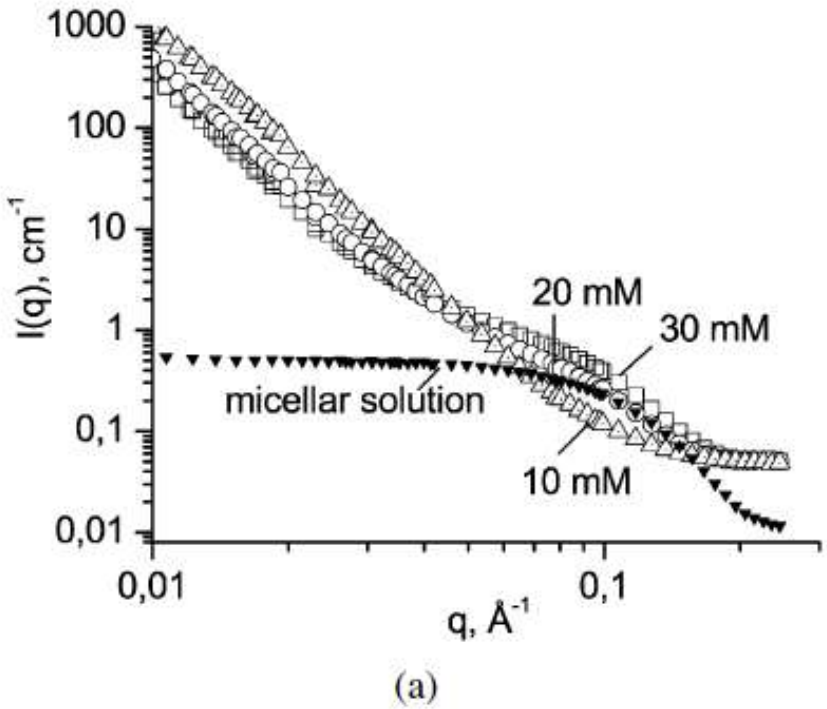
Dialysis

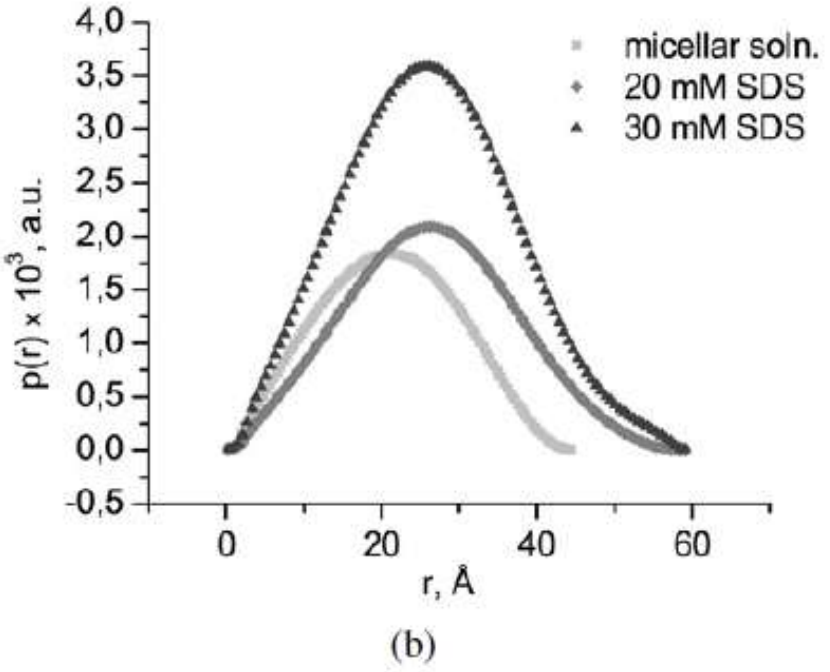


Partitioning of SDS in o/w emulsions determined by (a) ultrafiltration and (b) dialysis

- ✓ The monomer concentrations by UF were in the range of 1 mM (slightly above CMC) and by dialysis in the range of 1–2 mM (1-2xCMC).
- ✓ Micellar concentrations increased from 0.2 to 16.2 mM (UF) and 1.6-15.9 mM (dialysis).
- ✓ Interfacial SDS concentration rose with an increase of total SDS from 10 to 20 mM, but an increase of total SDS from 20 to 30mM did not affect the SDS concentration at the o/w interface.

Partioning of Emulsifiers: SDS by SANS



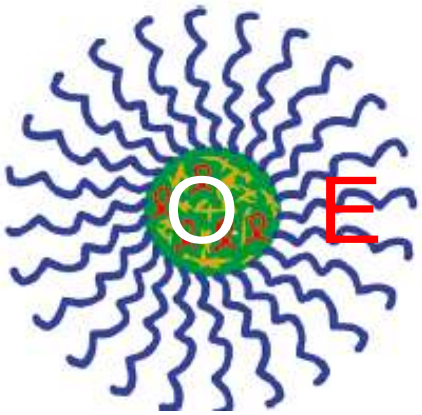


(a) Scattering curves and (b) pair distance distribution functions of emulsions with 10, 20 and 30 mM SDS and of a micellar solution of SDS in D_2O

Increasing SDS concentrations led to the appearance of a shoulder in high *q*-range - the presence of micelles

Partioning of Emulsifiers: Methods

Swollen micelle



Emulsifier shell (L) and oil core (R_{core}).

R_g and I(0)

$$R_{mic} = \sqrt{\frac{5}{3}} R_g, \qquad R_{core} = R_{mic} - L$$

$$V_{mic} = \frac{4}{3}\pi R_{mic}^3. \qquad V_{core} = \frac{4}{3}\pi R_{core}^3$$

$$\phi_{em} = \frac{V_{mic} - V_{core}}{V_{mic}} \qquad \phi_{oil} = 1 - \phi_{em}.$$

$$\Delta\rho_{mic} = \phi_{em}\Delta\rho_{em} + \phi_{oil}\Delta\rho_{oil}$$

$$I(0) = \eta \Delta\rho_{mic}^2 V_{mic}$$

$$c_{mic} = \frac{\phi_{em}\eta}{V_{mol} N_a}$$

Molecular volumes V_{mol}, scattering length densities per unit mass $\Delta\rho$, chain lengths L and cmc of SDS, CTAB and oil in deuterium buffer solution

	V_{mol} (Å^3)[a]	$\Delta\rho \times 10^{-10}$ (cm/g)[b]	L (Å)[c]	cmc (mmol/l)[d]
SDS	413	−5.95	16.7	0.8
CTAB	606	−5.76	21.7	0.096
Oil	1800	−6.63	–	–

Partioning of Emulsifiers: Micelle Structure

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

Fitted results of the SANS measurements

	$I(0)$ (cm^{-1})[a] mean ± s.d.[f]	R_g (Å)[a] mean ± s.d.[f]	R_{mic} (Å)[b] mean ± s.d.[f]	$\eta \times 10^3$[c] mean ± s.d.[g]	c_{mic} (mM)[d] mean ± s.d.[g]	c_{int} (mM)[e] mean ± s.d.[g]
SDS solution	0.57 ± 0.01	16.2 ± 0.1	20.9 ± 0.1			
Emulsions						
10 mM SDS	n.d.	n.d.	n.d.	0	0	9.2 ± 0.07
20 mM SDS	0.74 ± 0.04	20.3 ± 0.4	26.2 ± 0.5	2.75 ± 0.18	10.52 ± 0.69	8.68 ± 0.69
30 mM SDS	1.30 ± 0.04	20.3 ± 0.4	26.2 ± 0.5	4.83 ± 0.15	18.47 ± 0.59	10.73 ± 0.59
CTAB solution	1.69 ± 0.01	20.1 ± 0.1	25.9 ± 0.1			
Emulsions						
10 mM CTAB	n.d.	n.d.	n.d.	0	0	9.9 ± 0.01
20 mM CTAB	2.20 ± 0.1	27.9 ± 0.5	36.0 ± 0.6	3.32 ± 0.15	7.34 ± 0.04	12.56 ± 0.04
30 mM CTAB	3.3 ± 0.1	27.6 ± 0.4	35.6 ± 0.5	5.15 ± 0.16	11.85 ± 0.04	18.05 ± 0.04

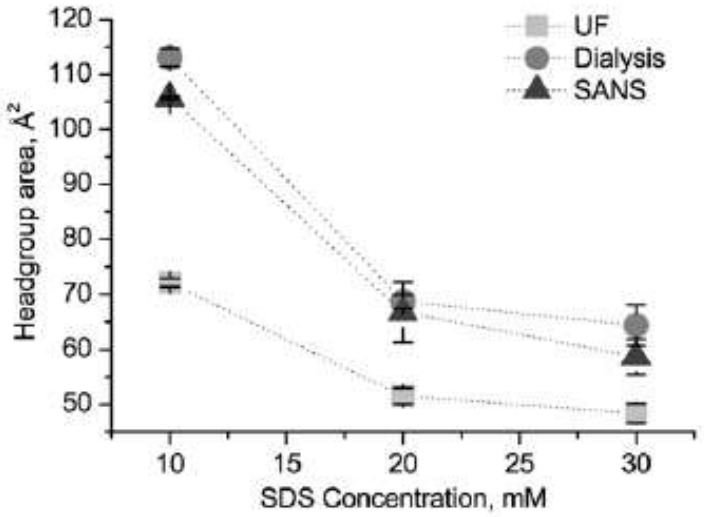
In the emulsion with 10 mM of emulsifier - no micelles were detected.

Radius of micelle in microemulsion is larger than in binary emulsifier/buffer system – incorporation of oil

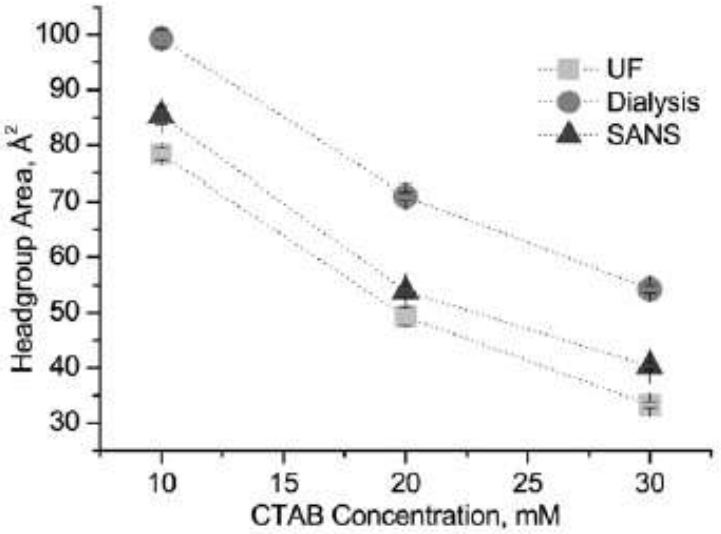
Area per Molecule at Oil Droplet Interface

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

SDS



CTAB



SANS has been successfully used for validation of dialisys and UF

Ultra filtration method is considered to be more accurate method - due to better control

Oehlke K., Garamus V. M., Heins A., Stöckmann H., Schwarz K.
"The partitioning of emulsifiers in o/w emulsions: A comparative study of SANS, ultrafiltration and dialysis"
Journal of Colloid and Interface Science, 2008, v. 322, P. 294–303

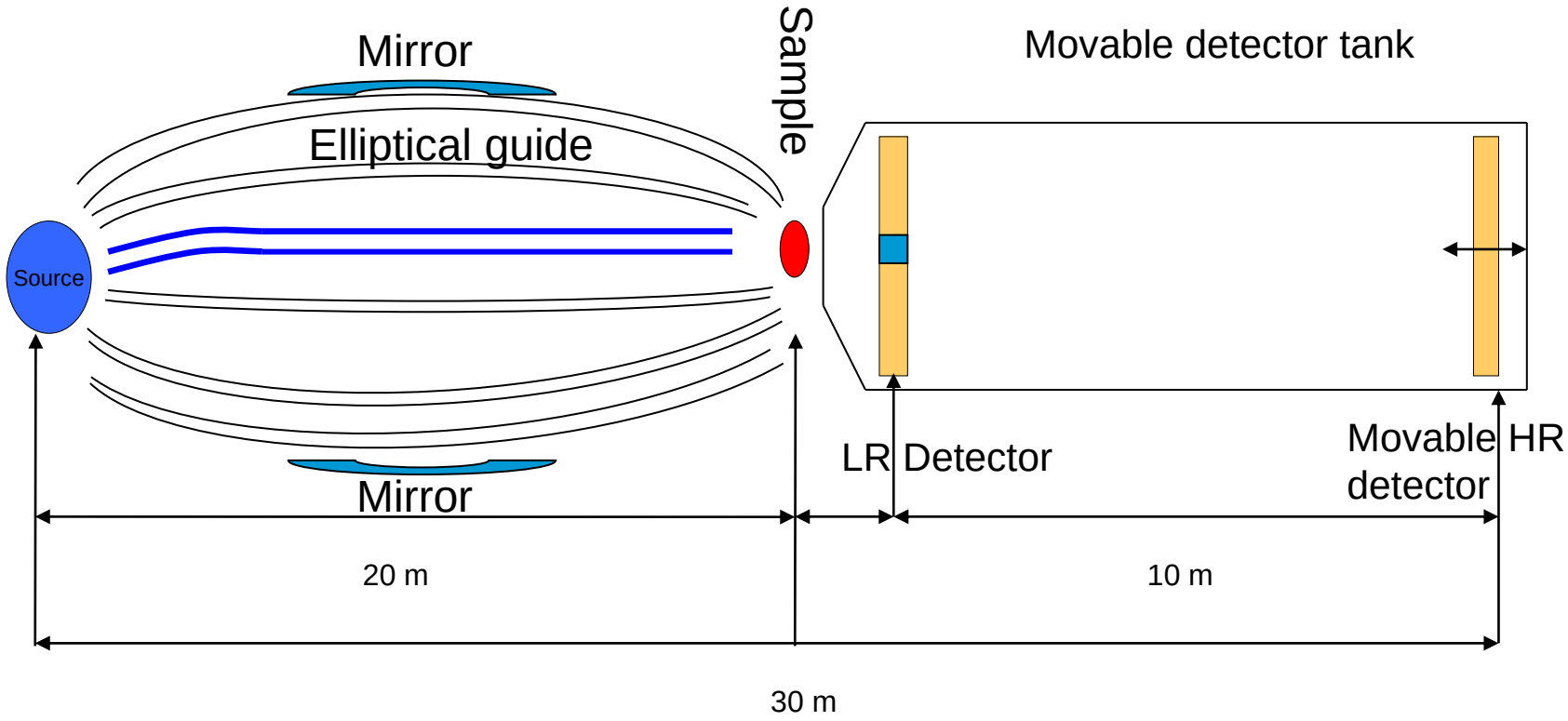
Small Sample SANS at ESS

Helmholtz-Zentrum Geesthacht
Zentrum für Material- und Küstenforschung

Beam sizes of 2 x 2 mm^2 or below (reduction to 50 x 50 μm^2)

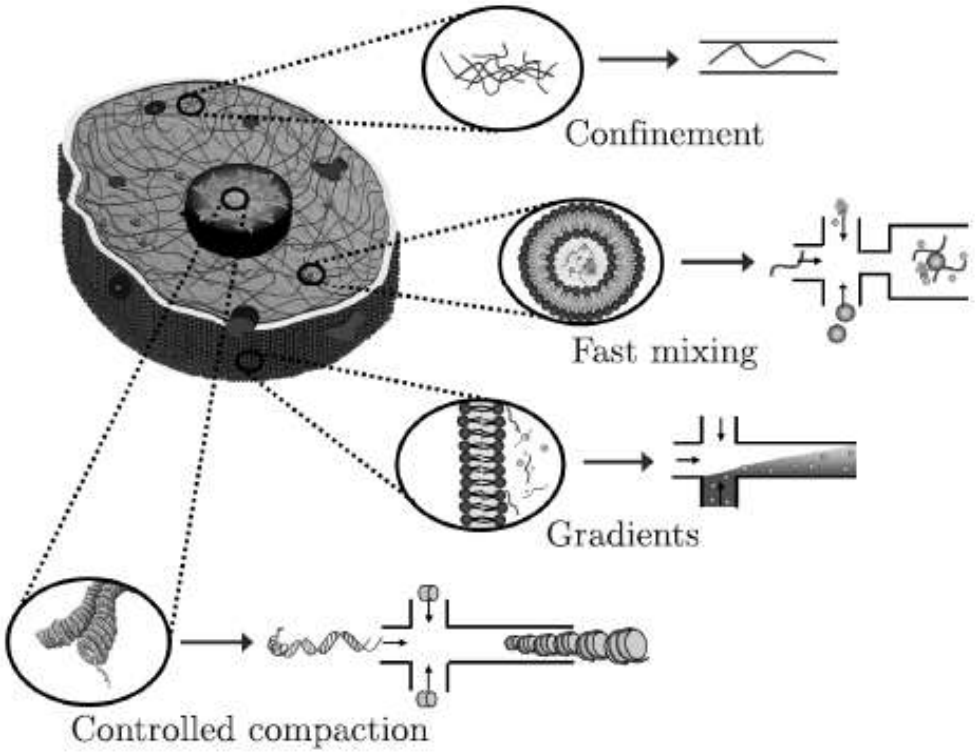
Q-range from 10^{-3} - 1 $Å^{-1}$

Speed of measurements as normal size sample (10x10 mm^2) at ILL

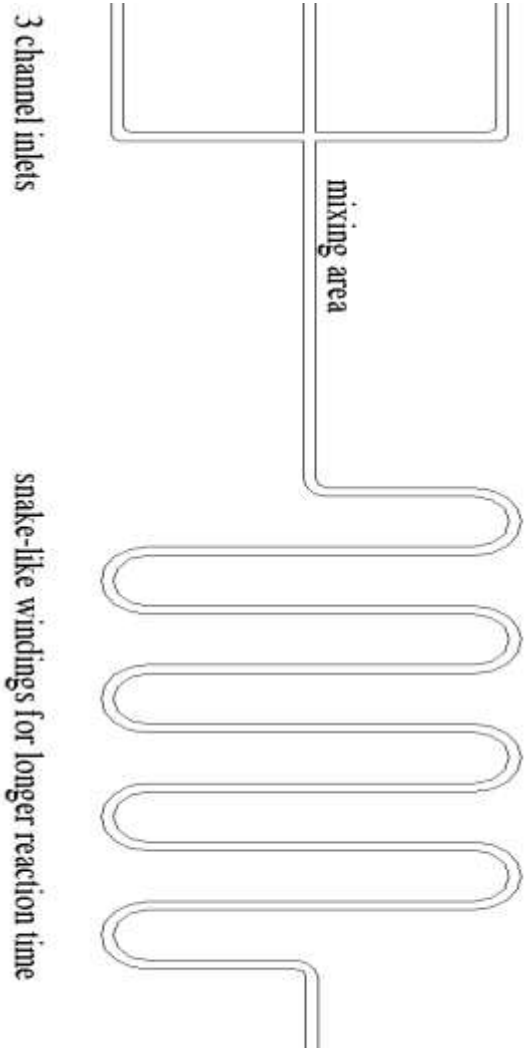


Sample position

Micro fluid chip



Evans et al., Bull. Pol. Ac.: Tech., 2007, 55, 217



Diameter of channel ~ 100 µm