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Lab Materials & Supplies

Detergents

A guide to the properties and uses of detergents in biological systems

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MERCK

MilliporeSigma is pleased to offer readily biodegradable surfactants as effective alternatives to Alkyl Phenol Ethoxylates (APEs).

Our new APEs product portfolio includes:

TRITON™ CG-110

Cat. No.	Description	Form	Mol wt	CMC	Transition Temp.	Pour Point	HLB
STS0005 – 100 mL STS0005 – 500 mL	nonionic	liquid	no test data available	1748 ppm (25°C)	cloud point: >100 °C (1 wt% actives aq solution)	-15 °C	

TERGITOL™ 15-S-40

Cat. No.	Description	Form	Mol wt	CMC	Transition temp	Pour Point	HLB
STS0002 – 100 g STS0002 – 500 g	nonionic	solid	1960 g/mol (approx.)	1314 ppm (25 °C)	cloud point: > 100 °C (1 wt% actives aq solution)	43 °C	18

ECOSURF™ EH-9

Cat. No.	Description	Form	Mol wt	CMC	Transition temp	Pour Point	HLB
STS0006 – 100 mL STS0006 – 500 mL	nonionic	liquid	no data available	1066 ppm (25 °C)	cloud point: 61 °C (10 wt% actives aq solution)	16 °C	12.5

TERGITOL™ 15-S-40 (70% AQ)

Cat. No.	Description	Form	Mol wt	CMC	Transition temp	Pour Point	HLB
STS0003 – 100 mL STS0003 – 500 mL	nonionic	liquid	1,960 g/mol (calculated)	1314 ppm (25 °C)	cloud point: > 100 °C (1 wt% actives aq solution)	5 °C	18

ECOSURF™ EH-9 (90% AQ)

Cat. No.	Description	Form	Mol wt	CMC	Transition temp	Pour Point	HLB
STS0012 – 100 mL STS0012 – 500 mL	nonionic	liquid	no data available	1066 ppm (25 °C)	cloud point: 64 °C (10 wt% actives aq solution)	-5 °C	12.5

TERGITOL™ TMN-100X (90% AQ)

Cat. No.	Description	Form	Mol wt	CMC	Transition temp	Pour Point	HLB
STS0004 – 100 mL STS0004 – 500 mL	nonionic	liquid	570 g/mol (calculated)	830 ppm (25 °C)	cloud point: 65 °C (1 wt% actives aq solution)	-6 °C	14.1

ECOSURF™ SA-9

Cat. No.	Description	Form	Mol wt	CMC	Transition temp	Pour Point	HLB
STS0007 – 100 mL STS0007 – 500 mL	nonionic	liquid	no data available	22 ppm (25 °C)	cloud point: 57 °C (1 wt% actives aq solution)	4 °C	11-13

TERGITOL™ 15-S-30

Cat. No.	Description	Form	Mol wt	CMC	Transition temp	Pour Point	HLB
STS0001 – 100 g STS0001 – 500 g	nonionic	solid	1,520 g/mol (calculated)	558 ppm (25 °C)	cloud point: >100 °C (1 wt% actives aq solution)	39 °C	17.4

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What are Detergents?

Detergents are amphipathic molecules that contain polar or charged hydrophilic groups (heads) at the end of long lipophilic hydrocarbon groups (tails) (**Figure 1**). They are also known as surfactants because they decrease the surface tension of water.

Figure 1. Structure of the anionic detergent sodium dodecyl sulfate (SDS), showing the hydrophilic and hydrophobic regions.

In contrast to purely polar or non-polar molecules, amphipathic molecules exhibit unique properties in aqueous media. Their polar groups form hydrogen bonds with water molecules, while the hydrocarbon chains aggregate due to hydrophobic interactions. At low concentrations, detergents exist as monomers in water. At a sufficiently high concentration, the **critical micelle concentration (CMC)**, the monomers start organizing themselves into non-covalent aggregates called micelles.[1-3] In **micelles**, the polar hydrophilic region of each detergent molecule is oriented toward the polar solvent (water) while the hydrocarbon regions form thermodynamically stable hydrophobic cores. **Figure 2** is a simple illustration of a micelle to demonstrate the orientation concept. Actual micelle structures are more complex and dynamic, and can change due to detergent concentration and solution composition.[4]

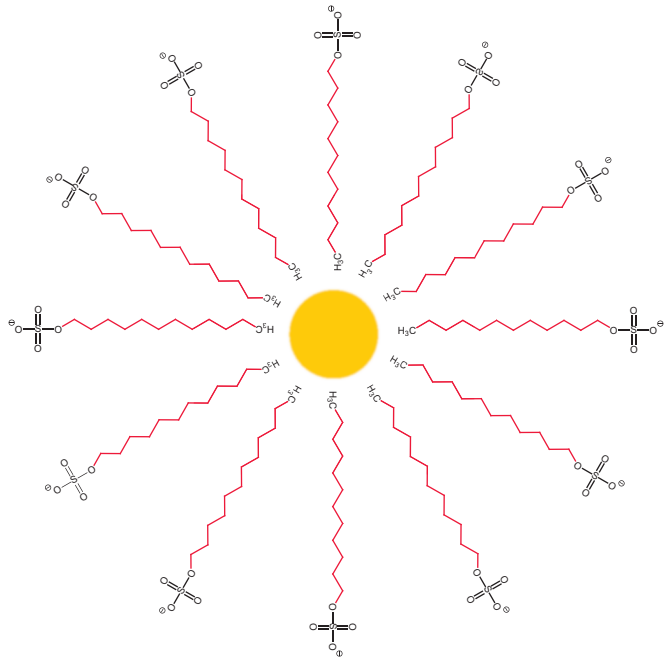


Figure 2. Simple illustration of an SDS micelle.

How Do Detergents Solubilize Membrane Proteins?

Detergents are widely used in biochemistry, cell biology and molecular biology. Common applications include cell lysis, solubilization of membrane proteins and lipids, protein crystallization, and reduction of background staining in blotting experiments.

Even though studying membrane proteins is a major challenge in protein biochemistry, they remain an important area of study due to their significant biological and pharmacological relevance. Understanding the structure and function of membrane proteins requires their careful isolation in the native form in a highly purified state. Since micelle-forming detergents provide an amphipathic environment that can mimic lipid bilayers, their use as solubilizing agents is essential for functional and structural investigations.

Biological membranes consist of phospholipids that contain two hydrophobic groups connected to a polar head. This molecular architecture allows phospholipids to assemble as lipid bilayers in which the hydrophobic chains face each other while the polar head groups face outward to the aqueous milieu (**Figure 3**). Proteins and lipids are embedded in this bilayer forming the **fluid mosaic model** (**Figure 4**) which was first proposed by Singer and Nicolson in 1972. Proteins are held in the lipid bilayer by hydrophobic interactions between the lipid tails and hydrophobic protein domains.[5] These **integral membrane proteins (IMP's)** (**Figure 4**) are not soluble in aqueous solutions as they aggregate to protect their hydrophobic domains, but are soluble in detergent solutions as micelles formed by detergents are analogous to the bilayers of the biological membranes.[6] Proteins are incorporated into these micelles via hydrophobic interactions. Hydrophobic regions of membrane proteins, normally embedded in the membrane lipid bilayer, are now surrounded by a layer of detergent molecules and the hydrophilic regions are exposed to the aqueous medium. This keeps the membrane proteins in solution. Complete removal of detergent could result in aggregation due to the clustering of hydrophobic regions and, hence, may cause precipitation of membrane proteins.[7]

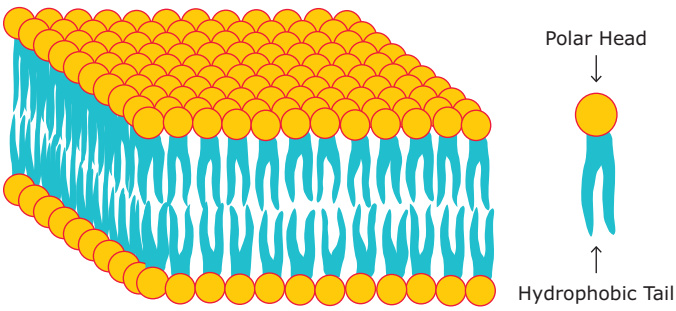


Figure 3. A phospholipid bilayer.

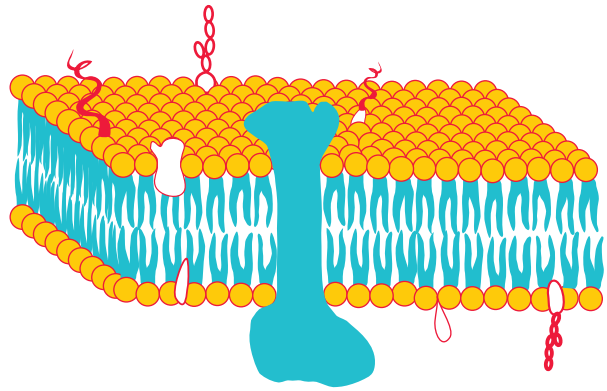


Figure 4. Fluid-mosaic model of a biological membrane.

Membrane solubilization by detergents can be described as a three stage process where the detergent-lipid-protein ratio is an important factor (**Figure 5**)[8]

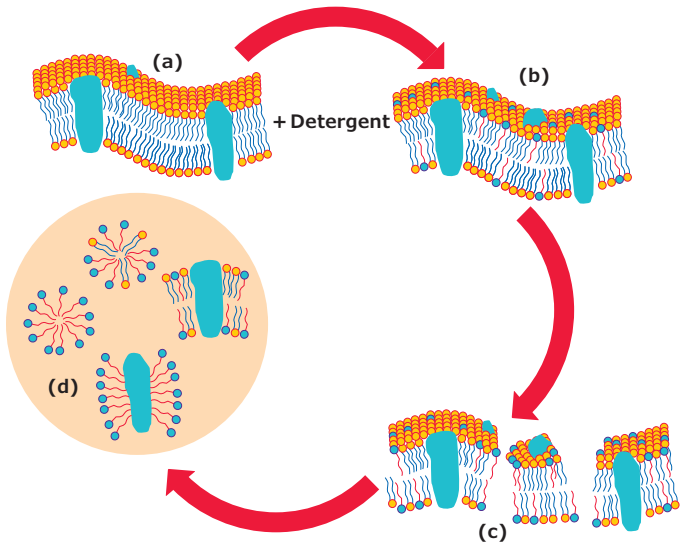


Figure 5. Stages in the solubilization of biological membranes by detergents. When low concentrations of a detergent are added to biological membranes (a), the detergent monomers (shown in red with single tails) perturb the membrane structurally by partitioning into the lipid bilayer (b). At concentrations equal to, or higher than the detergent's CMC, the lipid bilayer becomes saturated with detergent molecules and breaks apart generating lipid-protein-detergent mixed micelles (c).[8] A detergent/protein ratio of around 1-2 (w/w) is believed to be sufficient to solubilize IMPs to form lipid-protein-detergent mixed micelles.[9] A further increase of detergent concentration causes progressive delipidation of the lipid-protein-detergent mixed micelles. This leads to the formation of lipid/detergent and protein/detergent mixed micelles (d).[8] A solubilized IMP in a complex with a bound detergent is called a protein-detergent complex, PDC.[10] Typically, a detergent/protein ratio of around 10 (w/w) or higher will lead to complete delipidation.[11]

Classification of Detergents

Based on their structure, detergents can be broadly classified as:[12]

- Ionic
- Zwitterionic
- Non-ionic

Ionic Detergents

Ionic detergents contain anionic or cationic head groups and possess a net charge. Their hydrophobic tails are either straight hydrocarbon chains, as in sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB), or rigid steroidal groups, as in bile acid salts.[13] The size of an ionic detergent micelle is determined by the combined effect of hydrophobic attraction of the side chains and the repulsive forces of the ionic groups. Consequently, neutralizing the charge on the head group with increasing concentrations of a counter ion leads to a larger micellar size. Micellar size also increases with the increase in alkyl chain length. Ionic detergents are extremely effective in the solubilization of membrane proteins but are almost always denaturing to some extent.[6]

Bile acid salts are anionic detergents with backbones consisting of rigid steroidal groups, e.g., sodium salts of cholic acid and deoxycholic acid. Because of their planar structure, these molecules have a polar and a nonpolar face; as a result, their CMC's are high and their micelles are small, which makes them easy to remove by dialysis.[14] Bile acids are relatively mild detergents and are often less deactivating than linear-chain detergents with the same head group.[15] Unconjugated bile acid monomers have pKa values of approximately 5 - 6 and limited solubility at low pH values. However, conjugation of bile acids reduces the pKa and leads to a larger fraction of ionized molecules at any given pH. Since the ionized salt form is more soluble in water than the protonated acid form, conjugation enhances solubility at a low pH.[16]

Non-ionic Detergents

Non-ionic detergents contain uncharged, hydrophilic head groups that consist of either polyoxyethylene moieties, as in BRIJ® and TRITON™ Detergents, or glycosidic groups, as in octyl glucoside and dodecyl maltoside. Since non-ionic detergents break lipid-lipid and lipid-protein, but not protein-protein interactions, they are considered non-denaturing.[17] Thus, they are widely used in the isolation of membrane proteins in their biologically active form. Unlike ionic detergents, salts have minimal effect on the micellar size of non-ionic detergents.[6]

Detergents with polyoxyethylene head groups may contain alkylpolyethylene ethers with the general formula $C_nH_{2n+1}(OCH_2CH_2)_xOH$, or a phenyl ring between the alkyl chain and the ether group. TRITON™ X-100 detergent belongs to the latter class. It should be noted that detergents containing aromatic rings absorb in the ultraviolet region. They may interfere with spectrophotometric monitoring of proteins at 280 nm. Hydrogenated versions of these detergents are available, in which the aromatic rings are reduced to eliminate UV absorption. However, small amounts of unreacted material may be present in such reduced preparations. Alternatively, commercially available polyoxyethylene detergents with aliphatic hydrophobic moieties may be substituted for some aromatic polyoxyethylenes in certain applications. For example, TERGITOL™ 15-S-9, TERGITOL™ TMN-10, polyoxyethylene 10 lauryl ether, and polyoxyethylene 10 tridecyl ether may be generally substituted for TRITON™ X-100 in applications where UV invisibility is important. These detergents possess aliphatic hydrophobic moieties and therefore do not absorb significantly in the ultraviolet region.

Although polyoxyethylenes have significant cost advantage over synthetic non-ionic detergents such as the alkyl glycosides, the latter remain the detergents of choice in many applications for two main reasons. First, they are homogeneous with respect to their composition and structure. Second, several variations of alkyl glycosides containing different combinations of the hydrocarbon chain and the polar sugar group can be easily synthesized in pure forms. Subtle differences in the physicochemical properties of alkyl glycosides bearing various alkyl chains, attached to a glucose, maltose, or a sucrose head group, can be exploited for selective solubilization of membrane proteins.[18]

Zwitterionic Detergents

Zwitterionic detergents have characteristics of both ionic and non-ionic types. Like non-ionic detergents, the zwittergents do not possess a net charge, they lack conductivity and electrophoretic mobility, and do not bind to ion-exchange resins. However, like ionic detergents, they are efficient at breaking protein-protein interactions. Steroid-based zwittergents such as CHAPS are less denaturing than linear-chain zwitterionic detergents (e.g., dodecyldimethyldiamine oxide).[15]

Detergent Selection Table

Name	Description	CAS	MW (g/mol)	CMC
Anionic Detergents				
Alkyl Sulfates				
LDS, Lithium dodecyl sulfate	BioReagent, for molecular biology, suitable for electrophoresis, ≥ 98.5% (GC)	2044-56-6	272.33	8.2 mM
Niaproof® 4	~27% in H_2O	139-88-8	316.43	2.1 mM
SDS, Sodium dodecyl sulfate	≥ 98.0% (GC), dust-free pellets	151-21-3	288.38	7-10 mM
Sodium octyl sulfate	≥95%	142-31-4	232.27	
Alkyl Sulfonates				
1-Octanesulfonic acid sodium salt	BioXtra	5324-84-5	216.27	
Sodium 1-butanesulfonate	anionic detergent	2386-54-1	160.17	
Sodium 1-decanesulfonate	≥99.0% (T)	13419-61-9	244.33	
Sodium 1-heptanesulfonate	ion-pairing reagent for HPLC	22767-50-6	202.25	
Sodium hexanesulfonate	≥98% (elemental analysis)	2832-45-3	188.22	
Sodium pentanesulfonate	≥95% (elemental analysis)	22767-49-3	174.19	
Bile Acids and Salts				
Chenodeoxycholic acid	≥97% (TLC)	474-25-9	392.57	3 mM
Cholic acid	from ox or sheep bile, ≥98% (TLC)	81-25-4	408.57	
Deoxycholic acid	BioXtra, ≥98% (HPLC)	83-44-3	392.57	2-6 mM
Glycocholic acid hydrate	synthetic, ≥ 97% (TLC)	1192657-83-2	465.62	7.1 mM
Sodium cholate hydrate	Bioreagent, suitable for cell culture, ≥99% (HPLC)	206986-87-0	430.55	9-15 mM
Sodium deoxycholate	BioXtra, ≥98.0% (dry matter, NT)	302-95-4	414.55	2-6 mM
Sodium deoxycholate monohydrate	BioUltra, ≥99.0% (NT)	145224-92-6	432.57	2-6 mM
Sodium glycochenodeoxycholate	≥97% (HPLC)	16564-43-5	471.61	
Sodium glycocholate hydrate	≥ 97% (TLC)	338950-81-5	487.6	13 mM
Sodium glycodeoxycholate	BioXtra, ≥97% (TLC)	16409-34-0	471.61	2.1 mM
Sodium taurochenodeoxycholate	≥ 95% (TLC)	6009-98-9	521.69	4 mM
Sodium taurocholate hydrate	BioXtra, ≥95% (TLC)	345909-26-4	537.68	3-11 mM
Sodium taurodeoxycholate hydrate	BioXtra, ≥ 97% (TLC)	207737-97-1	521.69	1-4 mM
Sodium tauroursodeoxycholate	≥ 95% (TLC)	14605-22-2	521.69	4 mM
Ursodeoxycholic acid	≥ 99% (TLC)	128-13-2	392.57	7 mM
Other Anionic Detergents				
Docusate sodium salt	BioUltra, ≥99.0% (TLC)	577-11-7	444.56	
N-Lauroylsarcosine	≥ 95% (titration)	97-78-9	271.4	14.6 mM
N-Lauroylsarcosine sodium salt	30% aqueous solution, ≥ 97.0% (HPLC)	137-16-6	293.38	14.6 mM
Cationic Detergents				
Alkyltrimethylammonium bromide	≥95% (TLC)	8044-71-1		
Benzalkonium chloride	BioXtra	63449-41-2		
Benzethonium chloride	≥98% (HPLC)	121-54-0	448.08	
Benzyldimethylhexadecylammonium chloride	cationic detergent	122-18-9	396.09	
CTAB, Hexadecyltrimethylammonium bromide	for molecular biology, ≥ 99% (titration)	57-09-0	364.45	0.92 mM
Dimethyldioctadecylammonium bromide	≥98% (TLC)	3700-67-2	630.95	
Dodecyltrimethylammonium bromide	≥98%	1119-94-4	308.34	
Ethylhexadecyldimethylammonium bromide	≥98% (non-aqueous titration)	124-03-8	378.47	
Hexadecylpyridinium chloride monohydrate	meets USP testing specifications	6004-24-6	358.00	
Methylbenzethonium chloride	cationic detergent	25155-18-4	462.11	
TTAB, Tetradecyltrimethylammonium bromide	for ion pair chromatography, ≥99.0% (AT)	1119-97-7	336.39	4-5 mM

Aggregation Number	HLB	Cloud Point (°C)	Average Micellar Weight	Diagnostic Applications	Molecular Biology	Cell Culture	Electrophoresis/ Chromatography	Membrane Protein Solubilization	Enzymology	Antigen/Vaccine Preparation	Drug Delivery/ Liposomes	Mass Spectrometry	Microbiology
62		>100	17,000	•		•	•	•		•			
													•
62	40	>100	18,000	•	•	•	•	•	•	•	•		•
							•						
							•						
							•						
							•						
							•						
							•						
						•		•			•		
				•	•	•	•	•	•	•	•		
3-12	16		1,200-5,000	•	•	•	•	•		•	•		•
2.1			1,000			•				•			
2-3	18		900-1,300	•	•	•	•	•	•	•	•		
3-12	16		1,200-5,000	•	•	•	•	•		•	•		•
3-12	16		1,200-5,000	•	•	•	•	•		•	•		•
						•							
1.9			900			•				•			
2			900			•		•	•				
	20.1									•	•		
4			2,100			•	•	•					
6			3,100	•		•	•	•					
	20.1										•		
						•							
							•	•			•		
2			600		•		•	•					•
2			600		•		•	•					•
													•
						•							•
													•
											•		•
170	10		62,000	•	•	•					•		•
					•					•			
													•
													•
								•	•				•
						•							•
80			27,000	•	•				•				•

Detergent Selection Table (cont.)

Name	Description	CAS	MW (g/mol)	CMC
Non-Ionic Detergents				
1-Oleoyl-rac-glycerol	≥99.0% (TLC)	111-03-5	356.54	
Brij® 58	average Mn ~ 1124	9004-95-9	1122.0 (avg.)	0.004 mM
Brij® L23	Solution, 30 % (w/v) in H_2O	9002-92-0	1198.0 (avg.)	0.091 Mm
Brij® L4	average Mn ~362	9002-92-0	362 (avg.)	
Brij® O10	non-ionic surfactant	9004-98-2	709.00	
CYMAL-2®	≥99.0% (TLC)	260804-65-7	452.49	120 mM
CYMAL-5®	≥98.0% (TLC)	250692-65-0	494.57	2.4 - 5 mM
CYMAL-6®	≥99.0% (TLC)	228579-27-9	508.60	0.56 mM
Decaethylene glycol monododecyl ether	nonionic surfactant	9002-92-0	626.86	
Decyl β-D-glucopyranoside	≥98% (GC)	58846-77-8	320.42	2.2 mM
Decyl β-D-maltopyranoside	≥98% (GC)	82494-09-5	482.56	1.8 mM
Deoxy-BigCHAP, Big CHAP Deoxy	≥90% (HPLC)	86303-23-3	862.06	1.4 mM
Digitonin	free of water-insoluble constituents	11024-24-1	1229.31	<0.5 mM
Genapol® C-100	Major component: Polyoxyethylene (10) dodecyl ether	61791-13-7	627.0	0.075 mM
Genapol® X-080	Isotridecyl alcohol polyglycol ether with 8 EO	9043-30-5	552.78	0.06-0.15 mM
Genapol® X-100	Isotridecyl alcohol polyglycol ether with 10 EO	9043-30-5	641.0	0.15 mM
HECAMEG, Methyl 6-O-(N-heptylcarbamoyl)-α-D-glucopyranoside	≥ 97.0% (TLC)	115457-83-5	335.39	19.5 mM
Igepal® CA-630	nonionic, non-denaturing detergent	9002-93-1	603.0 (avg.)	0.083 mM
Igepal® CA-720	average Mn ~735	9036-19-5	734.95 (avg.)	
Kolliphor® P 188	oxyethylene, 79.9-83.7%	9003-11-6	7680-9510 (avg.)	17.9 mM
Kolliphor® P 407	oxyethylene 71.5-74.9 %	9003-11-6	9840-14600 (avg.)	3.97 mM
Kolliphor® EL	pH-range 6.0 - 8.0	61791-12-6	~ 2500	
MEGA-8, N-Octanoyl-N-methylglucamine	≥97% (GC)	85316-98-9	321.41	79 mM
MEGA-9, N-Nonanoyl-N-methylglucamine	≥98% (GC)	85261-19-4	335.44	25 mM
MEGA-10, N-Decanoyl-N-methylglucamine	≥ 98% (GC)	85261-20-7	349.46	6-7 mM
Methoxypolyethylene glycol 350	average mol wt 350	9004-74-4	350 (avg)	
N,N-Dimethyldodecylamine N-oxide	BioXtra, ≥99.0% (NT)	1643-20-5	229.40	1-2 mM
n-Dodecyl β-D-maltoside	BioXtra, ≥ 98% (GC)	69227-93-6	510.62	0.17 mM
n-Heptyl β-D-thioglucopyranoside	≥ 99% (GC)	85618-20-8	294.41	29 mM
n-Hexadecyl β-D-maltoside	≥ 99.0% (TLC)	98064-96-1	566.72	0.0006 mM
n-Nonyl-β-D-Glucopyranoside	≥97.0% (GC)	69984-73-2	306.40	6.5 Mm
n-Nonyl-β-D-thiomaltoside	≥ 98.0% (TLC)	148565-55-3	484.60	3.2 Mm
n-Octyl-β-D-maltoside	≥ 99.0% (HPLC)	82494-08-4	454.51	23.4 mM
n-Octyl-β-D-thioglucopyranoside	≥ 98.0% (GC)	85618-21-9	308.43	9 mM
n-Octyl-b-D-Glucopyranoside	≥98% (HPLC), 50 % (w/v) solution in H_2O	29836-26-8	292.37	18-20 mM
Nonaethylene glycol monododecyl ether	nonionic surfactant	3055-99-0	582.81	0.05 mM
Nonidet™ P40 Substitute	BioXtra, mixture of 15 homologues	9016-45-9	680 (avg.)	0.059 mM
Nonylphenyl-polyethyleneglycol acetate	for histology	54612-40-7	308.46	
Octaethylene glycol monododecyl ether	BioXtra, ≥98.0% (GC)	3055-98-9	538.75	0.11 mM

Aggregation Number	HLB	Cloud Point (°C)	Average Micellar Weight	Diagnostic Applications	Molecular Biology	Cell Culture	Electrophoresis/ Chromatography	Membrane Protein Solubilization	Enzymology	Antigen/Vaccine Preparation	Drug Delivery/ Liposomes	Mass Spectrometry	Microbiology
											•		
70	16	>100	79,000		•	•	•	•	•				
40	16.9	>100	48,000	•		•	•	•	•		•		
	9.7												
	12.4												
104			47,000				•	•					
47			23,200				•	•					
91			46,300				•	•					
							•	•					
							•	•					
69			33,300					•					
8-16			6,900 - 13,800					•					
60			70,000	•	•	•	•	•	•				
	14	89 – 92						•	•				
	13	>45						•					
88	13-14	82	56,000					•					
92			30,900					•					
100-155	13.4	63-67	60,300 - 93,500	•	•	•	•	•	•	•	•		
	14.6	85-90		•	•	•	•	•	•	•	•		
	>24	>100									•		
	18-23	>100									•		
	12-14										•		
				•		•		•	•		•		
				•		•		•	•		•		
				•		•		•	•	•	•		
76			17,400					•					
78-149			40,000-76,000	•		•	•	•	•	•	•		
27			8,000					•					
							•	•					
133			40,800				•	•					
								•					
84			38,000					•					
189			58,300			•		•	•				
27-100		≥100	7,900-29,200	•		•	•	•	•	•	•		
110			64,000		•			•					
132	13.5	45-50	90,000	•	•	•	•	•	•	•	•		
						•							
123			66,000				•	•	•				

Detergent Selection Table (cont.)

Name	Description	CAS	MW (g/mol)	CMC
Pluronic® F-127	powder, BioReagent, suitable for cell culture	9003-11-6	12600 (avg.)	3.97 mM
Pluronic® F-68	10%, sterile-filtered solution, BioReagent, suitable for insect cell culture	9003-11-6	8400 (avg.)	17.9 mM
Poloxamer 407	purified, non-ionic	9003-11-6	9840-14600 (avg.)	3.97 mM
Poly(ethylene glycol)	average mol wt 200	25322-68-3	200 (avg.)	
Polyoxyethylene (10) tridecyl ether	mixture of C11 to C14 iso-alkyl ethers with C13 iso-alkyl predominating	78330-21-9		
Polyoxyethylene (40) stearate	non-ionic surfactant	9004-99-3		
Polysorbate 20	tested according to Ph.Eur.	9005-64-5	1228 (avg.)	0.059
Polysorbate 60	tested according to Ph.Eur.	9005-67-8		0.0055 - 0.022 mM
Polysorbate 80	tested according to Ph.Eur.	9005-65-6	1310 (avg.)	0.012 mM
Saponin	for molecular biology, used as non-ionic surfactant, adjuvant	8047-15-2	1000-2000	0.001-0.01%
Span® 20	≥ 44.0 % (GC)	1338-39-2	346.47	
Span® 60	45 - 55 % (GC)	1338-41-6	430.63	
Span® 65	nonionic surfactant	26658-19-5	963.55	
Span® 80	nonionic surfactant	1338-43-8	428.62	
Span® 85	nonionic surfactant	26266-58-0	957.52	
Sucrose monolaurate	BioXtra, ≥ 97.0% (TLC)	25339-99-5	524.6	0.3 mM
Synperonic® PE/P84	≤1.0% water	9003-11-6		
TERGITOL™	MIN FOAM 1x	68551-14-4	640	34 ppm
TERGITOL™ NP-7	Type NP-7	127087-87-0	528	39 ppm
TERGITOL™ NP-9	Type NP-9	127087-87-0	616	60 ppm
TERGITOL™ NP-10	Type NP-10	127087-87-0	682	55 ppm
TERGITOL™ NP-40 (70%)	Type NP-40, 70% in H_2O	127087-87-0	1980	232 ppm
TERGITOL™ 15-S-7	Type 15-S-7	68131-40-8	508	38 ppm
TERGITOL™ 15-S-9	Type 15-S-9	68131-40-8	596	52 ppm
TERGITOL™ 15-S-30	Type 15-S-30	84133-50-6	1520	558 ppm
TERGITOL™ 15-S-40	Type 15-S-40	84133-50-6	1960	783 ppm
TERGITOL™ 15-S-40 (70%)	Type 15-S-40, 70% in H_2O	84133-50-6	1960	1314 ppm
TERGITOL™ TMN 6 (90%)	TMN-6, 90% in H_2O	60828-78-6	538	800 ppm
TERGITOL™ TMN 10 (90%)	TMN-10, 90% in H_2O	60828-78-6	683 (avg.)	1313 ppm
TERGITOL™ TMN-100X (90%)	TMN-100X, 90% in H_2O	60828-78-6	570	930 ppm
Tetraethylene glycol monododecyl ether	BioXtra, ≥98.0% (GC)	5274-68-0	362.54	
Tetramethylammonium hydroxide pentahydrate	≥97% (titration)	10424-65-4	181.23	
Thesit®	for membrane research	9002-92-0	583 (avg.)	0.09 mM
TRITON™ X-100	BioUltra, for molecular biology, ~10% in H_2O	9002-93-1	647.0 (avg.)	0.23 mM
TRITON™ X-100 reduced	Hydrogenated to reduce UV absorbance	92046-34-9	564.8	0.2-0.9 mM
TRITON™ X-102	non-ionic detergent	9002-93-1	757	0.33 mM
TRITON™ X-114	≤0.001% peroxides (as H_2O_2), ≤1% water	9036-19-5	536.0 (avg.)	0.2 mM
TRITON™ X-114, reduced	reduced, ≥ 99%	92046-34-9	564.79	
TRITON™ X-165 solution	69.0 - 71.0 % wt solids content	9036-19-5	911	0.43 mM
TRITON™ X-305 solution	70% in H_2O	9002-93-1	1526.0 (avg.)	0.65 mM
TRITON™ X-405 solution	70% in H_2O	9036-19-5	1967.0 (avg.)	0.81 mM

Aggregation Number	HLB	Cloud Point (°C)	Average Micellar Weight	Diagnostic Applications	Molecular Biology	Cell Culture	Electrophoresis/ Chromatography	Membrane Protein Solubilization	Enzymology	Antigen/Vaccine Preparation	Drug Delivery/ Liposomes	Mass Spectrometry	Microbiology
	18–23	>100				•					•		
	>24	>100		•		•		•			•		
	18-23	>100									•		
								•	•				
											•		
	16.7	76		•		•	•	•	•	•	•		
	14.9										•		
58	15	65	79,000	•		•	•	•	•		•		
				•	•	•				•	•		
	8.6										•		
	4.7			•							•		
	2.1 ±1.0												
	4.3										•		
	1.8						•						
								•	•				
	18.5												
	12.6	40		•									•
	12.0	20											•
	12.9	54			•	•							•
	13.2	63											•
	17.8	>100											
	12.1	37											
	13.3	60						•	•				
	17.4	>100											
	18	>100											
	18	>100											
	13.1	36											
	14.4	76						•	•				
	14.0	65						•	•				
							•					•	
					•		•	•	•		•		
75-165	13.5	66	80,000	•	•	•	•	•	•	•			
100-155	13.5	65	80,000	•	•	•	•	•	•	•			
	14.4	88			•	•							
	12.4	25				•	•	•					
		34				•	•						
	15.5	>100											
	17.3	>100											
	17.6	>100											

Detergent Selection Table (cont.)

Name	Description	CAS	MW (g/mol)	CMC
TRITON™ X-405, reduced	reduced	92046-34-9	564.8	
TRITON™ X-705 solution	70% in H_2O	9036-19-5		
TRITON™-CG-110	Alkyl Polyglucosides	68515-73-1		1748 ppm
TWEEN® 20	meets EP testing specifications	9005-64-5	1228 (avg.)	0.059 mM
TWEEN® 40	viscous liquid	9005-66-7	1284 (avg.)	0.027 mM
TWEEN® 60	nonionic detergent	9005-67-8		0.0055 - 0.022 mM
TWEEN® 65	45 - 65 % (GC)	9005-71-4		
TWEEN® 80	for molecular biology, syrup	9005-65-6	1310 (avg.)	0.012 mM
TWEEN® 85	≥ 50 % (GC)	9005-70-3		
Tyloxapol	BioXtra	25301-02-4		0.018 mM
Undecyl-β-D-maltoside	≥ 99.0% (TLC)	253678-67-0	496.59	0.59 mM
Zwitterionic Detergents				
ASB-14, Amidosulfobetaine-14	≥ 95% (HPLC)	216667-08-2	434.68	8
ASB-16, Amidosulfobetaine-16	≤5.0% (H_2O)	52562-29-5	462.73	
ASB-C7BzO	≥ 95% (HPLC)	565454-39-9	399.59	
CHAPS	100 mM solution	75621-03-3	614.88	8 mM
CHAPS hydrate	≥ 98% (TLC)	331717-45-4	614.88	8 mM
CHAPSO	BioXtra, ≥ 98% (TLC)	82473-24-3	630.88	8 mM
DDMAB, N-Dodecyl-N,N-(dimethylammonio)butyrate	≥ 94.0%	15163-30-1	299.49	4.3
SB3-8, Octyl sulfobetaine	≥ 98%	15178-76-4	279.44	390 mM
SB3-10, Caprylyl sulfobetaine	≥ 98%	15163-36-7	307.49	39 mM
SB3-12, Lauryl sulfobetaine	used for protein solubilization	14933-08-5	335.55	2.8 mM (20 mM Tris-HCl, pH 8.0, 0.1 M NaCl)
SB3-14, Myristyl sulfobetaine	≥ 99% (TLC), BioXtra	14933-09-6	363.6	0.16 mM
SB3-16, Palmityl sulfobetaine	≥ 98% (TLC)	2281-11-0	391.65	10 - 60 mM
Non-Detergents Sulfobetaines (NDSB)				
NDSB 195, Dimethylethylammoniumpropane sulfonate	≥ 97% (TLC)	160255-06-1	195.28	
NDSB 211, 3-[Dimethyl-(2-hydroxyethyl)ammonio]-1-propanesulfonate	≥ 98% (TLC)	38880-58-9	211.28	
NDSB 221, 3-(1-Methylpiperidinio)-1-propanesulfonate	≥97% (TLC)	160788-56-7	221.32	
NDSB 256, 3-(Benzyldimethylammonio) propanesulfonate	BioXtra, ≥99.0% (HPCE)	81239-45-4	257.35	
NDSB 256-4T, 3-(4-tert-Butyl-1-pyridinio)-1-propanesulfonate	≥ 98.0% (HPLC)	570412-84-9	257.35	

Aggregation Number	HLB	Cloud Point (°C)	Average Micellar Weight	Diagnostic Applications	Molecular Biology	Cell Culture	Electrophoresis/ Chromatography	Membrane Protein Solubilization	Enzymology	Antigen/Vaccine Preparation	Drug Delivery/ Liposomes	Mass Spectrometry	Microbiology
	18.4	>100											
		>100						•	•				
	16.7	76	*	•		•	•	•	•	•	•		
	15.6			•		•	•	•	•		•		
	14.9										•		
	10.5												
58	15	65	79,000	•		•	•	•	•		•		
	11										•		
		94.3			•	•					•		
71 (100 mM NaCl, 20 mM HEPES pH 7.5)			35,300				•	•					
≥23			10,000			•	•	•					
							•	•					
≤25			10,000			•	•	•					
10		>100	6,150	•		•	•	•	•	•	•		
10		>100	6,150	•		•	•	•	•	•	•		
11		90	7,000			•	•	•	•				
							•	•					
						•		•					
41			12,600			•		•					
55 - 87			18,500 - 29,100	•		•	•	•	•	•			
83 - 130			30200 - 47,300	•		•	•	•	•				
155			60,700			•	•	•				•	
These products do not form micelles							•	•					
							•	•					
							•	•					
							•	•					
							•	•					

General Properties of Detergents

Aggregation Number

This is the number of detergent monomers present within a micelle. It can be obtained by dividing the micellar molecular weight by the monomeric molecular weight.

$$\frac{\text{Micellar molecular weight}}{\text{Monomeric molecular weight}} = \text{Aggregation number}$$

Methods used to determine the molecular weight of micelles include gel filtration, light scattering and sedimentation equilibrium.[6]

Most detergents used for biochemical applications have aggregation numbers in the range of 50-100.[17] Bile acid salts have low aggregation numbers (e.g., CHAPS, CHAPSO, and Big CHAP have aggregation numbers of approximately 10), whereas the aggregation numbers of nonionic detergents tend to be much higher. In general, aggregation number increases as the length of the hydrocarbon chain increases. Increasing the temperature of ionic detergent solutions also results in higher aggregation numbers. Aggregation number decreases as the size of the hydrophilic group increases and upon the addition of hydrocarbons and polar compounds to the detergent solution.[6, 7]

Critical Micelle Concentration (CMC)

The CMC can be defined as the concentration above which monomers cluster to form micelles. Micellization actually occurs over a narrow concentration range rather than at a particular concentration.[7, 19, 20] Above the CMC, detergent monomers and micelles coexist in equilibrium, and increasing the detergent concentration results in an increase of the amount of detergent in micelles, while the concentration of the detergent monomers remains constant.[1] The CMC varies with conditions, including pH, ionic strength, temperature, as well as the presence of protein, lipid and other detergent molecules.[17] The CMC decreases with the length of the alkyl chain of the detergent and increases with the introduction of double bonds and other branched points (e.g., as observed in bile acid salts). Additives, such as urea, that break up water structure also increase the CMC. In ionic detergents, the CMC is reduced by increasing the concentration of counter ions, but is relatively unaffected by changes in temperature. Conversely, the CMC of non-ionic detergents is relatively unaffected by increasing ionic strength, but increases substantially with increasing temperature. From a practical point of view, a high CMC is desirable when dialysis is used for the removal of the detergent.[6]

Three of the most popular methods used to determine CMC are surface tension, light scattering, and dye solubilization. Surface tension decreases with the detergent concentration and reaches a minimum around the CMC value. Light scattering and the solubility of a hydrophobic dye increase with detergent concentration. The point of inflection on a graph obtained by plotting any of the three parameters vs the detergent concentration corresponds to the CMC of the detergent (**Figure 6**).

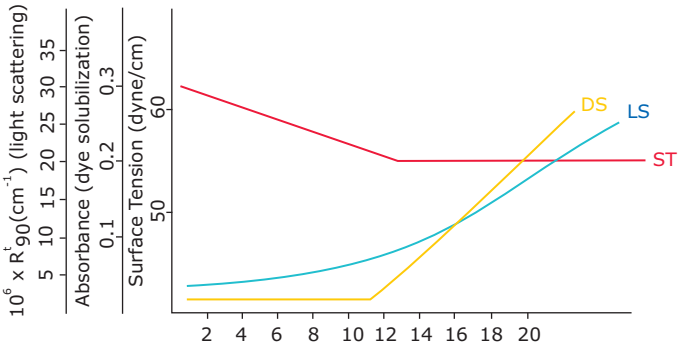


Figure 6. Representative results for determining the CMC of a surfactant by various methods. DS = dye solubilization; LS = light scattering; ST= surface tension.

Given the CMC, the concentration of the detergent and the aggregation number, it is possible to calculate the concentration of micelles in moles per liter using the following formula:

$$\text{Micelles} = \frac{(Cs - CMC)}{N}$$

where Cs is the bulk molar concentration of detergent and N is the mean aggregation number.[21]

Kraft Point

At very low temperatures, detergents remain mainly in an insoluble crystalline state and are in equilibrium with small amounts of dissolved monomer. Solubility of the monomeric detergent increases with temperature until the **critical micellar temperature (CMT)** is reached. At this temperature the detergent solution turns clear and the concentration of the detergent reaches its CMC value. The temperature at which all three phases — crystalline, micellar, and monomeric — exist in equilibrium is called the Kraft point (**Figure 7**). For most detergents, the **Kraft point** is identical to the CMT.[6]

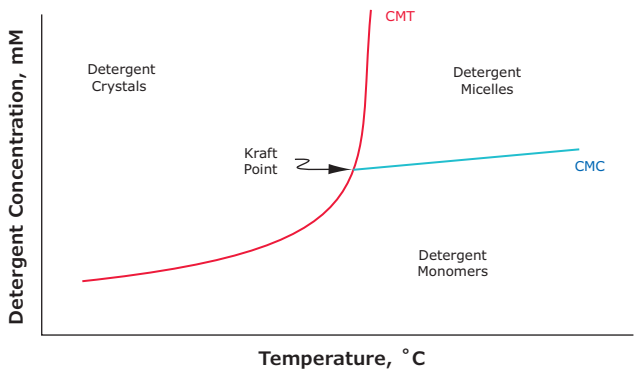


Figure 7. Temperature-composition phase diagram for detergent solutions.

Cloud Point

At a particular temperature above the CMT, non-ionic detergents become cloudy and undergo phase separation to yield a detergent-rich and a detergent-poor layers.[4,7,12] This temperature is called the **cloud point**. The phase separation is reversible upon cooling. Nonpolar additives (i.e., hydrocarbons) increase whereas polar compounds and salts decrease the cloud point.[7] A low cloud point may be useful in membrane protein purification.[22-24] Triton X-114, for example, has a cloud point around 22°C. This property allows for two-phase water/detergent extractions to separate water soluble proteins from membrane proteins.[22,25]

Hydrophile-Lipophile Balance (HLB)

The HLB is a measure of the relative hydrophobicity of the detergent. There is a good correlation between the HLB value of a detergent and its ability to solubilize membrane proteins, especially for solubilization by nonionic detergents.[12, 24] The most hydrophobic detergents have HLB numbers approaching 0, while the least hydrophobic ones have values reaching 20.[27] Detergents with HLB values in the range 12 to 20 are preferred for non-denaturing solubilization. Detergents in the higher end of the range are preferred for solubilization of extrinsic proteins. For simple, single-chain detergents, HLB can be determined by the following equation:

$$HLB = \Sigma H - \Sigma L + 7$$

where H is the contribution from the hydrophilic group and L is the contribution from the lipophilic group.[28,29]

It is also important to note that the HLB is additive. For example, when two detergents with HLB values of A and B are used, the following equation applies:

$$HLB\ (A+B) = (Ax+By)/(x+y)$$

where x and y are the percentages of each detergent. Provided there are no other factors influencing enzyme activity, using the above formula, two detergents can be selected to attain the desired HLB value.[30]

Summarizing the above properties, it is evident that the performance of a detergent is dependent on the following factors:

- Detergent concentration
- Ionic strength
- Length of the alkyl chain
- pH
- Presence of organic additives
- Purity
- Temperature

Removal of Unbound Detergents

Excess detergent is normally employed in solubilization of membrane proteins to ensure complete dissolution of the membrane and provide a large number of single protein molecule containing micelles. However, for further physicochemical and biochemical characterization of membrane proteins, it is often necessary to remove the unbound detergent.

The following is a brief description of four commonly used methods for detergent removal that take advantage of the general properties of detergents: e.g., hydrophobicity, CMC, aggregation number, and the charge.

Hydrophobic Adsorption

This method exploits the ability of detergents to bind to hydrophobic resins. Generally, a detergent-containing solution is mixed with a specific amount of the resin, and the mixture is allowed to stand at 4°C or room temperature. The resin with the bound detergent can be removed by centrifugation or filtration. This technique is effective for the removal of most detergents and especially suitable for detergents with low CMCs.[31] If the adsorption of the protein to the resin is of concern, the resin can be included in a dialysis buffer and the protein dialyzed.

Gel Chromatography

Gel chromatography takes advantage of the difference in size between protein-detergent, detergent-lipid, and homogeneous detergent micelles. In most situations, protein-detergent micelles elute in the void volume. The elution buffer should contain a detergent below its CMC value to prevent protein aggregation and precipitation. Since this method is based on separation based on size, parameters that influence micellar size (ionic strength, pH, and temperature) should be kept constant from experiment to experiment to obtain reproducible results.[6]

Dialysis

When detergent solutions are diluted below the CMC, the micelles are dispersed into monomers. Monomers are usually significantly smaller than micelles, and can be easily removed by dialysis. Dialysis is the most common form of detergent removal and typically requires dialyzing the protein detergent mixtures against detergent-free buffer (in about 200-fold excess). If a large dilution is not practical, micelles can be dispersed by other techniques such as the addition of bile acid salts. Dialysis is more practical with detergents having high CMCs and works best for those with low molecular weight/small cross-sectional area.[6]

Ion-exchange Chromatography

This method exploits the differences in charge between protein-detergent micelles and protein-free detergent micelles. When non-ionic or zwitterionic detergents are used, conditions can be chosen to retain protein-containing micelles on the ion-exchange resin, letting protein-free micelles pass through. The adsorbed protein can then be eluted by changing the ionic strength or pH of detergent-free buffer, or by washing with an ionic detergent.[6]

Guidelines for Choosing a Detergent

A membrane protein is considered solubilized if it is present in the supernatant after one hour centrifugation of a lysate or a homogenate at 100,000 x g. In most cases, the biological activity of the protein should be preserved in the supernatant after detergent solubilization. Hence, the appropriate detergent should yield the maximum amount of biologically active protein in the supernatant. Given the large number of detergents available today, choosing an appropriate detergent can be a difficult process. Some of the points outlined below can be helpful in selecting a suitable detergent.

- Survey the literature and try a detergent that has been used previously for the isolation and characterization of a protein with similar biochemical or enzymological properties.
- Consider the solubility of the detergent at the working temperature. For example, Dimethylpalmitylammonio) propanesulfonate (SB3-16) is insoluble in water at 4°C while TRITON™ X-114 Detergent undergoes a phase separation at room temperature.
- Consider the method of detergent removal. If dialysis is to be employed, a detergent with a high CMC is clearly preferred. Alternatively, if ion exchange chromatography is utilized, a non-ionic or a zwitterionic detergent is more suitable.
- Preservation of biological or enzymological activity may require experimenting with several detergents. Not only the type but also the quantity of the detergent used will affect the protein activity. For some proteins biological activity is preserved over a very narrow range of detergent concentration. Below this range the protein is not solubilized and above a particular concentration, the protein is inactivated.
- Consider downstream applications. Since TRITON™ X-100 Detergent contains aromatic rings that absorb at 260-280 nm, this detergent should be avoided if the protocols require UV monitoring of protein concentration. Similarly, ionic detergents should be avoided if the proteins are to be separated by isoelectric focusing. For gel filtration of proteins, detergents with smaller aggregation numbers should be considered.
- Consider detergent purity. Detergents of utmost purity should be used since some detergents such as TRITON™ X-100 are generally known to contain peroxides as contaminants.
- A variety of Molecular Biology Grade detergents are available for any research where contaminants such as DNase, RNase, and proteases are problematic.
- A non-toxic detergent should be preferred over a toxic one. For example, digitonin, a cardiac glycoside, should be handled with special care.
- For unknown reasons, specific detergents often work better for particular isolation procedures. For example, n-Dodecyl-β-D-maltoside has been found to be the detergent of choice for the isolation of cytochrome c oxidase. Hence, some “trial and error” may be required for determining optimal conditions for isolation of a membrane protein in its biologically active form.
- Sometimes it is difficult to find an optimally suited detergent for both solubilization and analysis of a given protein. In such cases, it is often possible to solubilize proteins with one detergent before replacing it with another that exhibits least interference with analysis.
- In some cases, it has been observed that the inclusion of non-detergent sulfobetaines (NDSBs) with detergents in the isolation buffer dramatically improves yields of solubilized membrane proteins.

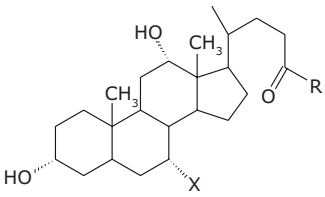
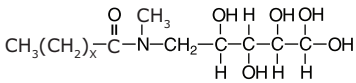
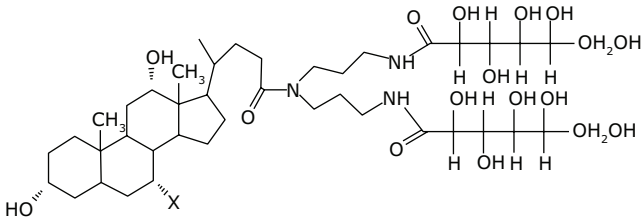
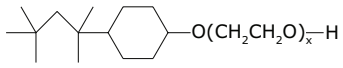
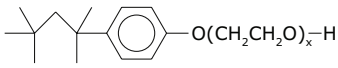
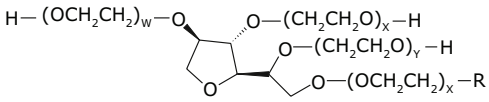
Non-Detergent Sulfobetaines

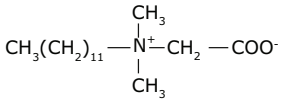
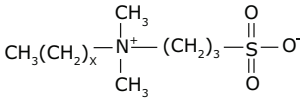
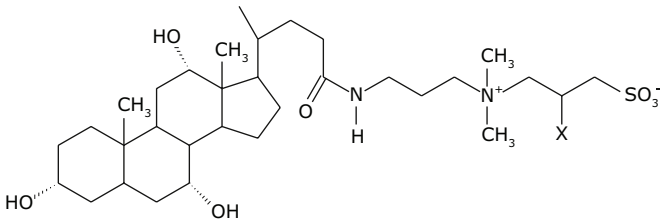
Non-Detergent Sulfobetaines, NDSBs, are zwitterionic compounds. Like detergent sulfobetaines (SB) – such as linear SBs (e.g., SB 3-16, SB 3-10, 3-12 and 3-14),[32] CHAPS,[33] and amidosulphobetaines (e.g., N-alkylamidopropyl-N,Ndimethylaminoalkyl-l-sulphonate) (34) – NDSBs carry the sulfobetaine hydrophilic head group. However, in contrast to SBs, the hydrophobic groups in NDSBs are too short for micelle formation even at concentrations as high as 1 M. Hence, NDSBs can be easily removed by dialysis and they can readily diffuse in chromatography matrices and polyacrylamide gels for electrophoretic applications.

NDSBs were first employed in native isoelectric focusing experiments to screen electrostatic interactions without increasing the conductivity.[35] Since then, they have found use in a wide range of applications including solubilization and crystallization of proteins,[35, 36] as well as renaturation and refolding of chemically and thermally denatured proteins.[37, 38] Due to their short hydrophobic groups and charge-screening effect, NDSBs prevent aggregation and result in higher yields of membrane proteins. Furthermore, NDSBs do not interfere with enzymatic assays involving chromogenic substrates bearing nitrophenyl groups and they do not inhibit the activities of enzymes such as β-galactosidase and alkaline phosphatase.[35] It is also noteworthy that NDSB-195, NDSB-211, and NDSB-221 do not absorb at 280 nm; therefore, they are compatible with protein purification procedures in which the protein concentrations are monitored by measuring absorbance at this wavelength.[48]

Appendix

Structure and Classification of Detergents

Detergent Class	General Structure	Examples
Alkyl glycosides	R-O-$(CH_2)_x$-CH_3	R = glucose x = 8, *n*-nonyl-β-D-glucopyranoside x = 7, *n*-octyl-β-D-glucopyranoside x = 6, *n*-heptyl-β-D-glucopyranoside x = 5, *n*-hexyl-β-D-glucopyranoside
	R -S-(CH_2)x-CH_3	R = maltose x = 11, dodecyl-β-D-maltoside x = 9, decyl-β-D-maltoside R = glucose, x = 7, octyl-b-D-thioglucopyranosid
Bile acids		x = H, R = O-Na+, sodium deoxycholate x = H, R = $NHCH_2CH_2SO_3$-Na^+, sodium taurodeoxycholate x = H, R = $NHCH_2CO_2$-Na^+, sodium glycodeoxycholate x = OH, R = O-Na^+, sodium cholate x = OH, R = $NHCH_2$ CH_2SO_3-Na^+, sodium taurocholate x = OH, R = $NHCH_2CO_2$-Na^+, sodium glycocholate
Glucamides		x = 8, MEGA-10 x = 7, MEGA-9 x = 6, MEGA-8
		x = H, Deoxy Big CHAP x = OH, Big CHAP
Poly-oxyethylenes, monodisperse and polydisperse		x = 9–10, reduced TRITON™ X-100 x = 7–8, reduced TRITON™ X-114
		x = 9–10, TRITON™ X-100, NP-40 x = 7–8, TRITON™ X-114
	$CH_3(CH_2)_Y$-$O(CH_2$ $CH_2O)_X$-H	y = 12, x = 8, GENAPOL® X-080 y = 12, x = 10, GENAPOL® X-100 y = 11, x = 8, $C_{12}E_8$ y = 11, x = 9, $C_{12}E_9$, THESIT®, LUBROL® PX y = 11, x = 10, GENAPOL® C-100 y = 11, x = 23, BRIJ® 35
	$HO(CH_2CH_2O)_X$-$(CH(CH_3)$-$CH_2O)_Y$-(CH_2CH_2O)-$_ZH$	x = 98, y = 67, z = 98, PLURONIC® F-127®
	 W + X + Y + Z = 20	R= $C_{11}H_{23}CO_2$- (laurate), TWEEN® 20 R=$C_{17}H_{33}CO_2$- (oleate), TWEEN® 80

Detergent Class	General Structure	Examples
Zwittergents	 pH ≥ 6	EMPIGEN® BB (*n*-dodecyl-N,N-dimethylglycine)
		x = 7, ZWITTERGENT® 3-08 x = 9, ZWITTERGENT® 3-10 x = 11, ZWITTERGENT® 3-12 x = 13, ZWITTERGENT® 3-14 x = 15, ZWITTERGENT® 3-16
		x = H, CHAPS x = OH, CHAPSO

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