

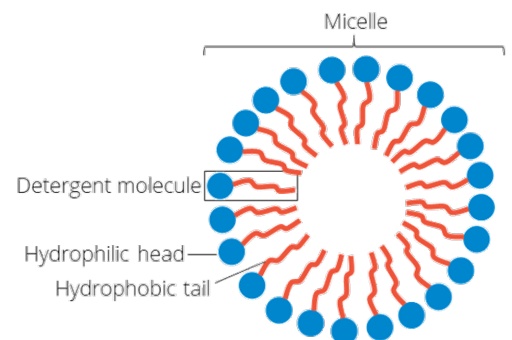
Detergents

Detergents are amphiphilic organic compounds containing well separated hydrophilic domains (heads) and hydrophobic domains (tails). Being able to decrease the surface tension of water, detergents are a class of surfactants.

Detergents are widely used in life science applications such as purification and solubilisation of proteins, to keep the protein in a solution and prevent precipitation. They are used for permeabilization, dissolution and solubilisation of membranes, as model to mimic membranes for *in vitro* studies, and as vehicles for drug delivery. Detergents are added to different lysis buffers and compatible with enzymatic cell lysis (lysozyme), sonication, cell press, freeze-thawing and others. They are also needed in protocols for purification of nucleic acids, where they solubilise protein and membrane fractions and allow to separate it from nucleic acids.

Detergents Physical Properties

When present at low concentration in water, detergent molecules associate in a monolayer situated at the interface between the liquid phase and the air. When the detergent concentration increases, stable structures called micelles are generated. These are typically spherical or ellipsoid aggregates with the hydrophilic heads exposed to the aqueous solution and the hydrophobic tails oriented inwards, towards the centre of the micelle. The process of micelle formation is driven by the hydrophobic effect related to a more favourable entropic contribution: a lower number of molecules of water is required for the solvation of the micelle compared to the solvation of non-associated detergent molecules.



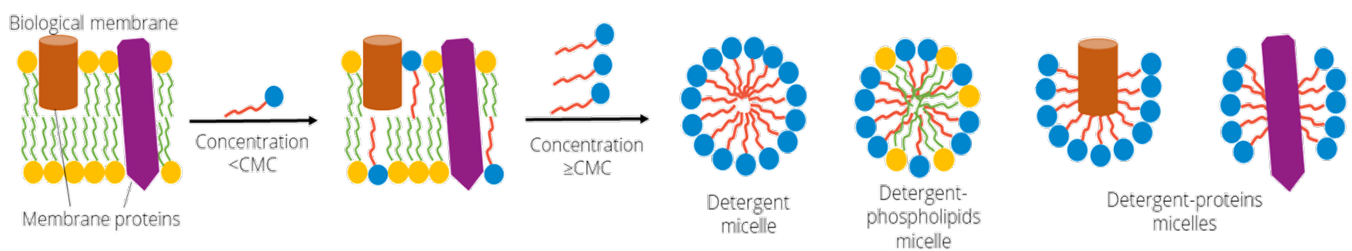
The concentration of detergent above which the micelle formation occurs is called critical micelle concentration (CMC), which can also be defined as the highest concentration of the detergent as free monomer in solution, since additional detergent molecules will only form micelles. CMC and micelle aggregation number are properties of a detergent depending on its chemical structure, temperature, pH, pressure, ionic strength, and purity. In general, CMC decreases at the increase of the detergent hydrophobicity (which is higher for long alkyl chains), and when electrolytes are added. CMC of non-ionic detergent is usually lower than for ionic detergents, and CMC of zwitterionic detergents is lower than CMC of ionic detergents. When working with membrane proteins the detergent concentration must be at least double the CMC.

The average number of detergent molecules present in a micelle is defined as micelle aggregation number. It increases with hydrophobicity and at higher temperatures. Spherical micelles are formed at small aggregation number, ellipsoid micelles when the aggregation number is higher. As for CMC, the micelle aggregation number depends on the detergent chemical structure, temperature, pH, pressure, ionic strength, and purity. Generally, the micelle aggregation number has values between 50-100 (with some exceptions such as CHAPS, CHAPSO and Big CHAP with aggregation number around 10).

The critical micelle temperature (CMT) is the temperature above which the micelle formation occurs. The Krafft point is a temperature at which monomeric detergent, micellar detergent and an insoluble crystalline phase coexist in equilibrium. Usually Krafft point and CMT coincide. The cloud point is the temperature above which the micelles of non-ionic detergents aggregate precipitating from the solution, which turns visibly turbid. It is increased by the addition of nonpolar compounds and decreased by the addition of polar compounds and salts. A low cloud point is convenient for biphasic extractions in membrane protein purification.

How Do Detergents Work?

As previously mentioned, detergents are employed to solubilise biological membranes and isolate proteins. Biological membranes are formed of a double layer of phospholipids with the polar heads on the exposed faces of the membrane and the two hydrophobic tails towards the inner part of the membrane. Membrane proteins are embedded within the double phospholipid layer, where hydrophobic interactions between the lipophilic phospholipids tails and protein hydrophobic domains take place. The way detergents act on membranes is shown in the scheme below.



At detergent concentration lower than CMC, molecules of detergent insert in the lipid bilayer of the membrane. When the CMC is reached, the lipid bilayer is saturated by the detergent, which causes lysis of the membrane. At this point, mixed detergent-phospholipids micelles, detergent micelles, and protein-detergent micelles are formed (where the hydrophobic domains of the protein are located towards the centre of the micelle and its hydrophilic domains towards the outer surface).

It is important to use the correct concentration of detergent to make sure that each individual protein is isolated in a single micelle, therefore usually an excess is employed. The optimal detergent concentration depends on the detergent physical properties, on the protein concentration and membrane characteristics.

Detergent Removal

After membrane lysis and protein solubilisation, the detergent should be partially or completely removed in order to isolate the extracted proteins for further experiments. The extraction of membrane proteins within the detergent micelles disrupts the native protein-lipid interactions causing possible protein inactivation. In order to restore the protein activity, the detergent can be replaced by phospholipids or other lipids that would form a bilayer artificial membrane.

The methods used for the detergent removal take advantage of its characteristics, such as hydrophobicity, CMC, aggregation number, and charge.

Detergent removal methods			
Hydrophobic adsorption	Size exclusion chromatography	Dialysis	Ion exchange chromatography
Detergents have the ability to bind to hydrophobic resins. Most detergents can be removed with this method, specifically the ones with low CMC. Usually a specific amount of resin is added to a solution containing the detergent, and the mixture is left standing at RT or 4 °C, to then be removed by filtration or centrifugation.	This type of chromatography takes advantage of the different sizes of protein-detergent, detergent-lipid, and detergent micelles, which elute at different times. The detergent concentration should be lower than CMC to avoid protein aggregation and all parameters affecting the micellar size, such as pH, salt concentration and temperature, should be maintained unvaried.	Dialysis is the most employed method for detergent removal, especially with high CMC and low MW. The detergent concentration is diluted below CMC, and the monomers, which are usually an order of magnitude smaller than the micelles, are removed. In cases where large dilutions are not convenient, the micelles are dispersed by adding bile acid salts.	This type of chromatography takes advantage of the charge difference between the protein-detergent and detergent micelles, with non-ionic or zwitterionic detergents. The protein-detergent micelles are retained on the resin, while the detergent micelles, being overall neutral, elute first. The protein-detergent micelles are then release from the resin by washes, or variations in pH or ionic strength.

Detergents Classification

Detergents can be classified in three broad groups based on the ionic character of the headgroup: ionic (anionic and cationic), non-ionic and zwitterionic. Their characteristics are summarised in the table below:

Detergent type		
Ionic	Non-ionic	Zwitterionic
Positively (cationic) or negatively (anionic) charged head group. The tails are straight hydrophobic chains (SDS, CTAB) or rigid steroidal groups (bile acid salts). Harsh, denaturing, they disrupt completely the cellular structure, inter- and intra-molecular protein-protein interactions. Used for protein separation in gel electrophoresis. It binds with the protein masking its charge. Micelle size results from the hydrophobic attractions between the tails and the repulsions between the charged heads. Bigger counter ions would cause an increase of the micelle size. CMC is reduced in media with high ionic strength and is poorly affected by temperature variations.	Non charged head groups, which are either polyoxyethylene moieties, as in TRITON™ and BRIJ®, or glycosidic based. Mild, non-denaturing (limited ability to disrupt protein-protein interactions): the solubilised proteins retain native subunit structure, enzymatic and non-enzymatic activity. They disrupt protein-lipid and lipid-lipid interactions. Used for the purification of enzymes or multimeric proteins in its native state. Micellar size is poorly affected by ionic strength. CMC increases at higher temperature and is poorly affected in media with high ionic strength.	The head group contains both a positive and a negative charge. Combined properties of ionic and non-ionic detergents: usually non-denaturing, they protect the native charge of proteins. In some cases, they can disrupt protein-protein interactions. Used for isoelectric focusing and 2D electrophoresis. They do not bind ion-exchange resins.

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Ionic

Detergent Name	Product Code	Cas No.	CMC (mM)	Aggregation No.	Uses	Denaturing
Triton® X-100	FP14128	9002-93-1	0.083	100-155	Chromatography; membrane protein solubilization	No
Dodecyl β-D-maltopyranoside	DD06199 D-8823 DD172113	69227-93-6	0.17	78-149	Cell lysis; outer-membrane protein solubilization; chromatography	No
Octyl β-D-glucopyranoside	DO05161 O-2000	29836-26-8	18-20	27-100	Membrane protein solubilization	No
Nonyl β-D-glucopyranoside	DN03173	69984-73-2	6.5	133	Membrane protein solubilization; electrophoresis; chromatography	No
Digitonin	OD09275 XD175416 XD175328 XD175329 D-3100 D-3200 D-3203	11024-24-1	<0.5	60	Protein solubilization; enzymology; chromatography	No
Glyco-diosgenin	DG63244	1402423-29-3	18	-	Synthetic version of digitonin	No
Brij® 35 Solution 30%	FB47169 FB175753	9002-92-0	0.091	40	Membrane protein solubilization; chromatography; electrophoresis	No
Polidocanol	FP32544 FP177516	3055-99-0	0.05	110	Membrane protein solubilization	No
Octyl β-D-thiogluco-pyranoside	DO06354 O-2710	85618-21-9	9	189	Membrane protein solubilization; enzymology	No
2-((4,4,5,5,5-Pentafluoropentyl)oxy)octyl β-D-maltopyranoside	DP171774	-	-	-	Membrane protein solubilization	No
2-((4,4,5,5,5-Pentafluoropentyl)oxy) dodecanyl β-D-maltopyranoside	DP171773	-	-	-	Membrane protein solubilization	No
Decyl glucoside	MD13516	68515-73-1	-	-	Membrane protein solubilization; enzymology	No
N-Decanoyl-N-methylglucamine (MEGA-10)	DD10404	85261-20-7	6-7	-	Membrane protein solubilization	No
N,N-Dimethyldodecylamine N-oxide (LDAO)	DD12118 FD46143	1643-20-5	1-2	76	Membrane protein solubilization, used in cleaning products, antibacterial properties	No
Polyoxyethylene castor oil	FP45353	61791-12-6	-	-	Liposomes	No
Pluronic F68	FP16303	9003-11-6	3.97	-	Liposomes; cell membrane stabilizer; anti-foaming agent	No
Saponins	OS29326 XS167592	8047-15-2	-	-	Natural surfactant	No
Tyloxapol	FT11312 FT157891	25301-02-4	0.018	-	Liposomes	No

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Anionic

Detergent Name	Product Code	Cas No.	CMC (mM)	Aggregation No.	Uses	Denaturing
Sodium N-Lauroylsarcosinate (Sarkosyl)	FS62988	137-16-6	14.6	2	DNA extraction; protein solubilization	Yes
Sodium dodecylsulfate (SDS)	FS15952	151-21-3	7-10	62	Protein solubilization; DNA extraction; electrophoresis	Yes
Cholic acid	FC09616 C-5900	81-25-4	-	-	Cell lysis; protein solubilization; extraction of membrane proteins; chromatography	Yes
Deoxycholic acid	FD11043	83-44-3	2-6	3-12	Cell lysis; protein solubilization; affinity chromatography	Yes
Sodium deoxycholate	FS12338	302-95-4	2-6	3-12	Cell lysis; protein solubilization; affinity chromatography	Yes
Chenodeoxycholic acid	FC09675 C-2900	474-25-9	3	-	Protein solubilization; cell culture	Yes
Glycodeoxycholic acid sodium salt	FG16281	16409-34-0	2.1	2	Protein solubilization; electrokinetic chromatography	Yes
Glycochenodeoxycholic acid sodium salt	FG15494	16564-43-5	-	-	Cell culture; lipid solubilization	Yes
Sodium glycocholate hydrate	FS75104	338950-81-5	13	-	Cell culture; enzymology; lipid solubilization	Yes
Tauroursodeoxycholic acid	FT03670	14605-22-2	4	-	Liposomes; lipid solubilization	Yes
Sodium taurodeoxycholate hydrate	FS45995	207737-97-1	1-4	6	Protein solubilization; capillary electrophoresis	Yes
1-Octanesulfonic acid sodium salt	FO10744	5324-84-5	-	-	HPLC analysis of peptides and proteins	Yes
1-Hexane sulfonic acid sodium salt	FH33348	2832-45-3	-	-	Ion pair chromatography	Yes

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Cationic

Detergent Name	Product Code	Cas No.	CMC (mM)	Aggregation No.	Uses	Denaturing
Cetyltrimethylammonium bromide (CTAB)	FC13320	57-09-0	0.92	170	DNA extraction; removal of polysaccharides from samples	Yes
Dimethyldioctadecylammonium bromide	FD75527	3700-67-2	-	-	Enzymology, protein purification	Yes
N-Dodecyl trimethylammonium bromide	FD54967	1119-94-4	-	-	Enzymology, protein purification	Yes
Tetradecyltrimethylammonium bromide (TTAB)	FT32264 T-6750	1119-97-7	4-5	80	Enzymology, ion pair chromatography	Yes

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Zwitterionic

Detergent Name	Product Code	Cas No.	CMC (mM)	Aggregation No.	Uses	Denaturing
CHAPS	FC12196	75621-03-3	8	10	Cell lysis; 2D electrophoresis; isoelectric focusing; glycoprotein electrophoresis; membrane protein solubilization; DNA extraction	No
Deoxy-bigCHAP	FD21057	86303-23-3	1.1-1.4	-	Membrane solubilisation; proteomic analysis	No
CHAPSO	FC46927	82473-24-3	8	11	Membrane protein solubilization, more soluble than CHAPS	No
Zwittergent 3-14, SB3-14	FT28075	14933-09-6	0.1-0.4	83	Membrane protein solubilization	No

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About Biosynth

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Biosynth is an innovative life sciences reagents, custom synthesis and manufacturing services company. We are by scientists, for scientists, securing supply chains with consistent quality, across the globe. We manufacture and source a vast range of chemical and biochemical products, and take pride in delivering products that others cannot. We are experts in complex chemistry, peptides and key biological raw materials. We provide a full range of products and services to support life science research and development, with more than half a million products in our research catalog and hundreds of complex manufacturing service projects.

The trusted supplier, manufacturer and partner for the pharmaceutical, life science and diagnostic sectors, along with customers across food, agrochemistry and cosmetics, we have facilities across three continents and a rapid global distribution network. Our main chemical research and manufacturing laboratories are in Switzerland, the United Kingdom, Slovakia and China, with peptide production in the USA and the Netherlands. Enzyme projects are based in Austria and biological IVD reagents in Ireland. Our R&D resources and production facilities are modern and versatile, allowing us to produce chemicals on the milligram to ton scale, and at ISO 9001 and GMP, with peptides at mg to multikilogram scale.

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