Calbiochem®

Detergents

A guide to the properties and uses of detergents in biology and biochemistry

Calbiochem®

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

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Cover image: the katydid is an *Orthopteran* insect closely related to grasshoppers and crickets. Although seldom seen due to their arboreal habitat, the male is often heard on summer nights singing his eponymous song "katy-did, katy-didn't". In North America, katydids typically survive the winter in the egg stage. *Photo credit: Scot Mitchell*

A word to our valued customers

As the leading supplier of a variety of quality detergents, the Calbiochem® brand has been recognized by many researchers all over the world for over 50 years. During this period, we have received a number of inquiries on the use of detergents, definitions, relevance of critical micelle concentration (CMC), cloud point, hydrophilic number, and how to select the most appropriate detergent. As a service to the research community, we are providing this guide to the use of detergents in biological systems. The background information and the selected bibliography provided here will hopefully serve the needs of the first time users of detergents as well as those of experienced investigators.

We have also included a section on a unique series of compounds known as Non-Detergent Sulfobetaines (NDSBs). As evident from the name, these compounds are not detergents and they do not form micelles. Structurally, NDSBs have hydrophilic groups similar to those found in zwitterionic detergents; however, they possess a much shorter hydrophobic chain. They have been reported to improve the yield of membrane proteins when used in conjunction with the traditional detergents and prevent aggregation during renaturation of chemically or thermally denatured proteins.

The discussion provided in this booklet is by no means complete. However, we hope it will help in the understanding of general principles involved in the use of detergents.

Table of Contents

Hydrophobic Interactions 1
What are Detergents? 2
Biological Membranes 3
How Do Detergents Solubilize Membrane Proteins? 5
Detergent-Lipid-Protein Ratios 7
Classification of Detergents 8
General Properties of Detergents 12
Removal of Unbound Detergents 16
Guidelines for Choosing a Detergent 18
Appendix 1: Non-Detergent Sulfobetaines 20
Appendix 2: CALBIOSORB™ Adsorbent 22
Selected Bibliography 29
Calbiochem® Detergents 37

Hydrophobic Interactions

Water forms a highly ordered network of intermolecular hydrogen bonds (Figure 1). It is the strength of all the hydrogen bonds combined that imparts the liquid properties to water. Polar or hydrophilic substances dissolve in water because they form hydrogen bonds and electrostatic interactions with water molecules. Non-polar or hydrophobic substances, on the other hand, are unable to form such interactions, and consequently, are immiscible with water. Addition of nonpolar substances to water disrupts intermolecular hydrogen bonding of water molecules and creates a cavity which is devoid of the water molecules. At the surface of the cavity, water molecules rearrange in an orderly manner (Figure 2). This results in a thermodynamically unfavorable decrease in entropy. To compensate for the loss of entropy, water molecules force the hydrophobic molecules to cluster and thus occupy the minimum space. This phenomenon is known as the **hydrophobic effect** and the "forces" between hydrophobic regions are called **hydrophobic interactions.**

Hydrophobic interactions play a major role in defining the native tertiary structure of proteins. Proteins consist of polar and non-polar amino acids. In water-soluble proteins, hydrophobic domains rich in non-polar amino acids are folded in together and thus are shielded from the aqueous environment. In membrane proteins, some hydrophobic regions that otherwise would be exposed to the aqueous environment are surrounded by lipids.

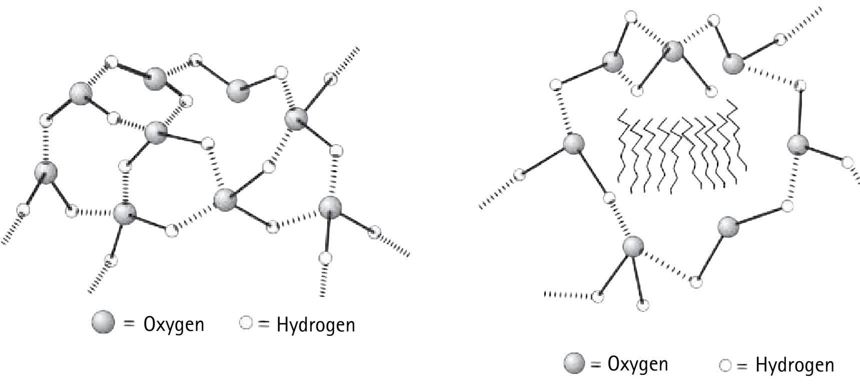

Figure 1: Inter-molecular hydrogen bonding in water.

Figure 2: Clustering of hydrocarbon molecules in water.

What are Detergents?

Detergents are amphipathic molecules that contain both polar and hydrophobic groups. These molecules contain a polar group (head) at the end of a long hydrophobic carbon chain (tail). In contrast to purely polar or non-polar molecules, amphipathic molecules exhibit unique properties in water. Their polar group forms hydrogen bonds with water molecules, while the hydrocarbon chains aggregate due to hydrophobic interactions. These properties allow detergents to be soluble in water. In aqueous solutions, they form organized spherical structures called **micelles** (Figure 3), each of which contain several detergent molecules. Because of their amphipathic nature, detergents are able to solubilize hydrophobic compounds in water. Incidentally, one of the methods used to determine the CMC (see page 12) relies on the ability of detergents to solubilize a hydrophobic dye. Detergents are also known as surfactants because they decrease the surface tension of water.

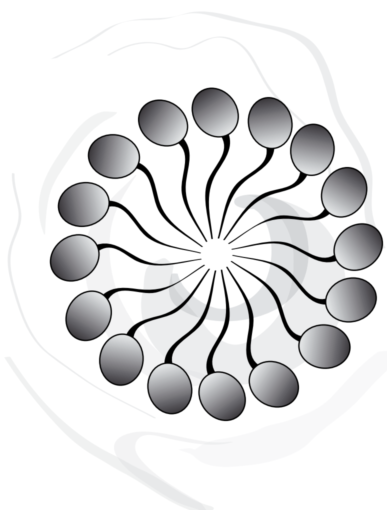

Figure 3: A detergent-micelle in water.

Biological Membranes

Biological membranes, composed of complex assemblies of lipids and proteins, serve as physical barriers in the cell and are sites of many signaling events. The majority of the lipids that make up the membrane contain two hydrophobic groups connected to a polar head. This molecular architecture allows lipids to form structures called **lipid bilayers** in which the hydrophobic chains face each other while the polar head groups face outward to the aqueous milieu (Figure 4). Proteins and lipids, like cholesterol, are embedded in this bilayer. This bilayer model for membranes was first proposed by Singer and Nicolson in 1972 and is known as the **fluid mosaic model** (Figure 5). **Integral membrane proteins** are held in the membrane by hydrophobic interactions between the hydrocarbon chains of the lipids and the hydrophobic domains of the proteins. These integral membrane proteins are insoluble in water but are soluble in detergent solutions.

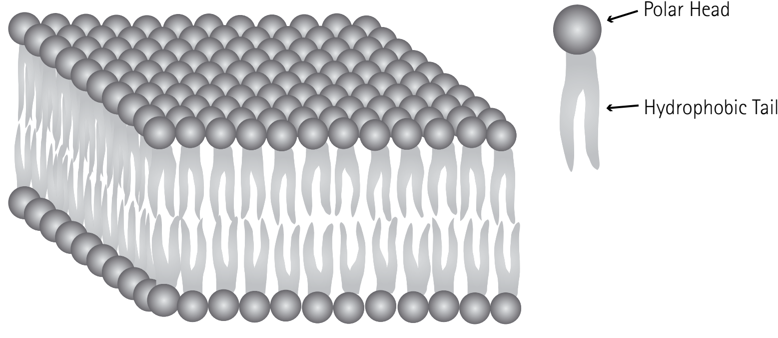

Figure 4: A phospholipid bilayer.

In order to understand the structure and function of membrane proteins, it is necessary to carefully isolate these proteins in their native form in a highly purified state. It is estimated that about one third of all membrane-associated proteins are integral membrane proteins, but their solubilization and purificaiton is more challenging because most of them are present in very low concentrations. Although membrane protein solubilization can be accomplished by using amphiphilic detergents, preservation of their biological and functional activities can be a challenging process as many membrane proteins are susceptible to denaturation during the isolation process.

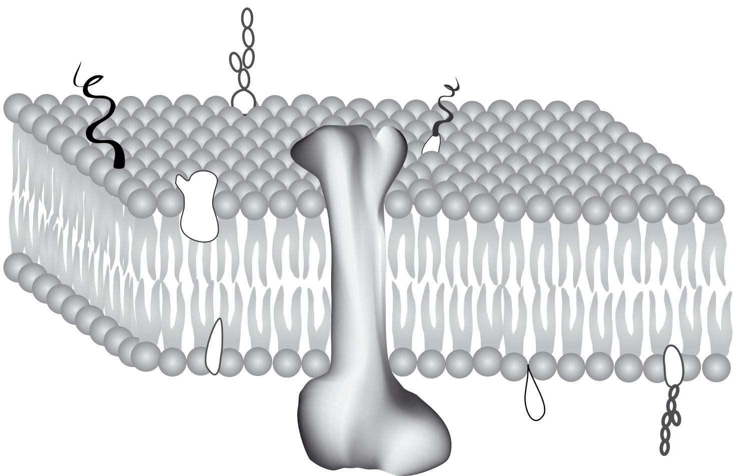

Figure. 5: Fluid-mosaic model of a biological membrane.

How Do Detergents Solubilize Membrane Proteins?

Detergents solubilize membrane proteins by mimicking the lipid-bilayer environment. Micelles formed by detergents are analogous to the bilayers of the biological membranes. Proteins incorporate 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 portions 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.

Although phospholipids can be used as detergents in simulating the bilayer environment, they form large structures, called vesicles, which are not easily amenable to isolation and characterization of membrane proteins. Lyso-phospholipids form micelles that are similar in size to those formed by many detergents. However, they are too expensive to be of general use in everyday protein biochemistry. Hence, the use of synthetic detergents is highly preferred for the isolation of membrane proteins.

Dissolution of membranes by detergents can be divided into different stages (Figure 6). At low concentrations, detergents bind to the membrane by partitioning into the lipid bilayer. At higher concentrations, when the bilayers are saturated with detergents, the membranes disintegrate to form mixed micelles with the detergent molecules. In the detergent-protein mixed micelles, hydrophobic regions of the membrane proteins are surrounded by the hydrophobic chains of micelles. In the final stages, solubilization of the membranes leads to the formation of mixed micelles consisting of lipids and detergents and detergent micelles containing proteins (usually one protein molecule per micelle). For example, solubilization of a membrane containing rhodopsin by digitonin leads to complexes containing one rhodopsin molecule per micelle consisting of 180 digitonin molecules. Other combinations of micelles containing lipids and detergents and lipid-protein-detergent molecules are possible at intermediate concentrations of detergent. Micelles containing protein-detergent molecules can be separated from other micelles based on their charge, size, or density.

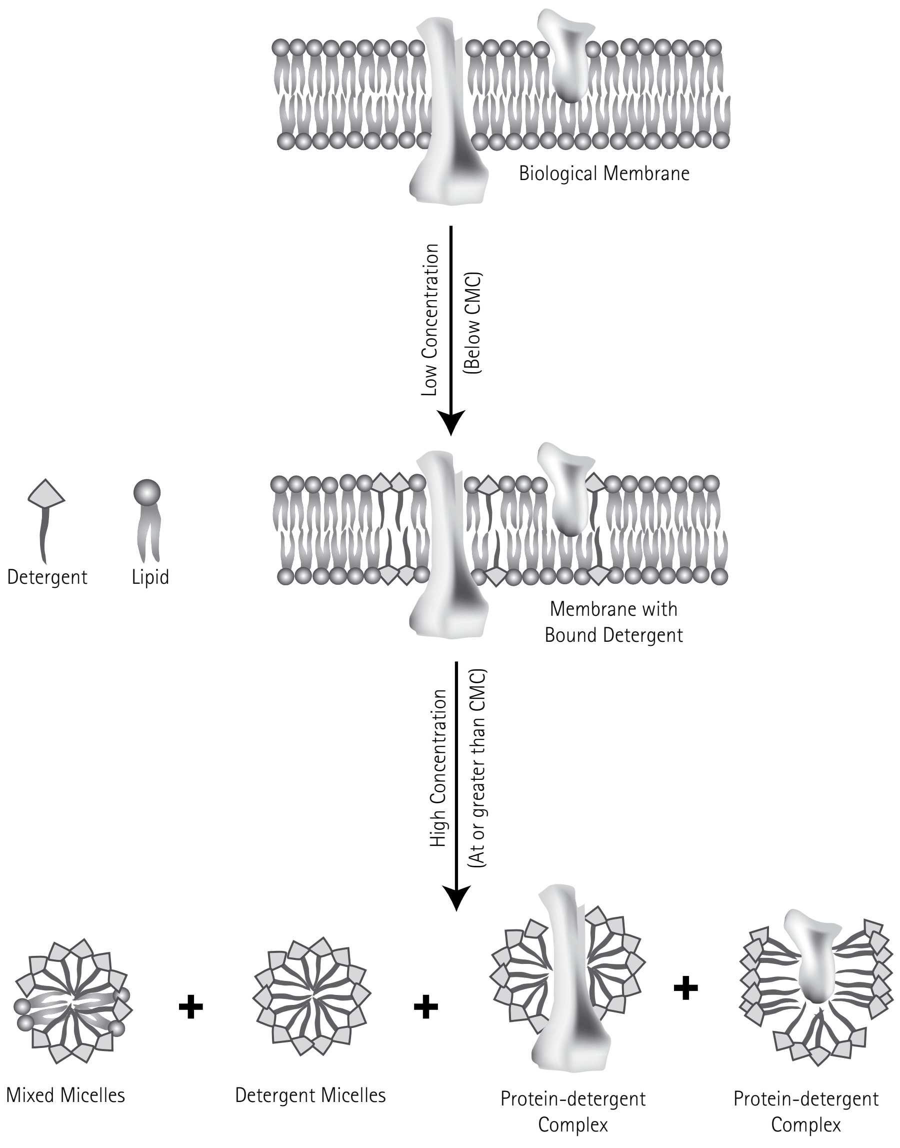

Figure 6: Stages in the dissolution of a biological membrane with detergents.

Detergent-Lipid-Protein Ratios

- It is an important factor for successful solubilization of membrane proteins.
- At low detergent concentration, monomers merely bind to the membrane, and there is minimal membrane perturbation.
- At higher detergent concentration, membrane lysis occurs and lipid-protein-detergent mixed micelles are generated.
- Much higher detergent concentration generates heterogeneous complexes of detergent, lipid, and protein. Progressive delipidation of lipid-protein-detergent mixed micelles occurs, which forces lipids to distribute among the increasing concentration of detergent micelles. This gives rise to lipid/detergent and protein/detergent mixed micelles.
- With increased detergent concentration, a steady state point is reached. Above this point solubilization does not increase any further and activity of the protein begins to decline.

Solubilization of the membrane is often accompanied by selective or differential solubilization of membrane lipids (due to asymmetric extraction of membrane lipids by detergents). This means that certain lipids could be enriched. For example, cholesterol, sphingomyelin, and glycolipids are enriched when red blood cells are extracted with TRITON® X-100 Detergent.

Classification of Detergents

A large number of detergents with various combinations of hydrophobic and hydrophilic groups are now commercially available. Based on the nature of the hydrophilic head group, they can be broadly classified as **ionic**, **non-ionic**, and **zwitterionic** detergents.

Ionic Detergents

Ionic detergents contain a head group with a net charge. They can be either negatively (anionic) or positively charged (cationic). For example, sodium dodecyl sulfate (SDS), which contains the negatively charged sulfate group, is an anionic detergent while cetyl trimethyl-ammonium bromide (CTAB), which carries the positively charged trimethylammonium group, is a cationic detergent. Furthermore, ionic detergents either contain a hydrocarbon (alkyl) straight chain as in SDS and CTAB, or a more complicated rigid steroidal structure as in sodium deoxycholate (see bile acid salts). There is a repulsion between the similarly charged polar groups of detergent molecules in a micelle. Therefore, the size of the 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.

Bile Acid Salts

Bile acid salts are anionic detergents containing a rigid steroidal hydrophobic group (e.g., sodium salts of cholic acid and deoxycholic acid). In addition to the anionic carboxyl group at the end of the short alkyl chain they also carry hydroxyl groups on the steroid structure. Thus, there is no well-defined polar head group. Instead, the bean shaped molecule has a polar and an apolar face.

Bile acid salts form small aggregates. They can be conjugated to taurine or glycine at the end of the carboxyl group. Unlike spherical micelles formed by alkyl ionic detergents, the micelles formed by bile acid salts are kidney shaped due to their rigid structure. As for ionic detergents, their micellar size is influenced by the concentration of the counter ion. Due to the low pK_a (5–6) of the unconjugated bile salt, and low solubility of bile acids, their utility is limited to the alkaline pH range. On the other hand, the pKa of conjugated bile acid salts is much lower, hence, they can be used over a broad pH range. Dihydroxy bile acid salts and deoxycholate are more effective than trihydroxy bile acid salts in membrane solubilization and in dissociation of protein-protein interactions. Trihydroxy bile acid salts are milder and are better suited for removal by dialysis.

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. In general, non-ionic detergents are better suited for breaking lipid-lipid and lipid-protein interactions than protein-protein interactions. Hence, they are considered non-denaturant and 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 the non-ionic detergents.

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 and NP-40 Detergents belong to the latter class (see Table I). Polyoxyethylene chains form random coils and are consequently farther removed from the hydrophobic core of the micelles. Detergents with shorter polyoxyethylene chains form aggregates and viscous solutions in water at room temperature, whereas those with longer chains do not aggregate. 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 also available, in which the aromatic rings are reduced and these detergents exhibit relatively low absorption at 280 nm.

Alkyl glycosides have become more popular as non-ionic detergents in the isolation of membrane proteins for several 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 (cyclic or straight chain) and the polar sugar group can be easily synthesized in pure forms. Third, subtle differences in the physicochemical properties of alkyl glycosides bearing various alkyl chains, attached to either to a glucose, maltose, or a sucrose head group, can be exploited for selective solubilization of membrane proteins.

Zwitterionic Detergents

Zwitterionic detergents are unique in that they offer the combined properties of ionic and non-ionic detergents. Like non-ionic detergents the zwittergents, including CHAPS and the ZWITTERGENT® Detergent 3-X series, 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. Zwittergents such as CHAPS are less denaturing than the ZWITTERGENT® Detergent 3-X series, possibly owing to their rigid steroid ring structure.

Custom Services

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or biospecialist

Types of Detergents: Main Features

<table>
<tr><th>Ionic Detergents</th><th>Non-ionic Detergents</th><th>Zwitterionic Detergents</th></tr>
<tr>
<td>Examples:
Anionic: Sodium dodecyl sulfate (SDS)
Cationic: Cetyl methyl ammonium bromide (CTAB)
• Contain head group with a net charge.
• Either anionic (- charged) or cationic (+ charged).
• Micelle size is determined by the combined effect of hydrophobic attraction of the side chain and the repulsive force of the ionic head group.
• Neutralizing the charge on the head group with increasing counter ions can increase micellar size.
• Useful for dissociating protein-protein interactions.
• The CMC of an ionic detergent is reduced by increasing the ionic strength of the medium, but is relatively unaffected by changes in temperature.</td>
<td>Examples:
TRITON®-X-100 Detergent
n-octyl-β-D-glucopyranoside
• Uncharged hydrophilic head group.
• Better suited for breaking lipid-lipid and lipid-protein interactions.
• Considered to be non-denaturants.
• Salts have minimal effect on micellar size.
• Solubilize membrane proteins in a gentler manner, allowing the solubilized proteins to retain native subunit structure, enzymatic activity and/or non-enzymatic function.
• The CMC of a non-ionic detergent is relatively unaffected by increasing ionic strength, but increases substantially with rising temperature.</td>
<td>Examples:
CHAPS
ZWITTERGENT® Detergents
• Offer combined properties of ionic and non-ionic detergents.
• Lack conductivity and electrophoretic mobility.
• Do not bind to ion-exchange resins.
• Suited for breaking protein-protein interactions.</td>
</tr>
</table>

General Properties of Detergents

Critical Micelle Concentration (CMC)

The CMC can be defined as the lowest concentration above which monomers cluster to form micelles. Alternatively, it is the maximum attainable chemical potential (concentration) of the monomer. In reality, micellization occurs over a narrow concentration range rather than at a particular concentration. The CMC decreases with the length of the alkyl chain and increases with the introduction of double bonds and other branched points such as would occur 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.

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 as well as 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 7).

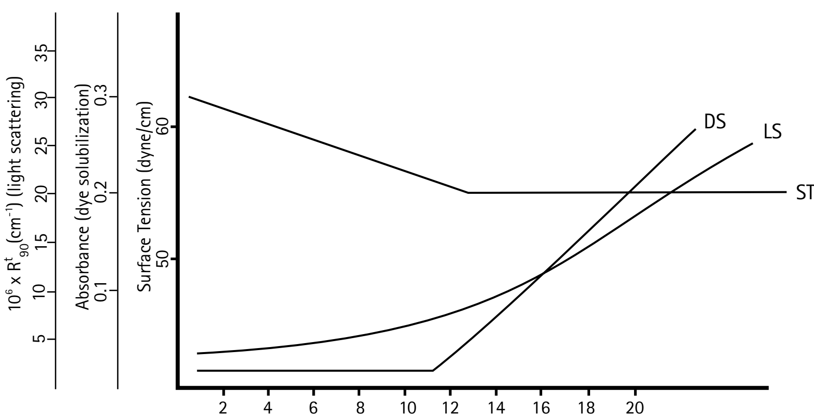

Figure 7: Representative results for determining the CMC of a surfactant by various methods.
Note: *DS = dye solubilization; LS = light scattering; ST= surface tension.*

Given the CMC, the concentration of the detergent and the aggregation number (see page 14), it is possible to calculate the concentration of micelles in moles per liter using the following formula:

$$[\text{micelles}] = (\text{Cs} - \text{CMC}) \div \text{N}$$

where Cs is the bulk molar concentration of detergent and N is the mean aggregation number. For example, a solution containing 35 mM of CHAPSO (M.W. = 630.9) in PBS buffer will have [(35 – 8) ÷ 11] or 2.45 mM of micelles.

Kraft Point

The temperature at which all the three phases—crystalline, micellar, and monomeric—exist in equilibrium is called the **Kraft Point** (Figure 8). At this temperature the detergent solution turns clear and the concentration of the detergent reaches its CMC value. For most detergents, the Kraft point is identical to the CMT. At very low temperatures, detergents remain mainly in an insoluble crystalline state and are in equilibrium with small amounts of dissolved monomer. As the temperature increases, more and more of the monomeric detergent goes into solution until the concentration of the detergent reaches the CMC. At this point it exists predominantly in the micellar form. The temperature at which the monomer reaches the CMC concentration is called **critical micellar temperature (CMT)**.

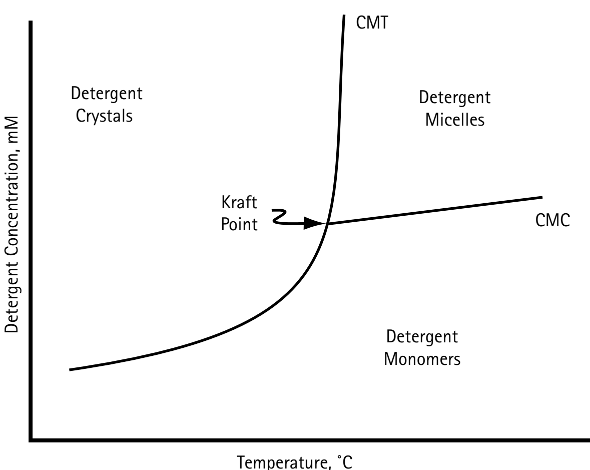

Figure 8: Temperature-composition phase diagram for detergent solutions.

Cloud Point

At a particular temperature above the CMT, non-ionic detergents become cloudy and undergo phase separation to yield a detergent-rich layer and an aqueous layer. This temperature is called the **cloud point**. Phase separation presumably occurs due to a decrease in hydration of the head group. For example, the Cloud Point of TRITON® X-100 Detergent is 64°C whereas that for TRITON® X-114 Detergent is around 22°C. Hence, TRITON® X-114 Detergent solutions are maintained cold. This property can be used to a particular advantage. Membranes can be first solubilized at 0°C and the solution can be warmed to about 30°C to effect the phase separation. This allows partition of integral membrane proteins into the detergent rich phase, which can be separated later by centrifugation.

Aggregation Number

This is the number of monomeric detergent molecules contained in a single micelle. It can be obtained by dividing the micellar molecular weight by the monomeric molecular weight. The molecular weight of micelles can be obtained from various techniques including gel filtration, light scattering, sedimentation equilibrium, and small-angle X-ray scattering. The micelles formed by bile acid salts tend to have low aggregation numbers while those formed by TRITON® have high aggregation numbers. Like micellar size, the aggregation number is also influenced by the ionic strength.

$$\frac{\textbf{micellar molecular weight}}{\textbf{monomeric molecular weight}} = \textbf{aggregation number}$$

Hydrophile-Lipophile Balance (HLB)

The HLB, hydrophilic-lipophilic balance, 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. The most hydrophobic detergents have a HLB number approaching zero, while the least hydrophobic detergents have values reaching 20. Detergents with a HLB value in the range 12 to 20 are preferred for non-denaturing solubilization. For example, solubility of D-alanine carboxypeptidase correlates well with the HLB number, while no correlation exists with CMC or surface tension. Detergents with HLB number between 12-14 were most effective (Umbreit and Stroninger, 1973*). Detergents

*Umbreit, J.N., and Strominger, J.L. 1983. *Proc. Natl. Acad. Sci. USA* **70**, 2997.

in the higher end of the range are preferred for solubilization of extrinsic proteins.

It is 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.

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

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.

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. This is to ensure complete dissolution of the membrane and to provide a large number of micelles such that only one protein molecule is present per micelle. However, for further physicochemical and biochemical characterization of membrane proteins, it is often necessary to remove the unbound detergent.

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

Hydrophobic Adsorption

This method exploits the ability of detergents to bind to hydrophobic resins. For example, CALBIOSORB™ Adsorbent is a hydrophobic, insoluble resin that can be used in batchwise applications to remove excess detergent. Generally, a solution containing a detergent 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. For further details, please refer to Appendix 2. This technique is effective for removal of most detergents. 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. However, this usually requires extended dialyzing periods.

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.

Separation by gel chromatography is based on size. Hence, parameters that influence micellar size (ionic strength, pH, and temperature) should be kept constant from experiment to experiment to obtain reproducible results.

Dialysis

When detergent solutions are diluted below the CMC, the micelles are dispersed into monomers. The size of the monomers is usually an order of magnitude smaller than that of the micelles and thus can be easily removed by dialysis. If a large dilution is not practical, micelles can be dispersed by other techniques such

as the addition of bile acid salts. For detergents with a high CMC, dialysis is usually the preferred choice.

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 so that the protein-containing micelles are adsorbed on the ion-exchange resin and the protein-free micelles pass through. Adsorbed protein is washed with detergent-free buffer and is eluted by changing either the ionic strength or the pH. Alternatively, the protein can be eluted with an ionic detergent thus replacing the non-ionic detergent.

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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.

1. First, survey the literature. Try a detergent that has been used previously for the isolation and characterization of a protein with similar biochemical or enzymological properties should be tried first.
2. Consider the solubility of the detergent at the working temperature. For example, ZWITTERGENT® 3-14 Detergent is insoluble in water at 4°C while TRITON® X-114 Detergent undergoes a phase separation at room temperature.
3. 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 detergent or a ZWITTERGENT® Detergent is the detergent of choice.
4. 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.
5. 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.
6. Consider detergent purity. Detergents of utmost purity should be used since some detergents such as TRITON® X-100 Detergent are generally known to contain peroxides as contaminants. The Calbiochem® PROTEIN GRADE® or ULTROL® GRADE detergents that have been purified to minimize these oxidizing contaminants are recommended.

7. A variety of Molecular Biology Grade detergents are available for any research where contaminants such as DNase, RNase, and proteases are problematic.
8. A non-toxic detergent should be preferred over a toxic one. For example, digitonin, a cardiac glycoside, should be handled with special care.
9. For as yet unknown reasons, specific detergents often work better for particular isolation procedures. For example, *n*-Dodecyl-β-D-maltoside (Cat. No. 324355) 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.
10. 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.
11. 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.

Appendix 1: Non-Detergent Sulfobetaines

A unique line of products, non-detergent sulfobetaines (NDSBs), are now available for protein chemists. NDSBs are zwitterionic compounds. Like ZWITTERGENT® Detergents, NDSBs carry the sulfobetaine hydrophilic head group. However, in contrast to ZWITTERGENT® Detergents, the hydrophobic group in NDSBs is too short for micellar formation even at concentrations as high as 1 M. Hence, they do not behave like detergents. NDSBs were first employed in native isoelectrofocusing gels to neutralize electrostatic interactions without increasing the conductivity. NDSBs are zwitterionic over a wide pH range and can easily be removed by dialysis. They do not absorb significantly at 280 nm. Recently, they have found use in several applications including isolation of membrane proteins and purification of nuclear and halophilic proteins. Presumably, the contribution from the short hydrophobic groups combined with the charge neutralization ability of the sulfobetaine group result in higher yields of membrane proteins. They have also been used in renaturation and refolding of chemically and thermally denatured proteins. NDSBs are shown to prevent protein aggregation and improve the yield of active proteins when added to the buffer during *in vitro* protein denaturation. It is hypothesized that the hydrophobic group, although short, interacts with the hydrophobic regions of the protein to prevent aggregation during renaturation. They have been used in renaturation of fusion proteins from inclusion bodies.

NDSBs do not interfere with enzymatic assays involving chromogenic substrates bearing nitrophenyl groups and they do not inhibit the activities of β-galactosidase and alkaline phospatase. In addition, NDSB-195, NDSB-211, and NDSB-221 do not absorb at 280 nm, making them compatible with protein purification procedures in which the protein concentrations are monitored by measuring absorbance at 280 nm.

Product	Cat. No.	M.W.
NDSB-195	480001	195.3
NDSB-201	480005	201.2
NDSB-211	480013	211.3
NDSB-221	480014	221.3
NDSB-256	480010	257.4
NDSB-256-4T	480011	257.4

You may download product data sheets for these products from our website:

Additional References:

Benetti, P.H., et al. 1998. *Protein Expr. Purif.* **13**, 283.

Blisnick, T., et al. 1998. *Eur. J. Biochem.* **252**, 537.

Chong, Y., and Chen, H. 2000. *Biotechniques* **29**, 1166.

Goldberg, M.E., et al. 1996 . *Folding & Design* **1**, 21.

Ochem, A., et al. 1997. *J. Biol. Chem.* **272**, 29919.

Vuillard, L., et al. 1994. *FEBS Lett.* **353**, 294.

Vuillard, L., et al. 1995. *Anal. Biochem.* **230**, 290.

Vuillard, L., et al. 1995. *Biochem. J.* **305**, 337.

Vuillard, L., et al. 1998. *Eur. J. Biochem.* **256**, 128.

Appendix 2: CALBIOSORB™ Adsorbent

Solubilization of membranes by detergents is essential for their characterization and reconstitution. However, subsequent removal of detergents, particularly the non-ionic detergents with low CMC values, is difficult to achieve (Jones, 1987; Allen, 1980). Dialysis, the most common method of detergent removal, usually requires about 200-fold excess of detergent-free buffer with three to four changes over several days. However, it is ineffective for removal of detergents of low CMC values. In addition, prolonged exposure to detergents during dialysis can damage certain membrane proteins (Jones, 1987). Gel filtration, another common method for detergent removal, is highly effective in the reconstitution of AChR (Mukerjee, 1967), (Ca^{2+}+ Mg^{2+})-ATPase (Andersen, 1983), and lactose transporters (Furth, 1980). However, it gives a broader size distribution of vesicles compared to the dialysis method (Popt, 1984). Therefore, an expeditious alternative in reconstitution studies is the prior removal of detergents by using a resin capable of effectively binding nondialyzable detergents of low CMC.

An excellent detergent removal product, CALBIOSORB™ Adsorbent, is a hydrophobic resin that is processed to eliminate unbound organic contaminants, salts, and heavy metal ions and is especially prepared for the removal of detergents from aqueous media. It is supplied in 100 mM Na_2HPO_4, pH 7.0, containing 0.1% sodium azide and can be easily re-equilibrated with any other suitable buffer prior to use.

Product	Cat No.
CALBIOSORB™ Adsorbent (50 ml)	206550
CALBIOSORB™ Adsorbent, Prepacked Column (5 ml resin bed + 5 ml buffer reservoir)	206552

References

Allen, T., et al. 1980 *Biochim. Biophys. Acta* **601**, 328.

Andersen, J., et al. 1983. *Eur. J. Biochem.* **134**, 205.

Furth, A., 1980. *Anal. Biochem.* **109**, 207.

Jones, O., et al. 1987. In: *Biological Membranes: A Practical Approach* (Findlay, J., and Evens, W., eds.) IRL Press, Oxford **139–177.**

Mukerjee, P. 1967. *Adv. Colloid. Interface Sci.* **1**, 241.

Popt, J., and Changeux, J. 1984. *Physiol. Rev.* **64**, 1162.

Table 1. Detergent Adsorption Capacity of CALBIOSORB™ Adsorbent

Detergent (mg detergent/ml resin)	Cat. No.	Mol. Wt.	Type	Adsorption Capacity (mg detergent/ ml resin)
Cetyltrimethylammonium Bromide (CTAB)	219374	364.5	Cationic	120
CHAPS	220201	614.9	Zwitterionic	110
Cholic Acid, Sodium Salt	229101	430.6	Anionic	73
n-Dodecyl-β-D-maltoside	324355	510.6	Non-ionic	66
n-Hexyl-β-D-glucopyranoside	376965	264.3	Non-ionic	78
n-Octyl-β-D-glucopyranoside	494460	292.4	Non-ionic	132
Sodium Dodecyl Sulfate (SDS)	428015	288.5	Anionic	94
TRITON® X-100 Detergent	648463	647.0 (Av.)	Non-ionic	157
TWEEN® 20, PROTEIN GRADE® Detergent	655206	1228.0 (Av.)	Non-ionic	122

Detergent adsorption capacities were measured by allowing 1.0 g of buffer-free CALBIOSORB™ Adsorbent to equilbrate at room temperature with an excess of detergent (10 ml of 2% in H_2O) for 24 h, then measuring the amount of unadsorbed detergent remaining in the supernatant by gravimetric analysis.

Protocol for Applications Using CALBIOSORB™ Adsorbent, Prepacked Columns

1. Equilibrate the column with 4 to 5 volumes of the sample buffer (e.g., 20 mM sodium phosphate buffer) to remove any sodium azide.
2. Apply the detergent-protein sample to the column.
3. Protein elution from the column may require several column volumes of buffer and can be monitored by UV absorption.

Protocol for Batch Applications Using CALBIOSORB™ Adsorbent

1. Wash CALBIOSORB™ Adsorbent to remove any sodium azide.
2. Calculate the amount of detergent to be removed. For example, 10 ml of 4 mM CHAPS solution contains 24.6 mg of CHAPS.
3. The amount of CALBIOSORB™ Adsorbent required for detergent removal can be determined by inserting the detergent specific adsorption capacity from Table 1 in the following equation:

$$\text{Amount of CALBIOSORB™ Adsorbent} = \frac{\text{Amount of Detergent (mg)}}{\text{Adsorption Capacity (mg/ml)}}$$

(i.e., 24.6 mg CHAPS requires about 0.22 ml of CALBIOSORB™ Adsorbent slurry)

4. Add CALBIOSORB™ Adsorbent directly to the detergent-protein solution. Incubate for 5 minutes at room temperature or for 45 minutes on ice with occasional gentle agitation.
5. Allow the resin to settle. Decant the detergent-free supernatant containing the protein.
6. Dialysis: CALBIOSORB™ Adsorbent may be added directly to a dialysis buffer to facilitate the removal of detergents with low CMC values and to decrease the time required for dialysis when using detergents with higher CMC values. This method is advantageous in that it prevents the adsorption of proteins by the resin.

A wide variety of application-specific pH and buffer compositions (e.g., HEPES, MOPS, PIPES, Tris, etc.) may be used.

Storage and Regeneration

Regeneration: Wash with methanol followed by exhaustive washing with water. Re-equilibrate with the desired buffer used in the experiment. (NOTE: exhaustive washing is essential to remove methanol from resin). CALBIOSORB™ Adsorbent columns can be used up to ten times before disposal. Regeneration of prepacked CALBIOSORB™ Adsorbent columns is not recommended.

Storage: Wash the resin with a buffer containing 0.1% sodium azide and refrigerate at 4°C.

When using either the batch or column method, lower ionic strength buffers may decrease the amount of protein absorption by the resin.

Detergent class	General structure	Examples
Alkyl glycosides	$R-O-(CH_2)_x-CH_3$ R -S-(CH2)x-CH3	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 = maltose x = 11, dodecyl-β-D-maltoside x = 9, decyl-β-D-maltoside R = glucose, x = 7, octyl-β-D-thioglucopyranosid
Bile acids	HO, CH_3, CH_3, O, R, HO, X	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 glycodeoxycho-late 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

Detergent class	General structure	Examples
Glucamides	$CH_3(CH_2)_x{-}C(=O){-}N(CH_3){-}CH_2{-}CH(OH){-}CH(OH){-}CH(OH){-}CH(OH){-}CH_2OH$	x = 8, MEGA-10 x = 7, MEGA-9 x = 6, MEGA-8
		x = H, Deoxy Big CHAP x = OH, Big CHAP

Detergent class	General structure	Examples
Poly-oxyethylenes, monodisperse and polydisperse	$-O(CH_2CH_2O)_x-H$	x = 9–10, reduced TRITON® X-100 x = 7–8, reduced TRITON® X-114
	$-O(CH_2CH_2O)_x-H$	x = 9–10, TRITON® X-100, NP-40 x = 7–8, TRITON® X-114
	$CH_3(CH_2)_y$-$O(CH_2CH_2O)_x$-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
	$HO(CH_2CH_2O)x$-$(CH(CH_3)$-$CH_20)y$-(CH_2CH_20)-$_zH$	x = 98, y = 67, z = 98, PLURONIC® F-127®
	$H-(OCH_2CH_2)w-O$; $O-(CH_2CH_2O)x-H$; $O-(CH_2CH_2O)y-H$; $O-(OCH_2CH_2)z-R$; O 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	$CH_3(CH_2)_{11}-N^{+}(CH_3)_2-CH_2-COO^{-}$ pH ≥ 6	EMPIGEN® BB (*n*-dodecyl-N,N-dimethylglycine)
	$CH_3(CH_2)_x-N^{+}(CH_3)_2-(CH_2)_3-SO_2-O^{-}$	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
	HO, CH_3, HO, CH_3, OH, O, N, H, CH_3, N^{+}, CH_3, X, SO_3^{-}	x = H, CHAPS x = OH, CHAPSO

Selected Bibliography

General Properties of Detergents

Amons, R., and Schrier, P.I. 1981. Removal of sodium dodecyl sulfate from proteins and peptides by gel filtration. *Anal. Biochem.* **116**, 439.

Ashani, Y., and Catravas, G.N. 1980. Highly reactive impurities in TRITON ® X-100 and BRIJ® 35: partial characterization and removal. *Anal. Biochem.* **109**, 55.

Banerjee, P., et al. 1995. Differential solubilization of lipids along with membrane proteins by different classes of detergents. *Chem. Phys. Lipids* **77**, 65.

Banerjee, P., et al. 1993. Differential solubilization of membrane lipids by detergents: coenrichment of the sheep brain serotonin 5-HT1A receptor with phospholipids containing predominantly saturated fatty acids. *Arch. Biochem. Biophys.* **305**, 68.

Brito, R.M., and Vaz, W.L.C. 1986. Determination of the critical micelle concentration of surfactants using the fluorescent probe N-phenyl-1- naphthylamine. *Anal. Biochem.***152**, 250.

Chang, H.W., and Bock, E. 1980. Pitfalls in the use of commercial nonionic detergents for the solubilization of integral membrane proteins: sulfhydryl oxidizing contaminants and their elimination. *Anal. Biochem.* **104**, 112.

Chattopadhay, A., and London, E. 1984. Fluorimetric determination of critical micelle concentration avoiding interference from detergent charge. *Anal. Biochem.* **139**, 408.

Expert-Bezancon, N., et al. 2003. Physical-chemical features of non-detergent sulfobetaines active as protein folding helpers. *Biophys. Chem.* **100**, 409.

Furth, A.H., et al. 1984. Separating detergents from proteins. *Methods Enzymol.* **104**, 318.

Helenius, A., and Simons, K. 1975. Solubilization of membranes by detergents. *Biochim. Biophys. Acta* **415**, 29.

Helenius, A., et al. 1979. Properties of detergents. *Methods Enzymol.* **56**, 734.

Hjelmeland, L.M., and Chrambach, A. 1984. Solubilization of functional membrane proteins. *Methods Enzymol.* **104,** 305.

Horigome, T., and Sugano, H. 1983. A rapid method for removal of detergents from protein solutions. *Anal. Biochem.* **130,** 393.

Lever, M. 1977. Peroxides in detergents as interfering factors in biochemical analysis. *Anal. Biochem.* **83,** 274.

Midura, R.J., and Yanagishita, M. 1995. Chaotropic solvents increase the critical micellar concentrations of detergents. *Anal. Biochem.* **228,** 318.

Neugebauer, J. M. 1990. Detergents: An overview. *Methods. Enzymol.* **182**, 239.

Racker, E., et al. 1979. Reconstitution, a way of biochemical research: some new approaches to membrane-bound enzymes. *Arch. Biochem. Biophys.***198**, 470.

Robinson, N.C., et al. 1984. Phenyl-sepharose mediated detergent exchange chromatography: its application to exchange of detergents bound to membrane proteins. *Biochemistry* **23**, 6121.

Singer, S.J., and Nicolson, G. L. 1972. The fluid mosaic model of the structure of cell membranes. *Science* **175**, 720.

Slinde, E., and Flatmark, T. 1976. Effect of the hydrophile-lipophile balance of non-ionic detergents on the solubilization of biological membranes and their integral b-type cytochromes. *Biochim. Biophys. Acta* **455**, 796.

Storm, D. R., et al. 1976. The HLB dependency for detergent solubilization of hormonally sensitive adenylate cyclase.
J. Supramol. Struct. **4**, 221.

Tanford, C. 1980. The Hydrophobic Effect: Formation of Micelles and Biological Membranes (2nd ed.), New York, Wiley.

Umbreit, J.N., and Strominger, J.L. 1973. Relation of detergent HLB number to solubilization and stabilization of D-alanine carboxypeptidase from *Bacillus subtillis* membranes. *Proc. Natl. Acad. Sci. USA* **70**, 2997.

Detergent-specific References

Zwitterionic Detergents

Abdullah, K.M., et al. 1995. Purification of baculovirus-overexpressed cytosolic phospholipase A2 using a single-step affinity column chromatography. *Protein Expr. Purif.* **6**, 291.

Cornelius, F., and Skou, J.C. 1984. Reconstitution of sodium-potassium ATPase into phospholipid vesicles with full recovery of its specific activity. *Biochim. Biophys. Acta* **772**, 357.

Fiedler, K., et al. 1993. Glycosphingolipid-enriched, detergent-insoluble complexes in protein sorting in epithelial cells. *Biochemistry* **32**, 6365.

Fricke, B., et al. 2000. Quantitative determination of Zwitterionic detergents using salt-induced phase separation of Triton X-100. *Anal. Biochem.* **281**, 144.

Fulop, M.J., et al. 1992. Use of a zwitterionic detergent for the restoration of the antibody binding capacity of immunoblotted *Francisella tularensis* lipopolysaccharide. *Anal. Biochem.* **203**, 141.

Hansel, A., et al. 1994. Isolation and characterization of porin from the outer membrane of *Synechococcus* PCC 6301. *Arch. Microbiol.* **161**, 163.

Hassanain, H.H., et al. 1993. Enhanced gel mobility shift assay for DNA-binding factors. *Anal. Biochem.* **213**, 162.

Iizuka, M., and Fukuda, K. 1993. Purification of the bovine nicotinic acetylcholine receptor α subunit expressed in baculovirus-infected insect cells. *J. Biochem.* (Tokyo) **114**, 140.

Lowthert, L.A., et al. 1995. Empigen BB: a useful detergent for solubilization and biochemical analysis of keratins. *Biochem. Biophys. Res. Commun.* **206**, 370.

Nguyen, T.D., et al. 1995. Solubilization of receptors for pancreatic polypeptide from rat liver membranes. *Am. J. Physiol.* **268**, G215.

Nollstadt, K.H., et al. 1989. Potential of the sulfobetaine detergent ZWITTERGENT® 3-12 as a desorbing agent in biospecific and bioselective affinity chromatography. *J. Chromatogr.* **497**, 87.

Paik, S.R., et al. 1993. The TF1-ATPase and ATPase activities of assembled alpha 3 beta 3 gamma, alpha 3 beta 3 gamma delta, and alpha 3 beta 3 gamma epsilon complexes are stimulated by low and inhibited by high concentrations of rhodamine 6G whereas the dye only inhibits the alpha 3 beta 3, and alpha 3 beta 3 delta complexes. *J. Bioenerg. Biomembr.* **25**, 679.

Rabilloud, T., et al. 1997. Improvement of the solubilization of proteins in two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* **18**, 307.

Rabilloud, T., et al. 1990. Amidosulfobetaines, a family of detergents with improved solubilization properties: Application for isoelectric focusing under denaturing conditions. *Anal Biochem.* **185**, 94.

Rhinehart-Jones, T., and Greenwalt, D.E. 1996. A detergent-sensitive 113-kDa conformer/complex of CD36 exists on the platelet surface. *Arch. Biochem. Biophys.* **326**, 115.

Riccio, P., et al. 1994. A new detergent to purify CNS myelin basic protein isoforms in lipid-bound form. *NeuroReport* **5**, 689.

Russell-Harde, D., et al. 1995. The use of ZWITTERGENT® 3-14 in the purification of recombinant human β-interferon Ser 17 (Betaseron). *J. Interferon Cytokine Res.* **15**, 31.

Schurholz, T., et al. 1992. Functional reconstitution of the nicotinic acetylcholine receptor by CHAPS dialysis depends on the concentrations of salt, lipid, and protein. *Biochemistry* **31**, 5067.

Schurholz, T. 1996. Critical dependence of the solubilization of lipid vesicles by the detergent CHAPS on the lipid composition. Functional reconstitution of the nicotinic acetylcholine receptor into preformed vesicles above the critical micellization concentration. *Biophys. Chem.* **58**, 87.

Spivak, J.L., et al. 1996. Isolation of the full-length murine erythropoietin receptor using a baculovirus expression system. *Blood* **87**, 926.

Stabel, T.J., et al. 1994. Periplasmic location of *Brucella abortus* Cu/Zn superoxide dismutase. *Vet. Microbiol.* **38**, 307.

Stark, R.E., et al. 1984. Physical studies of CHAPS, a new detergent for the study of visual pigments. *J. Phys. Chem.* **88**, 6063.

Tastet, C., et al. 2003. Structure-efficiency relationships of zwitterionic detergents as protein solubilizers in two-dimensional electrophoresis. *Proteomics* **3**, 111.

Valerio, M., and Haraux, F. 1993. Catalytic and activating protons follow different pathways in the H^+-ATPase of potato tuber mitochondria. *FEBS Lett.* **336**, 83.

Valerio, M., et al. 1993. The electrochemical-proton-gradient-activated states of FoF1 ATPase in plant mitochondria as revealed by detergents. *Eur. J. Biochem.* **216**, 565.

Wallace, A.V., and Kuhn, N.J. 1986. Incorporation into phospholipid vesicles of pore-like properties from Golgi membranes of lactating rat mammary gland. *Biochem. J.* **236**, 91.

Warren, B.S., et al. 1996. Purification and stabilization of transcriptionally active glucocorticoid receptor. *J. Biol. Chem.* **271**, 11434.

Xin, H.B., et al. 1995. Affinity purification of the ryanodine receptor/calcium release channel from fast twitch skeletal muscle based on its tight association with FKBP12. *Biochem. Biophys. Res. Commun.* **214**, 263.

Non-ionic Detergents

Bass, W.T., and Bricker, T.M. 1988. Dodecylmaltoside-sodium dodecylsulfate two-dimensional polyacrylamide gel electrophoresis of chloroplast thylakoid membrane proteins. *Anal. Biochem.* **171**, 330.

Begona-Ruiz, M., et al. 1994. An assessment of the biochemical applications of the non-ionic surfactant HECAMEG. *Biochim. Biophys. Acta* **1193**, 301.

Blochet, J.E., et al. 1993. Complete amino acid sequence of puroindoline, a new basic and cystine-rich protein with a unique tryptophan-rich domain, isolated from wheat endosperm by TRITON® X-114 phase partitioning. *FEBS Lett.* **329**, 336.

Dudek, R., et al. 1993. Effect of amphiphiles on nitric oxide synthase in endothelial cells. *Pharmacology* **48**, 374.

Dudeja, P.K., et al. 1995. Reversal of multidrug resistance phenotype by surfactants: relationship to membrane lipid fluidity. *Arch. Biochem. Biophys.* **319**, 309.

El-Kebbi, I.M., et al. 1994. Regulation of the GLUT1 glucose transporter in cultured myocytes: Total number and subcellular distribution as determined by photoaffinity labeling. *Biochem. J.* **301**, 35.

Englund, A.K., et al. 1995. Capillary and rotating-tube isoelectric focusing of a transmembrane protein, the human red cell glucose transporter. *J. Chromatogr.* **711**, 217.

Florke, R.R., et al. 1993. Differential insertion of insulin receptor complexes into TRITON® X-114 bilayer membranes: evidence for a differential accessibility of membrane-exposed receptor domain. *Eur. J. Biochem.* **211**, 241.

Franek, K.J., et al. 1994. Reliable method for the simultaneous detection of cytoplasmic and surface CD3 epsilon expression by murine lymphoid cells. *Cytometry* **17**, 224.

Ghebeh, H., et al. 1998. Development of an assay for the measurement of the surfactant Pluronic F-68 in mammalian cell culture medium. *Anal. Biochem.* **262**, 39.

Izawa, S., et al., 1993. Introduction of a series of alkyl thiomaltosides, useful new non-ionic detergents, to membrane biochemistry. *J. Biochem.* **113**, 573.

Kempf, A.C., et al. 1995. Truncated human P450 2D6: expression in *Escherichia* coli, Ni^{2+}-chelate affinity purification, and characterization of solubility and aggregation. *Arch. Biochem. Biophys.* **321**, 277.

Konig, N., and Zampighi, G.A. 1995. Purification of bovine lens cell-to cell channels composed of connexin 44 and connexin 50. *J. Cell. Sci.* **108**, 3091.

Konrad, R.J., et al. 1995. The heterotrimetic G-protein G_i is localized to the insulin secretory granules of β-cells and is involved in insulin exocytosis. *J. Biol. Chem.* **270**, 12869.

Lopez-Nicholas, J.M., et al. 1994. An octaethylene glycol monododecyl ether-based mixed micellar assay for lipoxygenase acting at neutral pH. *Anal. Biochem.* **221**, 410.

Mattsson, J.P., et al. 1994. Isolation and reconstitution of a vacuolartype proton pump of osteoclast membranes. *J. Biol. Chem.* **269**, 24979.

Mimura, K., et al. 1993. Change in oligomeric structure of solubilized Na^+/K^+-ATPase induced by octaethylene glycol dodecyl ether, phosphatidylserine and ATP. *Biochim. Biophys. Acta* **1145**, 63.

Moller, J.V., and le Maire, M. 1993. Detergent binding as a measure of hydrophobic surface area of integral membrane proteins. *J. Biol. Chem.* **268**, 18659.

Nederlof, P.M., et al. 1995. Nuclear localization signals of human and Thermoplasma proteasomal subunits are functional *in vitro. Proc. Natl. Acad. Sci. USA* **92**, 12060.

Nguyen, G., and Kruithof, E.K. 1993. A quantitative receptor assay using TRITON® X-114 for plasminogen activator binding proteins in solubilized membranes from human liver and placenta. *Anal. Biochem.* **208**, 277.

Nock, B., et al. 1993. Extracti-Gel D chromatography is a simple, efficient method of removing digitonin during receptor purification: Application to the κ_1 opioid receptor. *J. Neurosci. Methods* **50**, 353.

Ogiso, T., et al. 1994. Mechanism of enhancement effect of *n*-octyl-β-D-thioglucoside on the transdermal penetration of fluorescein isothiocyanate- labeled dextrans and the molecular weight dependence of water-soluble penetrants through stripped skin. *J. Pharm. Sci.* **83**, 1676.

Okamura, S., and Yamashita, S. 1994. Purification and characterization of phosphatidylcholine phospholipase D from pig lung. *J. Biol. Chem.* **269**, 31207.

Pierre, Y., et al. 1995. Purification and characterization of the cytochrome b6f complex from *Chlamydomonas reinhardtii. J. Biol. Chem.* **270**, 29342.

Previati, M., et al. 1994. Diacylgylcerol kinase activity in rat liver nuclei. *Cell Signal* **6**, 393.

Ramsby, M.L., et al. 1994. Differential detergent fractionation of isolated hepatocytes: biochemical, immunological and two-dimensional gel electrophoresis characterization of cytoskeletal and noncytoskeletal compartments. *Electrophoresis* **15**, 265.

Sivaprasadarao, A., et al. 1994. Solubilization and purification of the retinol-binding protein receptor from human placental membranes. *Biochem. J.* **302**, 245.

Slowiejko, D.M., et al. 1994. Sequestration of muscarinic cholinergic receptors in permeabilized neuroblastoma cells.
J. Neurochem. **62**, 1795.

Soulimane, T., et al. 1995. Three-dimensional crystals of cytochrome-c oxidase from *Thermus thermophilus* diffracting to 3.8 Å resolution. *FEBS Lett.* **368**, 132.

Strancar, A., et al. 1994. Extraction of TRITON® X-100 and its determination in virus-inactivated human plasma by the solvent-detergent method. *J. Chromatogr A.* **658**, 475.

Temkin, R.J., et al. 1993. Advantages of digitonin extraction to reveal the intracellular structure of rat glomerular podocytes for high resolution scanning electron microscopy. *Microsc. Res. Tech.* **26**, 260.

Ti, Z.C., et al. 1990. Purification of a membrane glycoprotein with an inositol-containing phospholipid anchor from *Dictyostelium discoideum. J. Biotechnol.* **16**, 233.

Virta, M., et al. 1995. Real-time measurement of cell permeabilization with low-molecular weight membranolytic agents. *J. Antimicrob. Chemother.* **36**, 303.

Wallace, P.G., et al. 1994. A method for the determination of the cellular phosphorylation potential and glycolytic intermediates in yeast. *Anal. Biochem.* **222**, 404.

Wong, P. 1993. The state of association of Band 3 of the human erythrocyte membrane: Evidence of a hexamer. *Biochem Biophys Acta* **1151**, 21.

Zardeneta, G., and Horowitz, P.M. 1992. Micelle-assisted protein folding. Denatured rhodanese binding to cardiolipin-containing lauryl maltoside micelles results in slower refolding kinetics but greater enzyme reactivation. *J. Biol. Chem.* **267**, 5811.

Ionic Detergents

Alba, F., et al. 1995. Properties of rat brain dipeptidyl aminopeptidases in the presence of detergents. *Peptides* **16**, 325.

Alba, F., et al. 1995. Comparison of soluble and membrane-bound pyroglutamyl peptidase I activities in rat brain tissues in the presence of detergents. *Neuropeptides* **29**, 103.

Almog, R., et al. 1990. A methodology for determination of Phospholipids. *Anal. Biochem.* **188**, 237.

Bhavsar, J.H., et al. 1994. A method to increase efficiency and minimize anomalous electrophoretic transfer in protein blotting. *Anal. Biochem.* **221**, 234.

Camilleri, P., et al. 1995. High resolution and rapid analysis of branched oligosaccharides by capillary electrophoresis. *Anal. Biochem.* **230**, 115.

Hassaan, A.M., et al. 1995. Calreticulin is the major Ca^{2+} storage protein in the endoplasmic reticulum of the pea plant (*Pisum sativum*). *Biochem. Biophys. Res. Commun.* **211**, 54.

Iwasaki, Y., et al. 1994. Purification and properties of phosphatidylinositol- specific phospholipase C from *Streptomyces antibioticus. Biochim. Biophys. Acta.* **1214**, 221.

Kantorow, M., et al. 1995. Conversion from oligomers to tetramers enhances autophosphorylation b lens α-A-crystallin. Specificity between α-A- and α-B-crystallin subunits. *J. Biol. Chem.* **270**, 17215.

Kapp, O.H., and Vinogradov, S.N. 1978. Removal of sodium dodecyl sulfate from Proteins. *Anal. Biochem.* **91**, 230-235.

Komuro, T., et al. 1993. Detection of low molecular size lipopolysaccharide contaminated in dialysates used for hemodialysis therapy with polyacrylamide gel electrophoresis in the presence of sodium deoxycholate. *Int. J. Artif. Organs* **16**, 245.

Muller G., et al. 1994. 42-Aminobenzamidotaurocholic acid selectively solubilizes glycosyl-phosphatidylinositol-anchored membrane proteins and improves lipolytic cleavage of their membrane anchors by specific phospholipases. *Arch. Biochem. Biophys.* **309**, 329.

Palmer, M., et al. 1995. Kinetics of streptolysin O self-assembly. *Eur. J. Biochem.* **231**, 388.

Rozema, Z., and Gellman, S.H. 1996. Artificial chaperone-assisted refolding of carbonic anhydrase. *J. Biol. Chem.* **271**, 3478.

Siler, D.J., and Cornish, K. 1995. Measurement of protein in natural rubber latex. *Anal. Biochem.* **229**, 278.

Spivak, W., et al. 1988. Spectrophotometric determination of the critical micellar concentration of bile salts using bilirubin monoglucuronide as a micellar probe. Utility of derivative spectroscopy. *Biochem. J.* **252**, 275.

Sundquist, B., et al. 1983. Assay of detergents by rocker electrophoresis in agarose gels containing red blood cells: "Rocker hemolysis". *Biochem. Biophys. Res. Commun.* **114**, 699.

Tadey, T., and Purdy, W.C. 1993. Effect of detergents on the eletrophoretic behavior of plasma apolipoproteins in capillary electrophoresis. *J. Chromatogr. A* **652**, 131.

Taipale, J., et al. 1995. Human mast cell chymase and leukocyte elastase release latent transforming growth factor β1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J. Biol. Chem.* **270**, 4689.

Non-Ionic Detergents

Non-Ionic Detergent	Cat. No.	MW[a] (anhydrous)	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	HLB
APO-10	178375	218.3	4.6	131	28,000	
APO-12	178377	246.4	0.568	2232	500,000	
Big CHAP	200965	878.1	3.4	10	8,800	
Big CHAP, Deoxy-	256455	862.1	1.1-1.4	8–16	10,500	
BRIJ® 35 Detergent, 30% Aqueous Solution	203724	1199.6	0.092	40	48,000	
BRIJ® 35 Detergent, PROTEIN GRADE®, 10% Solution	203728	1199.6	0.09	40	48,000	
$C_{12}E_8$	205528	538.8	0.110	123	66,000	
$C_{12}E_8$, PROTEIN GRADE® Detergent, 10% Solution	205532	538.8	0.110	123		13.1
$C_{12}E_9$, PROTEIN GRADE® Detergent, 10% Solution	205534	582.8	0.080			13.6
Cyclohexyl-*n*-hexyl-β-D-maltoside, ULTROL® Grade	239775	508.6	0.560	63	32,000	
n-Decanoylsucrose	252721	496.6	2.5	–		
n-Decyl-β-D-maltopyranoside, ULTROL® Grade	252718	482.6	1.6	–		
Digitonin, Alcohol-Soluble, High Purity	300411	1229.3	–	60–70	7000	
Digitonin, High Purity	300410	1229.3	–	60–70	7000	
n-Dodecanoylsucrose	324374	524.6	0.3	–		
n-Dodecyl-β-D-glucopyranoside	324351	348.5	0.130	200	70,000	
n-Dodecyl-β-D-maltoside, ULTROL® Grade	324355	510.6	0.1–0.6	98	50,000	
ELUGENT™ Detergent, 50% Solution	324707	–	–	–		13.1
GENAPOL® C-100, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	345794	627.0	–	–		14

Key: a: Average molecular weights are given for detergents composed of mixtures of different chain lengths. b: Temperature = 20–25°C

Non-Ionic Detergent	Cat. No.	MW[a] (anhydrous)	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	HLB
GENAPOL® X-080, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	345796	553	0.06–0.15	—		13
GENAPOL® X-100, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	345798	641.0	0.15	88	56,000	13–14
HECAMEG	373272	335.4	19.5			
n-Heptyl-β-D-glucopyranoside	375655	278.3	79	—		
n-Heptyl-β-D-thioglucopyranoside, ULTROL® Grade, 10% Solution	375659	294.4	30	—		
n-Hexyl-β-D-glucopyranoside	376965	264.3	250	—		
MEGA-8, ULTROL® Grade	444926	321.5	58	—	—	
MEGA-9, ULTROL® Grade	444930	335.5	19–25	—	—	
MEGA-10, ULTROL® Grade	444934	349.5	6–7	—	—	
n-Nonyl-β-D-glucopyranoside	488285	306.4	6.5	—	—	
NP-40, Alternative	492016	—	0.05–0.3			
NP-40, Alternative, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	492018	—	0.05–0.3			
n-Octanoylsucrose	494466	468.5	24.4	—	—	
n-Octyl-β-D-glucopyranoside	494459	292.4	20–25	84	25,000	
n-Octyl-β-D-glucopyranoside, ULTROL® Grade	494460	292.4	20–25	84	25,000	
n-Octyl-β-D-maltopyranoside	494465	454.5	23.4	84	38,000	

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Non-Ionic Detergent	Cat. No.	MW[a] (anhydrous)	CMC[b] (mM)	Aggregation No.	Average Micellar Weight	HLB
n-Octyl-β-D-thioglucopyranoside, ULTROL® Grade	494461	308.4	9	–	–	
PLURONIC® F-127, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	540025	12,500 (avg.)	4–11	–	–	
Saponin	558255					
TRITON® X-100 Detergent	648462	625 (avg.)	0.2–0.9	100–155	80,000	
TRITON® X-100, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	648463	625 (avg.)	0.2–0.9	100–155	80,000	
TRITON® X-100 Detergent, Molecular Biology Grade	648466	625 (avg.)	0.2–0.9	100–155	80,000	
TRITON® X-100, Hydrogenated Detergent	648465	631 (avg.)	0.25	100–155	80,000	
TRITON® X-100, Hydrogenated, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	648464	631 (avg.)	0.25	100–155	80,000	
TRITON® X-114, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	648468	537 (avg.)	0.35	–	–	
TWEEN® 20 Detergent	655205	1228 (avg.)	0.059	–	–	16.7
TWEEN® 20 Detergent, Molecular Biology Grade	655204	1228 (avg.)	0.059			
TWEEN® 20, PROTEIN GRADE® Detergent, 10% Solution	655206	1228 (avg.)	0.059	–	–	16.7
TWEEN® 80, PROTEIN GRADE® Detergent, 10% Solution	655207	1310 (avg.)	0.012	58	76,000	15

Key: a: Average molecular weights are given for detergents composed of mixtures of different chain lengths. b: Temperature = 20–25°C

Ionic Detergents

Ionic Detergent	Cat. No.	MW[a] (anhydrous)	CMC[b] (mM)	Aggregation No.	Average Micellar Weight
Cetyltrimethylammonium Bromide (CTAB), Molecular Biology Grade	219374	364.5	1.0	170	62,000
Chenodeoxycholic Acid, Free Acid	2204	392.6	–	–	–
Chenodeoxycholic Acid, Sodium Salt	220411	414.6	–	–	
Cholic Acid, Sodium Salt	229101	430.6	9–15	2.0	900
Cholic Acid, Sodium Salt, ULTROL® Grade	229102	430.6	9–15	2.0	900
Deoxycholic Acid, Sodium Salt	264101	414.6	4–8	4–10	1700–4200
Deoxycholic Acid, Sodium Salt, ULTROL® Grade	264103	414.6	2–6	3–12	1200–4900
Glycocholic Acid, Sodium Salt	360512	487.6	7.1	2.1	1000
Glycodeoxycholic Acid, Sodium Salt	361311	471.6	2.1	2	900
Glycolithocholic Acid, Sodium Salt	36217	455.6			
Glycoursodeoxycholic Acid, Sodium Salt	362549	471.6			
Lauroylsacrosine, Sodium Salt	428010	293.4	–	2.0	600
LPD-12	437600	2839.4	< 0.001		
Sodium *n*-Dodecyl Sulfate (SDS)	428015	288.4	7–10	62	18,000
Sodium *n*-Dodecyl Sulfate (SDS), High Purity	428016	288.4	7–10	62	18,000
Sodium *n*-Dodecyl Sulfate (SDS), Molecular Biology Grade	428023	288.4	7–10	62	18,000
Sodium *n*-Dodecyl Sulfate (SDS), 20% Solution	428018	288.4	7–10	62	18,000

Key: a: Average molecular weights are given for detergents composed of mixtures of different chain lengths. b: Temperature = 20–25°C.

Ionic Detergent	Cat. No.	MW[a] (anhydrous)	CMC[b] (mM)	Aggregation No.	Average Micellar Weight
Taurochenodeoxycholic Acid, Sodium Salt	580211	521.7	–	–	
Taurocholic Acid, Sodium Salt	580217	537.7	3–11	4	2100
Taurocholic Acid, Sodium Salt, ULTROL® Grade	580218	537.7	3–11	4	2100
Taurodeoxycholic Acid, Sodium Salt	580221	521.7	1–4	6	3100
Tauroursodeoxycholic Acid, Sodium Salt	580549	521.7	–	–	
Ursodeoxycholic Acid, Sodium Salt	672305	414.6			

Key: a: Average molecular weights are given for detergents composed of mixtures of different chain lengths. b: Temperature = 20–25°C.

Zwitterionic Detergents

Detergent	Cat. No.	MW[a] (anhydrous)	CMC[b] (mM)	Aggregation No.	Average Micellar Weight
ASB–C7BzO	182729	399.6			
ASB-14	182750	434.7			
ASB-14-4	182751	448.7			
ASB-16	182755	462.7			
ASB-C6Ø	182728	412.6			
ASB-C8Ø	182730	440.6			
CHAPS	220201	614.9	6-10	4-14	6150
CHAPSO	220202	630.9	8	11	7000
DDMAB	252000	299.5	4.3		
DDMAU	252005	397.7	0.13		
PMAL-B-100	528200	9000			
ZWITTERGENT® 3-08 Detergent	693019	279.6	330		
ZWITTERGENT® 3-10 Detergent	693021	307.6	25-40	41	12,500
ZWITTERGENT® 3-12 Detergent	693015	335.6	2-4	55	18,500
ZWITTERGENT® 3-14 Detergent	693017	363.6	0.1-0.4	83	30,000
ZWITTERGENT® 3-16 Detergent	693023	391.6	0.01-0.06	155	60,000

Key: a: Average molecular weights are given for detergents composed of mixtures of different chain lengths. b: Temperature = 20°C

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