anatrace

Technical Bulletin 101

Precipitation

During your membrane protein work, it's very frustrating when you prepare a solution containing detergent, buffer, salt, etc.; cool it to 4°C and it precipitates. It is especially annoying when it doesn't precipitate right away, but one to two days, or even a week later. Below are some possible reasons and troubleshooting suggestions:

Combination of reagents causes precipitation

Is the pH and ionic strength compatible with each of the components? The presence of an organic solvent as one component can often result in the precipitation of other components. Try making a solution of each component to test its solubility in water.

Microbial growth causes precipitation

Most solutions used in protein research are readily biodegradable. If you are using a nonionic detergent that is a sugar derivative it is easily degraded by microorganisms. It will probably take a few days to detect a precipitate or floating material in the solution. To avoid possible microbial contamination, new solution should be prepared daily whenever possible.

As a precaution, many researchers routinely filter their solutions through a 0.2 µm filter.

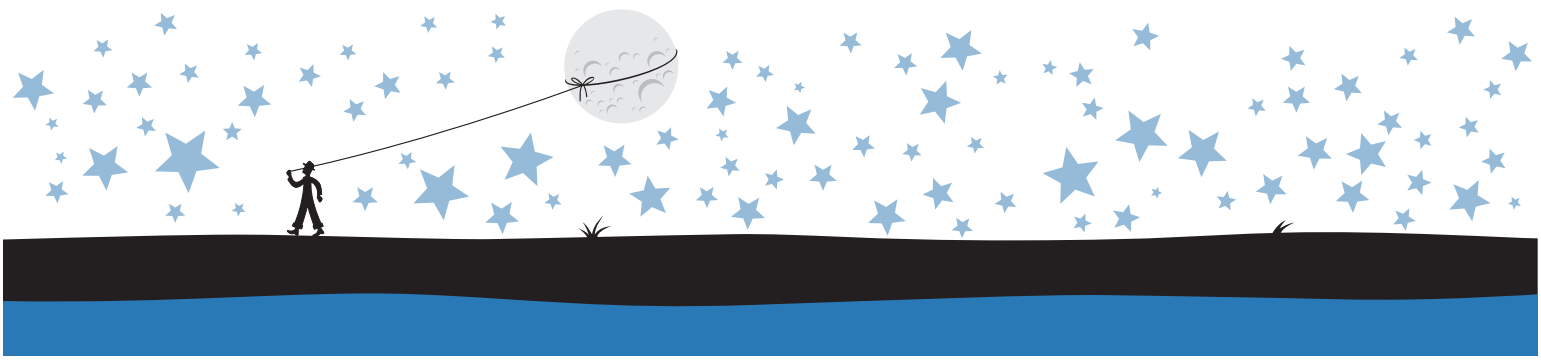
Presence of "starting"alcohol

Many alkyl glucosides and alkyl maltosides will contain some of the corresponding or starting alcohol, which is not completely removed during purification. When a concentrated solution of detergent is prepared and cooled to 4°C, a "milkiness" or cloudiness may become apparent due to precipitation of the alcohol. In some instances, such as extraction of a membrane protein, a slight cloudiness may not be a problem.

If you use Anagrade® detergents from Anatrace®, precipitation caused by starting alcohols should never happen because the level of starting alcohol is less than 0.005% (w/w). If you use our Sol-Grade® detergents, you may notice a slight cloudiness when you cool the solution. The level of corresponding alcohol can be as much as 0.05% for Sol-Grade detergents. It has been our experience that some other suppliers may have as high as 0.5% alcohol present, which will result in a very milky appearance.

Kinetic effect of detergent solubilization

Sometimes when Tetradecyl Maltoside is dissolved at a concentration of 20% (w/v) at room temperature and cooled to 4°C, a precipitate appears. However, if the detergent solution is heated to 50°C before cooling, the precipitate does not form. Possibly the Tetradecyl Maltoside "dissolves" as an aggregate at room temperature which reprecipitates when cooled. Thus, the detergent was never truly dissolved. This phenomenon has also been observed with HEGA®-10. If you think that you have observed this phenomenon, try heating the precipitated solution to 50°C for a few minutes and then cool the solution again. No precipitate should form.



Supersaturation

The detergent solution stored at 4°C may be supersaturated. Supersaturated solutions can exist for days or even weeks before precipitation occurs. The only recourse when supersaturation is confirmed is to reduce the detergent concentration.

Low and Moderately Soluble Detergents

Detergent	Product No.	Solubility (%)[1]	Temperature (°C)
Anameg®-7, Anagrade	A340	≥ 10	0 - 5
Big CHAP	B300	≥ 10	20
Big CHAP, Deoxy, Analytical Grade	B310	≥ 10	20
n-Decyl-β-D-Glucopyranoside, Anagrade[2]	D321	≥ 0.1	20
n-Decyl-β-D-Thioglucopyranoside, Anagrade[3]	D323	≥ 1	20
n-Dodecyl-β-D-Glucopyranoside, Anagrade	D318	practically insoluble in water	20
n-Dodecyl-β-D-Thiomaltopyranoside, Anagrade	D342	≥10	20
Fos-Mea®-12[2]	F212	≥ 0.01	20
HEGA®-10, Anagrade[2,4]	H110	≥ 10	0 - 5
n-Heptyl-β-D-Thioglucopyranoside, Anagrade	H301	≥ 10	0 - 5
n-Hexadecyl-β-D-Maltopyranoside, Anagrade[5]	H320	≥ 1	40
MEGA-9, Anagrade[2]	M325	≥ 5	5
n-Nonyl-β-D-Thioglucopyranoside, Anagrade	N335	≥ 0.05	0 - 5
n-Octyl-β-D-Galactopyranoside, Anagrade	O312	≥ 0.5	0 - 5
n-Octyl-β-D-Thioglucopyranoside, Anagrade	O314	≥ 0.8	0 - 5
Sodium Dodecanoyl Sarcosine, Anagrade	S300	≥ 10	20
n-Tetradecyl-N,N-Dimethylamine-N-Oxide, Anagrade	T360	≥ 1	20
n-Undecyl-β-D-Thiomaltopyranoside, Anagrade	U342	≥ 10	20

[1] Solubility may be somewhat greater than listed. Solubility limits listed as percent are weight/volume.
[2] Solubility of detergents in this series with shorter alkyl tails are greater than 20%.
[3] Solubility is 1:1 (v/v) methanol:water.
[4] This detergent must be heated briefly to 50°C to ensure the formation of a true solution.
[5] Measured at 40°C.

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