

SOP: SP030.2

Updated 9/22/22 modified by MCL

Percent SDS Determination in an Aqueous Solution

Materials and Reagents:

1. Sample to assay
2. Standards for curve (note 1)
3. Pipetman, P-10
4. Pipet tips, 10 μ l
5. Eppendorf tube, 0.65 ml
6. Pipetman, P-200
7. Pipet tips, 200 μ l
8. Methylene blue solution (note 2)
9. Glass capillary pipettor, 50 μ l
10. Glass pipet, 50 μ l
11. Chloroform, HPLC-grade
12. Vortexer
13. Eppendorf centrifuge
14. 96 well plate
15. Plate reader, with 655 nm filter

Protocol:

1. ____ Using P-10 pipetman and tip, add 10 μ l each of sample, standard, or blank to 0.65 ml eppendorf tubes.
2. ____ Using P-200 pipetman and tip, add 100 μ l of methylene blue solution to each eppendorf tube.
3. ____ Using a 50 μ l glass capillary pipettor, add 20 μ l of chloroform to each eppendorf tube.
4. ____ Close caps on all tubes and vortex vigorously.
5. ____ Centrifuge at 3000 x g, room temperature for 5 minutes.
6. ____ Using P-200 pipetman and tip, remove 90 μ l of aqueous (top) layer from each sample and standard, being sure not to collect any of the chloroform layer, and transfer to 96-well plate.
7. ____ Put plate in plate reader and read absorbance at 655 nm (note 3).
8. ____ Plot square root of SDS standards against square root of O.D.655 correlation coefficient and graph residuals linear regression. Interpolate sample SDS by squaring percent SDS on graph (note 4).

Notes:

- | Standards to use: | Appearance of standard: |
|-------------------|--|
| 0.1% SDS | CHCl ₃ layer is darker blue and clear, no visible granules. Only a slight color in aqueous layer. |
| 0.05% SDS | CHCl ₃ is dark blue and clear, no granules. Aqueous layer very light blue. |
| 0.01% SDS | Similar to 0.05% SDS. |
| 0.005% SDS | Similar to 0.01% SDS. |
| 0.0025% SDS | Aqueous phase a bit more color, both layers are clear. |
| 0.001% SDS | Aqueous phase a bit more color, both layers are clear. |
| 0.00075% SDS | Aqueous layer getting darker blue, CHCl ₃ getting lighter, both clear. |
| 0.0005% SDS | CHCl ₃ layer is very light aqua in color and clear. Aqueous layer is blue. |
| 0.00025% SDS | Both layers are about the same color, light aqua. Both clear. |

0.0001% SDS CHCl₃ layer is almost colorless, aqueous layer back to original color.
0.00005% SDS CHCl₃ layer is colorless and clear.
Water/Buffer (blank)

2. To make methylene blue solution: 12 mg/L in 0.01 M HCl with 0.02% NaN₃.
3. For Epoch plate reader using Gen5 software, use "SDS% Determination Protocol.prt", which can be found on the lab T:Drive
4. Percent SDS should be less than 0.01%

Reference:

Mukerjee. Use of Ionic Dyes in the Analysis of Ionic Surfactants and Other Organic Compounds. Analytical Chemistry. (28) 1956.