PEG/Ionic Liquid™ 2

HAMPTON RESEARCH
Solutions for Crystal Growth

Applications

Crystallization screen for biological macromolecules, where Polyethylene glycols are are the primary, and ionic liquid the secondary reagent, sampling a broad range of pH without an added buffer.

Features

- Samples a broad range of pH without an added buffer
- Polyethylene glycol 400, 1,000, 4,000, & 20,000 - primary reagent
- 12 unique ionic liquids - secondary reagent
- Vapor diffusion, microbatch, free interface diffusion

Refer to the enclosed PEG/Ionic Liquid 2 Reagent Formulation for more information.

General Description

Ionic liquids have been found effective as additives in protein crystallization, with different ionic liquids used to increase crystallization rates and crystal size.[1-4] The inclusion of ionic liquids in crystallization experiments has been reported to lead to less crystal polymorphism as well as less precipitation at higher precipitant concentrations.[2,5] Ionic liquids have been used as additives to produce crystals in reagents that had previously not resulted in crystallization and results suggest ionic liquids may be applicable for the solubilization and crystallization of membrane proteins.[2]

Ionic liquids are organic salts with melting points below 100°Celsius. They are thermally stable, nonflammable and demonstrate very low vapor pressure. Ionic liquids are soluble in a variety of organic and inorganic reagents and can be highly water soluble. Ionic liquids can demonstrate a degree of localized structuring about each ion compared to materials composed of disassociated ions, setting them apart from salt solutions.[5,6] Ionic liquids can participate in ionic, hydrophobic and hydrogen bond interactions. Ionic liquids are often chaotropic, composed of low symmetry ions with charge delocalization and weak intermolecular interactions.[1] These organic salts generally consist of combinations of organic cations and either an organic or inorganic anion. Ionic liquids have been demonstrated to suppress protein aggregation and significantly increase protein folding yields.[7,8] Ionic liquids have been reported to stabilize protein activity and structure.[9-11] The inclusion of the ionic liquid 1-n-Butyl-3-methylimidazolium tetrafluoroborate improved the thermal stability and solubility of integral membrane proteins for membrane proteomics study.[12]

Some ionic liquids, such as ethylammonium nitrate have water-like characteristics, including the capacity for hydrogen bonding and the promotion of micelle formation by some surfactants.[13] Many ionic liquids are also organic acids and have ionic character in addition to the hydrophobic behavior, which makes them unique and useful solvents in protein chemistry.

Variation of the anion and the cation as well as the utilization of both soft (formate and acetate) and hard anions (nitrate) in the reagents provides an additional dimension for evaluating the effects on ionic liquids on the solubility and crystallization of proteins. The screen contains 12 water soluble ionic liquids that comprise different cation (imidazolium, phosphonium, and pyridinium) and anion (sulfate, fluoroborate, fluoroacetate, sulfonate, bromide, tosylate, and chloride) structures for a diverse PEG and Ionic Liquid formulation for use in the crystallization screening of biological macromolecules.

PEG/Ionic Liquid 2 samples four different low molecular weight Polyethylene glycols (400, 1,000, 4,000, and 20,000) versus twelve ionic liquids, encompassing a broad range of pH without an added buffer. PEG/Ionic Liquid 2 is supplied in a 48 x 10 ml tube format. PEG/Ionic Liquid 2 is compatible with vapor diffusion, free interface diffusion, and microbatch crystallization methods. For research use only.

Sample Preparation

The protein sample should be homogenous, as pure as is practically possible (>95%), and free of amorphous material. Remove amorphous material by centrifugation or microfiltration prior to use. The recommended sample concentration is 5 to 25 mg/ml in dilute (25 mM or less) buffer. For initial screens, the sample should be free of unnecessary additives in order to observe the effect of the PEG/Ionic Liquid 2 reagents. However, agents that promote and preserve sample solubility, stability, and homogeneity can and should be included in the sample buffer. For additional sample preparation recommendations see Hampton Research Crystal Growth 101 - Preliminary Sample Preparation.

Manual Method - Sitting Drop Vapor Diffusion

1. Using two 24 well sitting drop crystallization plates, pipet the recommended volume (typically 500 to 1,000 microliters) of crystallization reagent from the tubes into the crystallization plates. PEG/Ionic Liquid 2 reagents 1 through 24 into the first plate and reagents 25 through 48 into the second plate. Use clean pipette tips, a new tip for each of the 48 reagents.

2. Using a clean pipette tip, pipet the desired volume of crystallization reagent (typically 0.5 to 2 microliters) from the crystallization plate reservoir to the sitting drop well.

3. Using a clean pipette tip, pipet the same volume (typically 0.5 to 2 microliters) of sample to the reagent drop in the sitting drop well. Work carefully but quickly to minimize evaporation from the drop and reservoir.

4. Seal the crystallization plate using an optically clear sealing film or tape.

Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) after setting the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after for up to 60 days, or until the drop dries out. Records should indicate whether the drop is clear, contains precipitate, and/or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a numerical scoring scheme (Clear = 0, Crystal = 1. Precipitate = 2). Figure 1 shows typical examples of what one might observe in a crystallization experiment.

PEG/Ionic Liquid™ 2

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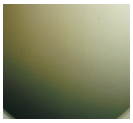
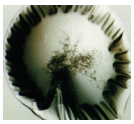
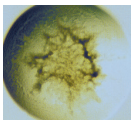
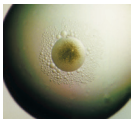
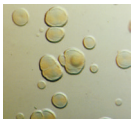
Figure 1
Typical observations in a crystallization experiment

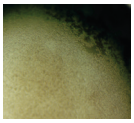

Clear Drop


Skin / Precipitate

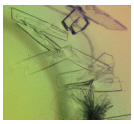

Precipitate


Precipitate / Phase

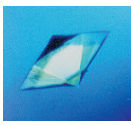

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Interpreting the Screen Results

Clear drops indicate that either the relative supersaturation of the sample and reagent is too low or the drop has not yet completed equilibration. If the drop remains clear after 3 to 4 weeks consider repeating the screen condition and doubling the sample concentration. If more than 35 of the 48 drops are clear, then consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold with sample buffer and repeat the screen condition. If more than 35 of the 48 drops contain precipitate and no crystals are present, then consider diluting the sample concentration in half by adding an equal volume of sample buffer to the sample and repeating the entire screen. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, additives, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing or UV optics to differentiate precipitate from microcrystals.

If the drop contains a macromolecular crystal the relative supersaturation of the sample and reagent is appropriate for crystal nucleation and growth. The next step is to optimize the preliminary conditions by varying Ionic Liquid and/or PEG concentration, screen pH, vary temperature between 4 and 30°C, screen additives, and evaluate other crystallization variables including sample construct, purity, stability, and homogeneity in order to achieve the desired crystal size and quality.

When sample quantity permits, set the screen in duplicate (4°C and 25°C) or triplicate (10°C and 20°C and 30°C) to evaluate the effect of temperature on crystallization. Compare the observations between the different temperatures to determine the effect of temperature on sample solubility. Different results in the same drops at different temperatures indicate that sample solubility is temperature dependent and that one should include temperature as a variable in subsequent screens and optimization experiments.

When sample quantity permits, set the screen using multiple drops and drop ratios, such as 1:2, 1:1, and 2:1. See Hampton Research Crystal Growth 101: Drop Ratio for details.

Formulation

Crystallization reagents are formulated using the highest purity chemicals, ultrapure water (Formulated in Type 1+ ultrapure water: 18.2 megaohm-cm resistivity at 25°C, < 5 ppb Total Organic Carbon, bacteria free (<1 Bacteria (CFU/ml)), pyrogen free (<0.03 Endotoxin (EU/ml)), RNase-free (< 0.01 ng/mL) and DNase-free (< 4 pg/µL)) and are sterile filtered using 0.22 micron filters into sterile tubes (no preservatives added). Store between -20 and 20°C. Best if used within 12 months of receipt.

Crystallization reagents can be reproduced using Hampton Research Optimize ™ polyethylene glycols and Ionic Liquid Screen or Custom Shop™ Ionic Liquid Screen individual reagents.

Recommended Reading

1. Introduction to protein crystallization. Alexander McPherson and Jose A. Gavira. Acta Crystallographica Section F Volume 70, Issue 1, pages 2–20, January 2014.
2. Optimization of crystallization conditions for biological macromolecules. Alexander McPherson and Bob Cudney. Acta Crystallographica Section F Volume 70, Issue 11, pages 1445–1467, November 2014.
3. Pusey, M.L., Paley, M.S., Turner, M.B., and Rogers, R.D. 2007. Protein crystallization using room temperature ionic liquids. Crystal Growth & Design. 74:787-793.
4. Hekmat, D., Hebel, D., Sebastian, J. Schmidt, M., and Weuster-Botz, D. 2007. Advanced protein crystallization using water-soluble ionic liquids as crystallization additives. Biotech. Lett., 29:703-1711.
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13. De Diego, T., Lozano, P., Gmouth, S. Vaultier, M., and Iborra, J.L. 2004. Fluorescence and CD spectroscopic analysis of the a-chymotrypsin stabilization by the ionic liquid 1-ethyl-3-methyimidazolium bis[(trifluormethyl)sulfonyl]amide. Biotechnol. Bioeng. 88:916-924.
14. Sun, L., Tao, D., Han, B., Ma, J., Zhu, G., Liang, Z., Shan, Y., Zhang, L., and Zhang, Y. 2010. Ionic liquid 1-butyl-3-methyl imidazolium tetrafluoroborate for shotgun membrane proteomics. Anal. Bioanal. Chem. DOI 10.1007/s0216-010-4381-5.
15. Evans, D.F., Yamauchi, A., Roman, R., and Casassa, E.Z. 1982. J. Colloids Interface Sci. 88:89-96.
16. Proteins in Ionic Liquids: Current Status of Experiments and Simulations. Christian Schröde, Top Curr Chem (J). 2017; 375(2): 25.
17. Ionic Liquids as Stabilization and Refolding Additives and Solvents for Proteins. Fujita K. Adv Biochem Eng Biotechnol. 2018 Jul 13. doi: 10.1007/10_2018_65.
18. Fujita K. (2018) Ionic Liquids as Stabilization and Refolding Additives and Solvents for Proteins. In: . Advances in Biochemical Engineering/Biotechnology. Springer, Berlin, Heidelberg.

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Tube #	Ionic Liquid	Tube #	Polymer	Tube #	pH ◊
1.	5% w/v 1,3-Dimethylimidazolium methyl sulfate	1.	30% v/v Polyethylene glycol 400	1.	5.6
2.	5% w/v 1,3-Dimethylimidazolium methyl sulfate	2.	25% w/v Polyethylene glycol 1,000	2.	3.7
3.	5% w/v 1,3-Dimethylimidazolium methyl sulfate	3.	20% w/v Polyethylene glycol 4,000	3.	4.0
4.	5% w/v 1,3-Dimethylimidazolium methyl sulfate	4.	15% w/v Polyethylene glycol 20,000	4.	3.8
5.	5% w/v 1-Butyl-3-methylimidazolium methyl sulfate	5.	30% v/v Polyethylene glycol 400	5.	3.6
6.	5% w/v 1-Butyl-3-methylimidazolium methyl sulfate	6.	25% w/v Polyethylene glycol 1,000	6.	2.7
7.	5% w/v 1-Butyl-3-methylimidazolium methyl sulfate	7.	20% w/v Polyethylene glycol 4,000	7.	2.7
8.	5% w/v 1-Butyl-3-methylimidazolium methyl sulfate	8.	15% w/v Polyethylene glycol 20,000	8.	2.6
9.	5% w/v 1-n-Butyl-3-methylimidazolium n-octylsulfate	9.	30% v/v Polyethylene glycol 400	9.	6.9
10.	5% w/v 1-n-Butyl-3-methylimidazolium n-octylsulfate	10.	25% w/v Polyethylene glycol 1,000	10.	5.4
11.	5% w/v 1-n-Butyl-3-methylimidazolium n-octylsulfate	11.	20% w/v Polyethylene glycol 4,000	11.	5.7
12.	5% w/v 1-n-Butyl-3-methylimidazolium n-octylsulfate	12.	15% w/v Polyethylene glycol 20,000	12.	5.6
13.	5% w/v 1-Ethyl-3-methylimidazolium ethyl sulfate	13.	30% v/v Polyethylene glycol 400	13.	7.2
14.	5% w/v 1-Ethyl-3-methylimidazolium ethyl sulfate	14.	25% w/v Polyethylene glycol 1,000	14.	7.7
15.	5% w/v 1-Ethyl-3-methylimidazolium ethyl sulfate	15.	20% w/v Polyethylene glycol 4,000	15.	7.9
16.	5% w/v 1-Ethyl-3-methylimidazolium ethyl sulfate	16.	15% w/v Polyethylene glycol 20,000	16.	7.9
17.	5% w/v 1-Ethyl-3-methylimidazolium tetrafluoroborate	17.	30% v/v Polyethylene glycol 400	17.	5.6
18.	5% w/v 1-Ethyl-3-methylimidazolium tetrafluoroborate	18.	25% w/v Polyethylene glycol 1,000	18.	3.8
19.	5% w/v 1-Ethyl-3-methylimidazolium tetrafluoroborate	19.	20% w/v Polyethylene glycol 4,000	19.	3.6
20.	5% w/v 1-Ethyl-3-methylimidazolium tetrafluoroborate	20.	15% w/v Polyethylene glycol 20,000	20.	3.4
21.	5% w/v 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate	21.	30% v/v Polyethylene glycol 400	21.	6.1
22.	5% w/v 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate	22.	25% w/v Polyethylene glycol 1,000	22.	4.1
23.	5% w/v 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate	23.	20% w/v Polyethylene glycol 4,000	23.	4.0
24.	5% w/v 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate	24.	15% w/v Polyethylene glycol 20,000	24.	3.5
25.	5% w/v 1-Butyl-3-methylimidazolium tetrafluoroborate	25.	30% v/v Polyethylene glycol 400	25.	5.8
26.	5% w/v 1-Butyl-3-methylimidazolium tetrafluoroborate	26.	25% w/v Polyethylene glycol 1,000	26.	3.9
27.	5% w/v 1-Butyl-3-methylimidazolium tetrafluoroborate	27.	20% w/v Polyethylene glycol 4,000	27.	3.9
28.	5% w/v 1-Butyl-3-methylimidazolium tetrafluoroborate	28.	15% w/v Polyethylene glycol 20,000	28.	3.4
29.	5% w/v 1-Butyl-3-methylimidazolium trifluoroacetate	29.	30% v/v Polyethylene glycol 400	29.	5.4
30.	5% w/v 1-Butyl-3-methylimidazolium trifluoroacetate	30.	25% w/v Polyethylene glycol 1,000	30.	3.6
31.	5% w/v 1-Butyl-3-methylimidazolium trifluoroacetate	31.	20% w/v Polyethylene glycol 4,000	31.	3.7
32.	5% w/v 1-Butyl-3-methylimidazolium trifluoroacetate	32.	15% w/v Polyethylene glycol 20,000	32.	3.4
33.	5% w/v 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate	33.	30% v/v Polyethylene glycol 400	33.	5.4
34.	5% w/v 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate	34.	25% w/v Polyethylene glycol 1,000	34.	3.2
35.	5% w/v 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate	35.	20% w/v Polyethylene glycol 4,000	35.	4.3
36.	5% w/v 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate	36.	15% w/v Polyethylene glycol 20,000	36.	5.2
37.	5% w/v Tetrabutylphosphonium bromide	37.	30% v/v Polyethylene glycol 400	37.	4.2
38.	5% w/v Tetrabutylphosphonium bromide	38.	25% w/v Polyethylene glycol 1,000	38.	2.7
39.	5% w/v Tetrabutylphosphonium bromide	39.	20% w/v Polyethylene glycol 4,000	39.	2.8
40.	5% w/v Tetrabutylphosphonium bromide	40.	15% w/v Polyethylene glycol 20,000	40.	2.8
41.	5% w/v Triisobutylmethylphosphonium tosylate	41.	30% v/v Polyethylene glycol 400	41.	2.7
42.	5% w/v Triisobutylmethylphosphonium tosylate	42.	25% w/v Polyethylene glycol 1,000	42.	2.4
43.	5% w/v Triisobutylmethylphosphonium tosylate	43.	20% w/v Polyethylene glycol 4,000	43.	2.5
44.	5% w/v Triisobutylmethylphosphonium tosylate	44.	15% w/v Polyethylene glycol 20,000	44.	2.5
45.	5% w/v 1-Butylpyridinium chloride	45.	30% v/v Polyethylene glycol 400	45.	6.0
46.	5% w/v 1-Butylpyridinium chloride	46.	25% w/v Polyethylene glycol 1,000	46.	4.9
47.	5% w/v 1-Butylpyridinium chloride	47.	20% w/v Polyethylene glycol 4,000	47.	5.0
48.	5% w/v 1-Butylpyridinium chloride	48.	15% w/v Polyethylene glycol 20,000	48.	4.8

Reagents formulated in Type 1+ ultrapure grade water

◊ Measured pH at 25ºC, no pH adjustment made to reagent

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Solutions for Crystal Growth

Sample: _____ Sample Concentration: _____

Sample Buffer: _____ Date: _____

Reservoir Volume: _____ Temperature: _____

Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ µl Additive _____ µl

1 Clear Drop
2 Phase Separation
3 Regular Granular Precipitate
4 Birefringent Precipitate or Microcrystals
5 Posettes or Spherulites
6 Needles (1D Growth)
7 Plates (2D Growth)
8 Single Crystals (3D Growth < 0.2 mm)
9 Single Crystals (3D Growth > 0.2 mm)

PEG/Ionic Liquid™ 2 - HR2-079 Scoring Sheet		Date:	Date:	Date:	Date:
1.	5% w/v 1,3-Dimethylimidazolium methyl sulfate, 30% v/v Polyethylene glycol 400				
2.	5% w/v 1,3-Dimethylimidazolium methyl sulfate, 25% w/v Polyethylene glycol 1,000				
3.	5% w/v 1,3-Dimethylimidazolium methyl sulfate, 20% w/v Polyethylene glycol 4,000				
4.	5% w/v 1,3-Dimethylimidazolium methyl sulfate, 15% w/v Polyethylene glycol 20,000				
5.	5% w/v 1-Butyl-3-methylimidazolium methyl sulfate, 30% v/v Polyethylene glycol 400				
6.	5% w/v 1-Butyl-3-methylimidazolium methyl sulfate, 25% w/v Polyethylene glycol 1,000				
7.	5% w/v 1-Butyl-3-methylimidazolium methyl sulfate, 20% w/v Polyethylene glycol 4,000				
8.	5% w/v 1-Butyl-3-methylimidazolium methyl sulfate, 15% w/v Polyethylene glycol 20,000				
9.	5% w/v 1-n-Butyl-3-methylimidazolium n-octylsulfate, 30% v/v Polyethylene glycol 400				
10.	5% w/v 1-n-Butyl-3-methylimidazolium n-octylsulfate, 25% w/v Polyethylene glycol 1,000				
11.	5% w/v 1-n-Butyl-3-methylimidazolium n-octylsulfate, 20% w/v Polyethylene glycol 4,000				
12.	5% w/v 1-n-Butyl-3-methylimidazolium n-octylsulfate, 15% w/v Polyethylene glycol 20,000				
13.	5% w/v 1-Ethyl-3-methylimidazolium ethyl sulfate, 30% v/v Polyethylene glycol 400				
14.	5% w/v 1-Ethyl-3-methylimidazolium ethyl sulfate, 25% w/v Polyethylene glycol 1,000				
15.	5% w/v 1-Ethyl-3-methylimidazolium ethyl sulfate, 20% w/v Polyethylene glycol 4,000				
16.	5% w/v 1-Ethyl-3-methylimidazolium ethyl sulfate, 15% w/v Polyethylene glycol 20,000				
17.	5% w/v 1-Ethyl-3-methylimidazolium tetrafluoroborate, 30% v/v Polyethylene glycol 400				
18.	5% w/v 1-Ethyl-3-methylimidazolium tetrafluoroborate, 25% w/v Polyethylene glycol 1,000				
19.	5% w/v 1-Ethyl-3-methylimidazolium tetrafluoroborate, 20% w/v Polyethylene glycol 4,000				
20.	5% w/v 1-Ethyl-3-methylimidazolium tetrafluoroborate, 15% w/v Polyethylene glycol 20,000				
21.	5% w/v 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate, 30% v/v Polyethylene glycol 400				
22.	5% w/v 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate, 25% w/v Polyethylene glycol 1,000				
23.	5% w/v 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate, 20% w/v Polyethylene glycol 4,000				
24.	5% w/v 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate, 15% w/v Polyethylene glycol 20,000				
25.	5% w/v 1-Butyl-3-methylimidazolium tetrafluoroborate, 30% v/v Polyethylene glycol 400				
26.	5% w/v 1-Butyl-3-methylimidazolium tetrafluoroborate, 25% w/v Polyethylene glycol 1,000				
27.	5% w/v 1-Butyl-3-methylimidazolium tetrafluoroborate, 20% w/v Polyethylene glycol 4,000				
28.	5% w/v 1-Butyl-3-methylimidazolium tetrafluoroborate, 15% w/v Polyethylene glycol 20,000				
29.	5% w/v 1-Butyl-3-methylimidazolium trifluoroacetate, 30% v/v Polyethylene glycol 400				
30.	5% w/v 1-Butyl-3-methylimidazolium trifluoroacetate, 25% w/v Polyethylene glycol 1,000				
31.	5% w/v 1-Butyl-3-methylimidazolium trifluoroacetate, 20% w/v Polyethylene glycol 4,000				
32.	5% w/v 1-Butyl-3-methylimidazolium trifluoroacetate, 15% w/v Polyethylene glycol 20,000				
33.	5% w/v 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate, 30% v/v Polyethylene glycol 400				
34.	5% w/v 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate, 25% w/v Polyethylene glycol 1,000				
35.	5% w/v 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate, 20% w/v Polyethylene glycol 4,000				
36.	5% w/v 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate, 15% w/v Polyethylene glycol 20,000				
37.	5% w/v Tetrabutylphosphonium bromide, 30% v/v Polyethylene glycol 400				
38.	5% w/v Tetrabutylphosphonium bromide, 25% w/v Polyethylene glycol 1,000				
39.	5% w/v Tetrabutylphosphonium bromide, 20% w/v Polyethylene glycol 4,000				
40.	5% w/v Tetrabutylphosphonium bromide, 15% w/v Polyethylene glycol 20,000				
41.	5% w/v Triisobutylmethylphosphonium tosylate, 30% v/v Polyethylene glycol 400				
42.	5% w/v Triisobutylmethylphosphonium tosylate, 25% w/v Polyethylene glycol 1,000				
43.	5% w/v Triisobutylmethylphosphonium tosylate, 20% w/v Polyethylene glycol 4,000				
44.	5% w/v Triisobutylmethylphosphonium tosylate, 15% w/v Polyethylene glycol 20,000				
45.	5% w/v 1-Butylpyridinium chloride, 30% v/v Polyethylene glycol 400				
46.	5% w/v 1-Butylpyridinium chloride, 25% w/v Polyethylene glycol 1,000				
47.	5% w/v 1-Butylpyridinium chloride, 20% w/v Polyethylene glycol 4,000				
48.	5% w/v 1-Butylpyridinium chloride, 15% w/v Polyethylene glycol 20,000				

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