Penn State RET in Interdisciplinary Materials

Teacher's Preparatory Guide

Complex Solubility Inquiry Lab

Purpose This lab is designed to help students understand solubility – how and why substances dissolve in solutions.

Objectives

- Students will collaborate in small groups to use prior knowledge in a new situation to make educated guesses about outcomes of experiments, and to propose explanations.
- Students will observe simulations of molecules to form a mental image of solvation.
- Students will draw molecular scale diagrams of the various processes discussed in the lab.
- Students will collect data and use critical thinking skills to form conclusions based on data.

Time required ~ Four 40 minute class periods – depends on which optional extensions are used.

Level High school chemistry

Teacher Background

These references provide background on thermoresponsive polymers and the Hofmeister series, and may be helpful reading for the instructor. These references are advanced, and most likely not appropriate for high school chemistry students. They may be appropriate for advanced AP students.

Gandhi, Arijit, Suma Oommen Sen, and Kalyan Kumar Sen. "Studies on Thermoresponsive Polymers: Phase Behaviour, Drug Delivery and Biomedical Applications." *Asian Journal of Pharmaceutical Sciences* 10.2 (2014): 99-107. *ScienceDirect*. 28 Aug. 2014. Web. 07 July 2016.

Jungwirth, Pavel, and Paul S. Cremer. "Beyond Hofmeister." *Nature Chemistry Nature Chem* 6.4 (2014): 261-63. Web.

Zhang, Yanjie, Steven Furyk, David E. Bergbreiter, and Paul S. Cremer. "Specific Ion Effects on the Water Solubility of Macromolecules: PNIPAM and the Hofmeister Series." *J. Am. Chem. Soc. Journal of the American Chemical Society* 127.41 (2005): 14505-4510. Web.

Center for Nanoscale Science www.mrsec.psu.edu

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Advance Preparation for Lab Part 1:

Materials

- 110 mg Poly(N-isopropylacrylamide) (available from Penn State – see note below)
- Ten 2 ml glass vials
- Deionized water
- 10 ml conical centrifuge tube
- Refrigerator
- Ice (crushed preferred)
- Sodium chloride
- Anhydrous sodium thiocyanate
- Anhydrous sodium sulfate
- Balance and weigh boats
- 10 ml graduated cylinder
- Thermometers or temperature probes for each group
- Hot plates for each lab group (may be shared between groups)
- 250 ml beakers (one per group)
- Tongs

The Poly(*N*-isopropylacrylamide) is available from Sigma Aldrich; however, a 10 gram sample is $250.0. Alternatively, you could contact Dr. Paul Cremer at Penn State (psc11@psu.edu) and request a 110 mg sample to prepare a lab kit.

Safety Information Students should exercise caution when working with glass and heating elements. Students should not over heat closed containers.

Prepare the following stock solutions with analytical balance:

- 11 ml of 10 mg/ml PNIPAM: dissolve 110 mg PNIPAM into 11 ml DI water (soluble in cold water – place in fridge for >4 hrs.)
- 10 ml of 1.2 M NaCl: dissolve 0.701 g NaCl in 10 ml DI water
- 10 ml of 1.2 M NaSCN: dissolve 0.973 g NaSCN in 10 ml DI water
- 10 ml of 0.72 M Na_2SO_4: dissolve 1.022 g Na_2SO_4 into 10 ml DI water

Prepare the following solutions by serial dilution:

NaCl Solution Concentration	Volume of previous solution	Volume of DI water	Total Volume
1.2 M (stock)			10.0 ml
0.8 M	6.67 ml	3.33 ml	10.0 ml
0.4 M	5.00 ml	5.00 ml	10.0 ml
0.0 M	0.00 ml	10.0 ml	10.0 ml

NaSCN Solution Concentration	Volume of previous solution	Volume of DI water	Total Volume
1.2 M (stock)			10.0 ml
0.8 M	6.67 ml	3.33 ml	10.0 ml
0.4 M	5.00 ml	5.00 ml	10.0 ml

Na_2SO_4 Solution Concentration	Volume of previous	Volume of DI water	Total

	solution		Volume
0.72 M (stock)			10.0 ml
0.48 M	6.67 ml	3.33 ml	10.0 ml
0.24 M	5.0 ml	5.0 ml	10.0 ml

Place 1 ml of each different concentration into a 2 ml vial. Add 1 ml of PNIPAM solution. Seal the vials with parafilm and store in a fridge until class.

Advance Preparation for Lab Part 2:

Materials

- NaCl (s)
- Na_2SO_4 (s)
- NaSCN (s)
- DI water
- 20 ml Purified Egg White
- Hot plate
- Disposable pipets
- Balance and weigh boats
- One 250 ml beaker
- 3 test tubes
- Stirring rod (or stir bar if stir/hot plate combo is available)
- Gloves and goggles

Processed Egg White:

Whisk the egg white of several eggs into a fine foam. Leave overnight in a narrow cylinder in the refrigerator. The liquid on the bottom is only slightly viscous and can easily be filtered despite its high protein content. A coffee filter is adequate. If the liquid contains no yolk and no added water, the filtrate is should be clear. The filtrate contains approximately 12% of proteins.[1] In my experience, one extra large egg typically produces just over 10 ml of processed egg whites. If additional water is added, egg whites may appear cloudy.

Safety Information Students should exercise caution when working with glass and heating elements. Students should not over heat closed containers.

Teaching Strategies

[1] Kunz, W., J. Henle, and B.w. Ninham. "'Zur Lehre Von Der Wirkung Der Salze' (about the Science of the Effect of Salts): Franz Hofmeister's Historical Papers." *Current Opinion in Colloid & Interface Science* 9.1-2 (2004): 19-37. Web.

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Required Background Knowledge:

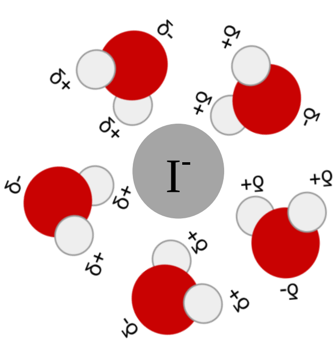
1. Intermolecular forces (dipole-dipole, dispersion, hydrogen bonding, etc.)
2. Properties of Water
3. Phases
4. Periodic Trends (atomic size, ion size)
5. Concentration and molarity basics
6. Basics about polymers
7. For AP Extension –
    a. Gibbs Free Energy
    b. Enthalpy
    c. Entropy
    d. Hess's Law

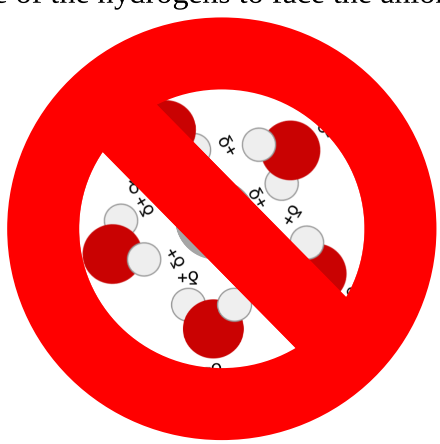
Unit Sequence:

- Prelab – Completed as HW before the lab.
- Day 1 → Prelab Discussion – Completed as a whole class discussion before the lab.
- Day 2 → Predict & Lab Part 1 - Completed in groups of two. Each group measures the LCST of two samples, and records the data on the board for the class to record. Then the data is plotted.
- Lab Part 1 Conclusion – Completed individually for HW.
- Day 3 and 4 → Lab Part 2 Inquiry Extension – Food Chemistry
- Lab Part 2 Lab Report – Completed for HW
- AP Extension – Connecting Entropy, Gibbs Free Energy, and solvation

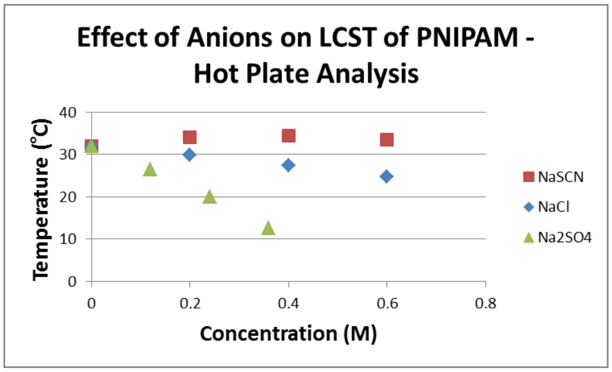
Misconception Alert - Prelab Animations

The animations used in the prelab occasionally show an anion surrounded by water molecules. According to the most recent research results, the correct formation is not for both of the hydrogens to face the anions, but for only one of the hydrogens to face the anion.





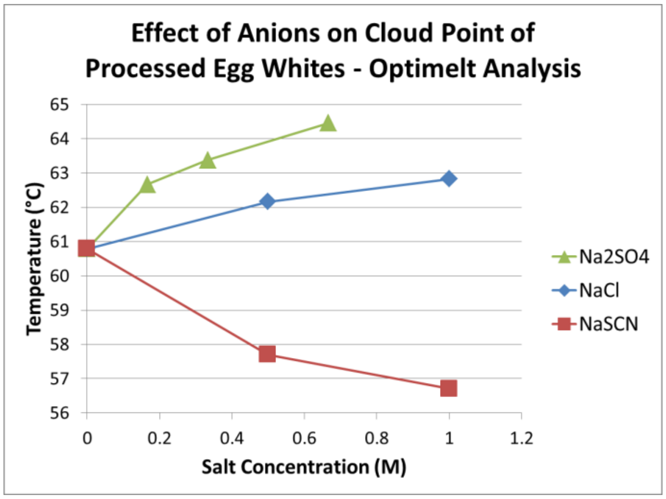
Lab Part 1 Expected Results



Lab Part 2 Inquiry Extension Teaching Tips

- This is an inquiry activity.
  - The purpose is to allow students to explore the material independently, to make mistakes, and to figure out how to fix them.
  - This is about process – I do not expect most groups to produce beautiful results. I do expect students to be coming up with ideas, and testing them, and when it doesn't work, figuring out how to improve their process.
  - Resist the urge to step in and explain everything.
  - If students are stuck for more than 5 minutes, or the lab period is running out of time, consider asking the students to explain what they've tried, to explain the problems they are having to you. When students have to put these things into words to explain it to someone else, it often clarifies their thought process for themselves. Prompts like, "What makes you say that?" "Why do you think that happened?" and, "What could you change to make that happen?"
  - If students get frustrated at lack of immediate results, remind them this is about the process. If they can show that they tried multiple logical methods in their lab report, their grades are going to be fine. Show them the lab report rubric before the lab starts to emphasize this.

- Useful info for the teacher:
  - The processed egg whites have a cloud point at around 60^{o}C. Provide students with test tubes without tops to make sure they do not overheat any closed glass containers.
  - The cloud point appears to change with the presence of salts according to a reverse Hofmeister series.
  - The cloud point is not reversible.



- Possible discussion topics for students to ponder, depending on what results they get.
  - Additional complications that arise when working with real proteins / samples that contain multiple proteins.
  - Why might some proteins follow the Hofmeister series (PNIPAM), while others (egg white proteins) do not?
  - Why is the clouding sometimes reversible, and sometimes irreversible?

Name: ___________________________ Date: _______________ Period:_____

Complex Solubility

Purpose

The goal of this lab is for you to be able to describe what happens on a molecular level when a substance dissolves, and to use this understanding to make predictions about the solubility of various solutions. A second goal is to introduce you to topics currently being investigated at top research universities.

Prelab

1. Review vocab: Look up the definition of the following terms in your textbook's glossary, and write the definition here.
   a. Solute
   ____
   b. Solvent
   ____
   c. Solution
   ____
   d. Polymer
   ____
   e. Monomer
   ____
   f. Phase
   ____

2. New vocab: Add the following terms and definitions to your flash card list.
   a. Salting In: the phenomenon of increasing solubility of a protein with increasing salt concentration
   b. Salting Out: The phenomenon of decreasing solubility of a protein with increasing salt concentration.
   c. Hydration shell: a shell of water molecules surrounding a dissolved ions.
   d. Strongly hydrated ions: Ions that do readily shed their hydration shells to bind to a polymer surface.
   e. Weakly hydrated ions: Ions that do readily shed their hydration shell to bind to the polymer surface.

3. Watch the animation of solvation of NaCl here: http://group.chem.iastate.edu/Greenbowe/sections/projectfolder/flashfiles/thermochem/solutionSalt.html[2]

4. Which element is represented by red? White? Blue? Grey?

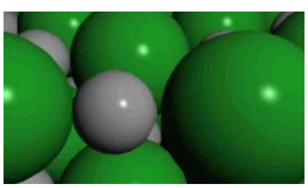
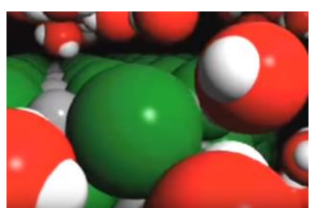
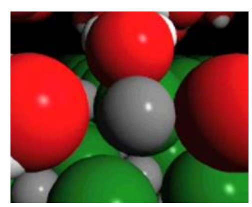
5. Describe how you decided whether the blue or grey represented sodium ions.

6. Look at the close up view of the blue hydrated ion. Are the red or white atoms facing the blue ion? Propose an explanation for this phenomenon.

7. Look at the close up view of the grey hydrated ion. Are the red or white atoms facing the blue ion? Propose an explanation for this phenomenon.

Prelab Class Discussion Prompts

1. In your lab groups, discuss prelab questions 4 – 7. Take turns speaking until your group agrees on a theory that explains what is scene in the animation. Be prepared to share your explanation with the class.
2. Watch this second video[3] (https://youtu.be/qXiEipXOj70) with the class. This video has no audio – only images. With your group, compose an informative narration to accompany the video. Use your phone / computer to record your narration. Be prepared to share with the class. Stuck? Break the video into three sections, based on the images below. Then describe what is occurring in each section.

3. In your lab group, read through the information on the next page, watch the video, and complete the Predict section.

[2] Chemical Education Research Group at Iowa State University
[3] Roy Tasker, VisChem Project

Complex Solubility Lab Part 1

Introduction

Solutions can contain many more molecules than the simple sodium chloride solutions in the prelab. In fact, many biologically relevant solutions contain thousands of molecules. For example, cells contain solutions that contain many different classes of molecules, such as proteins, lipids, sugars, salts, DNA, etc. In this lab, you will be working with solutions that contain a protein mimic, PNIPAM, and an anion, either SO_2^{2-}, Cl^-, or SCN^-. Proteins are polymers, molecules made of small repeating units called monomers. These chains can be over 100,000 units long, and so have many interesting properties in solutions. Since these molecules are so long, it can be helpful to image them as strings or chains.

Franz Hofmeister (1850 - 1922) was an early protein chemist who discovered that ions affect the solubility and stability of proteins by using purified egg whites. His research led to the ordering of anions by their ability to "salt in" or "salt out" proteins in aqueous solutions, known as the Hofmeister series. The mechanism for the Hofmeister series is still not completely understood, and it is currently the subject of research being performed at universities and research institutions around the world.

In the following lab, you will use the polymer Poly(*N*-isopropylacrylamide), or PNIPAM, as a substitute for protein. PNIPAM will be used since it is analogous to valine, which is one of the common amino acids. In the experiment, you will determine how salts at varying concentration have an effect on the temperature at which a protein (or in this case, a polymer) will precipitate out of solution.

Watch *this video* for an introduction to the Hofmeister series from a current research scientist.

Materials for each lab group

- Safety glasses and gloves
- Two vials containing a PNIPAM and salt mixture
- Thermometer or temperature probe
- Hot plate (may be shared between two groups if necessary)
- 250 ml beaker
- Crushed ice
- Tongs

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Predict

1. (a) When salted in, PNIPAM solutions are clear. Draw inspiration from the animations you viewed in the prelab, and sketch a picture of how you imagine a dissolved polymer is arranged.
(b) Salted out PNIPAM solutions are an opaque milky white. Once again, drawing inspiration from the animations you viewed in the prelab, sketch a picture of how you imagine a precipitated polymer is arranged. (Hint: review the Intro to Hofmeister video.)

Salted In

Salted Out

2. List the following ions from smallest to largest radius.
Na^+, Cl^-, SCN^-, SO_4^{2-}

3. Which will have the lowest charge density? Cl^-, SCN^-, or SO_4^{2-}? What two factors should you consider when making this determination?

4. Which do you think will have the highest charge density? Cl^-, SCN^-, or SO_4^{2-}? Hint: pay attention to the charges on the ions.

5. Of the three ions from question 4, which will form the strongest intermolecular interactions with water molecules upon dissolving? In other words, which ion is the most strongly hydrated?

6. Based on your answers to the previous questions, predict which ion will lower the LCST of PNIPAM most. Use full sentences to write a rationale for your prediction.

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7. Based on your answer to the previous questions, predict whether a high salt concentration or low salt concentration will affect the LCST of PNIPAM more. Use full sentences to write a rationale for your prediction.

Procedure

1. Obtain each of the lab materials (including the two vials assigned to your group by your teacher) and put on safety glasses and gloves. ***Do not open the vials under any circumstances.*** They must remain sealed in order to be reused.

2. Fill the 250 mL beaker with approximately 100 mL of tap water, and a small amount of crushed ice. The total volume of water and ice should not exceed 150 mL.

3. Gently stir the ice water bath with the thermometer or temperature probe until the temperature stabilizes. The temperature should drop to 5 - 10^0C.

4. Place both vials into the ice water bath and continue to gently stir for approximately one minute. This will ensure that the temperature of the vials is equal to the temperature of the bath.

5. Place the beaker and thermometer onto a hot plate and turn it on to a low setting. Continue to stir every 30 – 60 seconds and monitor both the temperature, and the state of the solution within the vials.

6. As the solutions approach the LCST, they may appear to contain cloudy “spots” which dissipate when stirred. This is due to an uneven temperature distribution within the vial. Continue to stir gently at this point.

7. Record the LCST temperature onto the data table the moment the solution appears cloudy, and it does not return to clear when mixed.

8. After the LCST has been recorded for both vials, remove them from the beaker with tongs and dry them off. If any of the labeling on the sides has washed off, use a permanent marker to relabel the vials before returning them to the teacher. They should be stored in a refrigerator.

9. Turn off the hot plate, dump the water from the beaker, and return all materials except for the hot plate. Allow time for the hot plate to cool before putting it away.

10. Record your lab group's data on the board. As other groups complete the data collection, fill in each of the LCST values into the chart and complete the corresponding graph using a different color for each salt. Then answer each of the analysis questions.

Data Collection: Record the data for your group on the board and in one of the tables below. As other groups begin to finish, record their data into the tables.

Phase Transition for PNIPAM with NaCl(aq)

Concentration (M)	Temperature (^oC)
0.0	
0.2	
0.4	
0.6	

Phase Transition for PNIPAM with NaSCN(aq)

Concentration (M)	Temperature (^oC)
0.0	
0.2	
0.4	
0.6	

Phase Transition for PNIPAM with Na_2SO_4(aq)

Concentration (M)	Temperature (^oC)
0.0	
0.12	
0.24	
0.36	

Analyze the Results

Use a graph to plot data points for each concentration of the three PNIPAM and salt solution mixtures. The points for each salt should be drawn using a different colored pencil. Be sure to include the color used within a legend. Make sure your plot axes are labeled.

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Lab Conclusion

1. Do your results support your predictions? Write a paragraph explaining. Include direct references to the data.

2. Extend: Understanding how ions affect the solubility of proteins has implications for many developing technologies such as gene delivery, smart drug delivery, and tissue engineering. This paper summarizes some of the current applications. Pick one application in the paper, read about it, and summarize the main idea. Be prepared to share with the class.

Lab Part 2 – Food Chemistry

Artificial polymers such as PNIPAM are not the only ones whose solubility is affected by the addition of salt. In fact, one common cooking debate centers on this idea. Many chefs debate whether salt should be added to eggs before or after cooking them, because they believe that the addition of the salt both alters the consistency of the eggs and lowers the temperature at which the eggs can be cooked. Gordon Ramsay, celebrity chef, in particular, advocates that eggs should only be seasoned after cooking.

With your group, design an experiment to test whether or not the addition of salt to egg white changes its properties. Start by considering your results from the previous experiments carefully, and forming a hypothesis with a rationale. Consider – What property in particular are you testing? Are you testing multiple salts, or multiple salt concentrations? After getting approval on your procedure from your teacher, perform your experiment. Note: Your teacher is only checking your procedure to ensure that it is safe. They will not check for correctness before you've completed the lab. You will turn in a lab report as a group. Use the usual lab report rubric to help you.

Materials Available to you:

- NaCl (s)
- Na_2SO_4 (s)
- NaSCN (s)
- DI water
- 20 ml egg white solution
- Hot plate
- Disposable pipets
- Balance and weigh boats
- One 250 ml beaker
- 3 test tubes
- Stirring rod
- Gloves and goggles

Clean Up:

At the end of the class period, all glassware must be cleaned and returned to its proper location. All used eggs, disposable pipets, and weigh boats should be thrown out.

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AP Extension – Connecting Entropy, Gibbs Free Energy, and Solvation

Learning Intention: Apply knowledge of spontaneity and entropy to deepen your understanding of solvation.

Directions: In your lab group, discuss the following questions together and then write down your own answers.

1. In order for a process to be spontaneous, ΔG must have what sign?

2. NaCl spontaneously dissolves in water. What sign does the ΔG for this process have?

3. Consider the lattice dissociation enthalpy of NaCl and the hydration enthalpy of the ions. Is dissolving NaCl endothermic or exothermic? (Confused? This website walks you through the calculation.)

Lattice energy of NaCl: -787 kJ/mol
Hydration enthalpy of Na^+: -406 kJ/mol
Hydration enthalpy of Cl^-: -378 kJ/mol

4. If ΔH is +, and ΔG is – for a particular process, what sign must ΔS have?

5. Why does NaCl dissolve in water?

6. Is dissolving salts always endothermic? If no, provide an example. Reference the chart on the next page.

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ENTHALPY OF SOLUTION OF ELECTROLYTES

This table gives the molar enthalpy (heat) of solution at infinite dilution for some common uni-univalent electrolytes. This is the enthalpy change when 1 mol of solute in its standard state is dissolved in an infinite amount of water. Values are given in kilojoules per mole at 25°C.

Reference

Parker, V. B., *Thermal Properties of Uni-Univalent Electrolytes*, Natl. Stand. Ref. Data Series — Natl. Bur. Stand.(U.S.), No.2, 1965.

Solute	State	$\Delta_{sol}H°$ kJ/mol	Solute	State	$\Delta_{sol}H°$ kJ/mol	Solute	State	$\Delta_{sol}H°$ kJ/mol
HF	g	–61.50	$LiBr \cdot 2H_2O$	c	–9.41	$KClO_3$	c	41.38
HCl	g	–74.84	$LiBrO_3$	c	1.42	$KClO_4$	c	51.04
$HClO_4$	l	–88.76	LiI	c	–63.30	KBr	c	19.87
$HClO_4 \cdot H_2O$	c	–32.95	$LiI \cdot H_2O$	c	–29.66	$KBrO_3$	c	41.13
HBr	g	–85.14	$LiI \cdot 2H_2O$	c	–14.77	KI	c	20.33
HI	g	–81.67	$LiI \cdot 3H_2O$	c	0.59	KIO_3	c	27.74
HIO_3	c	8.79	$LiNO_2$	c	–11.00	KNO_2	c	13.35
HNO_3	l	–33.28	$LiNO_2 \cdot H_2O$	c	7.03	KNO_3	c	34.89
HCOOH	l	–0.86	$LiNO_3$	c	–2.51	$KC_2H_3O_2$	c	–15.33
CH_3COOH	l	–1.51				KCN	c	11.72
			NaOH	c	–44.51	KCNO	c	20.25
NH_3	g	–30.50	$NaOH \cdot H_2O$	c	–21.41	KCNS	c	24.23
NH_4Cl	c	14.78	NaF	c	0.91	$KMnO_4$	c	43.56
NH_4ClO_4	c	33.47	NaCl	c	3.88			
NH_4Br	c	16.78	$NaClO_2$	c	0.33	RbOH	c	–62.34
NH_4I	c	13.72	$NaClO_2 \cdot 3H_2O$	c	28.58	$RbOH \cdot H_2O$	c	–17.99
NH_4IO_3	c	31.80	$NaClO_3$	c	21.72	$RbOH \cdot 2H_2O$	c	0.88
NH_4NO_2	c	19.25	$NaClO_4$	c	13.88	RbF	c	–26.11
NH_4NO_3	c	25.69	$NaClO_4 \cdot H_2O$	c	22.51	$RbF \cdot H_2O$	c	–0.42
$NH_4C_2H_3O_2$	c	–2.38	NaBr	c	–0.60	$RbF \cdot 1.5H_2O$	c	1.34
NH_4CN	c	17.57	$NaBr \cdot 2H_2O$	c	18.64	RbCl	c	17.28
NH_4CNS	c	22.59	$NaBrO_3$	c	26.90	$RbClO_3$	c	47.74
CH_3NH_3Cl	c	5.77	NaI	c	–7.53	$RbClO_4$	c	56.74
$(CH_3)_2NH_2Cl$	c	1.46	$NaI \cdot 2H_2O$	c	16.13	RbBr	c	21.88
$N(CH_3)_4Cl$	c	4.08	$NaIO_3$	c	20.29	$RbBrO_3$	c	48.95
$N(CH_3)_4Br$	c	24.27	$NaNO_2$	c	13.89	RbI	c	25.10
$N(CH_3)_4I$	c	42.07	$NaNO_3$	c	20.50	$RbNO_3$	c	36.48
			$NaC_2H_3O_2$	c	–17.32			
$AgClO_4$	c	7.36	$NaC_2H_3O_2 \cdot 3H_2O$	c	19.66	CsOH	c	–71.55
$AgNO_2$	c	36.94	NaCN	c	1.21	$CsOH \cdot H_2O$	c	–20.50
$AgNO_3$	c	22.59	$NaCN \cdot 0.5H_2O$	c	3.31	CsF	c	–36.86
			$NaCN \cdot 2H_2O$	c	18.58	$CsF \cdot H_2O$	c	–10.46
LiOH	c	–23.56	NaCNO	c	19.20	$CsF \cdot 1.5H_2O$	c	–5.44
$LiOH \cdot H_2O$	c	–6.69	NaCNS	c	6.83	CsCl	c	17.78
LiF	c	4.73				$CsClO_4$	c	55.44
LiCl	c	–37.03	KOH	c	–57.61	CsBr	c	25.98
$LiCl \cdot H_2O$	c	–19.08	$KOH \cdot H_2O$	c	–14.64	$CsBrO_3$	c	50.46
$LiClO_4$	c	–26.55	$KOH \cdot 1.5H_2O$	c	–10.46	CsI	c	33.35
$LiClO_4 \cdot 3H_2O$	c	32.61	KF	c	–17.73	$CsNO_3$	c	40.00
LiBr	c	–48.83	$KF \cdot 2H_2O$	c	6.97			
$LiBr \cdot H_2O$	c	–23.26	KCl	c	17.22			

5-86