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Project-Based Experiment in a Physical Chemistry Teaching Laboratory: Ion Effects on Caffeine Partitioning Thermodynamics

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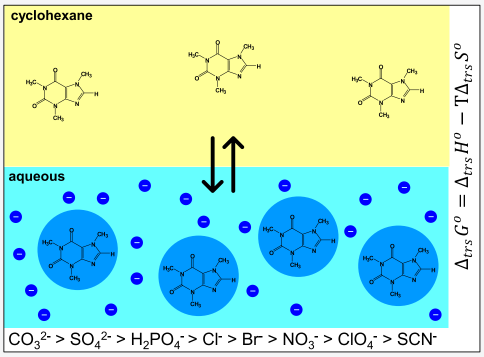

ABSTRACT: The Hofmeister series is a recurring trend that ranks ions' abilities to influence various aqueous processes in solution. Herein, a set of project-based experiments was developed for students in an upper-level physical chemistry laboratory to investigate the specific ion effects on the thermodynamics of caffeine partitioning between aqueous and cyclohexane phases. Students obtained the standard Gibbs free energy for caffeine partitioning in the presence of eight sodium salts in the aqueous phase at varied temperatures. The standard enthalpy and entropy for caffeine partitioning were obtained by plotting the standard Gibbs free energy vs temperature for each salt. Students then identified the trends in the thermodynamics for caffeine partitioning and explored the correlation between anions' properties and their influence on caffeine partitioning thermodynamic parameters. This project-based laboratory allowed students to gain a deep understanding of ion properties in solution and learn how the Hofmeister ions affect various aqueous processes. It also helped students internalize some fundamental concepts in thermodynamics such as standard Gibbs free energy, enthalpy, and entropy.

KEYWORDS: *Upper-Division Undergraduate, Physical Chemistry, Laboratory Instruction, Aqueous Solution Chemistry, Thermodynamics*

■ INTRODUCTION

In this report, we describe a project-based experiment to introduce the Hofmeister series to students in our upper-level physical chemistry laboratory. Project-oriented laboratory teaching has proven to be an effective approach in the Integrated Organic−Inorganic Laboratory and Biochemistry Laboratory courses in our department.[1−3] More recently, we began to transform physical chemistry laboratories into project-based courses. These laboratories give students some ideas about how scientific research is performed in laboratories. This experience is especially important for those students who are not involved in undergraduate research. To this end, we have incorporated experiments on the well-studied Hofmeister series into the teaching laboratory. The Hofmeister series[4,5] ranks ions abilities to affect a variety of processes such as micelle formation, polymer and protein aggregation, protein folding, protein crystallization, and enzyme activity. Research on the Hofmeister series has had tremendous impact on a broad range of studies including biological molecules, materials, physical chemistry, and related fields. Therefore, the Hofmeister series is chosen to be incorporated into a more research-based physical chemistry laboratory. Thermodynamics, spectroscopy, and computer simulations play important roles in probing the mechanisms of the Hofmeister effects. The typical order for the ability of anions to affect the properties of aqueous solutions follows:[6−8]

$$CO_3^{2-} > SO_4^{2-} > S_2O_3^{2-} > H_2PO_4^- > F^- > Cl^- > Br^- > NO_3^- > I^- > ClO_4^- > SCN^-$$

Ions on the left tend to decrease protein solubility in aqueous solution, while ions on the right generally increase protein solubility.[9−11] Chloride is considered as the dividing line between the two groups of ions in the series. Understanding the mechanisms for the Hofmeister effects is a fundamentally important problem in chemistry and biochemistry/biophysics. Students in chemistry/biophysical chemistry/biochemistry are introduced to solution related topics in their physical chemistry courses. However, the Hofmeister series is not usually covered in undergraduate physical chemistry curriculum. There are a few previous examples of teaching laboratories that examined the Hofmeister ions.[12,13] Our lab experiment described herein goes into more thermodynamic depth of the Hofmeister series using project-based activities in physical chemistry teaching laboratory.

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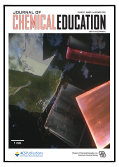

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Our upper-level physical chemistry teaching laboratories I & II enroll chemistry majors, biophysical chemistry majors, and chemistry minors (often time biology and biotechnology majors). Physical Chemistry Laboratory I focuses on thermodynamics, materials, and computational chemistry. Physical Chemistry Laboratory II focuses on quantum mechanics and spectroscopy. Students take either Physical Chemistry Laboratory I or II depending on their track of degree programs. Usually, the students who are on the track of pursuing an ACS certified B.S. in Chemistry (Chemistry, Biochemistry, and Chemical Education concentrations) take Physical Chemistry Laboratory II. The students who are in the General Program in Chemistry, Chemistry/Business, ACS certified degree in Materials Chemistry, and Chemistry Minors take Physical Chemistry Laboratory I. Biophysical Chemistry majors may choose to take either Physical Chemistry Laboratory course. The enrollment in Physical Chemistry Laboratory I is about twice as many as that in Physical Chemistry Laboratory II over the years. The laboratory described in this report is being taught in Physical Chemistry Laboratory I. In a typical year, we usually have two sections of 12−16 students enrolled in the course and two instructors. Each instructor teaches half of the laboratories individually. During a 2-week period, each instructor teaches one lab four times, and the students rotate through the two laboratories.

The laboratory described herein is designed to introduce the Hofmeister series to students through a collaborative research project that is carried out by the students in groups. It provides opportunities for students to participate in authentic research in a physical chemistry teaching laboratory. The students learn how to design experiments and answer scientific questions in a research setting. The learning goals of this laboratory for students in terms of chemistry concepts include the thermodynamics of caffeine partitioning, physical properties of ions such as size and hydration, and the correlation between ions' influence on caffeine partitioning and ion properties. The students work in groups to collect, analyze, and interpret data and write lab reports. The teamwork component of the laboratory helps the students become better team players and enhances their communication skills. The students are assigned research and review papers to read in the field of Hofmeister series and encouraged to search for more relevant literature. Reading the literature introduces the research topic to students and helps students understand the research questions they need to answer in the laboratory.

The students investigate the effects of eight sodium salts on the thermodynamics of caffeine partitioning between aqueous and cyclohexane phases. In the prelab, the students are introduced to the background of the research, experimental methods and procedures, expectations of the lab reports, and a list of suggested references for reading. In a 3-h lab session, two groups of 3−4 students each investigate one salt's influence on the thermodynamics of caffeine partitioning. The two laboratory sections carry out the experiments in eight groups during a 2-week period. Students in each group share their responsibilities and make sure that everyone in the group is fully engaged in the experiments. At the end of their lab sessions, all the groups save their data in a common Excel file on the departmental server that is accessible to all the students. The combination of student results collected by all the groups are used for lab reports. The students are expected to submit formal reports as groups in the style of *J. Phys. Chem. B* discussing the data on the eight sodium salts being studied. An important component of student lab reports is to explore possible correlations between the anion effects on the thermodynamics of caffeine partitioning and the intrinsic physical properties of the anions. Students may choose any relevant physical properties of anions to discuss in their lab reports after reading the assigned literature. This open-ended question gives the students an opportunity to learn a variety of ion properties such as hydration thermodynamics, limiting partial molar volume, surface tension increment, and polarizability and apply the knowledge to interpret their experimental data. The laboratory described herein was first developed in 2013, and it has served over 150 chemistry majors/minors in the undergraduate physical chemistry teaching laboratory. The data presented in this report were students' data collected during the lab in spring 2020.[14] The Results and Discussion section of this report reflects the level of students' understanding of their results and the Hofmeister series in general that is demonstrated in the lab reports.

A caffeine molecule, as shown in Figure 1, is chosen to be the model compound for Hofmeister studies in the laboratory.

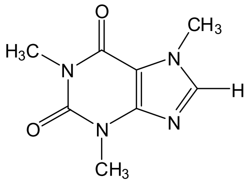

Figure 1. Chemical structure of a caffeine molecule.

Caffeine is suitable for studying multiple types of ion-solute interactions because it has a good mix of functional groups. As a key component in tea, coffee, energy drinks, and some soft drinks, caffeine is the most widely used psychoactive substance in the world.[15] Caffeine has significant biological relevance due to its pharmaceutical application as a mild central nervous system stimulant.[16−18] Moreover, caffeine resembles important biological purines such as adenine and guanine and some amino acids such as tryptophan.[19,20] The relevance of caffeine to biology and pharmaceuticals as well as our daily life makes it interesting to the students who have a variety of career and research goals.

■ EXPERIMENTAL PROCEDURES

Each group of 3−4 students was provided with a 20 mM caffeine solution in water and one of the eight sodium salts including Na_2CO_3, Na_2SO_4, NaH_2PO_4, NaCl, NaBr, $NaNO_3$, $NaClO_4$, and NaSCN. Each group was assigned to prepare a 1.0 M sodium salt solution. Table 1 showed the assignments of salts to each group in spring 2020. The students combined 1.5 mL of the provided caffeine solution and 1.5 mL of the prepared salt solution in a 15-mL Falcon tube to make the aqueous phase of the partitioning system. Three milliliters of cyclohexane was layered above the aqueous phase before the Falcon tube was placed in a water bath at the desired temperature. The tubes containing the aqueous and cyclohexane layers equilibrated for 5 min at the desired temperature, and then they were removed from the water bath and shaken for 1 min. After shaking, the tubes were returned to the water bath for an additional 5 min. There was a well-defined meniscus that separates the two phases once equilibrated. Students were instructed to carefully remove ∼2.5 mL of the cyclohexane layer for UV−vis analysis. The concentration of

Table 1. Assignments of Salts to Each Group in Spring 2020

Week 1 Tuesday		Week 1 Wednesday		Week 2 Tuesday		Week 2 Wednesday	
Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Na_2SO_4	NaBr	Na_2CO_3	$NaNO_3$	NaCl	$NaClO_4$	NaH_2PO_4	NaSCN

caffeine in the cyclohexane layer was determined from the absorbance at 273 nm with the molar extinction coefficient as 8508 cm^{-1} M^{-1} in cyclohexane.[21] UV−vis spectra of the cyclohexane layer were collected on an 845 Agilent UV−vis spectrometer at room temperature. The partitioning experiments were performed at five temperatures, approximately at 25, 30, 35, 40, and 45 °C in a fume hood. The temperature of the water bath was monitored by using a digital thermometer.

■ HAZARDS

Gloves and eye protection should be worn during lab sessions. Cyclohexane is a flammable liquid and potentially causes irritation to the skin and respiration system. The caffeine partitioning experiments should be carried out in a fume hood.

■ RESULTS AND DISCUSSION

Students learned how to measure the standard Gibbs free energy for caffeine partitioning from the aqueous to cyclohexane phase using a UV−vis spectrometer. Each group of students examined the influence of one sodium salt at 0.50 M on the partitioning thermodynamics. In a partitioning system, the transfer of caffeine from the aqueous to organic phase is an equilibrium process represented as

$$\text{caffeine (aqueous)} \rightleftharpoons \text{caffeine (organic)} \tag{1}$$

The partition coefficient is defined as the ratio of the concentration of the solute in the two phases.[13,22,23] The students determined the concentration of caffeine in the cyclohexane phase by monitoring the absorbance at 273 nm and using the rearranged Beer−Lambert equation

$$[\text{caff}]_{\text{cyclohexane}} = \frac{A}{\varepsilon l} \tag{2}$$

where A is the absorbance of caffeine in the cyclohexane phase at 273 nm, ε is the molar extinction coefficient of caffeine in cyclohexane (8508 cm^{-1} M^{-1}),[21] and l is the optical path length (1 cm). Higher caffeine concentration in the cyclohexane phase reflects greater transfer of caffeine from the aqueous phase. Since the original concentration of caffeine in the aqueous phase is 0.010 M and the volume of the aqueous and cyclohexane phases is identical, the concentration of caffeine in the aqueous phase at the equilibrium can be calculated by

$$[\text{caff}]_{\text{aqueous}} = 0.010 - [\text{caff}]_{\text{cyclohexane}} \tag{3}$$

The concentration of caffeine in the organic and aqueous phases can be used to determine the partition coefficient of caffeine, P, which is the equilibrium constant representing the distribution of caffeine between two phases:

$$P = \frac{[\text{caff}]_{\text{cyclohexane}}}{[\text{caff}]_{\text{aqueous}}} \tag{4}$$

The students calculated the standard Gibbs free energy for caffeine transfer at a specified temperature in the presence of sodium salts by using the following equation

$$\Delta_{\text{trs}}G^\circ = -RT \ln P \tag{5}$$

where R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature in Kelvin, and P is the partition coefficient of caffeine obtained by using eq 4. The raw data of absorbance of caffeine in the organic layer at 273 nm collected by the students in spring 2020 at varied temperatures together with calculated caffeine concentration in the organic phase, partition coefficient, and $\Delta_{\text{trs}}G^\circ$ can be found in Table S1 in the Supporting Information. The temperature dependence of $\Delta_{\text{trs}}G^\circ$ can be used to determine other thermodynamic values, $\Delta_{\text{trs}}H^\circ$ and $\Delta_{\text{trs}}S^\circ$, assuming $\Delta_{\text{trs}}H^\circ$ and $\Delta_{\text{trs}}S^\circ$ are constants over the given temperature range. The standard Gibbs free energy-temperature plots are fitted with a linear regression according to the Gibbs free energy relation:

$$\Delta_{\text{trs}}G^\circ = \Delta_{\text{trs}}H^\circ - T\Delta_{\text{trs}}S^\circ \tag{6}$$

The y-intercept and negative value of the slope correspond to the standard enthalpy, $\Delta_{\text{trs}}H^\circ$, and standard entropy, $\Delta_{\text{trs}}S^\circ$, for caffeine transfer, respectively. This method, analysis of temperature dependence for partitioning, has been previously used in the thermodynamic studies of hydrophobic dye molecules in a physical chemistry teaching laboratory.[13]

Student data collected in spring 2020 are shown in Figure 2. The guidance for the discussions that need to be covered in lab reports is given to students in the prelab lecture. Students read a significant amount of literature before they write their lab reports. The data in Figure 2 show that the standard Gibbs free energy for caffeine transfer, $\Delta_{\text{trs}}G^\circ$, decreases with increasing temperature for all the studied salts. The fitted thermodynamic parameters for caffeine transfer, $\Delta_{\text{trs}}H^\circ$ and $\Delta_{\text{trs}}S^\circ$, are listed in Table 2. Both the standard enthalpy and entropy for caffeine transfer are positive for all the salts. The data suggest that the transfer of caffeine from the aqueous phase to organic phase is an endothermic process due to the positive $\Delta_{\text{trs}}H^\circ$ values. The positive $\Delta_{\text{trs}}H^\circ$ can be rationalized by considering that energy is needed to remove the hydration water from caffeine as it is transferred across the aqueous−organic interface. The positive $\Delta_{\text{trs}}S^\circ$ values are attributed to the dehydration of caffeine that

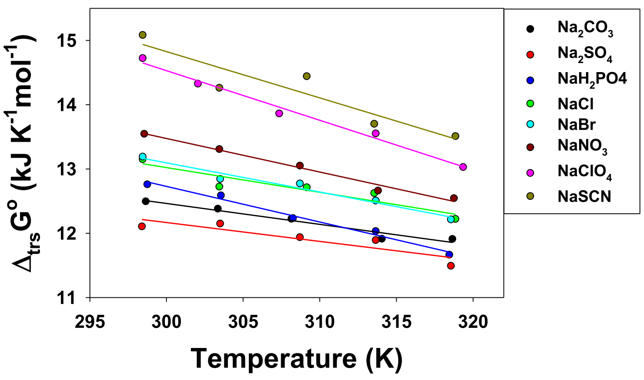

Figure 2. Student data collected in spring 2020: the standard Gibbs free energy for caffeine transfer from the aqueous to organic phase as a function of temperature in the presence of 0.5 M of sodium salts.

Table 2. Standard Enthalpy and Entropy for Caffeine Transfer and Their Contributions to the Overall Standard Gibbs Free Energy Together with the Limiting Partial Molar Volume and Hydration Gibbs Free Energy for the Anions

Anion	$\Delta_{trs}H°$ (kJ mol^{-1})	$\Delta_{trs}S°$ (J mol^{-1}K^{-1})	ζ_H (%)	ζ_{TS} (%)	$V_i^{\circ a}$ (cm^3 mol^{-1})	$\Delta_{hydr}G^{\circ a}$ (kJ mol^{-1})
CO_3^{2-}	22.23	32.5	69.62	30.38	6.7	−479
SO_4^{2-}	20.96	29.3	70.59	29.41	25.0	−1090
$H_2PO_4^-$	29.36	55.4	64.00	36.00	34.6	−473
Cl^-	24.56	38.5	68.18	31.82	23.3	−347
Br^-	26.72	45.4	66.38	33.62	30.2	−321
NO_3^-	29.14	52.2	65.19	34.81	34.5	−306
ClO_4^-	37.70	77.2	62.09	37.91	49.6	−214
SCN^-	36.46	72.1	62.92	37.08	41.2	−287

[a]Values of $V_i°$ and $\Delta_{hydr}G°$ are from ref 24.

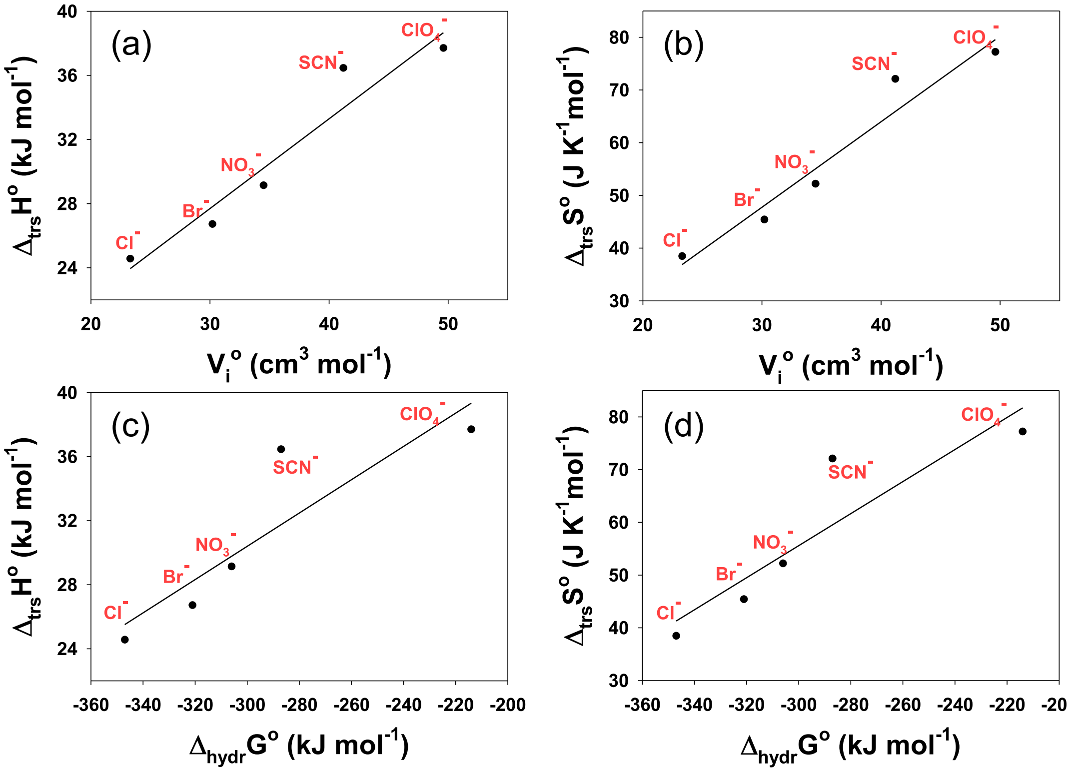

Figure 3. Correlation between the thermodynamic parameters for caffeine transfer and physical properties of weakly hydrated anions: (a) enthalpy for caffeine transfer vs $V_i°$; (b) entropy for caffeine transfer vs $V_i°$; (c) enthalpy for caffeine transfer vs $\Delta_{hydr}G°$; and (d) entropy for caffeine transfer vs $\Delta_{hydr}G°$.

creates a more "disordered" environment. As caffeine is transferred into the organic layer, the ordered hydration shell around the caffeine is released, and the entropy of the system increased. Both $\Delta_{trs}H°$ and $\Delta_{trs}S°$ being positive for each of the salts indicate that the caffeine transferring process from the aqueous to organic phase is an entropy driven process.

The specific anion effects on caffeine partitioning followed a direct Hofmeister series that the $\Delta_{trs}G°$ values at 298 K increase from the strongly hydrated CO_3^{2-} to the weakly hydrated SCN^-. The trend indicates that strongly hydrated anions promote caffeine transfer from the aqueous phase to the organic phase with lower $\Delta_{trs}G°$, while weakly hydrated anions solubilize caffeine in the aqueous phase and hinder the transfer process. The relative contributions of $\Delta_{trs}H°$ and $-T\Delta_{trs}S°$ to the overall Gibbs free energy for caffeine transfer at 298 K can be estimated by eqs 7a and 7b,[25−27] and the results are included in Table 2.

$$\zeta_H = \frac{|\Delta_{trs}H°|}{|\Delta_{trs}H°| + |T\Delta_{trs}S°|}; \qquad \zeta_{TS} = \frac{|T\Delta_{trs}S°|}{|\Delta_{trs}H°| + |T\Delta_{trs}S°|} \tag{7a,b}$$

The relative contributions of the $\Delta_{trs}H°$ and $-T\Delta_{trs}S°$ terms stay roughly constant across the Hofmeister series, and $\Delta_{trs}H°$ makes the major contribution to the overall $\Delta_{trs}G°$.

In order to understand the underlying mechanisms of the Hofmeister anion effects on caffeine partitioning thermodynamics, the students are expected to explore the correlations between the fitting thermodynamic parameters ($\Delta_{trs}H°$ and $\Delta_{trs}S°$) and the intrinsic physical properties of the anions. Limiting partial molar volume and hydration Gibbs free energy are the two properties of the anions chosen by many of the groups to discuss in their lab reports. The results show that the standard enthalpy and entropy for caffeine transfer for weakly hydrated anions are correlated to the limiting partial molar volume (Figure 3a, 3b). Limiting partial molar volume is a physical property of anions that is considered as the size of hydrated ion.[24] Large weakly hydrated anions can easily shed their hydration water to associate with the caffeine molecules and solvate the caffeine molecules into the aqueous phase. Therefore, more energy (higher $\Delta_{\text{trs}}H°$) is needed to dehydrate the caffeine molecules and transfer them to the organic phase when weakly hydrated anions are present in the aqueous phase. At the same time, the dehydration of caffeine molecules in the presence of large weakly hydrated anions creates more disorder (higher $\Delta_{\text{trs}}S°$) when caffeine molecules travel across the interface. Strongly hydrated anions follow a different mechanism compared to weakly hydrated anions. There are no correlations between the standard enthalpy and entropy for caffeine transfer and limiting partial molar volume found for the strongly hydrated anions (see Supporting Information Figure 1S). The hydration Gibbs free energy of anions is inversely proportional to anion size; therefore, the standard enthalpy and entropy for caffeine transfer are also correlated to the hydration Gibbs free energy of anions for weakly hydrated anions as shown in Figure 3c and 3d. There are no such correlations found for the strongly hydrated anions. Well hydrated anions are excluded from the caffeine surface and decrease caffeine solubility in the aqueous phase.[21] As a result, well hydrated anions decrease the standard Gibbs free energy for caffeine transfer and promote the transfer process to the organic phase.

Students make correlations between the thermodynamic parameters for caffeine partitioning and the physical properties of ions. These correlations help students understand why the trends in the ions' influence on caffeine partitioning occur in the system. The process of exploring the correlation encourages students to research the literature and learn a broad range of ion properties in aqueous solution. Except for the limiting partial molar volume and hydration Gibbs free energy discussed above, some other ion properties such as hydration entropy, polarizability, and surface tension increment (ability of ions to increase surface tension of water) are also examined by some groups in their lab reports. Asking an open-ended question of exploring the correlation allows students to choose any ion properties to discuss in their lab reports and apply what they learn from the literature to interpreting their own experimental results.

■ ASSESSMENT OF STUDENT LEARNING

Students are required to submit comprehensive formal lab reports in *J. Phys. Chem. B* style as groups to assess their learning in this lab. The students have a week and a half to write their reports after all groups complete data collection. Students collaborate in groups to analyze the data and write the lab reports. Each group distributes the duties among their group members, but everyone is responsible for their final reports. Group members check each other's work and proofread the final reports. The students recognize the trends in anion effects on caffeine partitioning and correlate the trends to the physical properties of anions. The open-ended discussions in the lab reports give students the freedom to research different aspects of the Hofmeister series and apply what they learn from the literature to interpreting their results. This laboratory provides students with an opportunity to experience the process of scientific research in real life. The students learn how to design experiments to answer research questions, measure partitioning thermodynamic parameters, analyze and interpret experimental data, and present their findings formally in writing. Our students were successful in understanding the trends in anion effects on caffeine partitioning thermodynamics. Some groups gained more in-depth comprehension of the Hofmeister series than others as shown in the discussions in their lab reports. In addition to the lab reports, students' understanding about the Hofmeister series is assessed in the lab final written exam. The students are asked to predict the effects of Na_2SO_4, NaCl, and NaSCN on the melting point of ribonuclease and explain their reasoning. Most of our students can predict the trend correctly and gave some reasonable explanation. Students' learning in this Hofmeister series laboratory is reflected in their lab reports and final exam.

This laboratory has been taught for eight years in our physical chemistry laboratory curriculum since it was developed in 2013. The undergraduate student who helped develop the experiments, Bradley Rogers, graduated from JMU in May 2014 and went on to pursue a Ph.D. in chemistry at The Penn State University. Currently, Bradley is a postdoctoral researcher at Penn State. The experiments described in this report were part of Bradley's research project for his honors thesis. Bradley's undergraduate honors thesis resulted in a peer-reviewed publication in *J. Phys. Chem. B.*[21]

■ CONCLUSIONS

The Hofmeister series widely exists in a variety of aqueous processes. The investigations of the underlying mechanisms for the Hofmeister effects and related solvation chemistry have drawn a great deal of attention in current literature. The experiments described in this work introduce solution chemistry of the Hofmeister series into undergraduate physical chemistry laboratory. The knowledge students gained by carrying out these experiments can be applied to understanding the ion effects on a broad range of aqueous processes such as protein solubility and stability in solution. Through this laboratory, students strengthen their learning skills in searching literature, designing experiments, using equipment, analyzing and interpreting experimental results, working in teams, and presenting their results in formal journal format. This work also allows the integration of faculty's research expertise into an upper-level teaching laboratory to enhance student learning.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.0c00961.

Students' raw data, correlation plots for strongly hydrated anions, and notes for instructors (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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