A Low-Cost Exercise Relating Glutamate Solubility to pH & Buffering Capacity

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ABSTRACT
The concept of solubility, and its relation to molecular structure, is important for understanding the properties of biological molecules in solution as they interact with the solvent and with each other. To demonstrate the relation between structure, solubility, and buffering capacity, we report a low-cost laboratory exercise using monosodium glutamate (MSG) as a model solute. Students observe how glutamate solution initially resists acidification and how glutamate solubility is first decreased and then restored with acidification. Students should interpret these observations relative to the structure of the solute. This exercise has been employed in introductory level courses for both biology majors and nonmajors and would also be appropriate for AP Biology.

Key Words: solubility; pH; buffer; introductory biology laboratory; organic functional groups; amino acid.

Introduction
Concepts explaining the aqueous solubility or insolubility of molecules contribute to an understanding of how biological molecules interact, either as solvated forms or as structures arising from hydrophobic interactions. Introductory college-level biology textbooks commonly explain a molecule’s solubility in terms of the distribution of electrons on its surface and its capacity to form stable interactions with water molecules: the “even” sharing of the valence electrons, occurring in nonpolar bonds, contributes to a molecular surface lacking sites at which water may stably associate, while polar covalent bonds within a molecule contribute to a “patchiness” of electron distribution on the molecular surface, generally corresponding to greater aqueous solubility. Amino, hydroxyl, carbonyl, and carboxyl functions contribute (to differing degrees) to molecular solubility. Among these functional groups, amino and carboxyl functions can bear full charges resulting from the association or dissociation of protons, respectively. Consequently, some soluble biological molecules have the potential to act as buffers.

Educational research has examined practices for teaching about the solubility of ions and molecules (Blake, 2003; Salinas and Reyes, 2014). Solubility concepts may be introduced through a series of “solubility rules” for inorganic compounds (Blake, 2003), emphasizing to greater or lesser degrees the roles of energetics and entropy (Eisen et al., 2014), and educational studies examining the teaching of solubility concepts have largely focused on introductory inorganic chemistry courses. Introductory biology courses have a similar need to introduce a sufficient minimum of concepts for understanding how it is that biological (principally organic) molecules behave in solution. Addressing this need, we have developed a low-cost, semiquantitative exercise for demonstration of the conditional solubility of a common biological molecule, glutamate, and its capacity to moderate pH change in solution. This exercise was developed for use in a college-level introductory biology course for majors, anticipating that the majority will not yet have completed an introductory college chemistry course.

The pedagogical goals of this exercise are to have students develop an understanding of the relation between molecular structure and aqueous solubility; understand how a solute can function as a buffer; and recognize that as a molecule’s structure is altered, through the accepting or donating of a proton, its solubility may change. In this pursuit, we would hope that the learners develop a connection between the microscopic (phenomena at the molecular level), the macroscopic (observed at the experiential level), and the symbolic (the structural representations used as models), as described by Bruck and colleagues (2009).

Background
Context in an Introductory Biology Course
Variations of this exercise have been used for over a decade during the early weeks of an introductory college biology majors’ course. Incoming students are not required to have completed college-level chemistry, though both high school biology and chemistry are recommended. Prior to this exercise, the students will have been introduced in lecture to the basics of atomic structure, and to polar and nonpolar covalent bonding, ionic bonding, hydrogen bonding, ion-dipole interactions, and the related concepts of solutions, pH, and buffering. This exercise is typically delivered during the week of lecture in which students are introduced to carbon-containing...
compounds, the conventions for interpreting organic structure notation, and the major functional groups commonly occurring in biological molecules, with carboxyl and amino functions being the most important for the purposes of this exercise. Specialized terminology describing glutamate's degree of protonation (monoprotic, diprotic, triprotic) and its overall charge state in solution (zwitterion) will not typically have been introduced in lecture; the instructor may choose to introduce these terms, though the exercise can be delivered effectively without specialized terminology. An introductory slide presentation reviewing major concepts and terminology, and providing an example of buffering in biological systems, is provided with the online version of this article as Supplement 1. A less in-depth version of this exercise has been used in a nonmajors' principles of biology course; in this setting the students are not introduced to details of organic structures, but instead to a more general idea that molecules can accept from or donate protons to solution (i.e., can act as buffers) and that this can impact their solubility.

The typical laboratory section size is 36 students, with students working in groups of three. The monosodium glutamate (MSG) experiment is accompanied by conventional demonstrations of the immiscibility of cooking oil and water, and the solvation of sugar in water and of butter in oil. In a separate demonstration, a saturated MSG solution is used to demonstrate that, despite its being MSG-saturated, the solution remains capable of dissolving other solutes like sucrose. All aspects of this lab are regularly delivered during a two-hour and fifty-minute laboratory section.

Glutamate in Solution

Glutamic acid has been used as a model molecule for the study of precipitation and crystallization from solution (Schöll et al., 2006); it has also been used as an educational model for analysis of protonation equilibria (Sarac & HadžI, 2021). The dynamics of reactive crystallization from a solution of MSG have been characterized by Borissova and colleagues (2005). Upon aqueous solvation, MSG dissociates to Na⁺ and monoprotic glutamate (Scheme 1A). This is the primary form in solution at pH between 4.6 and 9.7.

Addition of H⁺ as HCl to the MSG solution promotes the protonation of the side group carboxyl function (Scheme 1B). This protonation generates an overall electrically neutral zwitterion (predominant at pH 4.6 to 2.2), which forms a precipitate in solutions of 1M glutamate or greater. Crystallization results in a visually striking change in the appearance of the solution—the formation of an opaque, milky white precipitate. Additional protonation leads to the formation of the triprotic (+1) form, which has a greater solubility than the zwitterion form. Addition of sufficient HCl will cause the complete resolubilization in the 1M glutamate solution.

Scheme 1. Solvation of monosodium glutamate and glutamate structures with different degrees of protonation and solubility. (A) Solid MSG dissociates yielding the soluble monoprotic form, a dicarboxylate with a protonated amino function. (B) Addition of H⁺ (from left to right in the scheme) neutralizes the charge on the side group carboxyl function, forming a zwitterion (uncharged) with reduced solubility. Further protonation yields the triprotic (+1) form, which is more soluble than the zwitterion. Scheme 1B redrawn from Borissova et al. (2005).
Hazards
Food-grade MSG (CAS Registry Number 142-47-2) is a potential skin, eye, and respiratory tract irritant. Aqueous hydrochloric acid (3N in water, CAS Registry Number: 7647-01-0) is corrosive and can cause irritation and serious damage to eyes and skin. Students handling 3N HCl are required to wear personal protective equipment, such as gloves and goggles. A neutralizing agent (baking soda) should be kept on hand in the event of spills.

○ The MSG Experiment

Overview
Students use pH paper to monitor the pH change of unbuffered water and of 1M MSG solution in response to the stepwise addition of 1 mL aliquots of 3N HCl. The students should note both the estimated pH and any changes in the appearance of the MSG solution following the addition of each aliquot of HCl, and at the conclusion of the exercise, they should explain what they have observed relative to changes to the structure resulting from the increased abundance of protons. A detailed protocol for laboratory setup and delivery and practical observations are provided as Supplement 2.

Observing pH Change & Changes in Glutamate Solubility
Students may work individually or in groups; students handling 3 N HCl must wear gloves and eye protection. Each student or group should arrange segments of precut pH paper on the data collection sheet with parafilm strip (see Figure 1A). The use of pH paper permits delivery of this exercise in a laboratory that does not have a pH meter available for each student work station. This exercise has been tested (though not delivered in a laboratory class setting) using a pH meter; the pH meter will provide numerical data that reflects the pH changes shown by pH paper, and which may be suitable for a graphical summary. Consideration should be given to whether to use a pH meter if units are not available for each work space; traffic in the laboratory will increase as students carry their flasks to and from shared meters.

Students begin by preparing a 1M MSG solution of by adding 3.4 g MSG to 20 mL of distilled water in a 50 mL flask and swirling it until the MSG is dissolved. A flask containing 20 mL distilled water is also prepared. The data collection sheet (Supplement 3) lists the number of 1 mL aliquots of 3N HCl added over the course of the experiment, and students should arrange their pH paper segments on a parafilm strip next to the corresponding volumes. Using a 1 mL graduated disposable plastic transfer pipette, students test the pH of the unbuffered water and the 1M MSG solution by transferring a small volume of the liquid to a precut segment of pH paper and arranging these wetted segments next to the “Before HCl” labels on the data sheets. When testing the solution pH, it is recommended that students hold the pH paper in place on the data sheet with forceps while applying the tip of the transfer pipette, allowing the capillary action of the paper to draw a sufficient volume from the pipette.

Using a clean transfer pipette (used only for HCl), students add 1 mL 3N HCl to each flask, swirling to ensure mixing. The water and the 1M MSG solution are tested to observe the effect of the added HCl on pH, and the wetted pH paper segments are arranged at their respective locations on the data collection sheet (Figure 1A). It should be immediately evident that, while the pH of the unbuffered water dropped significantly, the HCl affected a smaller pH decrease in the MSG solution. From this point, the students should continue to add 1 mL aliquots of 3N HCl to the 1M MSG solution, swirling and testing the pH following each addition. The students should also observe the appearance of the solution as the acid is added and mixed, noting any changes. If a precipitate forms during mixing, the solids should be permitted to settle, and the pH should be tested using liquid collected at the surface.

Figure 1. Solution pH and glutamate solubility in response to added HCl. (A) Addition of 1 mL 3N HCl to unbuffered water results in a significant drop in pH, while the same addition to 1M MSG solution lowers the pH much less, though multiple aliquots of HCl overcome the buffering effect of glutamate in solution. (B) Acid impact on glutamate solubility. Initially, MSG is soluble (flask at left); stepwise addition of acid results first in reduced solubility (evidenced by precipitation, center flask) and then restored solubility (right flask).
Typical results are shown in Figure 1. Depending on the pH of the water used for preparing the solution, the 1M MSG pH may differ from the water control prior to addition of HCl. Addition of 1 mL 3N HCl to unbuffered water results in a large decrease in measured pH (Figure 1A). This large change is not observed in the MSG solution, which shows an estimated pH of 4 or 5 through the first five aliquots of 3N HCl. Depending on the students’ pipetting accuracy, a precipitate of the neutral zwitterion will first appear after the addition of four to five 1 mL aliquots of 3N HCl (Figure 1B). This precipitate will accumulate and persist through the addition of aliquots 9 or 10. Provided it is permitted to settle, the amount of precipitate at the bottom of the flask can then be seen to decrease with each subsequent aliquot after this point. Complete resolubilization of the precipitate will occur with the addition of aliquots 12 to 15 (Figure 1B), as the majority of the glutamate achieves the triprotic (+1) form. Students should note that, despite the initial resistance of the solution to the same degree of acidification of unbuffered water, the glutamate solution does become more acidic with continued addition of HCl (Figure 1A).

Discussing the Science

Prior to the exercise, it may be helpful to review the concepts of solution pH and buffering, and also of the relation between molecular structure and solubility. Important among these concepts is that only “free” protons in solution contribute to the measured pH, while protons bound in molecular structures do not.

During the exercise, as students note the resistance of the MSG solution to pH change, they may be challenged with the question “Where did the protons go?” and prompted to form an explanation with reference to the structure of the solute. Upon observing the formation of precipitated glutamate, students should attempt to explain how it is that the glutamate became less soluble with the continued addition of protons to the solution, noting that as protons (+1) associate with the carboxylate functions (−1) the charges are neutralized.

It is not uncommon for students to propose that the precipitate forms as a result of Na⁺ (from MSG) combining with Cl⁻ (from HCl); this can be used as an alternative hypothesis to the precipitation of the organic solute. Upon observing the resolubilization of the precipitated material accompanying continued acidification, students may be asked to judge which hypothesis (precipitated glutamate versus precipitated NaCl) best explains this observation, given the structures of these two materials.

Finally, upon observing the restored solubility of glutamate and its correspondence with a very acidic solution pH, students should further appeal to the structure of glutamate to explain how it could have become more soluble than the precipitated form. It might be noted that the stepwise changes in pH suggest that the different carboxyl functions have differing affinities for protons, and that, in solution, one site “fills” with protons before the other.

Students can be asked to speculate on what would happen to the structure of glutamate in the acidic solution if the process were reversed, if a strong base were added in aliquots.

Supplement 4 provides a short set of multiple-choice questions that might be used to assess student comprehension of this exercise’s major concepts.

Students’ Perception of Laboratory Exercise

Student perception of the exercise was assessed using a survey, in which students were asked to indicate the degree to which they agreed with three statements about the quality of the exercise. Students were asked to choose one response among the following options: strongly disagree, disagree, neutral, agree, strongly agree. Among students surveyed after the exercise, 90% agreed or strongly agreed with the statement that “the exercises improved my understanding of how a buffer works,” and 79% of the students agreed or strongly agreed that “the exercises improved my understanding of how molecular structure determines solubility.” “The instructions for the exercises were clear” was agreed or strongly agreed with by 87% of the students.

Supplement Material

Supplemental material is available with the online version of this article.

- Supplement 1: Introductory presentation, concepts, and terminology
- Supplement 2: MSG protocol
- Supplement 3: Data collection sheet
- Supplement 4: Assessment questions

References

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