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# Course-Based Undergraduate Research Experience (CURE) For Biochemistry Majors in Physical Chemistry

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## Abstract

*A course-based undergraduate research experience (CURE) was designed for biochemistry majors in physical chemistry, based on 15 years of prior experience. The course includes a theoretical background in classical thermodynamics and kinetics, problem-solving, and laboratory experiments. In the laboratory part, students follow a fairly comprehensive study of different proteins, from analytical evaluation by UV/VIS spectrometry, followed by transport measurements, differential scanning calorimetry, denaturation induced by chemical agents and by heat, and kinetics of denaturation. These laboratory proposals allow the student to gather all the information in a single study report, and different proteins can be included, giving rise to the need for the selection of specific methods by the students. As an example, we present results based on the C-phycocyanin protein extracted from Spirulina platensis.*

**Keywords:** *biochemistry, CURE, physical chemistry, proteins*

## 1. Introduction

Course-based undergraduate research experience (CURE) is a pedagogical proposal that seeks to introduce research early into the university curriculum. Its introduction in university courses must comply with being novel, potentially publishable, and whose results are not even known by teachers, who will act as research mentors. In the area of chemical physics, there is only one proposal designated for the upper-level physical chemistry laboratory course [1], for which participating students are expected to have completed one semester of physical chemistry.

The current approach to the dictation of physical chemistry for science university students considers the importance of mathematical and molecular models in the interpretation of experimental data, either to interpret phenomena at the molecular level, or as a way of predicting property values. Hand in hand with handling the concept of additive property and data processing based on molecular and mathematical models, it is possible to introduce research in physical chemistry, including uncertainty as to the final result, since both verification and non-verification of a model constitute transcendental results for a given system under study.

## 2. Experimental course proposal

The center of the proposal is the study of the thermodynamic properties of various processes associated with a given protein in an aqueous solution. The course is specifically designed for biochemistry majors, and is taught at the Faculty of Sciences of the University of the Republic (Uruguay) simultaneously with the courses of Biochemistry, Biophysics, and Organic Chemistry, after several semesters of Mathematics, General Chemistry, Biology General, and Analytical Chemistry. The course is one of the five physicochemical-themed courses taught for biochemistry majors, and it only includes the classic thermodynamics and kinetics topics.

The following laboratory practices (LP) are proposed in the course:

LP1. Crude protein extraction
LP2. Protein structure from PDB files
LP3. Kinetics of chemical denaturation
LP4. Kinetics of thermal denaturation
LP5. Chemical induction of protein denaturation
LP6. Thermal induction of protein denaturation

LP7. Differential Scanning Calorimetry (DSC)
LP8. Gibbs energy of transfer of peptides and amino acids

The course begins with obtaining a protein extract from natural sources (LP1), taking advantage of the knowledge they have on the theme of the Biochemistry course, which is carried out simultaneously during the semester. In the case of the C-phycocyanin (CPC) protein, it is obtained from Spirulina platensis contained in commercial products. The extraction procedure can be variable, and students will have a bibliography with different procedures, which they can use depending on the availability of materials in the laboratory. Once extracted, the students will characterize the extract by UV/VIS spectroscopy, and will use the absorbance as the additive property.
The importance of the additive properties is discussed in depth, demonstrating how it can be operated algebraically between different properties, as long as the condition of their linear variation with concentration is met. The concept of “specific extinction coefficient” is also introduced, when the exact molecular weight of the substance is unknown. Once the extracts have been obtained, they are stored at -20 °C as 100 µL aliquots for use throughout the semester.
An additional depth can be obtained from the use of the refractive index as an additive property for the measurement of the concentration of urea, a denaturing chemical agent, which will be used later in LP5. In this case, students will be able to discuss the physical meaning of the ordinate at the origin in the measurement (corresponding to pure water) and how this ordinate at the origin is set up in 0 depending on the instrument capability.
Students will search the Protein Data Bank (https://www.rcsb.org/) for the code that best approximates their protein (LP2). From the three-dimensional structure, students conclude about the main forces that maintain the native structure of the protein. They will also be able to obtain the value of the molecular weight of the different existing subunits of the CPC (known as α and β), and based on the specific extinction coefficient value obtained in LP1 and the data found in the literature [2], they will be able to conclude about of the quaternary structure of the protein.
Kinetic studies of the chemically (LP3) and thermally (LP4) induced unfolding process will allow studying the temporal profiles of the concentration of the native form of CPC until it reaches equilibrium. Chemical denaturing agents are used to unfold and alter protein structure, by disrupting water interactions and promoting hydrophobic protein and peptide solubilization. These agents mainly include urea and guanidine hydrochloride, and the students should suggest the appropriate chemical agent for the study [3], as well as the appropriate range of temperatures for the study of thermal denaturation. Likewise, they should develop the protocols for the study, based on the range of concentrations for which absorbance is an additive property. From the profile concentration vs. time, students must justify the choice of the appropriate data treatment method for the study, i.e. initial velocity method or integral method. In addition to the kinetic constants for each condition (in this case, as for an opposite mechanism, they calculate the sum ($k_1$+$k_{-1}$) value), both practices will allow knowing the time necessary to reach the equilibrium conditions necessary to carry out the following practices. Additionally, from the experimental data obtained in LP4 at different temperatures, students will be able to evaluate the applicability of the Arrhenius and Eyring models [4].
The chemical induction of the denaturation process (LP5) will be carried out from the experimental conditions obtained in LP3. Here the key concept to introduce is the ability to study a given process, in this case, the equilibrium *native protein* ↔ *denatured protein* from the effect of the concentration of the denaturing agent. Students will find it necessary to apply two different models: a molecular model called the “thermodynamic model of denaturation”, which considers the existence of two possible states for the protein, native and denatured, without detecting intermediate forms. On the other hand, the effect of the concentration of the denaturing agent on the Gibbs energy of the process is based on the mathematical model Linear Extrapolation Method (LEM), which has no molecular interpretation, from which the standard Gibbs energy for the process ($\Delta_{unf}G^o$) can be calculated by extrapolation a null chemical agent concentration.
In the case of LP6, the measurements are made directly using controlled-temperature baths. The equilibrium constants calculated will be analyzed to determine the applicability of the van't Hoff model. Students must select the temperature range for which this model is fulfilled, and from their molecular interpretation, calculate $\Delta_{unf}H^o$ and $\Delta_{unf}S^o$. From these values, students will calculate $\Delta_{unf}G^o$ and compare that value with the one obtained in LP5. Additional properties can also be calculated, as $\Delta_{unf}C_p{}^o$ and $T_m$, the transition temperature of unfolding.
In both LP5 and LP6, students calculate the equilibrium constants under different conditions of temperature and chemical agent, which, for the protein unfolding thermodynamic model, corresponds to the quotient ($k_1/k_{-1}$), so they can combine these data with those obtained in LP3 and LP4 to calculate the individual constants $k_1$ and $k_{-1}$.

From the values of $\Delta_{unf}H^{o}$, $\Delta_{unf}C_p{}^{o}$ and $T_m$ obtained in LP6, students will model the response of a thermogram obtained by differential scanning calorimetry (DSC), where they will locate its important characteristics [5]. It is a computational practice (LP7), using Excel spreadsheets, and that will allow an instance of integrating discussion.

Further insight can be achieved by introducing the concept of Gibbs energy of transfer, which considers the energy involved in the transfer of a molecule from water to a hydrophobic phase like 1-octanol (LP8). When the molecule in question is an amino acid or a peptide, the spontaneity of the process is linked to the preference for the location of that molecule inside the protein or on the surface. For this practice, students must select amino acids or peptides that are of interest to them, from the visualization of the PDB files, and look for the appropriate analytical technique to make the experimental measurements. It is about the determination of an equilibrium constant (and from it, the associated Gibbs energy), so it will be a practice totally open to the proposal of the students, and whose realization will depend on the existence of the necessary materials at the laboratory.

## 3. Learning goals

Various aspects of physical chemistry are envisioned as learning goals by the American Chemical Society (ACS) [6] and the American Society of Biology and Molecular Biology (ASBMB) [7]. Table 1 indicates the conceptual topics that the proposed course can meet.

Table 1. Conceptual topics suggested by ACS and ASBMB and covered in the proposed course

| **Conceptual topic** | **Laboratory practices** | **ASBMB / ACS** |
|---|---|---|
| Thermodynamics and equilibria | LP5, LP6, LP7, LP8 | ACS - Physical Chemistry |
| Chemical kinetics | LP3, LP4 | ACS - Physical Chemistry |
| Interdisciplinary applications | LP1, LP2 | ACS - Physical Chemistry |
| Biological Structures and Interactions | LP1, LP2 | ACS - Biochemistry |
| Biological Equilibria and Thermodynamics | LP5, LP6, LP7, LP8 | ACS - Biochemistry |
| Macromolecular structure and function | LP2 | ASBMB |

For physical chemistry courses, the ACS strongly recommends including examples of current scientific interest, making connections to others areas in chemistry, and studying interdisciplinary applications. A complete experimental course based on biochemical macromolecules is adequate to illustrate core concepts in physical chemistry.

## 4. Perspectives and conclusions

Although this course has been developed based on the C-phycocyanin protein, its extension in the format indicated for other proteins such or for self-assembling systems such as DNA is possible. In addition to allowing the inclusion of biochemical systems in the basic studies of physical chemistry, it is possible to coordinate the course with other courses that are carried out in the same semester, or in the preceding and subsequent semesters.

After 15 years in which this course has been progressively implemented, we have noticed improvements in the preparation of reports, and in the ability of students to integrate the different concepts, an aspect that is benefited by the use of the results of one practice for others. The course is of very low cost, since it is microscaled in its entirety, and if there is enough equipment (spectrophotometers, heating plates, etc.) it is possible to tend to individual experimentation. At the same time, due to microscale, it is possible to carry out the measurements in microwell plates [8], which allows making the necessary repetitions for each measurement and carrying out the data processing with the evaluation of experimental errors.

Finally, it should be noted that students must make a comprehensive report, which forces them to consider the results of all practices to make a general evaluation of the system under study, which facilitates the ability to integrate knowledge. Likewise, students can make their oral presentations at different events (intra-institutional, congresses) or, depending on their findings, publish their results.

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