New Perspectives in Science Education
International Conference
NEW PERSPECTIVES in SCIENCE EDUCATION

# Design of Inquiry-based Laboratory Projects for an Active Learning of (Bio)Chemistry, Focused on Problem Solving in a Professional Setting

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## Abstract

*Being aware that a change was needed in the style of laboratory teaching at our university our Educational Innovation Group TR4BIOCHEM (PIE22-067) is interested in the design and implementation of new inquiry-based laboratory activities, applicable to different subjects of the last courses of the degrees of the Faculty of Sciences. In this way, the learning process of disciplines such as biochemistry and analytical chemistry is transformed through a competency-based approach, making students to get more actively involved in their learning process, with the instructor as a mere "facilitator". Thus, many of these students have to face for the first-time issues that are common in a professional setting, such as the acquisition of reagents and materials, the assessment of the necessary instruments and equipment, the adaptation and scaling of experimental protocols, and the analysis of costs and operational feasibility, among others. This new approach motivates the students' interest, who being in the final phase of their studies, are particularly concerned about their upcoming incorporation into an increasingly demanding job market. This communication will present our experience from the last years at University of Malaga in the design and implementation of new teaching resources in which the hands-on laboratory work is just a part of a more complete sequence of learning activities.*

**Keywords:** *Problem-based learning, Laboratory experiments, Laboratory instruction, Hands-on learning, Bioanalytical chemistry, Undergraduate*

## 1. Changing the style of laboratory teaching towards inquiry-based laboratory projects

Paradoxically, laboratory practices in science laboratories at the university level largely ignore the principles of the scientific method. Often considered as a mere support to theoretical classes, their main objective is the development of certain technical skills and a very superficial applied knowledge of the subject by students. In addition, the much criticized "cookbook" style of expository instruction, is still highly widespread in the practical teaching laboratories of many universities, probably because it maximizes the number of students who can perform the activity, minimizing cost, time invested and instructor involvement [1].

In contrast to these classical approaches, inquiry-based teaching arises as a way to reformulate the work that the students carry out in the laboratories, moving toward a more cross-disciplinary learning. Inquiry-based laboratory projects make students, as scientists do, formulate questions, discuss protocols, take and analyse data, and draw conclusions [2].

Many Spanish universities develop educational innovation projects in order to improve their teaching-learning processes. University of Malaga (UMA) is not an exception, and within these programs our Educational Innovation Group TR4BIOCHEM (PIE22-067) has been working for several years in the implementation of new inquiry-based biochemistry laboratory experiments, focused to last course-chemistry and biochemistry undergraduate students. Three of these approaches are presented in the next sections of this chapter.

## 2. Transforming a classical laboratory practice of enzymatic analysis into a more ambitious learning experience

The enzymatic determination of glucose by means of the coupled reactions of glucose oxidase and peroxidase [3] is an affordable classical laboratory practice, carried out by many science university students worldwide to learn how enzymes can be used as analytical chemistry tools (Figure 1).

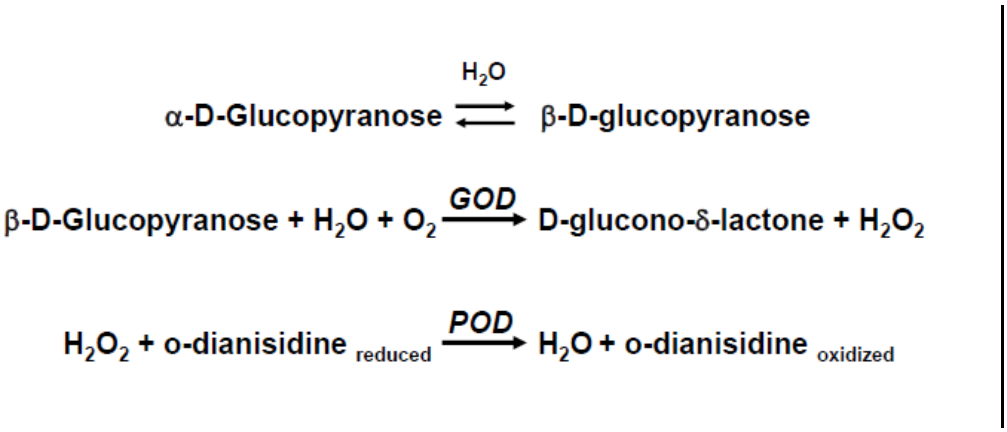


*Figure 1. Scheme of the enzymatic reactions used for the determination of glucose*
*GOD= Glucose oxidase; POD = Peroxidase*

This classical "cookbook" laboratory practice has been used for decades to teach enzymatic analysis to chemistry, biology and biochemistry undergraduate students at the UMA. According to the students' results and inspired by the commentaries from teachers and students over many years, we decided to transform this practical experiment into a more complete learning experience in which students could take a more active role. On one hand, the choice of carbonated beverages as problem solutions helps to raise the interest of students in knowing the glucose content of those beverages they consume. On the other hand, this experience is now presented as a way to compare different analytical methods, due to the kinetic characteristic of the glucose oxidase-peroxidase coupled enzymes system.

The kinetic constants of glucose oxidase allow the mentioned enzymatic reactions to be used in two different enzymatic analysis methods, the end-point and the kinetic one. The fact that they can be carried out in a single reaction mixture, shortens and decreases the costs of the experimental work, so that both methods can be performed by students in a 3-4 hours session. This is especially interesting from a biochemical and a pedagogical point of view, because it allows a critical discussion of the advantages and drawbacks of each method, providing students with some training in choosing which is the most suitable method, according to specific purposes. As mentioned, the choice of carbonated soft-drinks as problem solutions increases the interest of the students in the analyses, since many of them are regular consumers of these drinks and are concerned about the impact of their sugar-content on their health. In addition, comparison of the results obtained with coloured (cola refreshments) and uncoloured (tonic water) sodas is used to illustrate how the appearance of interferences may do a given analytical method unsuitable for a specific practical problem, providing students with some training in how to choose the best solution to a given experimental problem. Finally, the learning activities are designed to make students search for information in the literature, and to do calculation in the laboratory.

The learning activities of the practical project (fully described in [4]), include:

1. Introductory lecture in the classroom where the enzymatic analysis theoretical principles are explained by the teacher.
2. Students homework, aimed to make students think in what they will do in the laboratory and how they will do it, by answering some specific questions regarding the "tricky points" along the protocol. It also includes some numerical problems, specially buffers and enzymes solution calculations, in order to improve the students' numeric abilities. This homework is corrected by the instructor and returned before the laboratory session, so that students can use it as the experimental protocol.
3. Laboratory session. The previous learning activities help to minimize the laboratory time and resources, sometimes scarce and highly demanded at our institution. In this way, students can directly go through the different steps of the practical protocol in a short period of time (3-4 hours), collecting the experimental results they will use to elaborate the final report.
4. Final report, where students have to critically evaluate the convenience of one protocol against the other in terms of sensitivity, selectivity, repeatability, accuracy, interferences and time consumed. They need to search in the available bibliography in order to propose the use of alternative methods, and discuss their pros and cons for the measure of glucose concentrations in soft-drinks and other samples.

The above described new learning experience is now firmly established at our university, being used every year to teach enzymatic analysis to undergraduate Biochemistry (3rd year) and Chemistry (4th

year). The achievement of the Learning Goals by those students is very satisfactory. This has been evaluated by using an assessment test, composed by some multiple choice and short open-questions, before the introductory classroom lesson, and repeating this test after students have delivered their full report [4].
The students' perception of this laboratory protocol, has been evaluated by means of a Likert questionnaire, complemented with some other open answered questions. Our results indicate that this practical project, as a whole, is positively perceived by both, future biochemists and chemists, who have suggested that other practical lessons could be readapted according to this learning procedure.

## 3. Comparing two different educational approaches to the experimental teaching of a luminometric-based analytical method

Even though luminometry, the process of measuring light, is becoming increasingly used in many experimental and health sciences laboratories, the practical laboratory experiences that could be used to illustrate the applications of this technique are still scarce. Trying to overcome this shortage in the formation of our chemistry and biochemistry undergraduate students, we developed a new practical experiment based in the measurement of the light emitted in the enzymatic reaction catalysed by luciferase (Figure 2).
The bioluminescence phenomenon is easily recognized in some animals, such as fireflies, which emit light for the recognition and attraction of their partner in mating. This light is the result of a chemical reaction catalysed by the enzyme luciferase (EC 1.13.12.7), which requires the presence of the luciferin substrate and ATP as a cofactor.

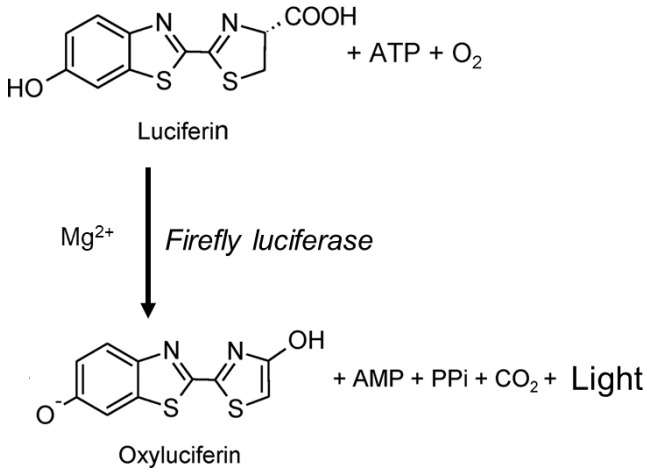


*Figure 2. Luciferase-catalyzed enzymatic reaction*

Since ATP is present in all life forms, its detection in drinking water is indicative of the presence of microbial contaminations. The correlation between the ATP concentration, measured by the luciferase reaction, and bacterial content is the basis for the development of some rapid methods to detect microbial contamination in drinking or stored water, skipping the long delays required by traditional microbiological methods [5]. The luminometric measurement of ATP for the detection of bacterial contamination in water is the basis of the new laboratory experiment implemented at the UMA with the objective of teaching to chemistry and biochemistry undergraduate students the use of bioluminescence in analytical chemistry. We have successfully carried it out in two different formats, a short protocol and a full PBL experience. The contextualization derived from the use of drinking water as problem samples greatly increased the students' interest.
Our results, described in [6] show that detection of microbial contamination in water by measuring ATP concentration with luciferase is a useful tool for teaching the use of bioluminescence to science undergraduate students. Whether as a short protocol carried out in a single laboratory session, or as a long PBL experience, the students' achievement of the learning objectives was very satisfactory. These learning goals included the correct use of the scientific literature, the application of luminometry to a real-world problem, the use of a luminometer, and the familiarization of the principles and applications of bioluminescence. Those objectives are related to some foundational scientific competencies that characterize the *Process of Science* included in the *Bioskills Guide* [7], such as evaluation and use of scientific information, critical thinking or data interpretation. The core

competency *Quantitative Reasoning*, including the performance of basic calculations, drawing of graphs and data presentation was also worked by students who had to set a calibration curve, establish the application range of a method, and interpolate their experimental data in order to get the ATP concentration of several water samples.

In addition, the learning goals for the PBL approach, included others derived from the more active role played by students in their learning process, aimed to make them undertake authentic real-world tasks, similar to those that they would find in their professional future. Among others, they developed some competencies for interacting and communicating the research results related to the *Communication and Collaboration* core competency, since they had to work in groups of 4-5 components, present their results to a diverse audience, write a scientific journal-quality report and summarize their conclusions in an “executive report” that could be understood by non-experts. With respect to skills of relevance to their future job performance, many of these students faced for the first-time common issues for a professional, such as the acquisition of reagents and materials, the assessment of the necessary instruments and equipment, the adaptation and scaling of experimental protocols, or the analysis of costs and operational feasibility, among others [6].

In conclusion, the luminometric measurement of the ATP concentration in water is a useful tool that can be used to work on different competencies, depending on the format employed, in terms of the student’s engagement. Between the two extreme examples here mentioned, either as a short protocol or as a PBL, different intermediate options could be used by educators to adapt this laboratory experiment to their course schedule and to meet the learning objectives to be achieved.

## 4. Developing a new CURE aimed to illustrate the early stages of drug discovery

The process of drug discovery is one of the topics covered within the subject "Pharmacological Biochemistry", in the 4th year-biochemistry grade at the UMA. Focused on the *blind screening* of enzyme inhibitors, as one of the most widely used strategies in pharmacology for the discovery of new drugs, this topic includes aspects that refer to both the *in vitro* identification of new inhibitors of a target enzyme by enzymatic analysis and *in silico* studies.

As a practical tool to teach this topic, we are currently working on the development of a new course-based undergraduate research experience (CURE), intended to be a hands-on introduction to the early stages of the drug discovery process [8]. After a theoretical introduction in the classroom by the instructor on the bases of the drug discovery process and the strategies for a *blind screening* of enzyme inhibitors, students work in groups of 4-5, facing a situation that resembles a real scenario found in the area of medicinal chemistry. Under the guidance of the responsible teacher, in the role of facilitator, the groups work as independent pharmaceutical laboratories that receive a letter stating the objective "To find new acetylcholinesterase inhibitors as drug candidates for the treatment of Alzheimer's disease". Guided by this challenging driving question, students are involved in a meaningful learning process focused on proposing solutions and carrying them out in a practical way, both *in vitro* and *in silico*.

After searching for information in the bibliographic databases, students propose solutions to the driving question and design protocols to carry them out. The experimental development includes a bibliographic search on the Alzheimer's disease and current therapies, the use of acetylcholinesterase inhibitors in the treatment of patients, and the possible enzymatic assays that could be used for the identification of new inhibitors of this enzyme. They select the Ellman’s procedure, based on the acetylthiocholine hydrolysis, reaction of the resulting thiocholine with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and formation of the yellow 5-thio-2-nitrobenzoic acid (TNB) anion [9] (Figure 3). With respect to the enzyme used in this *in vitro* screening, cost and commercial availability considerations make *T. californica* acetylcholinesterase the best choice.

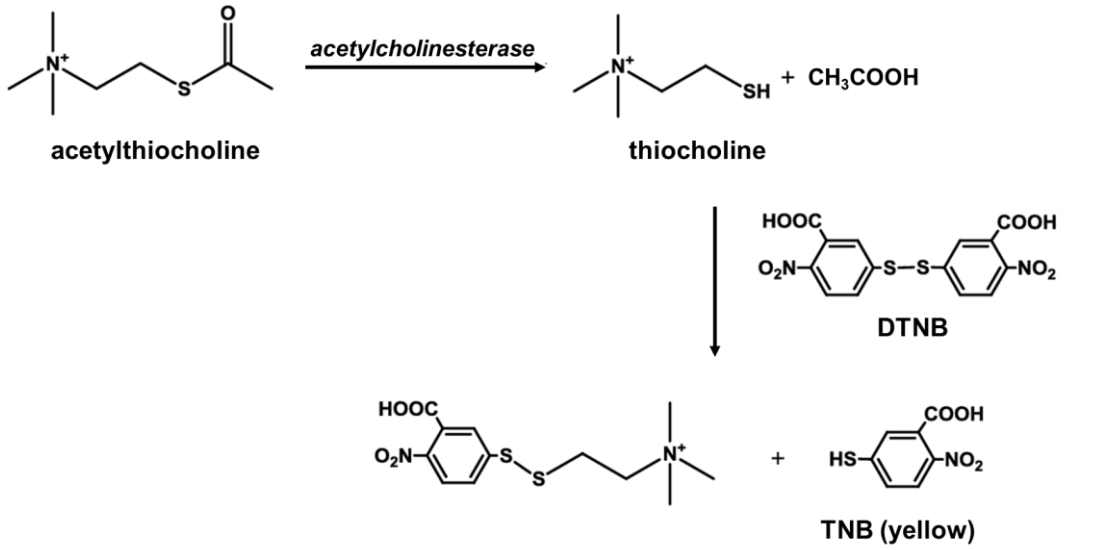


*Figure 3. Ellman's method, used for the in vitro detection of acetylcholinesterase inhibitors*

In a first report, groups summarize their findings, explaining what reagents and instrumentation are needed to carry out the *in vitro* screening for inhibitors of this enzyme. During the laboratory activities phase, students design the experimental protocol, make calculations of how the reagents are prepared, optimize the experimental protocols in the laboratory and finally perform the screening test. As a result of this process they identify an inhibitor of the enzymatic activity of the acetylcholinesterase among a group of unlabelled compounds provided by the instructor.

Once the *in vitro* part of this CURE is performed, groups begin with the *in silico* studies. In an introductory lecture in the classroom, the rational of the drug design using computational techniques is explained by the teacher. This helps students to get familiar with the informatics tools of statistical computing language R (PyMol, a molecular visualization software, and Bio3D, a software for the modelling and analysis of the structures). Then groups, working autonomously, proceed in the accomplishment of the following tasks [8]:

1. Workflow and scheme of the working hypothesis.
2. Modelling and representation of the acetylcholine binding site in the acetylcholinesterase from *T. californica.*
3. Modelling and representation of the binding site in *T. californica* acetylcholinesterase of the inhibitor they have found in the *in vitro* blind screening.
4. Modelling and representation of the structural alignment of *T. californica* acetylcholinesterase, used in the *in vitro* blind screening, and human acetylcholinesterase, which is the real target of the new drug.
5. Prediction of the inhibitory activity on the human enzyme of the potential new drug, based on the interaction of the inhibitor with *H. sapiens* acetylcholinesterase.

At the end of the CURE, students prepare both a final report and an oral presentation about the different stages of the project and the results obtained.

Although we are still working in the optimization of this CURE, our results indicate that it can contribute to make students develop skills related to the treatment of information and digital competence by using open-source big data applications, the learning to learn competence, or the competence in autonomy and personal initiative. Many of these skills have an intrinsic relationship with the future development of students as professionals in technical, scientific or academic positions. Preliminary results indicate that the implementation of this experience is very satisfactory, in terms of academic performance and students' perception.

## 5. Final conclusions

Our experience indicates that although inquiry-based approaches usually require more effort and time to develop, they are usually perceived very positively by students, who become more actively involved in their learning process and find this type of research experience very rewarding. In addition, these didactic resources are characterized by the promotion of some integrated inquiry tasks which require the use of transversal knowledge that, without being strictly in the field of biochemistry, result in a more integrated and complete training of the students. The choice of topics of study that are related to

aspects of real life or the daily professional practice, has increased the students' interest and motivation. As for teachers, they have also found these experiences to be demanding, but satisfactory in terms of the students' achievement.

Students appreciate having the opportunity to apply concepts in a real-world context, and consider that these laboratory experiments can prepare them to face a future professional scenario. In this sense, gamification provides a plus of interest to students, who gain confidence in their capability to apply their knowledge to solve problems in a professional setting. At a time when graduate employability is a key issue for higher education, as new graduates face a highly competitive and rapidly changing employment landscape, a shift from more traditional teaching systems to other thought-provoking learning approaches may help to promote those skills and attributes, most valued by employers.

## 6. Acknowledgements

This work was supported by the University of Malaga funds granted to the educational innovation projects PIE17-065, PIE19-086, & PIE22-067 (Educational Innovation Group TR4BIOCHEM). The commentaries and help of the University of Malaga biochemist and chemist undergraduate students in performing experiments and collecting data is greatly appreciated.

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