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Problems students experience with inquiry processes in the study of enzyme kinetics

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ABSTRACT
This case study describes a classroom-based questionnaire that was carried out with a group of 36 high school students (17–18 years old) in Catalonia. The aim was to examine the usefulness of questionnaires focused on scientific inquiry, both to evaluate students' inquiry abilities and for their potential as tools to improve the understanding of these processes. The questionnaire refers to procedural understanding within the field of enzyme kinetics. Rubrics for scoring the questionnaire were developed to standardise data analysis. Results showed ambiguous identification of the inquiry question and difficulties in formulating accurate hypotheses and identifying the independent variable. The greatest difficulties appeared in the control variables and the methodology design; misunderstandings related to the underlying scientific concepts were also identified. Questionnaires like the one used in this case study can be useful tools for formative assessment and allow fundamental aspects of scientific inquiry processes to be tackled in less time because they do not involve hands-on activities but instead combine scientific practices with core disciplinary ideas.

KEYWORDS
Procedural knowledge; inquiry abilities; assessment

Introduction and theoretical framework

Scientific inquiry in science education

Teaching and learning inquiry processes are fundamental in science education. A report by the Nuffield Foundation states that, 'The primary goal of science education across the EU should be to educate students both about the major explanations of the material world that science offers and about the way science works'. (Osborne and Dillon 2008, 8). Therefore, not only conceptual knowledge but also procedural knowledge has to be considered. In the PISA 2015 Framework, scientific literacy is described as: 'The ability to explain phenomena scientifically, evaluate and design scientific enquiry, and interpret data and evidence scientifically'. (OECD 2013, 7). From this perspective, the inquiry competence primarily refers to 'evaluate and design scientific inquiry' and to 'analyze and interpret data and draw appropriate conclusions' and it includes several inquiry abilities: defining scientific research questions, formulating hypotheses, applying appropriate scientific knowledge, proposing a way of exploring a given question scientifically, collecting data, analysing and interpreting data, and drawing appropriate conclusions (e.g. Ben David and Zohar 2009; Nowak et al. 2013).

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Despite the importance that current approaches to science education give to inquiry as an instructional outcome, research shows that difficulties related to the inquiry competence are not unusual among high school students. For example Schwichow et al. (2016) found that planning experiments is the most difficult process for students, and interpreting outcomes is easier than identifying controlled experiments. Other studies examine students' difficulties and show the positive effects of explicit knowledge about thinking strategies in defining research questions, formulating research hypotheses and controlling variables (Ben David and Zohar 2009), as well as increase of students' abilities to identify experimental variables when incorporating experimental case studies into a laboratory curriculum using an online course management system (Grunwald and Hartman 2010), or learners' progress to refine asking questions as an interactive and iterative process (Lombard and Schneider 2013).

Assessment of inquiry competence

The literature informs referents on how to deal with assessing the inquiry competence from two different standpoints: one referring more to the epistemological facet and the other to the procedural facet. One example of the former is the Views About Scientific Inquiry questionnaire (Lederman et al. 2014), while as an example of the second kind, Tamir, Nussinovitz, and Friedler (1982) proposed a tool called the PTAI (Practical Test Assessment Inventory) to assess student's ability to implement practical activities and to standardise and increase the reliability of the evaluation results. One integrative proposal that overcomes this split vision can be found in the PISA 2015 Framework (OECD 2013), which aims to evaluate not only understanding about scientific inquiry but also the skills that characterise scientific competence, and in particular the inquiry competence.

For the purposes of this case study, we have situated ourselves within this framework and designed a rubric (Appendix 2) that allows us to assess inquiry skills and examine students' difficulties in assessing procedural knowledge, not epistemic knowledge. Thus, the research question was: *What difficulties and misunderstandings do students show in inquiry processes in the study of enzyme kinetics?*

Methodology

Context

This study was carried out in a secondary school with a medium socioeconomic status and average scores in Catalonia. Data were collected in the 2014–2015 academic year from a group of 36 high school students aged 17–18. The curricular context was the study of enzymes and factors that influence enzyme activity, such as substrate concentration and the presence of inhibitors.

Data collection

The activity used a paper-and-pencil open-response questionnaire (Appendix 1) that uses the application of knowledge on enzyme kinetics in the context of the composition of medicines. Students are often told about the development of bacteria's resistance to antibiotics and the challenge this poses to fighting off infections. Following PISA 2015 Framework (OECD 2013), we can consider this questionnaire useful for assessing most of the inquiry abilities described in it. Table 1 relates eight questions in this questionnaire to the inquiry abilities being assessed when adapting the PISA criteria. Students wrote their answers during two half-hour sessions:

- First session: Individual activity that asked students to identify inquiry procedures in a given study. Students were asked to identify the research question, formulate the hypothesis, analyse the results and argue the conclusions (A&C). Students were also asked to identify the independent variable (IIV), the dependent variable (IDV) and the control variables (ICV), and to indicate which group is the control group (questions 1 to 7 on the questionnaire).

Table 1. Questions in questionnaire linked to scientific abilities.

Scientific competencies	Skills to describe scientific competencies (OECD 2013)	Questions
Evaluate and design scientific inquiry	Identify the question explored in a given scientific study	3
	Propose a way of exploring a given question scientifically	8
	Identifying variables including dependent, independent and control variables	5
	Common ways of abstracting and representing data using tables and graphs	1
	Describe and evaluate a range of ways that scientists use to ensure the reliability of data and the objectivity and generalisability of explanations. Consider importance of using replica and control groups and experimental investigations	6, 7
Interpret data scientifically	Analyse and interpret data and evidence scientifically and draw appropriate conclusions	2
Explain phenomena scientifically	Recall and apply appropriate scientific knowledge	2
	Offer explanatory hypotheses	4

- Second session: In a following activity, students working in teams of three to four had to write down their description of the methodological design proposal to obtain the data provided in the questionnaire (question 8 on the questionnaire).

Data analysis

In order to codify the students' responses, two rubrics were designed. Rubric 1 (Appendix 2), called QPTAI, is based on the PTAI by Tamir, Nussinovitz, and Friedler (1982) but adapted to this study. It was used to codify the students' responses on the individual activity (questions 1 to 7). It includes 6 categories: identification of the research question; formulation of the hypothesis (considering its formulation stated as a deduction, as suggested by Friedler and Tamir 1990; and referring to the model, as suggested by Windschitl, Thompson, and Braaten 2008); identification of the independent variable (IV), dependent variable (DV) and control variables; and data analysis and drawing well-founded conclusions. Each category has five hierarchical codes with an increasing level of ability and provides examples of students' answers. Each code is scored from 0 to 4, allowing a numerical score to be calculated when using the rubric. Preliminary assessments helped to refine the rubric and ground it in the data.

Rubric 2 was designed (Table 2) to codify the description of the methodological design proposals used to obtain the data (question 8). A combination of a partially inductive (open coding) and partially deductive (theoretically guided coding) approach was used for the emergence, definition and systematic application of codes and categories. Unlike Rubric 1, students' answers on Rubric 2 were coded instead of scored.

Table 2. Rubric 2. Analysis of partial processes and errors in methodology.

Partial processes considered in the methodology	11 teams
Two 6-tube groups are needed, one with clavulanic acid and the other without it	3
Tubes must contain penicillinase enzyme	1
Content of each tube is specified, which must include a certain penicillin concentration and always the same enzyme concentration	0
Controlled variables are detailed: enzyme concentration, pH, temperature	0
Reference is made to the control group (without clavulanic acid), either explicitly or implicitly	9
A replicate is proposed, with two 12-tube groups	3
Errors in the methodological proposals	
Using a number of tubes unrelated to the data collected.	5
Introducing bacteria in the tube	4
Generic reference to control variables, mentioning irrelevant ones (light, humidity)	2

Students' questionnaires were analysed individually by three people (the first author and two high school teachers from outside this research), and in the event of a discrepancy, the analysis was discussed in order to reach a consensus.

Results and discussion

Scores showed that students had difficulties in understanding the inquiry procedures (Figure 1). These results were coherent with previous research and showed that difficulties with inquiry abilities start from the initial process of identifying questions, and that planning experiments is the most difficult process for students, while interpreting outcomes is the easiest (Schwichow et al. 2016). Appendix 2 provides examples of students' answers and relates them to the Rubric 1 assessment codes. For example, if questions were formulated ambiguously (Lombard and Schneider 2013) or were vague, this means that students proposed questions related to the research, although only a very limited number referred to the concept of enzyme inhibitor. A common error was confusion between penicillin (the substrate) and penicillinase (the enzyme). For example *How does clavulanic acid act on penicillin? What is the difference between bacteria-produced penicillin and penicillin with clavulanic acid*? Students also formulate questions that cannot be answered with the data provided. Only a few students ($n = 6$) identified research problem and specified appropriate questions that suggest the methodological design.

Regarding the hypotheses, only 8 students formulated highly accurate ones as deductions, as suggested by Tamir, Nussinovitz, and Friedler (1982), with a description based on scientific concepts, as proposed by Windschitl, Thompson, and Braaten (2008). More common were answers that made predictions instead of formulating a hypothesis. In some cases, even though they were formulated deductively, the hypotheses showed conceptual misunderstandings, such as optimal pH and the inhibitor (*If we add clavulanic acid, the pH will change and, therefore, enzyme activity will also change*), while in other cases, the students were not even able to identify the results of the experiment (*When adding clavulanic acid to penicillin, hydrolisation speeds up; if the penicillin hydrolisation rate increases, then clavulanic acid has an enzyme effect*).

The best results were obtained in identifying the DV ($n = 28$ scored 3 or 4). This could be attributed to the fact that most students answered this question by copying the y-coordinate in the legend 'penicillinase enzyme activity', which is traditionally a DV, but without specifying what is being determined, which leads us to doubts as to whether they actually understood it or instead simply copied it mechanically.

Regarding identification of the IV, one-third of the students ($n = 12$) were unable to identify that *the presence or absence of clavulanic acid* is the independent variable. Answers such as *clavulanic acid concentration* or *amount of clavulanic acid* seemed to indicate that students did not understand that the inhibitor concentration is not modified, but that instead the IV is its presence or absence. Other answers showed that they did not differentiate between the two experimental processes and answered in reference to the one represented on the initial chart: *penicillin concentration*.

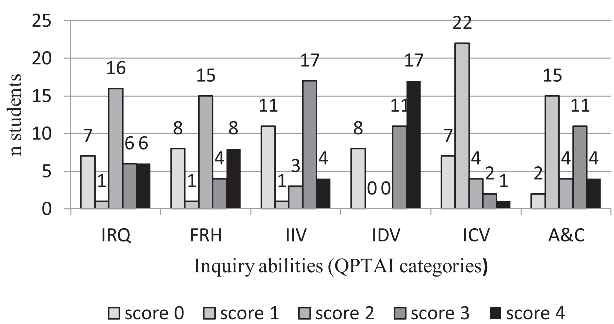

Figure 1. Individual activity results ($N = 36$).

The greatest difficulties in the individual activity appeared in the control variables. Three misunderstandings seem to be involved: the concept of control variables, the scientific ideas related to enzyme kinetics and biochemistry research methodologies. It is significant that some students mistook the control variables for the dependent variable or the control group: they confused what is controlled with what is measured. Seven students believed that the scientists were working with bacterial cultures.

Conceptual misunderstandings were observed in the data analysis and the argumentation of conclusions: one-third of the students offered an acceptable or good data analysis, although the complexity involved and the concepts they had to deal with hindered them from giving accurate answers, which were the exception (only 4 students), using, for example, the concept of inhibitor.

The results regarding the methodological design proposals formulated by the working teams are shown in Table 2, with the data analysis rubric. It is significant that only one of the eleven teams said that the tubes had to contain penicillinase enzyme. The other ten teams made no reference to this enzyme, although four teams suggested using 'bacterial culture'. This error, as discussed above, was also detected in the control variables. In fact, confusion in the practical processes even led one team to refer to inhibition zones on bacterial cultures in a solid medium.

Conclusions and educational implications

Even though inquiry has been part of the science curriculum for many years, students still struggle with procedural skills. In light of these results, we could assume that the inclusion of scientific practices is not a widespread phenomenon in classrooms, or that they are limited to activities with a low level of inquiry. The difficulties can also explain the fact that science content usually predominates in secondary school textbooks, which place little emphasis on scientific methodology, and textbooks determine what is taught in the majority of classrooms (Binns and Bell 2015). Following research should analyse the presence and characteristics of inquiry-based activities.

The usefulness of questionnaires, such as the one in this case study, for assessing most of the inquiry abilities described in the PISA 2015 Framework (OECD 2013) has been confirmed. These kinds of questionnaires can also be an opportunity to promote learning of the scientific inquiry processes in a way that is less time-consuming to implement than hands-on activities, as Wilke and Straits (2005) argue. This does not mean that hands-on activities are not essential to learning, however. What is more, these questionnaires accompanied by rubrics like the ones we used in this study to codify student answers could be appropriate instruments to promote formative assessment and better conceptual and procedural learning. Rubrics make it possible to obtain standardised quantitative data from the analysis of qualitative data and can be used by teachers to evaluate students and examine in which processes they show the most difficulties and their most common misunderstandings.

In light of our results, activities that address the learning of scientific practices linked to conceptual knowledge seem to be crucial. Questions that are ambiguously worded and confusion between the substrate, enzyme and inhibitor lead us to conclude that the students did not really understand the phenomenon. Inquiry skills depend on students' knowledge (Schwichow et al. 2016) and are a prerequisite to formulating research questions (Lederman et al. 2014; Lombard and Schneider 2013). Deficiencies in inquiry skills have been shown to be one of the underlying causes of the difficulties identified. Traditionally, in Catalonia, students have been trained to answer questionnaires, such as the one presented in this study, without necessarily relating them to a teaching unit, with the assumption that conceptual and procedural knowledge can be disassociated from each other. These questionnaires are often offered exclusively in the first section in textbooks, when the scientific methods are explained, but with a low level of conceptual demand. The challenge for students usually consists of applying the control of variables strategy (CVS) in different situations, and, for example, identifying the question that can be researched, the IV or the DV independent of the scientific content. In light of these results, it seems that this might encourage students to answer these questionnaires mechanically, without relating their answers to the conceptual content that underlies scientific inquiry. We believe that an approach that separates conceptual knowledge and procedural knowledge should be avoided.

Thus, when considering that 'a repetitive practice of CVS within different contexts might be especially effective for the development of robust CVS skills.' (Schwichow et al. 2016, 19), we believe that it is important to bear in mind that the conceptual content that underlies these contexts is meaningful and allows content knowledge to be promoted and transferred. It is worthwhile to repeat similar practices in each teaching unit to promote the co-development of inquiry skills and domain knowledge. In this vein, Zimmerman (2007) comments that,

> A number of studies that followed students through repeated cycles of inquiry and all phases of the investigation showed the co-development of reasoning strategies and domain knowledge. Either acquisition alone will not account for the development of scientific thinking. (207)

Therefore, these questionnaires may act as a tool to foster reflection processes related to the conceptual knowledge behind the questions posed. However, more research is needed to understand how questionnaires focused on scientific inquiry processes could promote both students' inquiry skill and conceptual knowledge when used in formative assessments. We agree with Schwichow et al. (2016) that 'further research is also needed to explore the interplay between student content knowledge and inquiry strategies' (23), and we hope that studies such as this one help to improve it.

Disclosure statement

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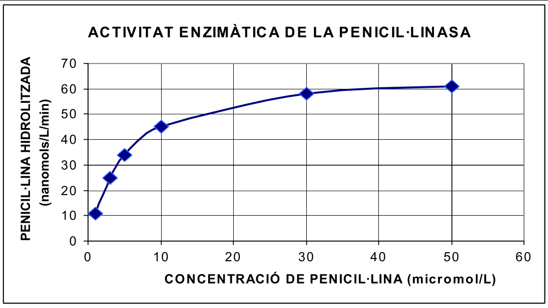
Appendix 1. Penicillin and clavulanic questionnaire

Penicillin is an antibiotic used against many bacterial infections, but some bacteria produce an enzyme called penicillinase that inactivates it. The following chart shows penicillinase enzyme activity. The table on the right gives penicillinase activity with the same penicillin concentrations, but with clavulanic acid added:



penicillin concentration (micro mole)	hydrolyzed penicillin (nanomol/min)
1	5
3	10
5	15
10	25
30	40
50	47

(1) Represent in the previous chart the enzyme activity curve in these conditions.
(2) What conclusions can be drawn from these research data? Give reasons for them. Why do you think clavulanic acid is added to many pharmaceutical products with penicillin?
(3) What question did researchers ask themselves when they carried out this research?
(4) What hypothesis did the researchers formulate? If possible, state it as a deduction.
(5) What are the independent and dependent variables in the experiment? What are the control variables?

Independent variable
Dependent variable
Control variables

(6) What can be considered the control group?
(7) Do you think researchers worked with two tubes only, one with clavulanic acid and the other without it, to get these results? Explain your answer.
(8) Describe the methodological design that made it possible to obtain these data.

Expected response

- Enzyme and substrate are available and the desired concentration can be measured
- The process must be carried out with the same enzyme concentration, varying only substrate concentration and the presence or absence of clavulanic acid
- *Conditions must be kept constant: pH, temperature, volume, penicillinase enzyme concentration*
- *Instruments are available to measure penicillin hydrolysed that results from penicillinase enzyme action*
- *From time to time, hydrolysed penicillin must be determined and rate, which is the VD, must be calculated.*
- It is not possible to work with two 6-tube groups only, because replicates must be done to confirm results.

Appendix 2. Rubric 1: QPTAI, rubric categories with *examples of students' answers*

Identify research question (IRQ)
0 Does not identify research problem or asks questions that cannot be answered.
Why does penicillin not always act? How can we make our pharmaceuticals more effective?
1 Confuses problem and hypothesis. *Maybe clavulanic will act as enzyme inhibitor.*
2 Identifies the problem only in a generic way or with conceptual errors. *How will clavulanic act on penicillin?*
3 Identifies the problem but does not specify questions to guide the design of the methodology.
4 Identifies the research problem and specifies appropriate questions that suggest the design of the methodology.
Will the presence of clavulanic acid decrease the penicillin hydrolisation reaction rate? Will it act as an inhibitor?

Formulate research hypothesis (FRH)
0 Does not formulate hypotheses, or formulates meaningless hypotheses.
1 Establishes hypotheses unrelated to the problem, or generic hypotheses. *With clavulanic acid, the pH is not optimal.*
2 Establishes ambiguous or wrongly formulated hypotheses, or simply makes predictions.
Clavulanic will impact enzyme activity. Clavulanic acid causes enzyme activity to change.
3 Establishes hypotheses in line with the problems, but they are inaccurate, not stated as a deduction or not based on scientific concepts.
4 Establishes accurate hypotheses, in line with the problem, stated as a deduction, with a description based on scientific concepts.
We know there are bacteria that produce the penicillinase enzyme, and also that there are substances that can act as inhibitors of enzyme activity (MODEL). Therefore, if clavulanic acid is a suitable inhibitor, if we add clavulanic acid (VI) to drugs with penicillin, then the penicillinase enzyme activity (DV) will be lower (or the penicillin hydrolisation reaction rate will decrease)

Identify independent variable (IVI)
0 Does not identify IV.
1 Confuses IV and DV. *The rate of antibiotic hydrolysis.*
2 Identifies IV, but also refers to other variables. *With or without clavulanic and with the same pH,*
3 Identifies IV, but only in a generic way. *Clavulanic acid.*
4 Identifies IV in a precise way. *The presence or absence of clavulanic acid.*

Identify dependent variable (IVD)
0 Does not identify DV.
1 Confuses IV and DV. *The presence or absence of clavulanic acid.*
2 Identifies DV, but also refers to other variables. *Rate of antibiotic hydrolysis and pH.*
3 Identifies DV, but only in a generic way. *Rate.*
4 Identifies DV in a precise way. *The rate of antibiotic hydrolysis.*

Control of variables other than IV (ICV)
0 Confuses control variables for control group or for dependent variable (what is controlled with what is measured).
1 Refers to meaningless variables in the research. *Light, pressure, humidity, bacterial culture medium, nutrients.*
2 Mentions control variables only in a generic way, without specifying them. *Same environmental conditions.*
3 Identifies control variables, but leaves out fundamental ones. Does not mention enzyme concentration.
4 Identifies control variables precisely with details.

Data analysis and drawing of well-founded conclusions (A&C)
0 Analysis and conclusions are not result-based or have neither analysis nor conclusions.
1 Analysis and conclusions with conceptual errors.
2 Conclusions similar to results, without data analysis.
3 Analysis and conclusions that refer to empirical results and related concepts, but incomplete.
4 Conclusions that consider empirical results and related concepts, and that show relationships between hypotheses and results.
The rate of penicillin hydrolysis is lower when clavulanic acid is present. So clavulanic acid is a penicillinase inhibitor. Clavulanic acid is added to many pharmaceutical products with penicillin because it increases the antibiotic's activity period.