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Instrumentation of Microscale Techniques for Biochemistry Teaching at FES Zaragoza, UNAM

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Abstract

Biochemistry education requires laboratory sessions where theoretical knowledge may be put on test. At the same time, there is always some risk due to exposure to toxic materials, dangerous chemicals storage and waste disposal. Compliance with new regulations to prevent environmental contamination may also constitute a real hindrance for biochemistry teaching as experimental science. Therefore, we have designed microscale techniques, in order to reduce costs as well as the negative impact of laboratory practical sessions due to risk and environmental contamination. To develop microscale techniques does not only mean to reduce equipment size and amount of the reagents that are required for the usual experiments. Microscale techniques serve particularly well as a motivating approach to experimental biochemistry teaching that produces highly motivated students at the same time that requires minor costs and decreases working time, laboratory space, amount of reagents and dangerous waste. We have demonstrated all these positive effects in biochemistry teaching and prompted the formal implementation of microscale techniques into the formal activities from the Cell and Tissue Biochemistry Laboratory I (BCT-I) from the Chemistry, Pharmacy and Biology (QFB) curricula at the National Autonomous University of Mexico (UNAM). First, we reviewed the BCT-I manual, choosing all the laboratory practices that might be microscaled. Then, we elaborated and validated all

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necessary protocols to analyse linearity, accuracy and reproducibility of the determinations, demonstrating that microscale techniques allow truthful results, comparable to full scale techniques.

Keywords
Biochemistry teaching, microscale techniques, experimental biochemistry, environment protection

1. Introduction

Since the ground-breaking discovery in 1828 demonstrating that biomolecules such as urea could be synthesised, starting with non-living elements, scientists have explored all biochemical aspects of life with increasing interest. Many mysteries of life have been revealed, showing the functioning of living beings at biochemical level. However, many more questions appear after an initial question has been answered. Biochemists have searched living processes with basis on a continuously increasing but never ending knowledge on biology, chemistry, physics and mathematics (McKee and McKee 2012). Thanks to modern technology, we enjoy now exceptional opportunities to learn every day more and to apply our knowledge to solve problems in diverse fields, such as agriculture, anthropology, pharmacy, genetics, medicine, odontology, veterinary, forensics, toxicology and others. Borders between these and many other sciences have become unclear and often arbitrary. Such big overlapping is due to distinctive physical and biological properties of the elements of life.

Subjects constituting the study program for students following the Chemistry, Pharmacy and Biology (QFB) curricula at the Faculty for Higher Education (FES), Zaragoza, UNAM gather many basic and advanced subjects on biomolecules, essential to sustain life as we know it. Forming QFB professionals is also directed to prepare capable professionals who may approach the study of life from many and diverse points of view, such as public health, ecology and all others demanding an increasing knowledge on

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functioning of life molecules and related clinical, pharmacology and technological aspects.

The Cell and Tissue Biochemistry Laboratory I (BCT-I) is organized for teaching of theoretical and practical knowledge, as well as to promote acquisition of experimental abilities. This kind of learning constitutes the first encounter for QFB students not only with biochemistry concepts but also with practical matters of diverse biology problems (Curricula QFB 2006). To turn this situation into a successful experience, students are assisted by experienced experimenters and scientists, able to solve biological problems through experimentation. To be approved at this level, learners need not only to master theoretical concepts but also to demonstrate that they are capable of performing experimental work according to the theoretical knowledge and practical abilities that they must possess at such level. Historically, science has evolved by hypothesis that must be proved with laboratory work (Molina *et al*., 2006). New hypothesis may contrast with established knowledge and testing them require experimenters with good handling of a number of laboratory techniques.

Objectives at laboratory teaching usually are: a) to illustrate theoretical knowledge, b) to teach experimental techniques and c) to promote scientific interest. No doubt, having an ample knowledge of laboratory methodologies greatly facilitates the problem-solving capacity that we want to give to our students. Confronting our students with the necessity to find adequate laboratory techniques to solve the problems that they face during their studies surely helps them to build up a good knowledge on experimental tools and the ability to use them correctly (Lacolla 2005). Additionally, during the process of learning laboratory techniques, efforts should be made to maintain a minimal risk for people as well as to reduce costs and to diminish production of chemical and biological waste. Good laboratory practices (GLP) are essential to guarantee the certainty of the results and to minimize or even avoid environmental contamination.

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2. Microscale instrumentation and validation

2.1 Antecedents

Laboratory work is usually expensive and may generate dangerous waste. Even big institutions have difficulties to satisfy the economic demands imposed by requirements of space, equipment, qualified personnel, laboratory organization and consumption of reagents, plastic and other materials. Minor institutions with shortage of economic resources are not able to provide high quality laboratory sessions and often choose to have only demonstrative sessions. Instead, we give our students the opportunity to perform the techniques by themselves, under proper supervision. Here, at our National University, we also take responsibility for laboratory work within a controlled environment, reducing to a minimal level the exposure to toxic materials and diminishing the generation of waste and environmental pollution that could be caused during our work.

2.2 Advantages of microscale techniques

To work with microscale instead of high-consuming, waste-generating techniques is a way to work in the biochemistry laboratory, using the smallest possible volumes of reagents without decreasing the quality of the experiments performed. Microscale techniques drastically reduce the amount of chemicals and laboratory materials, have lower cost and less requirement for expensive equipment. (Szafran *et al*., 1989, Singh *et al*., 1999, National Microscale Chemistry Centre, 2002). Among the obvious and environment-friendly advantages, we have:

- Minimized lab pollution.
- Lower release of toxic, carcinogenic or mutagenic products into the environment.
- Lower risk for accidents derived from fire and toxic substances (Smith *et al*., 2008).

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- Lower costs for experiment and number of students in the laboratory.
- A significant reduction (between 75 and 99 %) of chemical, biological and radioactive waste.

Didactic benefits and learning outcomes are considerable (Pesimo 2014). Techniques are easier to perform and thus, more experiments can be performed in less time with lower cost. Students become curious and are forced to work carefully through all steps of the experiments. Students receive full education, including awareness of the necessity to protect the environment and to work following GLP (New Hampshire Department of Environmental Services 2014). At the beginning, to acquire proper equipment for microscale techniques may represent an additional cost. However, after the initial expenses, costs for material and equipment are drastically reduced.

2.3 Instrumentation

First, we evaluate and diagnose which techniques could be developed as microscale techniques (Table 1).

No	Practical session	Reason to be selected
1	Standard curve	Basic method that can be validated and performed with minimal amount of reagents
2	Extraction and identification of reserve carbohydrates from animals and plants	Small volumes can be used during extraction and purification of starch and glycogen
3	Analysis of lipids from egg yolk	Small volumes can be used for extraction and purification Phosphates can be quantified It may be validated
4	Aminoacids	Small volumes can be used
5	Plasma proteins	Small volumes can be used for extraction and purification Proteins can be quantified by several methods It may be validated

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No	Practical session	Reason to be selected
6	DNA from wheat	Small volumes can be used for extraction and purification
7	Invertase kinetics	Small volumes can be used to study the influence of various factors that may modify the velocity of catalytic reactions Enzyme products can be quantified It may be validated

Table 1. Techniques that may be performed in microscale.

Then, we modify techniques that were found susceptible of modification to microscale conditions (Table 2). We tested various conditions and working volumes to find those allowing bigger saving of time and reagents while quality of work and results were as high as the original non-microscaled techniques.

	Objective	Importance	Reagent Saving
1	- To design and obtain standard curves - To determine albumin concentration in white egg	- Standard curves are basic tools for biochemistry students. - Methods to measure proteins are discussed in relation to protein properties.	50 %
2	- To extract and identify polysaccharides from various sources - To learn Lugol and Benedict methods	- The biological importance of reserve carbohydrates. - Students extract and purify glycogen from rat liver and starch from rice. - Students learn methods to identify hydrolysed and non-hydrolysed lipids.	48 %
3	- To extract lipids from egg yolk - Quantify phosphates from phosphorylated and non-phosphorylated lipids	- Physicochemical properties of lipids and methods for lipids extraction are discussed - Students get acquainted with the method of Kjeldahl for quantitative determination of nitrogen and methods for identification of phosphates.	69 %
4	- To determine pKa - To verify dipolar behaviour of aminoacids	- Students learn to identify aminoacids by pKa and ionic behaviour.	50 %

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	Objective	Importance	Reagent Saving
5	- To obtain albumin and globulin from blood - To quantify proteins by the method of Biuret	- Plasma composition and protein properties are analysed. Students learn the properties of plasma proteins, methods to fractionate plasma and to quantify its components	50 %
6	- To extract DNA from wheat germ - To evaluate DNA purity	- DNA can be isolated from wheat germ. - It facilitates learning of DNA properties and methods for extraction, purification and quantification of DNA.	50 %
7	- To analyse the velocity of enzymatic reactions - To analyse the ionic character of enzyme and substrate - To determine km and $V_{máx}$	- Biochemistry scholars must be acquainted with enzyme kinetics. Students learn to obtain km and $V_{máx}$ and analyse how pH, temperature and enzyme and substrate concentrations affect enzyme activity. - This session illustrates the participation of enzyme reactions in normal metabolism.	50 %

Table 2. Modified practical sessions

2.4 Validation

Most laboratory practices require certification by detecting and quantifying specific products. Validation is applied to demonstrate how accurate are the results obtained. Validation permits to recognize linearity, precision and accuracy, and when these measurements are within certain limits, it is right to affirm that both the results and the methods used to obtain the results are correct.

Analytical methods describe the sequence of activities, material resources and parameters that must be fulfilled to analyse the presence of specific products. Validation is a way to demonstrate that certain method fulfils its purpose with the required sensitivity and specificity (FDA 2014, Paquirigan and Beebe 2008).

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2.5 Linearity

It means that the results obtained are proportional to the concentration of the product, within certain intervals (FDA 2014, Paquirigan and Beebe 2008).

An analyst must prepare at least 5 concentrations of the reference chemical by triplicate. Triplicates can be prepared separately or by diluting the highest concentrated solution. Concentration in the middle must be equivalent to the analysed concentration. The interval between concentrations should be according to specifications of the method to validate. The slope of the regression line (b1), the Y intercept (b0), coefficient of determination (r^2) and the confidence intervals from the slope [CI (β1)] must be calculated. Intervals are closely related to the purpose of the method and are therefore, expressed as a percentage of concentration of the reference chemical. Concentration may be plotted in the X-axis vs the analytical response in the Y-axis. Acceptance criteria include: $r^2 \geq 0.98$ and IC (β1) $\neq 0$.

2.6 Precision

It refers to the degree of concordance between individual analytical results, when the procedure is repeatedly applied to different aliquots of a homogeneous sample. An analyst must prepare at least 5 concentrations of the reference chemical. Sextuplicates can be prepared separately or by diluting the highest concentrated solution. Standard deviation (S) and coefficient of variation (CV) of the analytical response must be calculated. Acceptance criteria in this case are CV ≤ 2.5% for physicochemical methods (FDA 2014, Paquirigan and Beebe 2008).

2.7 Accuracy

It refers to the degree of concordance between the results obtained and the reference value. An analyst must analyse a sample with the method to validate. The same analyst

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must prepare at least 6 samples to which the product to be analysed is added. It can be done for instance by utilizing half of the analytical sample originally required and adding the product obtained or a secondary reference until reaching 100%. Added samples must be analysed by the same analyst under similar conditions and using as reference the chemical or product added to the sample. The amount of the product is determined and the percentage of recovery from the product is calculated. It is necessary to calculate the arithmetical mean, SD, CV and CI from the percentage of recovery. Acceptance criteria demand 100% CI or an arithmetical mean from percentage of recovery between 97-103 % for chemical or spectrum photometric methods. The CV of the percentage of recovery must be < 3%.

2.8 Validation

Microscaled analytical methods (1, 3, 5 and 7 from table 2) were validated and the results demonstrated linearity, precision and accuracy. As an example, tables 3, 4 and 5 show data obtained when analysing phosphate content from egg yolk.

Values are within accepted limits. The method complies with linearity and specifications. Values in most levels are quite similar. The CV is elevated because absorbance is small, which means that the smallest variation would produce big changes. However, values observed are very similar. Therefore, the method is considered precise.

Repetition	Concentration (µg)	Absorbance
1a	2	0.012
1b	2	0.017
1c	2	0.017
2a	6	0.055
2b	6	0.048
2c	6	0.051

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Repetition	Concentration (µg)	Absorbance
3a	10	0.093
3b	10	0.083
3c	10	0.082
4a	20	0.166
4b	20	0.166
4c	20	0.162
5a	30	0.222
5b	30	0.252
5c	30	0.212

Table 3. Linearity

Slope (b_1)	0.008
y-intercept (b_0)	0.005
determination coefficient (r^2)	0.985
$S_{y/x}$	0.010
S_{b1}	0.000
superior IC (β_1)	0.008
inferior IC (β_1)	0.007

Table 4. Linearity

Repetition	Concentration levels				
	2 µg	6 µg	10 µg	20 µg	30 µg
1	0.012	0.055	0.093	0.166	0.222
2	0.017	0.048	0.083	0.166	0.252
3	0.017	0.051	0.082	0.162	0.212
4	0.019	0.054	0.082	0.166	0.24
5	0.017	0.055	0.082	0.169	0.231
6	0.017	0.054	0.088	0.166	0.231
$\bar{X}$	0.016	0.053	0.085	0.166	0.231

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Repetition	Concentration levels				
	2 µg	6 µg	10 µg	20 µg	30 µg
SD	0.002	0.003	0.004	0.002	0.014
CV	14.213	5.275	5.365	1.344	6.000

Table 5. Precision

2.9 Accuracy and protocols

Since results demonstrate that the method is linear and precise, it is concluded that the method is also accurate.

Once completed the validation procedure microscale protocols were elaborated after exhaustive literature review.

3. Teacher education as means for the implementation of the microscale

3.1 Antecedents

Scientific knowledge and technology advances are nowadays continuously increasing and evolving. Speed of changes and continuous testing demands highly capacitated professionals with great curiosity and devotion. Professional formation requires time and dedication as well as proper guidance and advice. To be a professional in science and education is a long life task. Teachers and scientist need to cultivate their knowledge permanently. Forming professionals with the right background and attitude must be considered the main objective of university teaching.

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3.2 Workshops

Three workshops were organized to present microscale techniques to teachers from BCT-1. Teachers learned the techniques, appreciated the easiness of execution and the critical points that should deserve more attention in order to keep precision and accuracy of the methods. During training, there was ample discussion on advantages and disadvantages of the new techniques. At the end, the entire group agreed to adopt the new techniques for teaching biochemistry in the BCT-1 program.

3.3 Limitations and contributions

Part of this work is dedicated to develop microscale techniques and to capacitate teachers to use them and instruct their students to use them well. Three workshops were offered to our teachers in order to train them properly. Nearly 100 % of our academic personnel are now acquainted with the new techniques. Our workshops also served to stimulate communication, teamwork, critical analysis and creativity among our colleagues. We all agree that updating and adapting our work in the teaching laboratories will contribute to improve the quality of teaching biochemistry at our university.

Seven new practical sessions were designed and included in the working schedule of BCT-1. All microscale techniques that we added to these sessions are validated. Saving time for the accomplishment of practical goals also facilitated explanation and comprehension of the subjects. New protocols allow to add additional goals such as being acquainted with additional instruments and to develop better routines for handling of materials and equipment.

Implementation of new protocols involves the acquisition of expensive materials and equipment and is time consuming. However, after the initial expenses, we noted an important reduction of costs. Reduction of risk and generation of chemical waste is also noteworthy. An important goal is to provide our students not only with good work

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routines and successful approaches to biological and socioeconomic problems but also with a permanent concern for the environment and toxicological risks derived from handling chemicals. In this way, we try to contribute to educate and train well-prepared professionals to produce a positive impact in our society.

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