The enzymatic determination of glucose in carbonated beverages: an useful tool for the undergraduate students to learn the basis of enzymatic analysis and the comparison of two analytical methods

Ángel Luis García-Ponce, *Universidad de Málaga, Andalucía Tech (Spain)*
Beatriz Martínez-Poveda, *Universidad de Málaga, Andalucía Tech (Spain)*
Ángel Blanco-López, *Universidad de Málaga, Andalucía Tech (Spain)*
Miguel Ángel Medina Torres, *Universidad de Málaga, Andalucía Tech (Spain)*
Ana Mª Rodríguez Quesada, *Universidad de Málaga, Andalucía Tech (Spain)*

Abstract

The importance of enzymatic analysis in biochemistry, clinical chemistry and food chemistry is undoubted. The course "Applied Biochemistry" in our Faculty is aimed to undergraduate students of Chemistry and Biochemistry. In this subject, the principles and applications of enzymatic analysis are presented to the students, who receive a theoretical introductory lecture in the classroom before they carry out an experiment that should be feasible to be solved in a short laboratory period. The experimental protocol here presented, based on the enzymatic determination of glucose in carbonated beverages, has been implemented at the University of Málaga and it has been optimized according to the students' results and commentaries along the last years. It aims to illustrate basic issues relating enzymatic analysis, including its potential application to food chemistry.

Although there are several enzymatic methods that can be used for the determination of glucose, we selected the one based on the coupled reactions of glucose oxidase (GOD; EC 1.1.3.4.) and peroxidase (POD; EC 1.11.1.7.) because the kinetic constants of glucose oxidase allow the mentioned enzymatic reactions to be used in both, the end point and the kinetic enzymatic analysis methods. In this way, data for two different protocols for the determination of glucose concentration are obtained by the students from a single reaction mixture. Students construct a calibration curve for each method using a glucose standard solution, and use them to determine the glucose concentration in the problem solutions.

The inclusion of replicate samples in the determination of the glucose concentration of an "ideal problem" (glucose in purified water) is used to illustrate the principles of statistics in the lab, and comparison with the "real value" allows an estimation of the accuracy of each method. The evaluation of glucose concentration in four carbonated beverages: coloured coke and uncoloured tonic sodas (regular or sugarless in both cases) makes student to recognise the appearance of interferences that should be either avoided or eliminated. Since all samples are analysed by means of end-point and kinetic methods, students can discuss the applicability of each method to these specific analytical problems. They are also encouraged to compare both analytical methods in terms of sensitivity, selectivity, accuracy, and time consumed.

Chemistry and Biochemistry undergraduate students having performed this experiment in our laboratories have found it formative, interesting and challenging.