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# An Automatic Grading System For a Laboratory Experiment Class: Kinetic Determination of Furfural as a Parameter of Food Quality

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**Abstract:** In this work, we present the application of the automatic grading system, Goodle GMS (Grading Management System), to an analytical chemistry lab exercise based on large amounts of experimental data. The lab-case study used to show the potentiality of this grading system is the kinetic determination of furfural, a widely used parameter for food quality control. Goodle GMS is a Web-based application that has been extensively used in engineering courses and, more recently, in analytical chemistry courses, to automatically assist the instructor in the grading process. Goodle GMS allows the students to submit their results to a server via a Web-based form from any networked computer and receive their grade, feedback and an indication of where mistakes were made. The proposed lab example, the determination of furfural, was achieved following kinetically the Winkler's reaction by photometric measures at 585 nm.

## Introduction

Worldwide education institutions have started using learning management system (LMS) packages of the so-called Web 2.0 that offer collaborative tools and additional features at the service of education [1]. The use of this technological framework in education reduces the student mobility requirements and enables a closer interaction between teacher and students, using different tools such as forums, e-mail, FAQ (frequently asked questions), immediate course material delivery, warnings, grades, planning, calendars, etc. In Kapur and Stillman [2]; Poindexter and Heck [3]; Heck, Poindexter and García [4], an overview of the possibilities offered to educators is presented.

Most widely used LMS offer several evaluation capabilities such as multiple-choice questions (MCQs). Some examples of application in chemistry courses can be found in [5–7]. However, although MCQs are useful tools, they are not appropriate for the assessment of tasks requiring numerical manipulation, calculation, and complex logical processes that bring together conclusions from different sets of data, possibly very large, such as practical analytical chemistry exercises developed in a laboratory.

In previous works, we have developed a novel Web-based education tool named Goodle GMS (Grading Management System), for automating the collection and assessment of practical exercises [8–11]. Goodle GMS is based on executing the student's answers, as code, inside an ad-hoc developed program by the instructor to analyse and grade the student's work. The student's answer together with the instructor

grading criteria is coded using the same programming language. In our case, Matlab was used, as it provides an appropriate framework for the presenting calculations. This architecture provides a flexible tool in which different problems/practices can be evaluated. This Web-based system has been extensively used in engineering courses, and more recently an analytical chemistry application to determine the nitrite concentration in water samples by flow injection analysis (FIA) based on the Griess reaction, has been presented [12].

In most Goodle GMS applications, the amount of experimental data used in the majority of the laboratory experiments, or the number of parameters that defined the particular problem for each student was manageable, and in general is included in the instructor evaluation code or in the student solution. However, in certain laboratory experiments, the amount of experimental data could be very large, as in kinetic determination techniques. In these cases, the raw data cannot be included in an appropriate manner as in previous applications of Goodle GMS.

An important feature of kinetic determination techniques is that they are based on relative measures of signal, and therefore absolute values of absorbance, intensity of fluorescence, potential, for instance, are not relevant. Thus, it is only necessary to accurately measure the rate of change of the signal, which is not affected by turbidity, coloration, initial fluorescence and dirt of the cell, for example. In this way, a great number of interferences are eliminated, so the selectivity is greater. The application of kinetic methods for the analysis of chemical systems can be carried out along the duration of a reaction, although often they are applied to its beginning. The use of kinetic methods in analytical chemistry is a valid alternative for those procedures based on chemical reactions that do not meet the requirements to be used in equilibrium, i.e. those chemical reactions that are not quick, complete or with

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defined stoichiometry. Thus, kinetic methods can be based on slow or not quantitative reactions, since the reaction speed can be measured only in a fraction of the reaction.

Currently, methods based on the measurement of the reaction rate have been consolidated due to the advance in the last years of the instruments with the incorporation of electronic systems that ensure higher degrees of precision and accuracy. Thus, kinetic methods have gained in accuracy, precision, safety, instrumental accessibility and low cost, so they are widely used in clinical chemistry routine analyses [13, 14].

Kinetic methods do not require the measurement of the absolute value of the parameter chosen to follow the reaction, but the measurement of the variation of this parameter as a function of time. Thus, a great amount of data is generated, and as it was mentioned above, the previous version of Goodle GMS was not suitable.

Motivated by these issues, in this work we focus on the development and application of Goodle GMS in an advanced analytical chemistry laboratory experiment based on large amounts of experimental data. Goodle GMS has been modified allowing the instructor to upload to the server any amount of text files to be used by the automatic evaluation program. In particular, it has been applied to a laboratory practice involving the kinetic determination of furfural (FUR), a parameter of food quality.

The analytical control of furfuraldehydes is a useful tool in the food and related industries. The presence of these compounds is related to the evaluation of the quality of the processing method used and to the organoleptic characteristics of the final product as well. FUR has been used as a potential indicator of temperature abuse and inadequate time of storage in different foods and related materials [15]. The kinetic determination of furfural practice considered is made by photometric measures at 585 nm by using the Winkler's reaction. In this work, we use the computer program ESCIN [16] to analyse the kinetic data of the FUR reaction.

### Learning Objectives

In kinetic methods the quality of the results depends highly on the precision in the determination of the interval time in which the measurements are going to be performed, hence, a strict control of all factors with influence on the reaction rate, such as temperature, pH, ionic strength or solvent composition is necessary to obtain reproducible results.

Time is the differentiating feature between equilibrium and kinetic methods. It is a variable that must be strictly controlled in all and each one of the steps of a kinetic method. It is not only important to accurately measure the time in which analytical signals (measurement time) arise, but it is also essential to control the duration of the initial operations (previous time). In addition, it is also important to rigorously know how this variable influences the data acquisition system.

The acquisition and processing of data is an essential step in this methodology. On one hand, the transduced signal must be accurately taken as a function of the time. On the other hand, because no simple mathematical relationship exists between these signals and the analyte concentration, a rather complex calculation process is necessary, depending on the type of applied kinetic method (fixed time, variable time, tangent...).

Regarding this issue, the computer program ESCIN [16] can be used with satisfactory results. This Matlab software allows

obtaining the mathematical relationship between the signal and time data, as well as carrying out the study of the rate equations, the temperature influence or influence of the concentration, among others. In this work, ESCIN is used to analyse the FUR reactivity. ESCIN is designed in Matlab environment and can be used to determine the maximum slope section for the kinetic curve; that is, the period of time with the higher rate between a starting value and a final one; and to calculate the kinetic curve slope between an initial time and a final time.

In addition to determining the kinetic parameters of the experiment, in this practice the students also have to analyse these parameters to obtain the problem concentration. To this end, the students will use the ACOC software [17, 18]. ACOC is a useful software package for statistical analysis by linear-regression written for the Matlab programming environment, intended to perform the necessary calculations for the linear adjustment of the experimental data of instrumental analytical chemistry laboratories.

Both learning objectives will be assessed using Goodle GMS [8–11]. Goodle GMS allows the students to submit their results to a server via a Web-based form from any networked computer and receive their grade, feedback, and an indication of where mistakes may have been made.

### Experimental

**Apparatus:** A Varian Cary 50 Bio spectrophotometer was used for all measurements, acquisition of kinetic data, storage and analysis of spectrophotometric data. A Varian single cell Peltier accessory was used for temperature control.

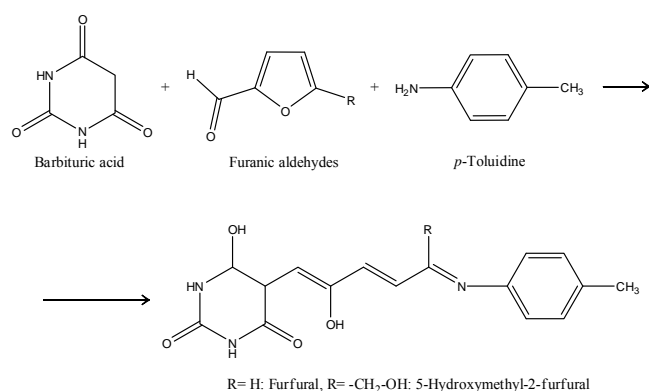
**Reagents and Solutions:** A derivatization reagent consisting on a mixture of barbituric acid (0.05 g, Fluka), *p*-toluidine (7.63 g, Panreac), glacial acetic acid (3 mL, Panreac), ethanol 96% (50 mL, Panreac) and diluting to the mark to 100mL with deionized water was prepared. A stock furfuraldehyde (Merck) solution of concentration of 100  $\mu\text{g mL}^{-1}$  was prepared by accurately weighting 0.010 g of furfuraldehyde and diluting with 100mL of ethanol 96%. A diluted solution of furfuraldehyde containing 6  $\mu\text{g mL}^{-1}$  was prepared from the stock furfuraldehyde solution by diluting with ethanol 96%.

**Laboratory Experiment Description:** The determination of FUR in alcoholic beverages was carried out in the laboratory by the Winkler's derivatization reaction (Figure 1), following a modification of the original procedure [19]. FUR is the principal product of the hydrolysis of pentoses. This compound and 5-hydroxymethyl-2-furfural (HMF) seem to be related with browning processes of food [15]. Both are used as indicators of temperature abuse or storage time in diverse food [20, 21].

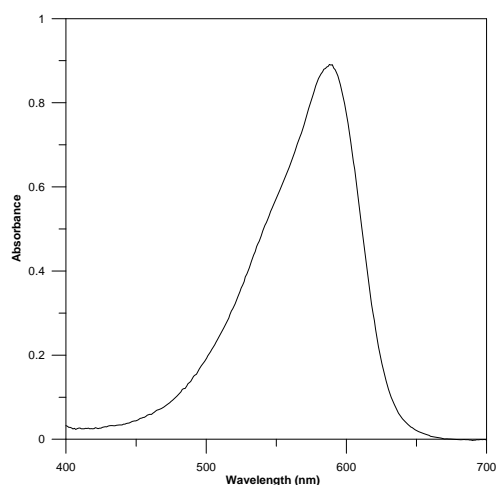
The student work was organized in two stages.

Stage 1: This stage took place in the lab, where the students performed the experiments. Each one of them was provided with an information sheet, which described the experimental procedure. Firstly, they had to prepare the derivatization reagent. After that, different external standards to establish the calibration curve according to the external standard calibration method were prepared, for further determination of the content of FUR in the unknown sample and, finally, they had to record the necessary raw data to subsequently perform the necessary calculations to obtain the FUR concentration in the problem sample. The second part of the students work consisted in performing the calculations related to the different parameters necessary for the FUR determination.

Regarding the experimental part of the students work, it was carried out as follows. Initially, the students have to prepare the derivatization reagent which consists on a mixture of barbituric acid (0.05 g), *p*-toluidine (7.63 g), glacial acetic acid (3 mL) and ethanol 96% (50 mL). The mixture was placed in a volumetric flask of 100.0 mL sonicated for 15 min and, finally, diluted to the mark with deionized



**Figure 1.** Winkler's reaction.



**Figure 2.** Absorption spectrum of the product formed after 4 min of the beginning of the reaction. FUR concentration  $1.44 \mu\text{g}\cdot\text{mL}^{-1}$ .

water. Once the derivatization reagent was prepared, 2 mL of it were placed in a 3 mL quartz cell. Then, different volumes of a FUR solution of known concentration were added to obtained solutions with concentrations between  $0 - 2.0 \mu\text{g}\cdot\text{mL}^{-1}$  and, finally, these solutions were diluted up to a final volume of 3 mL with ethanol 96%. In these conditions, the derivatization reaction took place (Figure 1) and, after 4 minutes, the spectrum of the product formed was recorded (Figure 2). This product presents an absorption maximum at 585 nm. Therefore, the kinetic method for the FUR determination was performed by measurement of the absorbance at 585 nm versus time. An example of the kinetic curves obtained for different concentrations of FUR is shown in Figure 3. The determination of FUR was performed in alcoholic beverages. For this, volumes between 100 and 1000  $\mu\text{L}$  were taken and the same procedure as for the standard solutions was applied.

Stage 2: Once students have been carried out the experimental work and obtained the corresponding raw data in ASCII format, they have to calculate the stretch with the maximum slope of the kinetic curves and analyse the results to obtain the problem concentration. This step was carried out in the computer laboratory. The kinetic curves for the FUR determination are plotted in Figure 3. The slope will be the analytical signal in each case. To this end, students have to use the ESCIN software, as it is described below. Firstly, the time interval (from  $t_1$  to  $t_2$ ) with the maximum slope is calculated employing the automatic mode and the data corresponding to one of the replicates of the most concentrated standard. As previously mentioned it is necessary to provide the software the number of points that this interval must own (a 30 points windows size as been selected in this case) and also the total number of data points among which you wish calculate the interval (from point 1 to point 481, as the kinetic has been recorded during 4 minutes (240 seconds) taken data each 0.5

seconds) (Figure 4). In this way, ESCIN provides the students a figure with the kinetic curve and the selected stretch for the slope calculation (Figure 5), as well as the data corresponding to the initial and final time, the intercept, the slope and the correlation coefficient of the fitting in the slope calculation. Afterwards, using the manual mode of ESCIN, in which the calculated interval must be supplied (Figure 4), the slopes corresponding to the rest of samples are calculated in the optimum interval. The software returns the selected regression slope as well as its correlation coefficient.

For this particular experiment (highest slope from the very beginning of the reaction), this kinetic determination method is known as a method of initial rate. In this method, in the initial stretch, the slope gets its maximum value. However, there are other interesting kinetic determinations, which can present this maximum value in other sections of the kinetic curves (i.e. due to the existence of an induction period) [22]. Therefore, the use of the ESCIN software becomes important, given that it allows obtaining the maximum value of the slope in any stretch of the kinetic curve.

Using ESCIN the students obtain the analytical signal data (slopes) to establish the external standard calibration plots, which allow them to calculate the concentration of FUR in the unknown samples, using the ACOC software [17, 18]. In the next step, the students must submit their data in the Web-based system Goodle GMS, as it is described below.

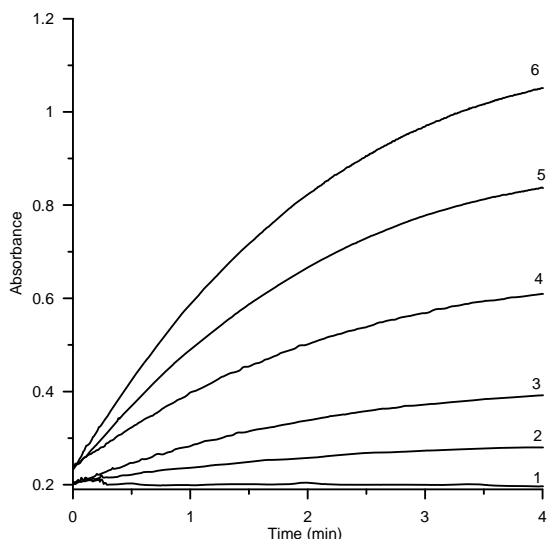
### Goodle GMS Work

After the students carry out the practice, they have to submit the results to Goodle GMS. To this end, each student registered on the server with his or her identification number and a password and provided a text following a particular syntax described below. For more information about Goodle GMS, the reader may refer to [8–11].

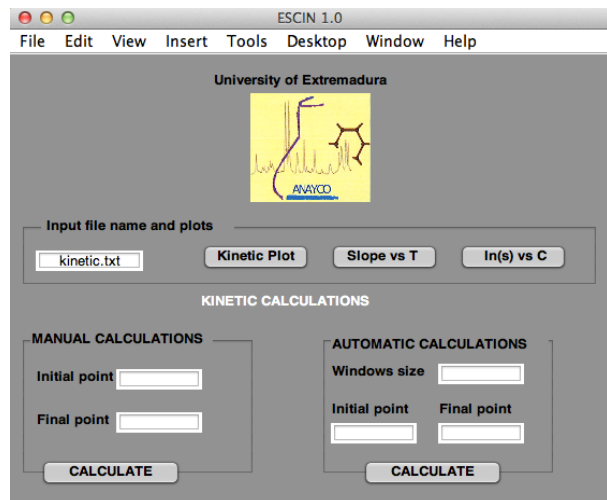
To correctly evaluate this work, Goodle GMS needs not only the final results, but also the experimental data obtained in the laboratory. In previous applications of Goodle GMS to analytical chemistry practices, the students uploaded this data as part of their results, in particular, the pairs of experimental values corresponding to the solutions of the standards and the responses of the replicates of the problem sample [12]. In addition, the instructor uploaded to the evaluator a list of the problem concentration of each student to evaluate the results. In this case, however, the raw data; that is the kinetic response, is too large to be included in the student's response. Instead, a new functionality of Goodle GMS is used, which is the possibility of uploading auxiliary files to the server that can be read when the evaluation program is executed. In particular, in the practice carried out, the instructor uploaded the ASCII file obtained by each student of the most concentrate standard, along with a table that provided to each student the particular filename.

With this data, the evaluation program can calculate the time period (from  $t_1$  to  $t_2$ ) with the maximum slope and these results will be compared with the values provided by the students. For example, in the case of our experiments in which we have recorded the absorbance from time 0 to 4 min taken a measure each 0.5 s, the ASCII file obtained had 481 rows. From row 1 to 481 there are the recorded kinetic data (one datum each 0.5 s).

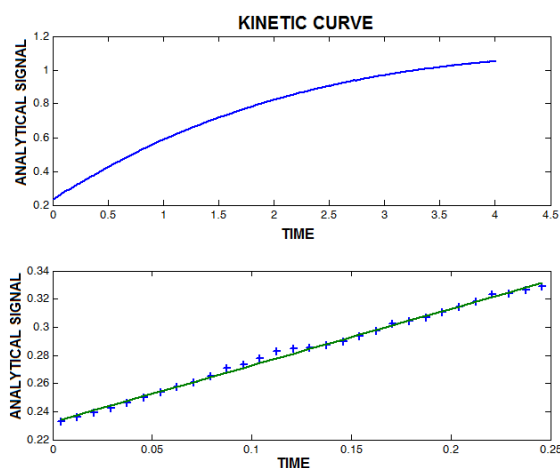
The students upload the calculated interval ( $t_1$ ,  $t_2$ ) with the highest slope and, in addition, the experimental values as in the previous work [12], in particular the 18 pairs of experimental values ( $x_i$ ,  $y_{ij}$ ), corresponding to the 18 standard solutions (6 standards with 3 replicates each), the responses of the three replicates of the unknown sample ( $y_1$ ,  $y_2$ ,  $y_3$ ), the dilution (dil) and, finally, the calculated concentration of FUR in the unknown sample ( $x_p$ ) as well as the associated uncertainty ( $e_p$ ). To evaluate the concentration and the uncertainty, the students had to take into account the dilution. Therefore, they also have to submit the dilution factor they used, which is a variable that the code needs to perform the correct calculation of the FUR concentration in the unknown sample. The uncertainty is given by  $t_{s_n}$ , for a t-Student value with  $n-2$  degrees of freedom and a significance level of 95%. See Figure 6.



**Figure 3.** Kinetic curves corresponding to the external standard calibration solutions: 1, 0  $\mu\text{g}\cdot\text{mL}^{-1}$ ; 2, 0.190  $\mu\text{g}\cdot\text{mL}^{-1}$ ; 3, 0.480  $\mu\text{g}\cdot\text{mL}^{-1}$ ; 4, 0.960  $\mu\text{g}\cdot\text{mL}^{-1}$ ; 5, 1.44  $\mu\text{g}\cdot\text{mL}^{-1}$ ; 6, 1.91  $\mu\text{g}\cdot\text{mL}^{-1}$ .



**Figure 4.** Initial window of ESCIN software.



**Figure 5.** Kinetic curve corresponding to the first replicate of the most concentrate FUR standard and selected stretch for the slope calculation. Automatic mode of ESCIN software. FUR concentration, 1.91  $\mu\text{g}\cdot\text{mL}^{-1}$ ; number of points in the interval, 30.

Note that it is also possible to upload more than one file for each student; that is, to upload the kinetic response of each of the experiments carried out. In this case, the students only would have to provide the dilution and the results. However, the evaluation code would be much more complex, so we have chosen a mixed approach to demonstrate both options.

Students submitted their laboratory experiment results via the Google GMS interface using the code structure shown below:

```
Interval = [t1 t2];
Standards = [x1 y11 y12 y13;
x2 y21 y22 y23;
x3 y31 y32 y33;
x4 y41 y42 y43;
x5 y51 y52 y53;
x6 y61 y62 y63];
Replicates = [y1 y2 y3];
Dilution = dil;
Concentration = [xp ep];
Group = ID;
```

### Evaluation Code Design

To use Google GMS, a particular evaluation code in Matlab has to be developed by the instructor for each different exercise. The results submitted by the students in text form are executed as Matlab code to generate in the workspace a set of variables with the initial and final time for slope calculation, standards, replicates, dilution and the FUR concentration in the problem sample. The server executes this code and then the evaluation code designed by the instructor for the exercise. The evaluation code is written in Matlab. Google GMS generates a string ID variable in the workspace to identify each student. The evaluation code must generate two variables that are stored in Google GMS database and can be accessed afterward by the student, one numeric variable with the grade and a string variable in which comments in html format are stored. All Matlab functions and toolboxes are available. In particular, the evaluation code makes the calculations using ESCIN [16, 23] and ACOC [17, 18] and the student's raw data to establish their "right" answers.

To apply ESCIN, the evaluation code retrieves the kinetic data from the appropriate ASCII file of the students. To this end, is very important that the instructor keeps track of the files generated by each of the students. The ASCII files are included in Matlab's path, so they can be processed using standard Matlab file functions, such as fopen and fscanf, taking into account the particular structure of the files generated by the experimental equipment. The evaluation code that retrieves the problem concentration and filename for a particular student is shown below. Google GMS generates the string variable dni with the ID, while the instructor must provide the ID, problem concentration and filename for each student organized in a pair of Matlab variables, as follows:

```
% ID, problem concentration and filename data that must be included
in the evaluation code by the instructor
StudentData = [8773001 21; 79306956 19];
StudentFileName(1).fname = 'niro16.csv';
StudentFileName(2).fname = 'niro1.csv'; % Matlab code to
retrieve the problem concentration and filename for a particular
student. Google GMS generates the string variable dni with the ID
DNI = str2num(dni);
student = find(StudentData(:, 1)==DNI);
filename = StudentFileName(student).fname;
problem concentration = StudentData(student, 2);
```

The student's calculated interval and concentration are then compared with the right answers to generate a grade and some comments in text form. In this particular exercise, the grading criterion was established taking into account the precision of the determination of the problem sample, the calculations, and the correct expression of the uncertainty associated to the results. If the error was

Figure 6. Goodle GMS results submission page.

below 1%, the students grade was 10 (the maximum grade); if the error was between 1 and 2%, the grade was 9; 8 for errors between 2 and 5%; 6 for errors between 5 and 10%; 4 for errors between 10 and 15%; and, finally, 2 for errors higher than 15%. This grade was modified subtracting 1 point if the result or the associated uncertainties were not expressed correctly. Besides, the selection of the data points interval selected to calculate the slope is as well evaluated. Students reporting a wrong stretch selection are penalized by subtracting 1 point to their final grade. Regarding the unknown sample, each student has a different one, and the evaluation code obtains the corresponding concentration based on his or her ID. Note that the grading criteria can be easily modified by the instructor changing the evaluation code (see the Supporting Materials).

## Conclusions

In this work, we have presented the application of the automatic grading system, Goodle GMS, to an analytical chemistry lab exercise based on large amounts of experimental data, in particular to the kinetic determination of furfural. The results demonstrate how it is possible to grade this class of experimental practices using the new Goodle GMS functionality that allows the instructor uploading auxiliary files that can be accessed by the evaluation code. This particular scheme broadens the experimental exercises to which this particular evaluation paradigm can be applied.

Should the reader be interested in testing and using the application by freely creating auto-evaluated exercises (or using the code presented in this work) and assigning them to their students, it is possible to do so on a fully functional application running on a publicly available server. Please contact the authors by email to gain password access.

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**Supporting Material.** The evaluation code developed for the FUR determination in alcoholic beverages by a kinetic

method using the Winkler's reaction is provided as supporting material (<http://dx.doi.org/10.1333/s00897142551a>).

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