v. 2, n. 2, 2025

International Journal of
Exact
Sciences

MÉXICO: XXXIV NATIONAL CHEMISTRY OLYMPIAD, 2025: SYNTHESIS OF FLUORESCEIN, SOLVENT-FREE REACTION

Acceptance date: 18/08/2025

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Abstract: In this work, we present the experiment performed by the students participating in the XXXIV National Chemistry Olympiad, held from February 19 to 23, 2025, in the city of Querétaro, Querétaro, Mexico. A total of 123 high school students took part, representing 31 federal entities into which the Mexican Republic is divided. In this experiment, the students synthesized fluorescein via a double electrophilic aromatic substitution reaction, followed by elimination steps, starting from one equivalent of phthalic anhydride and two equivalents of resorcinol. The product was isolated and purified by successive washes with cold water. Its identification was carried out by thin-layer chromatography (TLC), which allowed the differentiation of the product from the starting materials based on their respective *Rf* values.
Keywords: fluorescein synthesis, green chemistry education, thin-layer chromatography, statistical analysis of chromatographic data, confidence interval estimation.

INTRODUCTION

From February 19 to 23, 2025, the XXXIV National Chemistry Olympiad was held in Querétaro City, Querétaro, Mexico, organized by the Mexican Academy of Sciences in collaboration with the Universidad Autónoma de Querétaro (UAQ). The practical examination was conducted at the Faculty of Chemistry of UAQ. A total of 123 high school students participated, representing all 31 federal entities of the Mexican Republic. Participants were assigned to two levels: Level A (47 students), whose curricula included courses in organic chemistry, and Level B (76 students), who had not undertaken such coursework.

The competition spanned four days. On the first day, students completed three theoretical examinations covering different areas of chemistry, with difficulty levels ranging from multiple-choice questions to complex problem-solving exercises. On the second day, participants took the "International Exam", a five-hour theoretical test of greater complexity. The third day was dedicated to the practical examination.

For the organic chemistry practical component, the top 26 students from the first day's theoretical results were selected. Based on the combined scores of the four theoretical examinations and the practical assessment, the top sixteen students were identified. These finalists received four months of intensive training, after which the four highest-ranking students were chosen to represent Mexico at the 2025 International Chemistry Olympiad (57th IChO), to be held from July 5 to 14, 2025, in Dubai, United Arab Emirates, and at the XXIX Ibero-American Chemistry Olympiad, to be held in October 2025 at the Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico.

Since 2003, the National Chemistry Olympiad in Mexico has integrated thin-layer chromatography (TLC) into its experimental program for the identification of organic compounds—either synthesized during the examination or as unknowns—through spot tests and verification of *Rf* values. In this edition, the experimental data obtained by the 26 students in the organic chemistry practical examination were subjected to statistical analysis to determine the mean, standard deviation, and three distinct confidence intervals.

Moreover, an essential objective of these academic events is to foster awareness among high school students of the relevance of sustainable chemistry, grounded in the twelve principles of green chemistry proposed by Paul Anastas and John Warner [1,2].

BACKGROUND

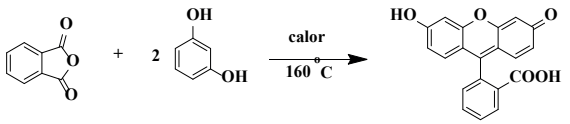
Fluorescein is a fluorescent dye synthesized via the reaction of phthalic anhydride with resorcinol in the presence of an acid catalyst, such as sulfuric acid. This transformation, which involves two Friedel–Crafts reactions, is carried out at temperatures between 180 and 200 °C and was first reported by Baeyer [3]; it is also performed at the industrial scale [4]. The use of alternative acid catalysts, such as zinc chloride or methanesulfonic acid, has been reported to accelerate the Friedel–Crafts reaction [5,6]. The resulting product is a deep red crystalline solid that exhibits intense fluorescence under ultraviolet light. Microwave-assisted syntheses of fluorescein have also been described [7].

To ensure that the students undertaking this examination were familiar with the basic concepts of thin-layer chromatography (TLC) and the variables influencing good practice in this technique, general information was provided beforehand. The main advantage of TLC is its ability to distinguish reactants from products based on their molecular structure, offering benefits over melting point determination—which is limited to solid compounds and often restricted in educational institutions by the availability of instrumentation.

Both TLC and melting point determination are fundamental techniques for the identification of organic compounds, particularly for assessing purity and identifying unknown substances. TLC stands out as a rapid, low-cost method that enables the separation and visualization of the components of a mixture, while melting point determination provides a characteristic physical constant that aids in the identification of solid compounds and the evaluation of their purity.

Together, these techniques yield complementary information on the identity and purity of organic compounds: TLC enables a rapid assessment of the number of components and their relative mobility, whereas melting point determination provides a precise measurement of a key physical property to confirm compound identity and degree of purity [8]. In addition, the students were provided with information on the twelve principles of green chemistry. The complete version of the examination, in the edition intended for instructors, can be accessed at the following link: https://nube.quimica.unam.mx/index.php/s/zFwwUaCLDHTIMIh.

Reaction



Scheme 1. Reaction for the synthesis of fluorescein

PROCEDURE

In a Pyrex test tube (14.7 cm height × 2 cm internal diameter), 0.3 g (2.7×10^{-3} mol) of resorcinol, 0.2 g (1.35×10^{-3} mol) of phthalic anhydride, and 0.1 g (7.3×10^{-4} mol) of zinc chloride as catalyst were weighed. A magnetic stir bar was added, and the reaction mixture was homogenized.

The tube was placed in a sand bath maintained at 140–150 °C for 30 min. The thermometer bulb and the reaction mixture were positioned at the same height. After the desired time, the tube was removed from the sand bath, allowed to cool, and 2 mL of acetone were added. The mixture was stirred and gently heated for 2–3 min until homogeneous, then allowed to cool.

The reaction mixture was transferred to a 50 mL beaker containing 20 g of an ice–water mixture. The beaker was placed in an ice bath and kept under vigorous stirring for 20 min until a pasty solid formed. After this period, the gummy material transformed into a suspended solid. The mixture was left to stand for

an additional 20 min, maintaining the beaker in the ice bath.

The product was isolated by vacuum filtration using a Büchner funnel (5 cm internal diameter) fitted with a rubber adapter and connected to a 50 mL side-arm (Kitasato) flask. The solid was washed three times with 10 mL portions of cold distilled water. Drying was performed under continuous vacuum for 15 min. The product was analyzed by thin--layer chromatography (TLC).

STATISTICAL ANALYSIS OF THE *RF* VALUES OF THE STARTING MATERIALS AND PRODUCT

Two instructors performed the experiment and the thin-layer chromatography (TLC) procedure on twelve separate occasions in order to obtain sufficient data for statistical analysis. This allowed the calculation of the mean *Rf* values for resorcinol, phthalic anhydride, and the product, fluorescein. On the day of the examination, statistical analysis was performed on the *Rf* values reported by the 28 students who carried out the experiment [9,10].

For the evaluation of the participants, the examination also included questions regarding the reaction they had performed and which of the twelve principles of green chemistry were applied in the process.

RESULTS OBTAINED

The students completed this experiment within 2.5 hours. During the procedure, some issues arose with the heating in the sand baths, and only two of the 28 students who took the examination encountered difficulties that prevented them from completing the entire experiment. However, as yield was not evaluated, this did not constitute a drawback. Of all the students who carried out the examination, ten obtained crystalline products.

Thin-Layer Chromatography (TLC). Statistical Analysis of *Rf* Values

Figure 1 shows one of the chromatoplates prepared by the instructors prior to the laboratory session.

Rf phthalic anhydride **A** = 0.3 / 3.9 = 0.076
Rf resorcinol **R** = 3.2 / 3.9 = 0.82
Rf fluorescein **F** = 2.8 / 3.9 = 0.72

Figure 1. Representative TLC plate showing the separation of phthalic anhydride (A), resorcinol (R), and fluorescein (F). Mobile phase: hexane/ethyl acetate (30:70, v/v).

Twelve repetitions of the experiment were performed (Table 1) to obtain statistical parameters (Table 2), such as the mean and the sample standard deviation, with the aim of evaluating the results obtained by the students.

Repetition	*Rf* (R)	*Rf* (A)	*Rf* (F)
1	0.810	0.078	0.710
2	0.820	0.076	0.692
3	0.770	0.076	0.692
4	0.770	0.076	0.692
5	0.820	0.076	0.692
6	0.820	0.076	0.710
7	0.820	0.076	0.710
8	0.800	0.060	0.710
9	0.800	0.060	0.720
10	0.800	0.070	0.720
11	0.820	0.080	0.740
12	0.800	0.080	0.740

Table 1. *Rf* values determined for each repetition by the instructors. R refers to resorcinol, A to phthalic anhydride, and F to fluorescein.

Substance	Mean	Std. Dev.	CV (%)
R	0.804	0.018	2.28
A	0.074	0.007	9.32
F	0.711	0.017	2.43

Table 2. Statistical parameters obtained from the experiment.

The evaluation consisted of assigning a maximum of 60 points, distributed as 20 points for each *Rf* value determined. For each *Rf*, 20 points were awarded if the value obtained was within the interval ; 15 points if within ; 10 points if within ; and 0 points if it fell outside these confidence intervals.

ASSESSMENT OF RESULTS

To verify whether the results obtained by the students (Table 3) are consistent with those from the reference experiment (Table 2), statistical parameters were calculated for both data sets (Table 4). Subsequently, a two-sample *t*-test was applied to compare the means and determine whether significant differences exist between the two groups (Table 5).

Based on the results of the independent two-sample *t*-test, it was determined that the results obtained by the students are not statistically comparable to the control sample, as significant differences were found between the two groups. Therefore, it was decided to evaluate the students using their own mean and standard deviation as an internal reference (Table 6). The variability observed in both samples could be attributed to factors such as the quality of the reagents, the preparation of the mobile phase (eluent), and differences in experience between students and instructors.

CONCLUSIONS

The proposed experiment provided students with an engaging introduction to organic synthesis by enabling them to carry out electrophilic aromatic substitution reactions (Friedel–Crafts type) and elimination reactions. The use of fluorescein, which exhibits intense fluorescence under ultraviolet light, allowed students to directly observe this property when visualizing the chromatoplates under a UV lamp. Throughout the procedure, students applied techniques aligned with the principles of green chemistry, fostering a more sustainable approach to laboratory practice.

As anticipated, the *Rf* values obtained by the students did not exactly match those determined in advance by the instructors, making it necessary to conduct a comparative statistical analysis to assess the consistency of the results obtained by the students. Beyond its instructional value, this experiment also played a key role in identifying the 16 highest-performing participants, who formed the national preselection. Following an intensive training program, the four most outstanding students represented Mexico at the 2025 International Chemistry Olympiad in Dubai, earning two bronze medals and one honorable mention.

Student	R	F	A
1	0.35	0.60	0.10
2	0.35	0.45	0.10
3	0.38	0.50	0.10
4	0.33	0.43	0.08
5	0.48	0.63	0.13
6	0.33	0.45	0.10
7	0.25		0.08
8	0.33	0.45	0.10
9	0.40	0.58	0.13
10		0.58	0.13
11	0.40		0.13
12	0.35	0.45	0.10
13	0.40	0.60	
14	0.33	0.38	0.10
15	0.38	0.48	
16	0.43	0.53	
17	0.40	0.60	0.13
18		0.50	0.08
19	0.40	0.50	0.10
20	0.30	0.40	0.13
21	0.35		0.13
22			
23	0.35	0.48	
24	0.55	0.73	0.13
25	0.35	0.40	0.13
26	0.30	0.35	

Table 3. *Rf* values obtained by the students. R refers to resorcinol, A to phthalic anhydride, and F to fluorescein.

Substance	Mean	Std. Dev.	CV (%)	N
R	0.369	0.062	16.9	21
F	0.503	0.094	18.7	21
A	0.111	0.019	17.5	20

Table 4. Statistical parameters calculated from the results obtained by the students.

Substance	***t* (experimental)**	***t* (critical, α = 0.05, two tailed)**	**df**	***p*-value**
R	29.76	2.05	26.67	5.41×10^{-22}
F	9.75	2.07	22.39	1.61×10^{-9}
A	-7.39	2.06	24.35	1.15×10^{-7}

Table 5. Results of the independent two-sample *t*-test.

Student	R (interval)	R (score)	F (interval)	F (score)	A (interval)	A (score)	Total
1	±1σ	20	±2σ	15	±1σ	20	55
2	±1σ	20	±1σ	20	±1σ	20	60
3	±1σ	20	±1σ	20	±1σ	20	60
4	±1σ	20	±1σ	20	±2σ	15	55
5	±2σ	15	±2σ	15	±2σ	15	45
6	±1σ	20	±1σ	20	±1σ	20	60
7	±2σ	15		0	±2σ	15	30
8	±1σ	20	±1σ	20	±1σ	20	60
9	±1σ	20	±1σ	20	±2σ	15	55
10		0	±1σ	20	±2σ	15	35
11	±1σ	20		0	±2σ	15	35
12	±1σ	20	±1σ	20	±1σ	20	60
13	±1σ	20	±1σ	20		0	40
14	±1σ	20	±1σ	20	±1σ	20	60
15	±1σ	20	±2σ	15		0	35
16	±1σ	20	±1σ	20		0	40
17	±1σ	20	±1σ	20	±1σ	20	60
18		0	±1σ	20	±2σ	15	35
19	±1σ	20	±1σ	20	±1σ	20	60
20	±3σ	10	±2σ	15	±2σ	15	40
21	±1σ	20		0	±2σ	15	35
22		0		0		0	0
23	±1σ	20	±2σ	15		0	35
24	±3σ	10	±3σ	10	±2σ	15	35
25	±1σ	20	±2σ	15	±2σ	15	50
26	±2σ	15	±2σ	15		0	30

Table 6. Test results for the *Rf* values determined by the students.

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