An instrument-free classification of phenolic compounds using ferric chloride reagent to improve organic chemistry teaching and learning

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Abstract

The chemistry of functional groups is an essential component of basic organic chemistry courses in the undergraduate level. A rapid qualitative method to classify the type of phenolic compounds for the improvement in organic chemistry teaching and learning has been developed. The solution of $FeCl_3$ was used as the reagent. The different colors of the phenolic-Fe^{3+} complexes solutions were observed in the different types of phenolic compounds. The phenolic compounds with *ortho*-dihydroxy and *ortho*-trihydroxy groups specifically obtained the dark green color of the solutions. In this paper, we present a low-cost detector, designed to classify the types of phenolic compounds based on visual observation of colors with a simple enough concept and operation to be used as a teaching tool.

Keywords: Classification, Phenolic compounds, Ferric chloride, Organic chemistry

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Introduction

The chemistry of functional groups is an essential component of basic organic chemistry courses in the undergraduate level. In order to supplement the materials covered in lectures, functional group test methods can be very effective in the understanding of the lecture materials [1-5]. In this work, we were interested in developing test methods for the phenolic function. Phenolic compounds are importantly secondary metabolites produced in plants [6-13]. These include chalcones, flavonoids, coumarins, anthraquinones, xanthones and anthocyanins [14-15]. Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables. These beneficial effects have been attributed to their antioxidant activity which can protect the human body from free radicals which are believed to be the cause of cardiovascular disease, cancer, inflammatory activity and neurodegenerative disorders [16-24].

Generally, there are two laboratory procedures for analyzing phenolic compounds including total phenolic content using Folin-Ciocalteu spectrophotometric quantitative assay and visually colorimetric screening assay using ferric chloride ($FeCl_3$) solution [25, 26]. The ferric chloride test is commonly used to determine the presence of phenols in samples. In the experiment, a few drops of ferric chloride solution are added to the sample to give a red, blue, green, or purple solution, indicating the presence of phenols. However, this method has not been used to classify the types of phenolic compounds [27, 28]. Therefore, this paper reports an experiment for students when they classify and identify types of phenolic compounds using a colorimetric screening assay with ferric chloride reagent, they could be observed and detected by their eye together with the option of spectrophotometric measurements. This activity provides an excellent avenue to engage students in organic chemistry concepts involving types of phenolic compounds in a more visually appealing approach.

Materials and Methods

Activity overview

Ultraviolet spectra were recorded on a Specord-210 Plus UV-Vis spectrophotometer. All the analytical grade organic solvents and chemicals used in this study were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The test method is a simple and rapid qualitative method to classify the types of phenolic compounds using ferric chloride ($FeCl_3$) solution to improve organic chemistry teaching and learning. Samples of phenolic compounds were used in the student experiment. Students worked in pairs. Experiment 1 can be carried out in one 3-h lab period and Experiment 2 in two 3-h lab periods.

Experiment 1: Visually observation method

The ferric chloride solutions (1% w/v) were prepared in ethanol. A mixture of 1 mL of 1% $FeCl_3$ and 1 mL of 10 mM methanolic solution of the phenolic compound or sample were made in a white palette and mixed for 5 minutes. After incubation, the colors of phenolic–Fe^{3+} complexes were observed and visually compared with that of the reference solution. A solution of methanol and 1% $FeCl_3$ (1:1) was used as the reference solution.

Experiment 2: UV-Vis spectrophotometric method

After incubation for 5 minutes, the solution from Experiment 1 was measured using a UV-Vis spectrophotometer with a full scan mode from 200-900 nm. The results of the phenolic–Fe^{3+} complexes data were compared with the corresponding reference solution.

Hazard

Only very low concentrations of the samples were employed to minimize chemical hazard. However, methanol and ethanol are low boiling and highly flammable liquids, the experiment should be done in a fume hood or in a well-ventilated area. In addition, safety goggles, gloves and appropriate clothing are needed at all times.

Results and Discussion

The detailed information including a student handout, flow charts, copies of actual spectra and reports of student assessment, is provided in the Supporting Information. The Fe^{3+} forms many colored complexes with ligands including phenolic compounds which are good electron donors. This reaction occurred when the phenolic compound donate electron donor atom (hydroxyl group, -OH) to metal ion or radical form complexes to metal chelation and reduced metal-induced oxidative reaction from Fe^{3+} to Fe^{2+} [29, 30]. In this paper, we present a low-cost detector, designed to classify the types of phenolic compounds based on visual observation of colors with a simple enough concept and operation to be used as a teaching tool. The data collected were observed by students and provided the basis for discussion and lectures on type of phenolic compounds principles. The phenolic standard compounds including phenol, catechol, resorcinol, hydroquinone, protocatechuic acid, caffeic acid, *p*-hydroxybenzoic acid, gallic acid, and protocatechualdehyde were used in the experiment. The solution of 1% $FeCl_3$ in ethanol was used as the control solution (yellow solution). The results are shown in Figure 1.

The effects of the different substitutions of the hydroxyl group on the aromatic ring were studied. The phenolic compounds contained *ortho*–dihydroxy (catechol) and *ortho*–trihydroxy groups showed a significant color change from yellow solution to dark green solution (Figure 1). On the other hand, phenolic compounds contained only one or two hydroxyl groups (*meta*- and *para*-substitution) showed nonsignificant color change when compared with control solution.

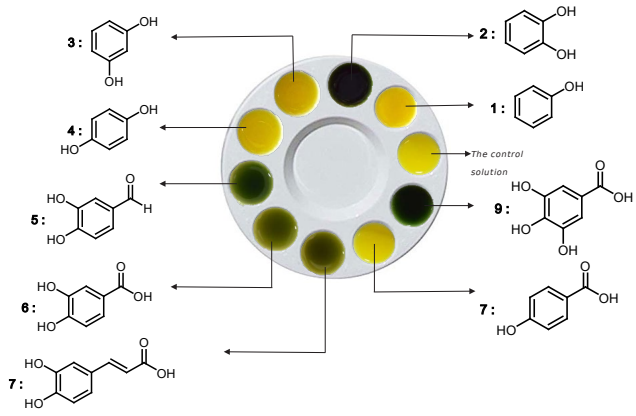

Figure 1. The visually observation the color of phenolic–Fe^{3+} complexes phenolic including phenol (**1**), catechol (**2**), resorcinol (**3**), hydroquinone (**4**), protocatechualdehyde (**5**), protocatechuic acid (**6**), caffeic acid (**7**), *p*-hydroxybenzoic acid (**8**), and gallic acid (**9**) in white palette, comparing color of phenolic-Fe^{3+} complexes with the control solution.

The effects of other substituents on the catechol moiety including formyl, carboxyl, and α,β-unsaturated carboxyl groups showed different color shade in a dark green solution. Protocatechualdehyde showed deep yellow green solution and protocatechuic acid also showed light yellow green solution while caffeic acid observed moderate yellow green solution. In addition, the influence of the number of hydroxyl groups on the colors of the phenolic-Fe^{3+} complexes were studied. The *p*-hydroxybenzoic acid, protocatechuic acid, and gallic acid were use in the experiment. From the result in Figure 1, this method also classified the type of phenolic in these groups. All phenolic-Fe^{3+} complexes with *ortho*-dihydroxy and *ortho*-trihydroxy groups observed the absorbance at 650-700 nm which could not classified the type of phenolic compounds (Figure 2).

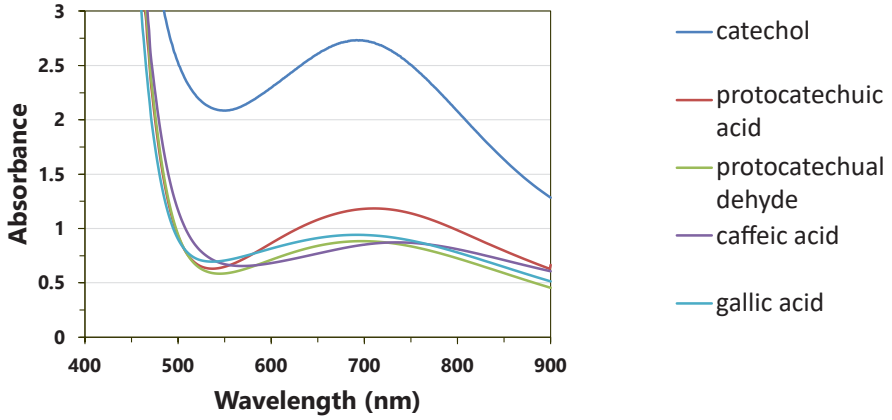

Figure 2: The UV-Vis absorbance spectra of Phenolic–Fe^{3+} Complexes in phenolic compounds contained *ortho*–dihydroxy (catechol) and *ortho*–trihydroxy group including catechol, protocatechualdehyde, protocatechuic acid, caffeic acid and gallic acid.

Created a new education innovation for teaching organic chemistry

We created activities for classify phenolic compounds using ferric chloride solution. These activities were separated into two parts, the first part was a visual observation activity for teaching students in functional group topic. The phenolic-Fe^{3+} complexes were developed different color change according to type of phenolic compounds. And the second part, the UV-Vis spectrophotometric was use to result from part one. The first activity can be carried out in one 3-h laboratory period and the second in two 3-h laboratory periods. Moreover, student work sheet and the flowchart guideline were developed for instructional student assistant in organic chemistry subject (Supporting Information). These activities were therefore an ideal high school or undergraduate experiment to improve organic chemistry teaching and learning.

Conclusions

The classification of types of phenolic compounds based on colors visual observation was reported. Phenolic compounds contained *ortho*–dihydroxy and *ortho*–trihydroxy groups developed the most significant in the color change. A rapid qualitative method is therefore an ideal for high school or undergraduate to improve organic chemistry teaching and learning.

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