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Communication

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"Supermarket Column Chromatography of Leaf Pigments" Revisited: Simple and Ecofriendly Separation of Plant Carotenoids, Chlorophylls, and Flavonoids from Green and Red Leaves

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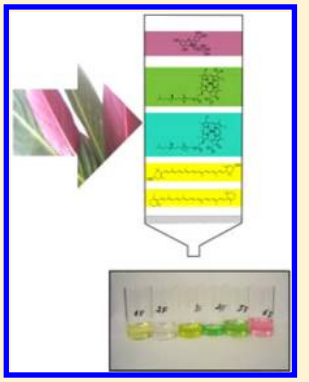
Supporting Information

ABSTRACT: A simple and ecofriendly procedure was developed in order to prepare extracts from red and green leaves. This procedure enables the separation of yellow, green, and red band pigments and optimizes the previously reported baking soda "supermarket column". The same extract also led to a novel and colorful potato starch column, which can efficiently separate β-carotene, xanthophylls, chlorophylls *a* and *b*, and flavonoids. Thus, two simple and attractive "supermarket columns" are presented that may be implemented either in university or high-school practical classes. The challenges associated with separation of these pigments, which have diverse physical and chemical properties, will improve the expected learning outcomes of these activities.



KEYWORDS: *High School/Introductory Chemistry, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Chromatography, Dyes/Pigments, Green Chemistry, Plant Chemistry, Separation Science, First-Year Undergraduate/General*

Plant pigments are privileged molecules for chemical education because they may have a crucial value for the student's image of chemistry in the real world.[1,2] They are well known both for the beautiful colors of the vegetal world and for their human nutritive value.[3] Apart from their essential roles in plant biochemistry, these molecules are also associated with increased health benefits and industrial applications.[3−5] The typical pigments of plant leaves are the green chlorophylls and yellow-orange carotenoids. They occur in chloroplasts playing essential functions in photosynthesis. Certain plant leaves also have significant flavonoids content, in particular anthocyanins that give leaves beautiful red/purple colors.[6] Color variations in leaves may be determined by season, nutrients, pH, and chemical interactions.[5,6]

The traditional separation of chloroplast pigments in green leaves through column chromatography techniques is highly recommended as an activity that motivates young students, because attractive green and yellow bands are developed in the columns.[7−13] However, this well-known experiment usually involves complex procedures, requiring the use of special laboratorial techniques/equipment and the manipulation of toxic materials. These drawbacks have limited the use of this experiment to university laboratory classes. Simpler and greener techniques are required to extend this interesting activity to high-school laboratory classes.

Kimbrough described an original "supermarket column chromatography" for separating pigments from red and green leaves using baking soda as adsorbent and common solvents as eluents.[14] Further developments of this experiment are now reported. Two new and more efficient columns giving yellow, green, and red bands are described. The extensive studies of the Kimbrough experiment, which led to the development of these new columns using green chemistry principles, are described in the Supporting Information (Part I). The results obtained in these studies showed that this interesting experiment, although simple and capable of separating flavonoids from the chloroplast pigments, fails to split green and yellow chloroplast pigments. Preparing a solid extract could circumvent this experimental drawback. A suitable solid extract was obtained by simple and sustainable procedures that avoid pigments degradation. The use of this novel approach led to an excellent optimization of the Kimbrough baking soda "supermarket column". Attempts have also been made to adapt this novel extract to columns packed with the previously reported cornstarch or powered sugar adsorbents;[8,9] however, these columns clogged during elution of the anthocyanins. The use of potato starch as adsorbent led to a novel and more efficient "supermarket column" that meets all the initial goals.

The optimized baking soda column is reported in this paper as well as the novel potato starch column, as two alternative or subsequent methods to separate not only carotenoids and chlorophylls but also anthocyanins obtained from red and green leaves (detailed procedures may be found in Supporting Information, Part II). These two attractive and challenging "supermarket columns" may be implemented either at university or high-school practical classes. Lipophilic and

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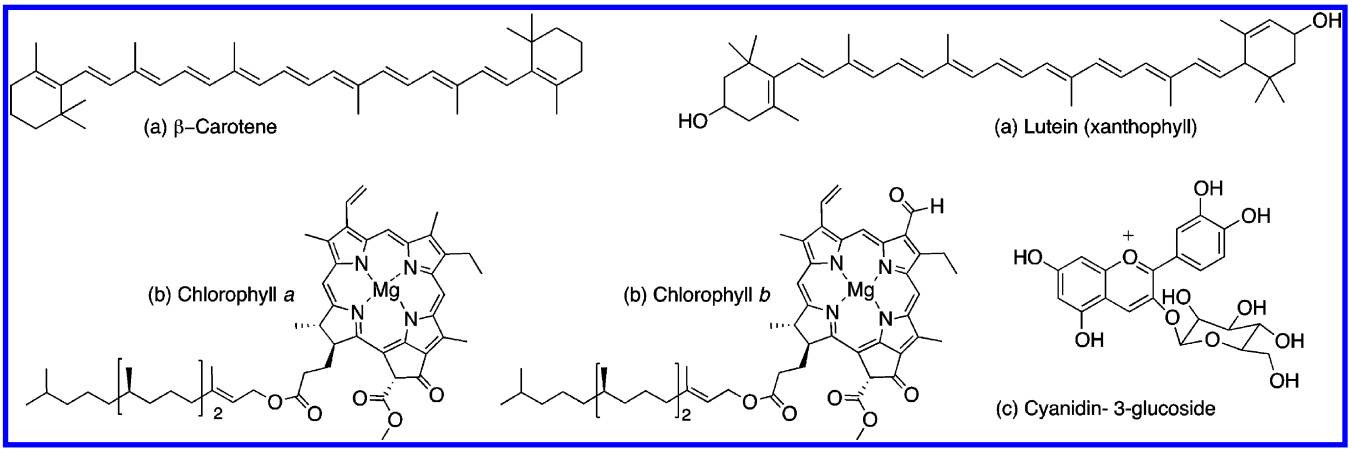

Figure 1. Representative molecules of red and green leaves:[3] (a) carotenoids (β-carotene and lutein); (b) chlorophylls (chlorophylls *a* and *b*); (c) anthocyanins (cyanidin-3-glucoside).

hydrophilic properties, as well as a wide range of molecular polarities, are well represented by these green and red leaf pigments (Figure 1). These properties may be readily associated with their structures balancing the prevalence of nonpolar hydrocarbon units or polar nitrogen and oxygen groups present in their molecules. Thus, the challenges associated with the extraction and separation of this series of pigment molecules will improve the conceptual understanding provided by these activities.

■ EXPERIMENT

Fresh *Stromanthe sanguinea* (Figure 2a) leaves were cut with scissors and crushed using a mortar and pestle. The leaf pigments were extracted by maceration in acetone. The crude extract, obtained by filtration, was treated with a desiccating agent (kitchen salt or anhydrous sodium sulfate, Figure 2b). After elimination of salt by filtration, adsorption onto the surface of commercial potato starch led to a solid extract (5−10 min, Figure 2d) ready to be directly applied onto a baking soda (Procedure A) or potato starch column (Procedure B). A disposable syringe (20 mL) was packed with one of these adsorbents and the solid extract was applied on the top of the packing, immediately after pre-elution the column with petroleum ether. The solvents chosen to elute pigments were petroleum ether 40−60 °C, mixtures of petroleum ether 40−60 °C/acetone, acetone, ethanol, and saturated aqueous baking soda solution, in an adaption of previous procedures.[13,14] The fractions were isolated according to the visual variations in the color of the separated bands. The composition of these collected fractions was evaluated by paper chromatography using a mixture of petroleum ether 40−60 °C/acetone 90:10 as mobile phase. The pigments were identified by their characteristic colors and polarity (Figure 2c).

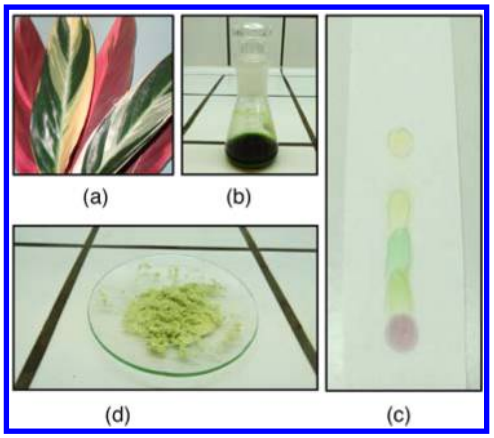

Figure 2. (a) *Stromanthe sanguinea* "tricolor"; (b) crude extract under anhydrous sodium sulfate; (c) paper chromatography obtained from liquid (b); (d) solid extract prepared by adsorption of the liquid (b) on the surface of potato starch.

Procedure A: A syringe was packed with baking soda. Elution was started with petroleum ether and changed to a mixture of petroleum ether/acetone 90:10 after elution of the yellow band. A 75:25 mixture eluted the green bands. Acetone and ethanol were subsequently passed through the column to remove minor polar pigments. Saturated aqueous baking soda solution eluted anthocyanins, identified by the red color developed after adding aqueous 1 M HCl.

Procedure B: The syringe was packed with potato starch. Elution was started with petroleum ether and changed to a mixture of petroleum ether/acetone 90:10 after elution of β-carotene. The eluent was changed again to a mixture of petroleum ether/acetone 75:25 to elute chlorophyll *b*. Acetone was passed through the column to remove minor pigments. Ethanol (or additional acetone) was used to elute flavonoids. This last fraction turned red with addition of aqueous 1 M HCl.

■ HAZARDS

Cornstarch, potato starch, and sugar are common household products with no adverse health effects expected from inhalation, ingestion, and contact with skin or eyes (in the doses described). Sodium bicarbonate (baking soda) may cause mild irritation by contact with the skin and eyes or by inhalation. In large doses, it may cause gastrointestinal disturbances when ingested. Acetone and petroleum ether are skin and lung irritants. These solvents should be manipulated in a hood, using protective gloves and goggles. They are also flammable liquids, so no exposed flames should be used in the laboratory when these experiments are being performed. The glass material should be handled with care and safety goggles and gloves should be used by students.

■ RESULTS AND DISCUSSION

Many introductory chemical concepts can be readily demonstrated by the green and red leaf pigments, with the aid of their

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beautiful colors. Additionally, this experiment provides an excellent opportunity to introduce foundations of chromatography, offering a memorable demonstration to young students of the wide scope of this technique.

The extraction of lipophilic and hydrophilic pigments from leaves and their application onto a chromatography column add solubility challenges that will allow students to understand molecular polarity, intermolecular forces, and solubility concepts. The analysis of the liquid extract by paper chromatography (Figure 2c) will allow teachers to introduce the principles of chromatography. They should stimulate students to envisage the elution process in the column chromatography. The importance of a gradual increase in the polarity of solvents entering the column must be emphasized. This will help students to understand the need to eliminate acetone and water from the crude extract. The natural hydration capacity of kitchen salt or anhydrous sodium sulfate was used to achieve the elimination of residual water (Figure 2b). Simple adsorption on to finely divided solid starch facilitated rapid evaporation of the acetone leading to a suitable extract (Figure 2d) for application onto the column. Concepts of adsorption and hydration may be discussed with the students and can be supported with several examples chosen from every day life.

Visual analysis of the chromatographic separation of pigments will subsequently enable the correlation of molecular structures (Figure 1) with pigment affinities for mobile and stationary phases (Figures 3 and 4). This practical exercise will serve to demonstrate foundations of chromatography.

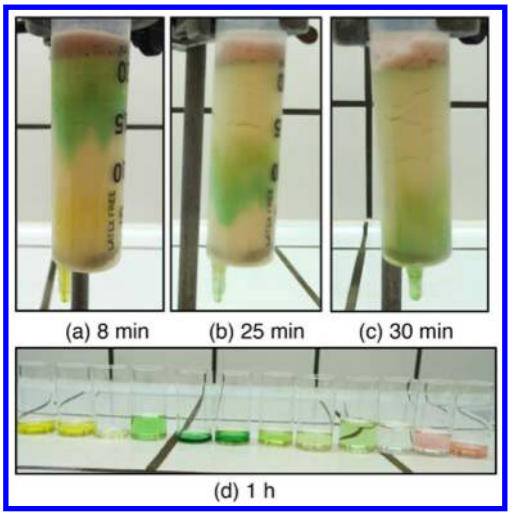

Figure 3. (a) Baking soda column 8 min after elution with petroleum ether; (b) same column immediately before elution with petroleum ether/acetone 75:25; (c) same column during elution with petroleum ether/acetone 75:25; (d) all the fractions collected in this experiment.

Red, green, and yellow bands were separated on both columns by elution with petroleum ether (Figure 3a and 4a). Anthocyanins, the water-soluble pigments, are highly adsorbed onto the neutral starch applied on the top of the two columns and these molecules are readily identified by their pink coloration. Strong hydrogen bonds are expected to be established between the starch and the hydroxyl-rich anthocyanins. Carotenoids and chlorophylls, the lipophilic pigments, were displaced at different rates by the petroleum ether. Green chlorophylls moved more slowly than lesser polar

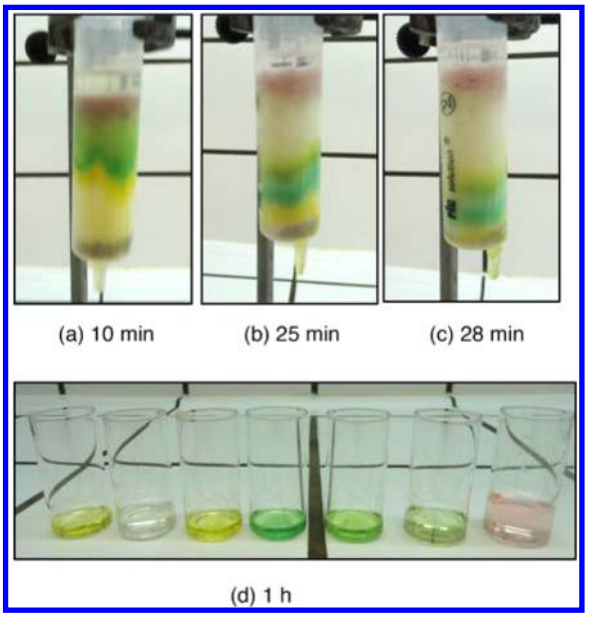

Figure 4. (a) Separation of β-carotene and xanthophylls on a potato starch column by elution with petroleum ether; (b and c) Separation of xanthophylls, chlorophyll *a* and *b* on the same column by elution with mixture of petroleum ether/acetone 90:10; (d) all the fractions collected from leaves (2a).

carotenoids. The hydrocarbon phytyl chain of chlorophylls gives them solubility in this mobile phase, but the polar nitrogen and oxygen functional groups are responsible for the substantial adsorption on both columns. The yellow carotenoids moved more quickly than chlorophylls. The higher solubility of carotenoids in petroleum ether is due to the predominance of hydrocarbon chains in their structures. Baking soda columns separate carotenoids efficiently from chlorophylls, but separation of β-carotene and xanthophylls was not achieved by elution with petroleum ether (Figure 3a). In the potato starch column, the same solvent eluted the nonpolar β-carotene, while xanthophylls were retained by starch (Figure 4a) due to the presence of the oxygen polar groups (Figure 1a).

By increasing the polarity of the eluent to a mixture of petroleum ether/acetone 90:10, three well-defined yellow (xanthophylls), blue-green (chlorophyll *a*), and yellow-green (chlorophyll *b*) bands were obtained (Figure 4b). This mixture separated xanthophylls (Figure 4c) and chlorophyll *a*, whereas a mixture of petroleum ether/acetone 75:25 was required to elute chlorophyll *b*. Chlorophyll *b* moved more slowly than chlorophyll *a* due to the presence of a more polar aldehyde group in this structure (Figure 1b). In the baking soda column, separation between chlorophylls *a* and *b* was moderate and it was very difficult to collect the two chlorophylls separately (Figure 3b–d). The elution of anthocyanins could be achieved with acetone or ethanol in the potato starch column, but saturated baking soda aqueous solution was necessary in the baking soda column. These results demonstrate that potato starch columns led to excellent separations of *Stromanthe sanguinea* leaf pigments. Small differences in the content of the collected fractions were obtained when different *Stromanthe sanguinea* plants were used (Figures 4d and 5a,b).

A different flow rate was observed in these two columns, but in both cases the elution may be completed in 1 h with columns. A 40–60 min period is required for preparation of the extract and column packing. The time required to perform each

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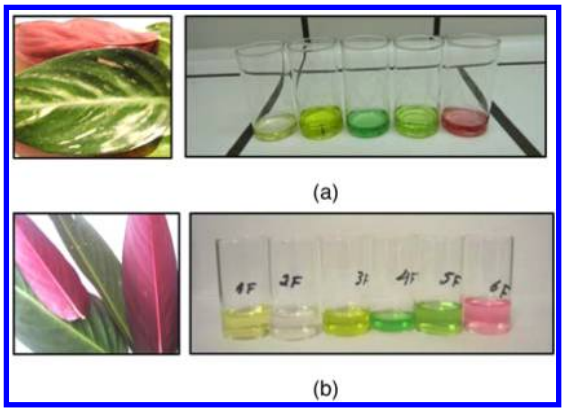

Figure 5. (a–b) Main fractions collected from potato starch columns when leaves of different *Stromanthe sanguinea* plants were used.

of these two activities is a practical class of 2–3 h. For high-school classes of 60–90 min duration, two sessions may be used. The extract may be prepared in the first session, but it must be maintained at 8 °C and protected from moisture and light, and the column separation session must be performed within the next 24–48 h. The choice between these column chromatography techniques, and the use of both columns, must depend on the time available, objectives defined for the practical classes and the laboratory pedagogy adopted. For university-level classes, the superior potato starch column chromatography must be implemented in introductory chemistry courses. Organic chemistry curriculum may benefit from a guided inquiry laboratory project by incorporating the developmental studies described in the Part I of Supporting Information.[15]

■ SUMMARY

The traditional experimental activity of column chromatography of chloroplast pigments from green leaves was improved. The new approach, using red and green leaves, leads to higher visual impact and involves simpler and greener procedures. To achieve these goals, a simple and ecofriendly procedure was developed to prepare extracts of red and green leaves. Two efficient and challenging "supermarket columns", that may be useful either at the university or high-school practical classes, were established exhibiting yellow, green, and red bands. In particular, an excellent separation of β-carotene, xanthophylls, chlorophyll *a* and *b*, and flavonoids could be obtained by a novel potato starch column chromatography. The challenges associated with the additional separation of flavonoids will broaden the learning experience of this activity and allow teachers to design laboratory pedagogies for exercising student critical thinking.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details of our developmental studies; experimental procedures for activities that can be implemented in high school or university classes. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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