A microscale experiment for organic chemistry: column chromatography of pigments of *Capsicum frutescens*

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A microscale column chromatography using a Pasteur pipette as column, silica gel as adsorbent, and hexane-dichloromethane-methanol as eluting solvent was done to separate the colored pigments of red pepper (*Capsicum frutescens*). Four very distinct bands were collected: a yellow band was first eluted followed by a light orange, the major red colored band, and a light brown band.

Keywords: microscaling, column chromatography, *Capsicum frutescens*

**COLUMN CHROMATOGRAPHY IS AN ANALYTICAL OR SEPARATORY procedure based on reversible sorption and desorption of components of a mixture on a preparative scale** [1]. The importance of such technique cannot be overlooked in the undergraduate laboratory, but the cost of equipment and reagents is a major consideration. A study done to replace silica with sand as adsorbent in the separation of plant pigments was excellent in large scale [2]. Still, the amounts of reagents required have raised some safety concerns that have forced some to forego column chromatography experiments in the undergraduate laboratory. Experiments are being looked into like caffeine being isolated from tea using microscale column chromatography, to cut-down amounts of reagents being used [3].

This paper describes a microscale experiment on column chromatography for the undergraduate organic chemistry course. We have, for many years, removed this from our curriculum due to the cost of reagents, safety considerations and the time allotment for the experiment. We have now found that the separation of colored pigments of *Capsicum frutescens* using microscale column chromatography is an easily workable and fast technique that has reduced amounts of reagents to micro quantities.

*Capsicum frutescens*, locally known as *siling labuyo*, contains several brightly colored pigments. A related species, *Capsicum annum*, is reported to contain the following pigments: a major red colored pigment known to be a fatty acid ester of capsanthin (1) and a minor red colored pigment fatty acid ester of capsorbin (2). It also contains the yellow pigment β-carotene (3) [4]. *C. frutescens* was chosen on the basis of its availability.

**EXPERIMENTAL**

**Preparation of the Column.** Commercially available Pasteur pipette (150 mm) or a 10 cm glass tube (19 mm in diameter) drawn at one end was used as a column. A cotton plug was placed at the bottom and the column was clamped to an iron stand. A slurry of 0.75 g silica gel in 5 mL hexane/dichloromethane (1:1) was prepared, stirring to eliminate air bubbles. The mixture was swirled to suspend silica gel in the solvent, then it was immediately poured into the Pasteur pipette. The solvent was allowed to drain until it...
Figure 2. Column chromatography using Pasteur pipette

Four bands were eluted: yellow, light orange, red and light brown. The most non-polar component pigment was the yellow band, being the first to be eluted from the column. The light orange component was second followed by the red band considered the major component because of the color intensity and the volume of the eluent. A fourth band which is the most polar was light brown.

The procedure can be carried out within a single 3-h laboratory period with the elution completed in thirty (30) minutes. It brought excitement to the students seeing the brightly colored yellow and the wide major red bands distinctly descending the column. Efficiency of the column depends also on how well the students prepared the column. Air bubbles in the column can be avoided by tapping the column lightly while being packed. The solvent system for elution should always be freshly prepared.

CONCLUSION

The four major component pigments of *Capsicum frutescens* can be isolated in the organic laboratory class using simple and low-cost microscale column chromatography. The separation of components can be explained on the basis of polarity, adsorptivity and solubility. The set-up can be easily assembled by the students and the experiment can be finished in a 3-hour laboratory class.

REFERENCES