Continuous Solid Phase Extraction with Juice Pigments

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Introduction:

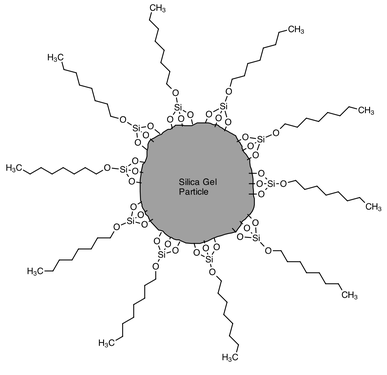
This experiment focuses on the continuous separation of pigments from organic fruit juices. Juice pigments are used in various types of natural food dyes. Juice pigments can be separated by a process known as Solid Phase Extraction (SPE). SPE is traditionally done with one column and has four basic steps: equilibrating, loading, washing, and rinsing the column (**Figure 3**). This can be a time consuming process when it requires that large amounts of material be separated. With the use of the Semba® Biosciences Octave 100 (**Figure 1**), the SembaPro script software, and its valve block working together these instruments are able to run and control up to eight columns simultaneously. The Octave 100 allows for flexibility in running different methods of liquid chromatography. Because of this we were able to create a new continuous SPE method, separating target pigments from organic fruit juices. This application represents a novel use for the instrument beyond its primary design. This method would utilize four columns; each column being in a different stage of separation and rotating through the stages. This rotation of stages effectively allows for continuous separation of pigments from non-pigment compounds, like sugars, in organic juices. Using a continuous process can free up time and allow for large amounts of juice to be separated.

Figure 1:Semba® Biosciences Octave 100 system.

The top unit is the control module that will trigger the switching of solvents that are going into the valve block. The middle unit consists of the pumps that, which can pump a different solvent mixture at different rates into each column. The bottom unit is the valve block system that allows for the switching of solvents going in and out of the columns.

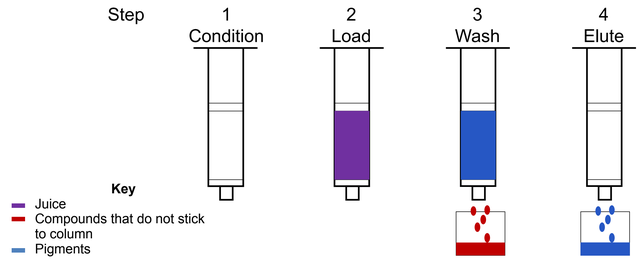
In order to make this process with one column into a continuous process, three columns are added. Each of these columns undergoes each step done in the single column process, but each of the four columns will be in a separate step of the process. With no two columns in the same step at the same time. This process can be seen in **Figure 4**.

Figure 2: C8 packing material, tiny sized beads with C8 hydrocarbon tails



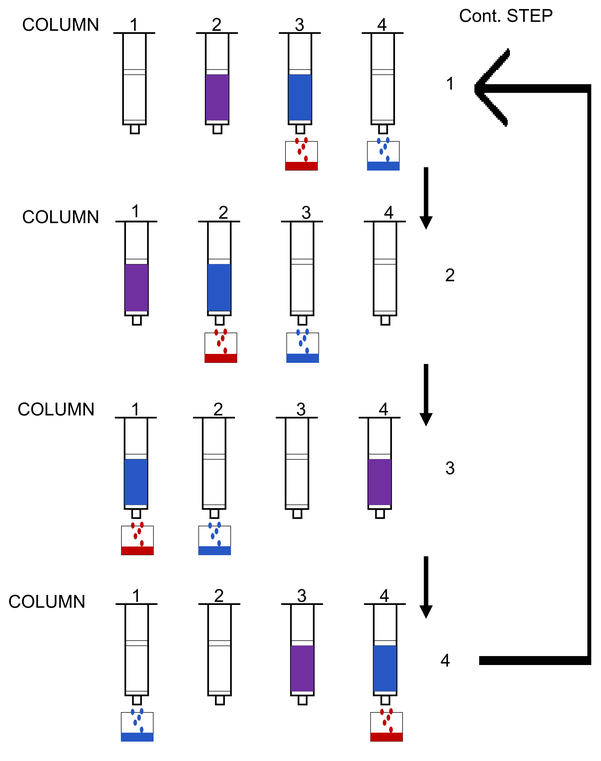
In all cases, columns are packed with tiny sized beads that have hydrocarbon tails on them (**Figure 2**). The pigments that are being separated are attracted to these and thus stay inside the column when the column is flushed with water. Everything else that isn't attracted to these beads will get flushed out of the column and will be in the waste fraction. All the waste is environmentally friendly. It consists of the remains of the juice minus the pigments and water. This waste usually gets dumped down the drain.

Figure 3: Single column separation



The column is preconditioned with a water wash **(Step 1)**. The filtered juice is injected into the column **(Step 2)**. The pigments are attracted to these particles and therefore stick to the column. The column is flushed with a water to remove all compounds except for the pigments from the column **(Step 3)**. The column is then flushed with a mixture of methanol and ethyl acetate that will remove the pigments from the column **(Step 4)**. This final wash is collected and the eluted mixture is evaporated off so all that remains are the pigments. This is the method seen above in **Figure 3**.

Figure 4: Continuous separation using four columns



The continuous separation method uses the same steps as in the single column method, except that each step in the single column method happens to a different column in step 1 of the continuous **(Cont. Step 1)**. The next step in the continuous method will shift the steps one column over **(Cont. Step 2)**. This will happen again two more times **(Cont. Step 3) & (Cont. Step 4)**. Then, it will revert back to step 1 in the continuous method and repeat until stopped.

Figure 5: Table of Starting, Ending, and Percent of Starting Masses

Juice	Starting Mass	Ending Mass	Percent of Starting Mass
Cranberry Run 1	0.6717g	0.0727g	10.82%
Cranberry Run 2	0.6717g	0.0586g	8.72%
Blueberry	33.81g*	1.209g	3.046%

*Note: mass calculated based on juice used

In the table above the masses of cranberry and blueberry powder was taken. After running the juices through the continues SPE method the ending masses were taken and the resulting percentages were calculated; this mass should be mostly pigments. The percentages of the starting masses are typical of the percent of pigments that are in fruit juices. Through other research, cranberry has shown to have ~6% of the mass being pigments.

The data above implies that the pigments of cranberry juice are being separated from the juices, but the separation of these pigments isn't perfect yet. Further method development is needed to insure the complete or nearly complete separation of pigments from the cranberry juice. The data of the blueberry juice shows that the method developed for separating pigments from the blueberry juice was a success. No more method development is needed for that specific juice. Further work on the method development in combination with the Semba® Bioscience Octave 100 instrument and software will allow other pigments to be separated from other organic juices. Some of these juices could include grape, orange, apple, pomegranate, and many more. The pigments from these various juices could provide vibrant colors that could be used to naturally dye food and food products.

Future work could include:

1.Finish method development of cranberry juice separation to insure that only pigments are being separated.

1.More juices to test separation of pigments

1.Method development for each new juice being tested, including filtering techniques before separation can be done.

Figure 6: Pigments

Cranberry pigments on right.

Blueberry pigments on left

References:

Product literature from Semba® Biosciences, (www.sembabiosciences.com)

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