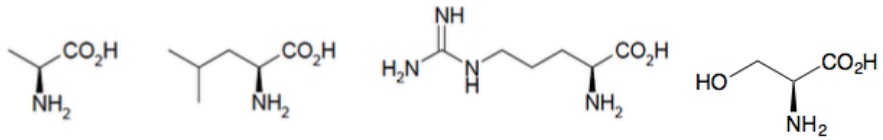
KJE2003 Lab 3: TLC OF AMINO ACIDS -
PRINCIPLES OF RETENTION AND DETERMINATION OF UNKNOWN

Learning goals:

1. Be able to conduct TLC analysis
2. Understand and explain relative retention times based on compound and column polarity.
3. Understand and explain eluent strength and how to tune mobile phase composition to obtain optimal separation resolution.
4. Be able to identify unknown compound by TLC comparison of known standards.

Chemicals and risk assessment:



(+)-*L*-Alanin (Ala) | (−)-*L*-Leucin (Leu) | (+)-*L*-Arginin (Arg) | L-Serin (Ser)

1-butanol	H226, 303, 315, 318, 335, 336	P261, 280, 305+351+338	
eddiksyre	H226, 314	P280, 305+351+338, 310	
ninhydrin	H302, 315, 319, 335	P261, 305+351+338	

(eddiksyre = acetic acid) Please look up the MSDS sheets for each chemical to identify H and P sentences above, and **fill out the Job Safety Analysis form *before* the lab starts.**

Equipment

Capillary tubes 200 mL beaker TLC plates Filter paper watch glass

Procedure

– **Preparation for this experiment involves having seen the introductory video lecture on TLC from MIT which is available on Fronter under the "Resources" drop-down menu.** The lab will start with a quiz from the video lecture.

– Each group prepares a solution of an amino acid from above selection in water (ca 5 mg in 1-2 mL water). We should then have four known solutions of different amino acids and one solution of unknown amino acid.

To think about now:
Can you rank the given amino acids in order of decreasing polarity based on the structures?
What relative migration distances would you predict to observe in the TLC analysis (qualitatively)?

– Make a solution of the unknown sample as above.

– Prepare a 5 x 10 cm silica TLC plate for analysis of 5 samples.

To think about now:
What is important to consider when preparing a TLC plate for analysis?
How far from the bottom should be base line be drawn?
Why should you use pencil and not a pen?

– Apply each sample 2-3 times on each spot and remember to wash the capillary tube between spottings to avoid cross contaminations.

– Elute the TLC plate with a solvent system consisting of *n*-butanol: acetic acid : water in a ratio of 3 : 1 : 1 (v/v). Let the plate dry in the fume hood. Visualize the plate using a ninhydrin solution. Use tweezers to dip the plate in the ninhydrin solution, let it drip off on a paper towel and then dry it gently with a heat **gun in the fume hood.**

To think about now:
How can you tune the given solvent system to increase its polarity?
...to decrease its polarity?
What are the relative polarities of butanol, acetic acid and water?
Find out what the structure of ninhydrin is and how it works?
Why is it chosen in this analysis?

– Determine the retardation factor R_f (using two significant digits) for each amino acid and determine the identity of the unknown sample.

To think about now:
Explain the relative migration distances! Do they fit with your predictions?

– Prepare three more TLC plates for analysis of 1 sample each. Spot an amino acid of choice (the same one on all three plates). Calculate retardation factors.

Run one plate in *n*-butanol: acetic acid : water in a ratio 6 : 1 : 1 (v/v).
Run the second plate in *n*-butanol: acetic acid : water in a ratio 3 : 2 : 1 (v/v).
Run the third plate in *n*-butanol: acetic acid : water in a ratio 3 : 1 : 2 (v/v).

– Record your observations and explain.