Name: ______________________

Odorants as pH Indicators

Background

Fresh baked bread? Hot chocolate? Dirty socks? Cat litter box? When you smell something, you are smelling chemicals. More specifically, you are smelling chemicals called *odorants*. These odorants trigger receptors at the top of your nasal cavity, which your brain eventually interprets as an *aroma* or *odor*.

Some aromas can be explained by a single odorant. For example, "white vinegar", the type commonly found in home kitchens, is a 5% solution of acetic acid (CH_3COOH) in water, and its aroma is solely due to acetic acid[1]. Most real aromas, including baked bread and chocolate, result from the combination of dozens of volatile chemicals. Put another way, no single odorant compound smells just like "chocolate" all by itself.

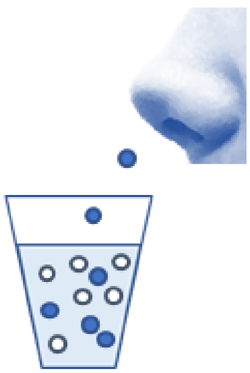

Figure 1. Cartoon of a volatile compound (acetic acid, filled circle) and a non-volatile compound (sugar, open circle) dissolved in a glass of water

Odorants are *volatile* chemicals (a portion will be airborne). Chemicals that are not volatile (e.g. sugar) do not reach your nasal cavity, and have no smell (see Figure 1). The 'sweet' aroma of caramel is due to new odorants formed during heating of sugar, not the sugar itself. Not all volatiles are odorants. For example, water is volatile at room temperature, as is evidenced by the fact that water drops will evaporate. However, water has no smell, and is therefore not an odorant.

Even though they are volatile, most odorants in foods and beverages are not in the gas phase. For example, in a cup half-filled with vinegar, a small amount of acetic acid will volatize and enter the *headspace* – the space above the liquid. At room temperature, less than 1 out of 100000 acetic acid molecules will be in the headspace, with the rest still in the vinegar[2]. This ratio is typical of most odorants, and it's useful for cooks! If the situation was reversed, we would only get one intense sniff of our food or drink, and then all of the aroma would be used up!

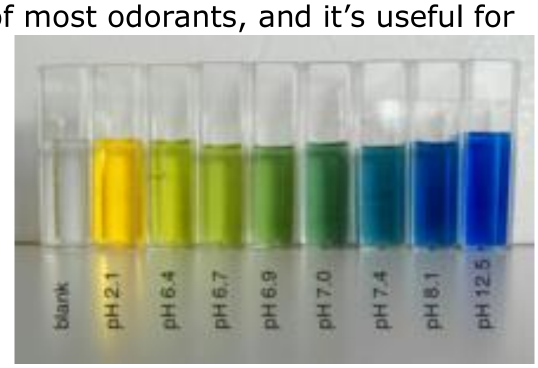

Figure 2. Bromothymol blue, an example of a color-changing pH indicator (Photo courtesy of Gregor Trefalt; Wikimdeia Commons)

Acetic acid can also be used as an "odorous" pH indicator. Typically, pH indicators are chemicals that <u>change color</u> when the pH of a solution changes. For example, bromothymol blue will appear yellow at a pH < 6 (acid), green between pH 6-8 (neutral), and blue at pH >8 (basic). The reason pH indicators change color is because they are weak acids and bases themselves, and their ability to absorb light changes as they gain or lose protons (H^+).

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[1] Technically, acetic acid is not a simple odorant – it is also perceived by your sense of touch, causing an irritating sensation. For simplicity, we will treat both the odorous and tactile sensations caused by acetic acid as a smell.

[2] At low acetic acid concentrations, like vinegar, the relative proportion of acetic acid in the headspace and the liquid will be constant even if the acetic acid concentration is changed. This is described by Henry's Law, which is discussed in other lectures.

The situation is similar for acetic acid, except that instead of a color change, its volatility changes with pH. At low pH, acetic acid exists primarily in its neutral, acidic form (CH_3COOH, [HA]). As the pH increases acetic acid, it will lost a proton to form its conjugate base, acetate (CH_3COO^-, [A-]). The acid dissociation constant (pK_a) of acetic acid (4.5) describes the pH at which acetic acid and acetate exist in a 50-50 ratio. This relationship is described by the Henderson-Hasselbach equation:

$$pH = pK_a + \log_{10}\left(\frac{[A^-]}{[HA]}\right)$$

As a rule, some neutral compounds are volatile, but charged compounds like acetate (CH_3COO^-) are always non-volatile (and thus don't have a smell). The effect of pH on acetic acid and acetate is depicted in Figure 3.

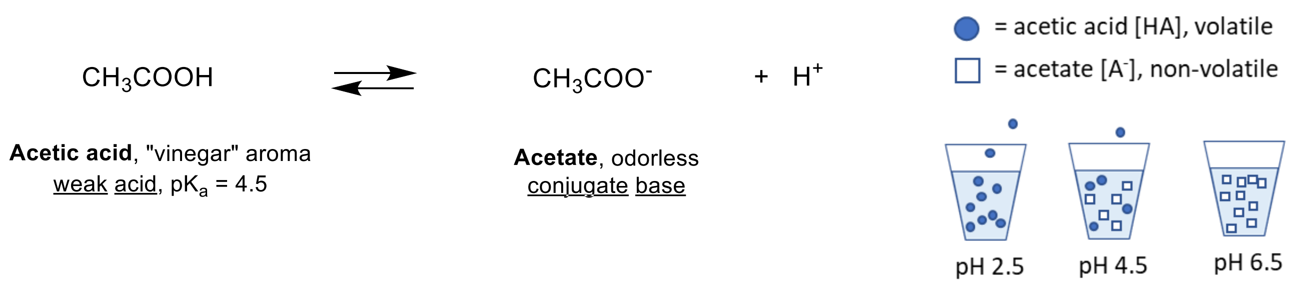
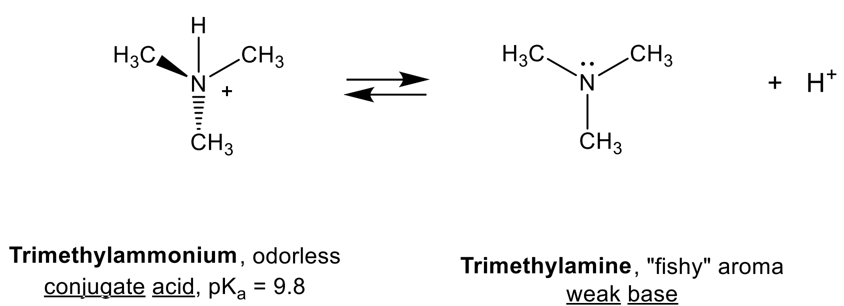


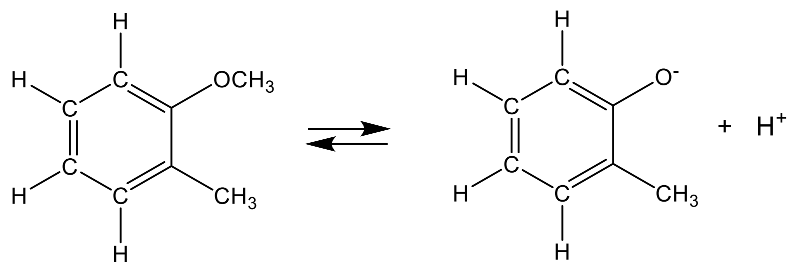
Figure 3. Acetic acid can lose a proton to form its conjugate base, acetate (left). The acetate form is favored when pH is greater than pK_a (i.e. pH > 4.5). Acetic acid is volatile, and acetate is non-volatile, and the acetic acid concentration in the headspace of a glass of vinegar will decrease with increasing pH.

Many other foods and beverages contain acidic or basic odorants.

For example, liquid aminos and soy sauce are both produced by breaking down soy protein into smaller compounds called amino acids. For soy sauce, this breakdown is through fermentation, while in liquid aminos a non-fermentative process is used. Both processes also produce basic odorants with "fishy, rotten" aromas, e.g. trimethylamine:



As a second example, roasting coffee beans generates many odorants, especially acidic odorants like guaiacol, which smells 'smoky' in its neutral form.



Guaiacol, "smoky" aroma
weak acid, pK_a = 10

Guaiacolate, odorless
conjugate base

Investigation Question
How will the odor of an acetic acid solution (white vinegar) change at low pH vs. high pH?

Hypothesis
Make a hypothesis statement about the investigation question. Be sure to justify it with reasoning.

Pre-lab Questions

1. If you have a glass and filled it partially with vinegar, what is the "headspace" of the glass? What would you find in the headspace?

2. Make a y vs. x sketch of the expected concentration of acetic acid in the headspace (y-axis) vs. the solution pH (x-axis) for pH values 3, 5, 8, and 10.5

3. How do you expect the coffee to smell after it is brewed? List 5 aroma descriptors (Hint: "coffee" is not an ok descriptor 😊. But, "smoky" would be an ok descriptor.)

Materials (per group)

- 5, 125 mL Erlenmeyer flasks with stoppers (or 200 mL jars with lids)
- 200 mL each of the following, in flasks or jars appropriate for pouring
  - Water
  - pH 3 buffer
  - pH 5 buffer
  - pH 8 buffer
  - pH 10.5 buffer
- Graduated cylinders or other equipment to measure 5-50 mL of liquids
- 50 mL White (distilled) vinegar
- 2 tbsp Instant coffee
- 20 mL Liquid aminos (soy sauce can be substituted, if needed)
- Paper towels (for spills)

Safety

- Always wear safety goggles when handling chemicals in the lab.
- Wash your hands thoroughly before leaving the lab.
- Do not consume lab solutions, even if they're otherwise edible products.
- Food in the lab should be considered a chemical not for consumption.
- In the event of any of the solutions spilling on your skin, immediately rinse the area with water and inform the instructor.
- When you have completed the lab, clean up materials and dispose of any chemicals as follows:
  - Solutions can be dumped down the drain; flasks can be rinsed out.
  - Spills on the lab bench and/or on the floor should be cleaned up with paper towels.
  - Students should wash their hands thoroughly before leaving the lab.

EXPERIMENT 1 – ODORANT INTENSITY IN SIMPLE SOLUTION VS. PH

Procedure

1. Put on your safety equipment.
2. Obtain 5 clean flasks. Label the flasks as follows: 'Water', 'pH 3', 'pH 5', 'pH 8', 'pH 11'.
3. Using a graduated cylinder, measure 25 mL of water, and pour it into the flask marked "water".
4. Repeat Step 3 for the pH buffers and their corresponding flasks. At the end, you should have five flasks, each containing 25 mL of either water or one of the buffers.
5. Using a graduated cylinder, add 5 mL of vinegar to each flask.
6. Stopper (cover) the flasks and swirl.

Aroma Evaluation

7. In each group, 1-2 people will be the “Sniffers”, one person will be the “Presenter”, and one person will be the “Recorder”. The Presenter will sit with the flasks several feet away from the Recorder and Sniffer.
8. The Sniffer will hold the ‘Water’ with vinegar flask. The presenter will keep the other pH adulterated flasks.
9. The Presenter will select one of the pH-adjusted flasks at random, show the label to the Recorder, and then give the flask to the Sniffer. The Sniffer should <u>not</u> look at the label.
10. The Sniffer should swirl the flask, remove the stopper, sniff the solution by wafting the aroma toward their nose, then tell the Recorder how strong the vinegar aroma is on a scale of 0 (no aroma, the smell of water alone) to 10 (as strong as the Water w/vinegar flask).
11. The Recorder notes the sniffer’s rating.
12. The Sniffer then hands the pH-adjusted flasks back to the Presenter.
13. The Presenter repeats steps 3-5 with a different pH-adjusted flask each time until four flasks have been sniffed and evaluated.
14. The Presenter, Sniffer, and Recorder then change roles, until all group members have sniffed the samples and evaluated the vinegar aroma.

Data

Data Table 1: Vinegar aroma vs. pH					
		Vinegar intensity, 0-10 scale: Reference: Pure water = 0, vinegar+water tube odor = 10			
Solution		Sniffer 1 (name)	Sniffer 2 (name)	Sniffer 3 (name)	Average
pH 3					
pH 5					
pH 8					
pH 10.5					

EXPERIMENT 2 – ODORANT PERCEPTION IN COMPLEX MIXTURES VS. PH

Procedure

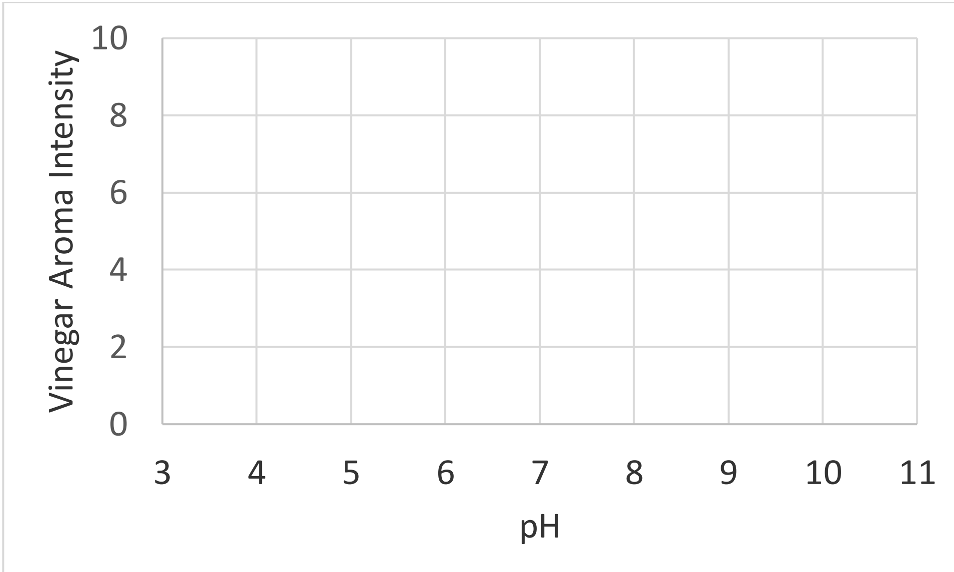
1. Keep your safety equipment on.
2. Rinse your flasks with clean water until they no longer smell like vinegar.
3. Using a graduated cylinder, add 25 mL of pH 3 buffer to the 'pH 3' flask and 25 mL of pH 10.5 buffer to the 'pH 10.5' flask. The other three flasks can be set aside.
4. Using a graduated cylinder, add 5 mL of liquid aminos to the pH 3 flask and to the pH 10.5 flask.
5. Stopper the flasks and swirl.
6. Each group member sniffs each solution, and records what they think it smells like in Data Table 2 (1-3 word descriptions).
7. Clean the flasks with water, and repeat the experiment in Steps 4-6 using 1.5 tsp (5 g) of instant coffee.

Data

Data Table 2: Odor character vs. pH for liquid aminos and instant coffee				
		Odor description (1-3 words per sniffer)		
Solution	Sample	Sniffer 1 (name):	Sniffer 2 (name):	Sniffer 3 (name):
pH 3	Liquid aminos			
pH 10.5	Liquid aminos			
pH 3	Instant coffee			
pH 10.5	Instant coffee			

Analysis: Experiment 1

1. Create a X-Y plot, as shown below, of Vinegar Aroma Intensity (average) vs. pH.



2. Explain your graph:

Analysis: Experiment 2

1. How did the "smoky" aroma of the low pH instant coffee compare to the high pH instant coffee? Does this mean that these smoky odorants are more likely to be acids or bases? Why?

2. How did the "fishy, rotten" aroma of the low pH liquid aminos compare to high pH liquid aminos? Does this mean that these rotten-smelling odorants are more likely to be acids or bases? Why?

Conclusions

1. What does it mean for a compound to be volatile? What does it mean for a compound to be an odorant? Do foods and beverages have more volatiles, or more odorants?

2. What is a pH indicator? What properties could be used to determine pH?

3. You are a flavor scientist at a major food company (yes, this is a real career path!) They want to create an alkaline bottled coffee (pH of 9) because consumers are interested in alkaline products. Based on your knowledge of aroma chemistry, explain why you think this idea is inadvisable.

4. Beyond pH, what other factors could affect the volatility of odorants, and a food or beverage aroma? Here's a couple of factors to consider.
   - *The volatility of compounds increases as temperature increases. How would this affect the amount of flavoring you would want to put into hot chocolate vs. chocolate ice cream?*

   - *Most odorants are non-polar – they dissolve well in oil, but not in water. Based on this, do you think you would need to use more chocolate flavor in skim chocolate milk or whole chocolate milk to have the same aroma intensity?*