

# To Smell or Not To Smell, That is the Ester

**Developers:**

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**Topic Area:**

Polymers: The effect of carboxylesterases on residual monomers.

**Grade Level:**

High School

**Discipline:**

Chemistry and Biology

**Goals:**

Students will be introduced to the concepts of polymerization. They will additionally explore the progressive steps followed in the determination of an approach to a scientific problem.

**Objectives:**

A student upon completion of this lab, will be able to:

- explain the basic concept of polymerization
- understand the basic functioning of enzymes
- apply the concept of “brainstorming” to approach scientific questions
- understand the undesirable effect of residual monomers in polymer production
- prepare a crude preparation of carboxylesterase and test its effectiveness in reducing the residual monomer in an emulsion such as paint.

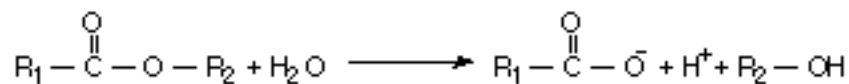
**Background Information:**

Polymerization is a very important chemical process which produces many products found in our daily living. Artificial fibers (nylon, dacron), synthetic rubber, plastics (vinyl, polyethylene, PVC pipes), chewing gum, starch, wool and paint are just a few polymers. Polymerization is brought about by the joining of small molecules to form long chains containing a repeating group. These small molecules are referred to as monomers. The joining of monomers can be achieved by the processes of addition polymerization or condensation polymerization. Chemists vary the use of catalysts, the choice of monomers and the selection of other conditions to control the reaction. This project involves water-based polymers formed by addition polymerization. Addition polymerizations are carried out with water being used as the diluent solvent. These polymers may be emulsions, where the solids are suspended, or solutions, where the monomers and polymers are dissolved in water.

One problem that exists with the polymerization process is that residual monomers, that remain at the completion of the product, produce an unpleasant odor. There are various methods for reducing the residual monomer content such as steam stripping and the addition of chemical “chasers”. These can reduce the residual monomer content to about 25 ppm. However, this small amount still leaves odor which is a cause for concern.

Carl Frederick and John Udinsky of the Spring House Division of Rohm and Haas Company, have recently introduced a method for reducing the carboxylester content of an emulsion polymer. This method involves the use of carboxyesterase from pig livers to eliminate the presence of the odor-causing carboxyl ester.

Carboxylesterases are enzymes widely distributed in animals, plants, and microorganisms. They catalyze the hydrolysis of carbonic acid esters to carbonic acid anion and alcohols:



**Teacher's Note:**

During my stay at Rohm and Haas, Dr. Dougherty and I attempted to develop a quick and reproducible lab exercise that could be carried out with typical high school lab equipment. Due to the complexity of the enzyme extraction, we found it necessary to streamline the procedure so that the results that you get will not represent the data obtained with total purification of the enzyme.

Additionally, we decided to expand on the thought process used in our approach to the problem. The technique of brainstorming has the potential for widespread application- not just in the sciences. This is represented by activity #1.

**Activity 1:**

**Brainstorming Exercise**

Discuss the standard scientific method

Introduce industry's scientific method: **plan; do; check; act**

Propose question or problem:

Does the use of a crude carboxylesterase preparation from pig liver reduce the residual monomer in an emulsion?

**Brainstorming Rules:**

- Sit in a circle
- Assign one person to be a scribe
- Each person states one question pertinent to the problem
- You may pass if you do not have a question
- Continue going around until everyone passes
- After questions have been compiled, copy and distribute them to students.
- Have the students organize the questions into groups with similar themes.
- Decide on criteria for prioritizing the groups
- Prioritize your groups of question
- Decide on a work plan
- Implement the plan

**Sample Questions:**

1. Is there a quick possible purification method and emulsion test?
2. Which of the various procedures do we follow?
3. Should we check with an enzyme expert?
4. Should we make a flow chart to list procedures and time constraints?
5. Why are there so many steps and what does each step do?
6. Why so many different temperatures and what was the reason for their selection?
7. What is the mechanism for the carboxylesterases – ester interaction?
8. What would the cost be to industry to implement this procedure?
9. Why does pork liver work better than bovine or chicken liver?
10. Is there a chemical synthesis procedure to make carboxylesterase?
11. What is the environmental impact of such a procedure?
12. Are there any ethical questions associated with this activity?
13. Will this be reproducible in a high school lab?
14. Can we involve an enzyme manufacturer?
15. From concept to commercial production, what is the time line envisioned?
16. In examining the available products, can we use substitution?
17. Are any of the raw materials toxic?

18. Are there any health benefits to the consumer as the result of the success of this operation?
19. From the marketing point of view, how much is the consumer willing to pay for odor improvement?
20. Is this relevant to your school's students?

We grouped these questions into six different categories: procedure and complexity, SHE (safety, health, environment) issues, background information, cost and manufacturing, teaching application, expectations of the enzyme.

Our next step was to prioritize these groups, which we did as follows in descending order: 1) procedures and complexity, 2) expectations for the enzymes, 3) background information, 4) SHE issues, 5) cost and manufacturing, 6) applications for teaching.

Our first step was to visit the enzyme experts at the Rohm & Haas Spring House Laboratories. This helped us get a better feel for the overall scheme of the project. From these discussions we arrived upon the following procedure.

### **Activity 2:**

#### **Crude Preparation of Carboxylesterase**

##### **Materials Needed:**

a blender	an emulsion such as paint
1 kg pig liver	centrifuge
0.9% saline solution	Tris buffer or pH 7 buffer*
centrifuge tubes	amyl acetate or vinyl acetate**

\*Selection of a buffer is dependent on the choice of an emulsion. Be sure the buffer does not cause the emulsion to coagulate.

\*\*If you use vinyl acetate (VA) to spike the emulsion instead of amyl acetate (AA), you can quantitatively determine the percentage left after hydrolysis by the enzyme by using the method listed in Activity 3. Vinyl acetate should be handled with proper ventilation, preferably in a hood.

### **Procedure:**

1. Weigh out 1 kg of pig liver.
2. Dice and wash with cold physiological saline (0.9% NaCl solution) to removed excess blood.
3. Rinse several times with water to remove some of the salt. (We found that the salt tends to cause the emulsion to precipitate). Blot dry and keep cold.
4. Homogenize in a blender with six volumes Tris buffer, pH7 buffer, or water.
5. Homogenize on high for about five minutes
6. Transfer the homogenate into a series of centrifuge tubes and spin 1 hour.
7. Discard the pellet, and pool the supernatant. (Refrigerate the supernatant if you can't continue.)
8. To make a 2% amyl acetate solution, use 98 mL of latex paint and add 2 mL of amyl acetate (banana oil) and mix.
9. Ask the students to take a slight whiff (using proper techniques) and rate it from 1 (odorless) to 10 (very smelly). Save this as your control.
10. Prepare another sample containing 450 mL latex paint and 10 mL amyl acetate. Add 40 mL of supernatant containing the carboxylesterase. (2% AA solution)
11. Compare odor against the control every 30 minutes. Rate on a 1-10 scale.
12. You may graph your ratings with odor on the X axis and time on the Y axis.

### **Questions:**

Was there a change in odor? If so, why? If not, how can you explain your results?

### **Activity 3:**

## **Method for Determining % Vinyl Acetate in Emulsion Polymers**

### **I. Raw Materials**

1. 90 mL methanol
2. Acid Solution 1:  
270 grams DI water  
600 grams glacial acetic acid  
30 grams sulfuric acid
3. Titrating Solution:  
16.7 grams potassium bromate  
60.0 Potassium bromide  
X grams DI water to a total solution of 1000 mL

### **II. Preparation of Acid Solution**

1. To a one-half gallon wide mouth jar add 270 grams DI water
2. Mass and add 600 grams glacial acetic acid
3. Slowly add 30 grams sulfuric acid
4. Stir the mixture gently for two (2) minutes and hold for Step IV.1.

### **III. Preparation of Titrating Solution:**

1. To a 1000-mL volumetric flask add 16.7 grams of Potassium bromate.
2. Add 60 grams Potassium bromide
3. Add enough DI water to make one (1) liter of solution. Shake until a uniform solution is obtained.
4. Place the solution in a dark area until needed. Cap flask tightly.

### **IV. Titrating Procedure For Determining Vinyl Acetate**

1. To a 4-ounce wide mouth jar add 10 mL of acid solution (I).
2. Add 90 mL methanol
3. Mass accurately 2.6 grams of sample containing vinyl acetate
4. Gently agitate the above solution. Continue agitation during titration.
5. Place titrating solution (II) into a burette graduated to 0.1 mL.
6. Begin titrating solution into the 4-ounce bottle until a color change is noted (pale yellow).
7. When the color change persists uniformly, this is the end point.
8. Record the mL of titrating solution used.
9. Only when the sample size is 2.6 grams, the % VA is equal to the mL of titer used (e.g., 1.5 mL titer solution (II) = 1.5% VA.

### **Safety Guidelines:**

See the inside of the back cover.

### **Safety Precautions:**

Students should be reminded of all safety instructions including but not limited to: wearing safety goggles and aprons, no tasting the chemicals or products, and washing hands after clean up.

### **References:**

Frederick, Clay Bruce and Udinsky, John Richard, "Method For Reducing The Carboxylester Content of An Emulsion Polymer," U.S. Patent application.

Jung, Wolfgang and Krisch, Klaus, "The Carboxylesterases/amidases of Mammalian Liver And Their Possible Significance," *Critical Reviews in Toxicology*, Vol. 3, pp. 371-434, 1975.

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