Original Research Article

Solubilization of Benzyl Acetate by Polysorbate 80: A Thin Layer Chromatography Study

Kacey Song[1*], Hyoju Kim[2*], Audrey Lee[3], Gurnho Park[4], Gaeun Jang[5], Benjamin Yu[6], Minsuk Kim[7], Suji Kim[8], Zara Guerra Arthur[9], Hailey Gu[10]

[1]Coppell High School, 185 W Parkway Blvd, Coppell, TX 75019, United States; [2]Lone Star High School, 2606 Panther Creek Pkwy, Frisco, TX 75033, United States; [3]Westmoore High School, 12613 S Western Ave, Oklahoma City, OK 73170, United States; [4]Canyon Crest Academy, 5951 Village Center Loop Rd, San Diego, CA 92130, United States; [5]Richmond Hill High School, 500 Wildcat Blvd, Richmond Hill, GA 31324, United States; [6]Centennial High School, 6901 Coit Rd, Frisco, TX 75035, United States; [7]James M. Bennett High School, 300 E College Ave, Salisbury, MD 21804, United States; [8]Westview High School, 4200 NW 185th Ave, Portland, OR 97229, United States; [9]North Garland High School, 2109 W Buckingham Rd, Garland, TX 75042, United States; [10]Augusta University, 1120 15th St, Augusta, GA 30912, United States

ABSTRACT

In today's society, we are exposed to many chemicals, either natural or synthetic, which can cause allergic reactions on the skin. To investigate the solubilization of benzyl acetate, which can cause allergic reactions in high concentrations, we decided to use Polysorbate 80 owing to its availability, cost-effectiveness, and versatile applicability as a surfactant. In this paper, we tested two methods: one is to observe the minimum amount of Polysorbate 80 required to fully dissolve different concentrations of benzyl acetate in an aqueous solution. The other experiment was conducted to study the interaction between Polysorbate 80 and benzyl acetate using thin-layer chromatography (TLC) and spotting trials. Polysorbate 80's durability is exemplified by how difficult it is to separate mixtures that contain benzyl acetate. In two different experiments, the amount of Polysorbate 80 and the different ratios of heptane and ethyl acetate (EA) were tested. The results indicated strong intermolecular interactions between Polysorbate 80 and benzyl acetate. It was concluded that increasing the amount of Polysorbate 80 led to increased intermolecular interaction. Through the experimentation process, it was discovered that Polysorbate 80 is a strong surfactant, retaining benzyl acetate in solution. Moreover, it was concluded that the higher the ratio of heptane to ethyl acetate (EA), the lower Rf value of benzyl acetate.

Keywords: Polysorbate 80; Benzyl Acetate; Thin Layer Chromatography; Polarity; Micellization

INTRODUCTION

Surfactants are amphiphilic molecules that have a hydrophilic head and a hydrophobic tail, and the structure allows them to decrease the surface tension of a liquid by adsorbing at the interfaces of air-water or oil-water (1). By breaking the interactions between water molecules, surfactants lower the surface tension,

Corresponding author: Hailey Gu, E-mail: dgu@augusta.edu.

Accepted March 11 2026
*These authors contributed equally.
https://doi.org/10.70251/HYJR2348.42110114

forming micelles when the concentration is high (2). An example of a widely used surfactant is Polysorbate 80, a nonionic surfactant from a fusion of sorbitol, dehydrated monooleate, and ethylene oxide (3). Since its discovery, Polysorbate 80 has been widely used as an excellent surfactant. We hypothesized that we could use Polysorbate 80 to enhance the solubilization of hydrophobic compounds. Benzyl acetate was chosen as a model hydrophobic compound because it is commercially available, relatively safe to handle under precautions, and suitable for evaluating solubilization and TLC mobility with Polysorbate 80. Benzyl acetate, an ester produced by coupling benzyl alcohol and acetic acid, is commonly used in food products, cosmetics, fragrances, cleaning agents, biocides, and more (4). Though naturally derived, benzyl acetate can still have negative effects on the human body. Short-term exposure to the human body through inhalation and ingestion can cause irritation to the eyes and respiratory tract, central nervous system depression, and can cause potential unconsciousness if the exposure exceeds the occupational exposure limit (OEL) (5). Long-term exposure to benzyl acetate could result in dryness of the skin and potential effects on the kidneys, such as degeneration and regeneration of renal tubule epithelium (6, 7). Symptoms of exposure can lead to nausea, vomiting, headache, fatigue, dizziness, and lack of coordination. Benzyl acetate also impacts the environment in both aquatic and land. If released into the water, it can harm aquatic life, soil, and sediments (8). Furthermore, benzyl acetate can form vapor through the process of evaporation. At the temperature of 20°C, the molecules will escape from the liquid to the air and cause long-term health effects if people inhale the vapor or come into contact with the benzyl acetate. To test the solubilization of benzyl acetate in aqueous solutions, we decided to use Polysorbate 80 as our surfactant. First, we tested how much Polysorbate 80 must be added to dissolve benzyl acetate in water. Afterward, we used TLC to see if there were any Rf value changes due to Polysorbate 80 inhibiting benzyl acetate from moving in TLC. We observed that Polysorbate 80 was able to help dissolve benzyl acetate in water and reduce the movement of benzyl acetate on the TLC plate.

METHODS AND MATERIALS

Solubilization of Benzyl Acetate with Varying Polysorbate 80 Concentrations

1 mL of neat benzyl acetate (1.17 M, based on density 1.08 g/mL and molecular weight 150 g/mol), and 1 mL of EA, (2.04 M, based on density 0.902 g/mL and molecular weight 88 g/mol), were placed in a 50 mL beaker. This corresponds to a benzyl acetate concentration of approximately 3.50 M. Separately, a solution of 5 mL of water and 0.1 mL of benzyl acetate (0.21 M, calculated using density of 1.08 g/mL and molecular weight of 150 g/mol) was prepared in a small glass vial. In a graduated cylinder, a 7:1 ratio solution of 7 mL of heptane and 1 mL of EA was prepared. For consistency, the corresponding EA molarity in the mixture was roughly 0.18 M. As for TLC plate preparation, a horizontal line was drawn 0.5 cm from the bottom, and perpendicular dash marks at the center and left were marked. Then on the TLC plate, a glass capillary tube was used to spot our benzyl acetate and EA on the left dash. The Polysorbate 80 solution (0.042 M at 0.3 mL addition) was spotted on the center dash. Then, the TLC plate was placed into a 100 mL beaker, and the heptane solution was poured until it reached the line. A 250 mL beaker was placed over a 100 mL beaker to prevent solvent evaporation. Once the solvent traveled ¾ from the bottom of the TLC plate, the TLC plate was removed and left to dry before analyzing it with a UV lamp. The Rf value of the Polysorbate 80 and benzyl acetate was then calculated to be 0.41 and 0.99, respectively. With a pencil, the dragging of two solutions and the distance the solvent traveled were marked. The Rf values were then calculated by measuring the distance between the line 0.5cm (about 0.2 in) from the bottom to the top of dragging marks. The same procedure was applied to varying concentrations of Polysorbate 80, conducted approximately 30 times for each condition.

Thin-Layer Chromatography Analysis with Varying Heptane:Ethyl Acetate Ratios

1 mL of benzyl acetate and 1 mL of EA were placed in a 50 mL beaker. Separately, a solution of 5 mL of water and 0.1 mL of benzyl acetate (0.21 M, calculated using density of 1.08 g/mL and molecular weight of 150 g/mol) was prepared in a small glass vial In a graduated cylinder, various ratios of heptane: EA were prepared, ranging from 1:1 to 9:1. For ratios lower than 5:1, the volumes were doubled. For example, a 4:1 ratio was prepared by mixing 8 mL of heptane and 2 mL of EA in a graduated cylinder. The Rf value for the ratio 5:1 was 0.15 for Polysorbate 80 and 0.99 for benzyl acetate. After labeling the TLC, the EA and benzyl acetate solution were spotted on the left dash mark, and the Polysorbate 80 solution was spotted in the middle. After spotting, the TLC was put under a UV light with a wavelength of 254 nm to visualize the spotting on the TLC plate in

order to take a picture. Then, the TLC was placed in the 100 ml beaker, and the heptane solution was poured until it reached the horizontal line. The 250 mL beaker was placed over it (to prevent it from evaporating). Finally, after the heptane solution rose about ¾ of the TLC, it was taken out and let dry. The new spotting under the UV light was analyzed, and RF values were noted for both the acetate solution and Polysorbate 80 solution. The same procedure was applied to varying ratios of benzyl acetate and EA solution, conducted approximately 30 times for each condition.

Materials and Reagents

Benzyl acetate (Mystic Moments), ethyl acetate (EA; CCS Consolidated Chemical & Solvents), heptane (Chemboys), and polysorbate 80 (Hznxolrc) were used as received without further purification. Thin-layer chromatography (TLC) was performed on silica gel plates (Lichen Cottage). TLC spotting was carried out using glass capillary tubes (AAdvance Instruments), and chromatograms were visualized under ultraviolet light using a handheld UV lamp (254 nm). Glassware used in the experiments included 50 mL beakers (ULAB) and 10 mL capped glass vials (Kesell). Liquid handling was performed using disposable transfer pipettes (3 mL; MAQIHAN) and 1 mL needleless syringes (Dfpty).

RESULTS AND DISCUSSION

Benzyl acetate and water did not mix in the absence of Polysorbate 80 and showed similar results for addition of 0.1 mL of Polysorbate 80. Mixing was first observed when 0.3 mL of Polysorbate 80 was added (Table 1). This corresponds to a Polysorbate 80 concentration of approximately 0.042 M, calculated using a density of 1.064 g/mL and a molecular weight of 1310 g/mol. Under this condition, the final benzyl acetate concentration was approximately 1.21 M. The total solution volume for each trial was 5.8 mL, including water, benzyl acetate, and Polysorbate 80. To prove this observation, we used thin-layer chromatography (TLC) techniques that could analyze benzyl acetate behavior in solvent systems. As the proportion of EA, a polar solvent, increased, the Rf values of benzyl acetate increased proportionally from 0.498 ± 0.0149 at a 9:1 heptane: EA ratio to 0.939 ± 0.0282 at a 1:1 ratio, indicating greater movement up the TLC plate. The low standard deviation indicates high precision of the TLC measurements. This shows that the spotting technique was consistent throughout the trials. The highest Rf value (0.939 ± 0.0282) was observed using a 1:1 heptane: EA ratio, and a 0.04–0.08 decrease in Rf for each incremental increase in heptane proportion. A 7:1 heptane: EA ratio was chosen as our standard ratio between heptane and EA as it allowed clear visualization of the spots on TLC plates after development (Figure 1). A 0.6 Rf value was consistently produced from this ratio when applying a solution with no Polysorbate 80. When 0.3 mL of Polysorbate 80 was added, dragging occurred, decreasing the Rf value to 0.41, indicating that Polysorbate 80 forms strong intermolecular bonding with benzyl acetate (Table 2) (Figure 2).

Table 1. *Benzyl acetate solubility in water with Polysorbate 80. This table shows how much Polysorbate 80 is needed to dissolve benzyl acetate in 1 mL of water.*

0 mL of P80 (0 M)	**0.1 mL of P80** (0.014 M)	**0.3 mL of P80** (0.042 M)	**1 mL of P80** (0.14 M)
			

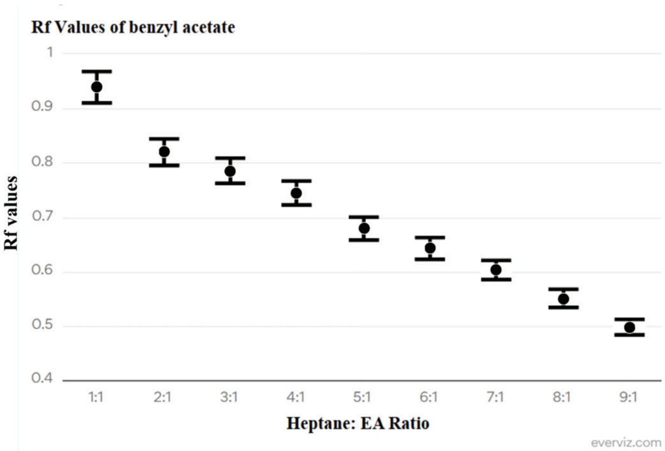

Figure 1. *Effect of different ratios of Heptane to EA to Rf value for benzyl acetate. This shows how the proportion of EA has a positive correlation with the Rf value of benzyl acetate, as seen through the decreasing linear graph.*

Table 2. *Rf value of benzyl acetate. As the solvent system changed from 9:1 (heptane: ethyl acetate (EA)) to 1:1, the Rf value of benzyl acetate increased from 0.498 ± 0.0149 to 0.939 ± 0.0282. This represents an increase of approximately 88%. The consistent increase in Rf values across each solvent ratio shows a strong relationship between solvent polarity and compound mobility. As the amount of ethyl acetate (EA) increased, benzyl acetate traveled farther up the TLC plate.*

Heptane: EA Ratio	Rf Values of benzyl acetate (EA + BA)
1:1	0.939 ± 0.0282
2:1	0.820 ± 0.0246
3:1	0.785 ± 0.0236
4:1	0.745 ± 0.0224
5:1	0.679 ± 0.0204
6:1	0.642 ± 0.0193
7:1	0.602 ± 0.0181
8:1	0.551 ± 0.0165
9:1	0.498 ± 0.0149

0 mL of P80 (0 M)	**0.1 mL of P80** (0.014 M)	**0.3 mL of P80** (0.042 M)	**0.7 mL of P80** (0.098 M)	**1 mL of P80** (0.14 M)	**2 mL of P80** (0.28 M)
Rf value of benzyl acetate (P80): 0.52 (± 0.016)	**Rf value of benzyl acetate (P80):** 0.51 (± 0.015)	**Rf value of benzyl acetate (P80):** 0.35 (± 0.12) **(due to dragging)**	**Rf value of benzyl acetate (P80):** 0 (± 0)	**Rf value of benzyl acetate (P80):** 0 (± 0)	**Rf value of benzyl acetate (P80):** 0 (± 0)

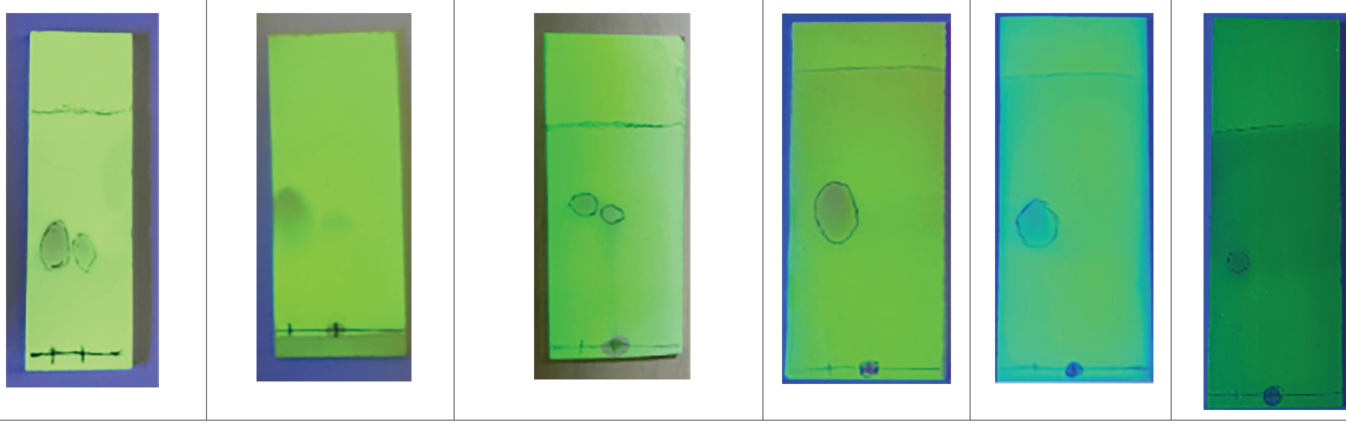

Figure 2. *Polysorbate 80 influence on benzyl acetate: Left spot is benzyl acetate without adding Polysorbate 80. The middle spot is a mixture of Polysorbate 80 and benzyl acetate. At 0.3 mL of Polysorbate 80, 0.042 M, spots are being dragged due to Polysorbate 80 forming intermolecular bonding with benzyl acetate. Starting from 0.7 mL of Polysorbate 80, 0.098 M, benzyl acetate has no movement on the TLC plate.*

CONCLUSION

Polysorbate 80 was tested for its capability to allow hydrophobic substances, such as benzyl acetate, to interact with water. After adding Polysorbate 80, it was observed that benzyl acetate was able to become fully mixed with water starting at 0.3 mL of Polysorbate 80, showing that a certain concentration of this surfactant is needed. The 0.3 mL solubilization threshold is consistent with Polysorbate 80 approaching its critical micelle concentration (CMC), at which micelles can encapsulate benzyl acetate and increase its apparent solubility in water. To visually showcase this interaction, thin-layer chromatography (TLC) techniques were used to analyze benzyl acetate within various solvent systems. While benzyl acetate displayed consistent Rf values of 0.6 on its own, applying 0.3 mL of Polysorbate 80 resulted in spot dragging, showing a stronger intermolecular interaction between benzyl acetate and the surfactant. While increased viscosity or adsorption effects could contribute to reduced mobility, these factors would not fully explain the consistent change in Rf behavior across solvent systems. Polysorbate 80 forms micelles above the critical micelle concentration (CMC), which is about 0.012-0.015 M. The concentration of 0.042 M exceeds the reported CMC range of 0.012-0.015 M, showing micelle-mediated solubilization. This micelle formation shows a strong correlation with the reduced movement observed in TLC, indicating that intermolecular interactions are likely the leading cause. The use of heptane: ethyl acetate (EA) solvent ratios also played a role in the movement of benzyl acetate, as it increased the Rf values as proportions of ethyl acetate (EA) increased. This study demonstrates the capability of Polysorbate 80 to solubilize hydrophobic benzyl acetate, reducing its mobility in TLC through intermolecular interactions. A limitation of this study is the use of only one surfactant, Polysorbate 80, being tested because other surfactants, such as Tween 20 or Triton X-100, may have stronger interactions with benzyl acetate, leading to different Rf behaviors on TLC plates. Furthermore, alternative UV-based equipment, such as UV-Vis spectroscopy, could have been utilized to measure the solubility quantitatively. This study can lead to future research with alternative surfactants being tested for effectiveness in solubilizing hydrophobic compounds and related applications.

ACKNOWLEDGEMENTS

We would like to thank Woojoo Park, Eileen Pak, Gaeun Huh, Elisha Kim, Timothy Park, and Sara Zhao for helping us check grammar, sentence flow, and template.

CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Laurén S. What are surfactants and how do they work? Biolin Scientific. Available from: https://www.biolinscientific.com/blog/what-are-surfactants-and-how-do-they-work (accessed on 2025-05-29).
2. Halliday HL. The fascinating story of surfactant. *J Paediatr Child Health*. 2017; 53: 327–333. https://doi.org/10.1111/jpc.13500
3. NCPAP vs. intubation for premature babies. Available from: https://www.bomimed.com/resources/pages/files/Clinical%20Insider%20-%20Oct%202015%20Website.pdf (accessed on 2025-05-29).
4. Laurén S. What is critical micelle concentration? Biolin Scientific. Available from: https://www.biolinscientific.com/blog/what-is-critical-micelle-concentration (accessed on 2025-05-29).
5. Polysorbate 80 – an overview. ScienceDirect Topics. Available from: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/polysorbate-80 (accessed on 2025-05-29).
6. Surfactants. Chemical Safety Facts. Available from: https://www.chemicalsafetyfacts.org/chemicals/surfactants/ (accessed on 2025-05-29).
7. Benzyl acetate. Lanxess. Available from: https://lanxess.com/en/products-and-brands/products/b/benzyl-acetate (accessed on 2025-05-29).
8. National Toxicology Program. Toxicology and carcinogenesis studies of benzyl acetate (CAS No. 140-11-4) in F344/N rats and B6C3F1 mice (feed studies). *Natl Toxicol Program Tech Rep Ser*. 1993; 431: 1–285.
9. ICSC 1331: Benzyl acetate. International Labour Organization. Available from: https://chemicalsafety.ilo.org/dyn/icsc/showcard.display?p_lang=en&p_card_id=1331&p_version=2 (accessed on 2025-05-29).
10. Agency for Toxic Substances and Disease Registry (ATSDR). Draft toxicological profile for toluene. 2015. Available from: https://www.atsdr.cdc.gov (accessed on 2025-05-29).