Optimization of Microencapsulation Composition of Menthol, Vanillin, and Benzyl Acetate inside Polyvinyl Alcohol with Coacervation Method for Application in Perfumery

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Abstract. One of many applications of essential oils is as fragrance in perfumery. Menthol, benzyl acetate, and vanillin, each represents olfactive characteristic of peppermint leaves, jasmine flowers, and vanilla beans, are commonly used in perfumery. These components are highly volatile, hence the fragrance components will quickly evaporate resulting in short-lasting scent and low shelf life. In this research, said components have been successfully encapsulated simultaneously inside Polyvinyl Alcohol (PVA) using simple coacervation method to increase its shelf life. Optimization has been done using Central Composite Diagram with 4 independent variables, i.e. composition of menthol, benzyl acetate, vanillin, and tergitol 15-S-9 (as emulsifier). Encapsulation efficiency, loading capacity, and microcapsule size have been measured. In optimized composition of menthol (13.98 %w/w), benzyl acetate (14.75 %w/w), vanillin (17.84 %w/w), and tergitol 15-S-9 (as emulsifier), encapsulation efficiency of 97.34% and loading capacity of 46.46% have been achieved. Mean diameter of microcapsule is 20.24 µm and within range of 2.011-36.24 µm. Final product was achieved in the form of cross linked polyvinyl alcohol with hydrogel consistency and orange to yellow in color.

1. Introduction
Indonesia with its tropical climate has been a place for various plants that yield essential oils. Essential oils is widely used as main components in perfumery. Every perfume consists of 3 main components, top notes which are the most volatile, middle notes, and base notes which are the least volatile. Its nature as volatile component makes essential oils can not release its fragrant aroma for relatively long period.

Encapsulation is one of many method to retain essential oils scent. In general, there are 3 methods of utilizing encapsulation technology, there are chemical (e.g. interfacial polycondensation, in situ polymerization), physico-chemical (coacervation, sol-gel encapsulation), and mechanical (spray drying, solvent evaporation). Coacervation is the most suitable encapsulation method to apply in food and beverage, pharmacy, cosmetics, and fragrances and flavors.

Limonene has been encapsulated inside chitosan with complex coacervation method. Lutensol as surfactant has been reported to decrease particle size. Martins encapsulated thyme oil inside polylactide using 3 kind of surfactants with various hydrophilic-lypophilic balance (HLB), Tween 20 (HLB 16.4), Tween 80 (HLB 15.5) and Tergitol 15-S-9 (HLB 13.3). Encapsulation using Tergitol 15-S-9 yields the best efficiency (65%). Polyvinyl alcohol (PVA) has been used to encapsulate santosol...
oil with simple coacervation method using glutaraldehyde as crosslinking agent. Higher concentration of sodium sulphate as coacervating agent has been reported to increase microcapsule wall thickness, hence slower release of santosol oil7.

In this research, our group has recently encapsulated menthol, benzyl acetate, and vanillin simultaneously inside PVA with aqueous phase simple coacervation method and Tergitol 15-S-9 as surfactant. Composition of menthol, benzyl acetate, vanillin, and Tergitol 15-S-9 was optimized to achieve optimum encapsulation efficiency and loading capacity. Optimization has been done using Response Surface Method (RSM) with Central Composite Diagram (CCD) experiment design. RSM is equipped with statistical tools to determine the significance of a factor over a response. The evaluation of factors using the RSM uses experimental design in order to distribute the selected variables within the boundaries of the design8.

2. Materials and Methods

2.1. Materials
Sodium sulfate (ACS reagent, ≥99.0%, anhydrous, powder) as coacervating agent, glutaraldehyde solution (grade II, 25% in H2O) as crosslinking agent, Tergitol 15-S-9, l-menthol (≥99%, FCC, FG) as emulsifier, benzyl acetate (≥99%, FCC, FG), vanillin (≥97%, FCC, FG), and Mowiol 4-98 (Mw 27,000) were all shipped from Sigma Aldrich Singapore.

2.2. Methods
2.2.1 Preparation of Microcapsule
PVA solution was made by adding 6g of Mowiol 4-98 into 100 ml aqua dm at 60°C and stirred at 250 rpm for 30 minutes. Sodium sulfate solution was made by adding 6g of sodium sulfate into 50 ml aqua dm and then added to PVA solution. A mixture of menthol, benzyl acetate, vanillin, and tergitol was added into solution and then stirred at 750 rpm for 5 minutes. After 5 minutes, the temperature of solution was raised to 70°C to induce coacervation. Then, 6 ml of glutaraldehyde solution is added and then stirred at 500 rpm and heated at 70°C for 60 minutes. After that, solution was cooled and sedimented for 24 hours. After 24 hours period, the solution separated into PVA hydrogel and aqueous phase. Aqueous phase was then analyzed using GC FID.

2.2.2 Particle size measurement
Particle size was measured using Beckman Coulter LS 100 Q. PVA microcapsules suspension was collected before it formed hydrogel. Microcapsule suspension was dispersed in aqua dm and then analyzed.

2.2.3 Gas chromatography analysis
GC analysis was done using Shimadzu GC-FID, with DB-5 column, oven temperature at 200°C, injector temperature at 110-160°C, detector temperature at 200°C, N2 50kPa, H2 70 kPa, and air 50kPa. 600 ppm sample was prepared by diluting aqueous phase with chloroform and vaporizing with turboevaporator-nitrogen.

3. Results and Discussion

3.1. Optimized composition of menthol, benzyl acetate, vanillin, and tergitol 15-S-9
Numerical optimization has been done using Design Expert 7.0, a statistical software from StateEase, to reach optimum encapsulation efficiency and loading capacity by adjusting composition of menthol, benzyl acetate, vanillin, and tergitol 15-S-9 with CCD experimental design. The low value is was to 1, intermediate value was set to 1.5, high value is set to 2, and total run was 30 runs. Encapsulation efficiency was set to 1 (as maximum value), and loading capacity was set between 0.32123 (as
minimum value achieved in experiment) and 0.5 (at value more than 0.5, there will be encapsulated components on the surface of microcapsule)\textsuperscript{9}. The result is shown in Table 3.1.

**Table 3.1** Optimized value of menthol, benzyl acetate, vanillin, and tergitol

| Menthol (%w/w) | Benzyl acetate (%w/w) | Vanillin (%w/w) | Tergitol (%w/w) | Encapsulation efficiency (%) | Loading capacity (%) |
|----------------|-----------------------|-----------------|-----------------|-----------------------------|---------------------|
| 13.98          | 14.75                 | 17.84           | 13.4            | 97.34                       | 46.46               |

As shown above, vanillin was needed in much higher concentration compared to benzyl acetate and menthol. The reason for this is because vanillin is the most water-soluble components (1 g/100 ml) compared to benzyl acetate (<0.1 g/100 ml) and menthol (<0.046 g/100 ml). In aqueous phase coacervation, the more a component solubilized in water, the less it will be encapsulated inside the polymer. In this case, higher vanillin concentration was needed to increase vanillin that can be encapsulated inside polyvinyl alcohol.

### 3.2 Microcapsule particle size

Particle size analysis has been done using different concentration of tergitol as surfactant. This surfactant was used to modified the size of microcapsules. Table 3.2 shows the result.

**Table 3.2** Microcapsule particle size

| Surfactant (%w/w) | Mean diameter (µm) |
|-------------------|--------------------|
| 19.28             | 17.35              |
| 13.4              | 20.24              |
| 9.64              | 22.63              |

As shown at Table 3.1, higher concentration of surfactant yields smaller particles. The reason is with higher concentration of surfactant, surface tension will decrease and wider oil-water interface is achieved, hence smaller particle size\textsuperscript{10}.

### 3.3 Microcapsule hydrogel analysis with ATR-FTIR

![ATR-FTIR analysis](image)
If we compare between Figure 3.1 (a) and (b), the graphs look almost identical except for the existence of vanilla peak between wavelength 1500-1600 cm⁻¹ on figure 3.1 (a). Figure 3.1 (c) is the substarction form (a) and (b) and so the peak for menthol and vanillin become more visible. The peak for benzyl acetate can not be detected and the existence of benxyl acetate is confirmed with GC analysis in the following section.

3.4 Release profile of menthol

![Figure 3.1](image_url) (a) Microcapsule sample, (b) Hollow microcapsule sample, (c) Subtraction of a and b.

If we compare between Figure 3.1 (a) and (b), the graphs look almost identical except for the existence of vanilla peak between wavelength 1500-1600 cm⁻¹ on figure 3.1 (a). Figure 3.1 (c) is the subtraction form (a) and (b) and so the peak for menthol and vanillin become more visible. The peak for benzyl acetate can not be detected and the existence of benxyl acetate is confirmed with GC analysis in the following section.

![Figure 3.2](image_url) Release profile of menthol.
- Standard menthol
- Encapsulated menthol

Standard menthol is unencapsulated pure menthol and release data is collected by analytical balance periodically. Release data for encapsulated menthol is collected by GC Headspace analysis. The gradient for encapsulated menthol is -0.0265 and this is almost identical to the gradient for standard menthol which is -0.0271. This shows that encapsulation for menthol is not slowing down the release rate significantly. It may be because the evaporation rate of pure menthol is identical to the diffusion rate of menthol through PVA matrix. Further research on those two topics is expected to be done.
3.5 Release profile of benzyl acetate

![Benzyl acetate graph](image)

**Figure 3.3** Release rate of benzyl acetate.
- Standard benzyl acetate
- Encapsulated benzyl acetate

Standard benzyl acetate is unencapsulated pure benzyl acetate and release data is collected by analytical balance periodically. Release data for encapsulated benzyl acetate is collected by GC Headspace analysis. The gradient for encapsulated benzyl acetate is -0.0439 and this is significantly lower compared to the gradient for standard benzyl acetate which is -0.0903. This shows that encapsulation for benzyl acetate is significantly slowing down the release rate. It may be because the evaporation rate of pure benzyl acetate is much more slower compared to the diffusion rate of benzyl acetate through PVA matrix. Further research on those two topics is expected to be done.

3.6 Release profile of vanillin

![Vanillin graph](image)

**Figure 3.4** Change of area of vanillin FTIR spectra.

Release rate of vanillin is done using FTIR. Figure 3.4 shows the change of vanillin area over six days. Instead of getting lower, the absorbance of vanillin is getting higher. This shows that the release rate of vanillin is much more slower compared to the release rate of menthol and benzyl acetate, hence the increasing of vanillin composition in microcapsule over the period of six days.
3.7 Release rate of water

![Figure 3.5 Change of area of water FTIR.](image)

Qualitative analysis of released water is done by FTIR. If figure 3.5 compared to figure 3.4, it is clear that hydrogel microcapsule contains considerable amount of water, i.e. area for water is ranged around 60-90, while area for vanillin is ranged around 0.2-0.8. From day 5 to day 6, the change of area becomes significantly constant compared to the previous days and it shows that water content within the hydrogel microcapsule has reached its limit to be released in ambient temperature.

3.8 Morphology of hydrogel microcapsule

![Figure 3.6 Microcapsules morphology.](image)

Figure 3.6 is taken with confocal laser scanning microscope. It is shown that menthol and benzyl acetate can be encapsulated simultaneously inside one microcapsule. Another thing to note is that both menthol and benzyl acetate are homogeneously distributed inside the microcapsules. As for vanillin, we can not detect its existence with this microscope. Existence of vanillin in microcapsules has been proven with FTIR analysis.

4. Conclusion

Our research group has successfully encapsulated menthol, benzyl acetate, and vanillin inside polyvinyl alcohol with encapsulation efficiency as high as 97.34% and loading capacity as high as
46.46%. These results is higher compared to several previous research, namely vanillin inside polysulfonate (45%)[11] and menthol inside gum arabic and gelatin (93%)[12]. The mean diameter microcapsules size with optimized composition is 20.24 µm has been achieved. Encapsulation can significantly slow down the release rate of benzyl acetate, but not for menthol. Benzyl acetate and menthol can be encapsulated simultaneously and homogenously distributed inside one microcapsule.

5. References

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