Encapsulation of Volatile Citronella Essential Oil by Coacervation: Efficiency and Release Study

M A Manaf, I Subuki, J Jai, R Raslan and A N Mustapa

Faculty of Chemical Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

Abstract. The volatile citronella essential oil was encapsulated by simple coacervation and complex coacervation using Arabic gum and gelatin as wall material. Glutaraldehyde was used in the methodology as crosslinking agent. The citronella standard calibration graph obtained with R2 of 0.9523 was used for the accurate determination of encapsulation efficiency and release study. The release kinetic was analysed based on Fick’s law of diffusion for polymeric system and linear graph of Log fraction release over Log time was constructed to determine the release rate constant, k and diffusion coefficient, n. Both coacervation methods in the present study produce encapsulation efficiency around 94%. The produced capsules for both coacervation processes were discussed based on the capsules morphology and release kinetic mechanisms.

1. Introduction

These guidelines, written in the style of a submission to IOP Conf. Ser.: Mater. Sci. Eng., discuss how few reported works on the formulation of Citronella oil (CO) claimed that the active ingredient has only 3 minutes to 6 hours retained in any formulation of solution [1-3]. Encapsulation is one of the recent methods to preserve or retain the high value volatile compounds in several chemical solutions. The basic form of encapsulation is by emulsifying the target compounds in an aqueous solution by entrapping the oil drops in a carrier material [4]. Common goals in the development of encapsulated essential oil are to protect the compounds from degradation or from losses by evaporation, to achieve a controlled release, and to facilitate handling [5].

Encapsulation by coacervation is a phase separation of one or many hydrocolloids from the initial solution. It involves the deposition of the newly-formed coacervate phase around the active ingredient suspended or emulsified in the same reaction media under specific pH, temperature or composition of the solution [6, 7]. Chemical agent or cross-linking may require strengthening the capsules. Simple coacervation involves the use of single polymer such as gelatin or ethyl cellulose. While, complex coacervation involves neutralization of two oppositely charge polymer in aqueous solution. The widely used method is neutralization between negatively charge Arabic gum and positively charge gelatin [8]. The release behavior of the core material from the capsules was studied by using a generalized equation that is based on Fick’s law of diffusion for a polymeric system as Eq. 1. Where (Mt / M∞) is the fractional release at time t. Mt is percent release at specific time while M∞ is total material release, k is a constant characteristic of the polymer encapsulated and n is diffusion coefficient.

\[
\frac{Mt}{M\infty} = ktn
\]
2. Procedure

2.1 Preparation of Citronella Standard Graph.
A stock solution of 2 mg/ml of citronella standard solution was prepared in chloroform. Working solutions were prepared by diluting the stock solution with the same solvent to contain 2, 5, 10, 20, 40, 60, 80 and 100 µg/mL. Each solution was injected into GC-MS system and the concentration of citronella against peak area was obtained for each sample.

2.2 Encapsulation of Citronella oil by Simple Coacervation
For simple coacervation method, 60 mL Arabic gum solution was prepared at 12.5 % w/v concentration and mixed using magnetic stirrer at 250 rpm and temperature 27°C. The Arabic gum solution was first mixed with 4 mL citronella essential oil and 10 mL deionized water. To enhance coacervation stage, the mixture was added with 90 mL deionized water and mixed at speed 500 rpm, temperature 50°C for 20 minutes. pH of the mixture was adjusted to 4.5 by dropwise addition of 10% acetic acid solution. Then, 100ml distilled water was added and emulsified at stirring speed of 250 rpm, temperature below 10°C in 60 minutes. Twenty ml 25% of glutaraldehyde solution was added as crosslinking agent. pH was altered to value of the mixture of 9.7 by 10% sodium hydroxide solution addition the stirring was kept at the speed of 250 rpm, in 30 minutes, below 10°C to stabilize the microcapsules. The emulsion was put in separation funnel for 24 hours.

2.3 Encapsulation of Citronella oil by Complex Coacervation
In the present complex coacervation method, two wall materials used which are gelatin and Arabic gum. In the study, on to one ratio of gelatin and Arabic gum was practiced. It was reported produced spherical, scattering, maximum encapsulation efficiency with relatively equal size capsules. The gum Arabic solution was prepared at 12.5 % w/v concentration and mixed using magnetic stirrer at 250 rpm and room temperature. The gelatin solution was prepared at 12.5 % w/v concentration and mixed using magnetic stirrer at 250 rpm and temperature 40°C. The 30 mL 12.5 % gum Arabic solution was first mixed with 4 mL citronella essential oil and 5 mL deionized water at speed 500 rpm, temperature 50°C for 20 minutes. Then, the 30 mL gelatin solution was mixed together with the emulsion at room temperature, stirring speed of 500 rpm for another 20 minutes. After that, 195 ml distilled water was added to the emulsion, then continued agitating in 30 minutes, at temperature 50°C and same stirring speed 500 rpm. To enhanced coacervation, pH of the mixture was adjusted to 4.5 by dropwise addition of 10% acetic acid solution. Then, 200 ml distilled water was added and emulsified at stirring speed of 250 rpm, temperature below 10°C in 60 minutes. 20 ml 25% of glutaraldehyde solution was added as crosslinking agent. The stirring was kept at the speed of 250 rpm, in 30 minutes, below 10°C to stabilize the microcapsules. The emulsion was put in separation funnel for 8 hours.

2.4 Determination of Encapsulation Efficiency (EE)
The encapsulation process efficiency was calculated as Eq. 2. In this study, the amount of un-capsulated CO was determined by analyzing the amount of excess citronella oil presented at each layer for both process after the separation.

\[
\text{EE (\%)} = \left( \frac{\text{Initial CO} - \text{un-capsulated CO}}{\text{Initial CO in emulsion}} \right) \times 100
\]  

(2)

2.5 GC-MS Analysis
The CO presence was determined by using inert GC-MS Agilent 7890A Series of GC system, USA. The analysis methods were referred to Xiao et al. [12]. For the upper layer, before analysis, 1 g of the capsules layer were suspended in a flask containing 10 ml n-hexane. The mixture was shaked gently and allowed to settle. The hexane layer was collected and added with 5 g of anhydrous sodium sulfate to remove water. After mixed well the mixture, the supernatant was collected and filter to be used for GC-MS analysis. The same procedure was applied for the middle and bottom layer with 20 ml sample, 20 ml n-hexane and 10 g of anhydrous sodium sulfate.
2.6 Determination of Release Kinetic

Generalized equation developed from Fick’s law of diffusion was used to analyze the release behavior of the citronella from capsules. By using this equation, the representative plot of ‘Log (fractional release)’ as a function of ‘Log (release time)’ was developed by using the data to determine the release kinetic characterization. The constants n and k, both are best determined by means of a Log-Log plot of Mt/M∞ versus time as Eq.3. The slope of this plot is the dimensionless exponent, n and the intercept on the Y-axis gives the release rate constant, k [9].

\[
\log (Mt/M\infty) = n \log t + \log k
\]  

To determine Mt, the procedure was conducted according to Siow and Ong [11] and Dong et al. [6]. 0.5 g of wet capsules were placed into six separated sealed containers which each of it contained 10 ml of n-hexane. The suspensions were put at room condition at stirring speed of 50 rpm. Two ml samples were taken out once from one suspension at 30, 60, 120, 180, 240, and 300 minutes by using syringe. For determination of M∞, the 0.5 g of wet capsules were suspended in same solvent and extracted in ultrasonic for 10 minutes. Then, the collected samples were filtrated before used for GC-MS analysis.

2.7 Capsules Morphology Study

Morphology of the capsules were examined under light microscope, (MEIJI Techno, RZ, Jepan) which incorporated with a monitor display. The wet capsules were put on a microscope slide and observed under objective magnification of 10x. Image was captured and determined the size using the Video Toolbox Pro software.

3. Result and Discussion

3.1. Encapsulation Efficiency

Preliminary calibration experiments were carried to determine the citronella compound presence in the polymeric solution. The standard graph was constructed based on GC-MS peak area versus the citronella oil concentration from 2 µg/ml to 100 µg/ml as in Fig. 1. The standard calibration graph is a linear graph with R2 is 0.9523. The same citronella standard graph was used for release kinetic study in order to determine the emitted citronella to the medium at specific time.

Encapsulation efficiency data for both simple and complex coacervations are shows at Table 1. The present study shows that both process able to provide good encapsulation process with encapsulation efficiency around 94%. According to Yeo et al.[15] encapsulation efficiency increases with increasing polymer concentration. High concentration of polymeric solution, provide high viscosity media and faster the solidification of the dispersed phase contributed to reducing porosity of the microparticles as well. In the both coacervation process, equal polymeric solution concentration was applied which is 12.5 %w/v Arabic gum solution and 12.5 %w/v gelatin solution. Compare to other method, this concentration is high enough to produce good encapsulation efficiency.

Figure 1. Citronella standard graph of GC-MS peak area over varies concentration of citronella oil
**Table 1. Encapsulation Efficiency of Simple and Complex Coacervation**

| Efficiency                      | Simple coacervation | Complex coacervation |
|---------------------------------|---------------------|----------------------|
| CO presence in bottom layer (g) | 0.0929              | 0.0042               |
| CO presence in middle layer (g)  | -                   | 0.1059               |
| CO presence in upper layer (g)   | 0.1271              | 0.0911               |
| Encapsulation Efficiency (%)     | 93.9116             | 94.4218              |

*CO refer to Citronella oil.*

3.2. Oil Release Study

Figure 2 shows the plots of percentage of citronella release from polymeric wall system over time for simple coacervation and complex coacervation. Simple coacervation shows higher percent release at specific time compare to complex coacervation. This is partly because the low molecular weight polymer is more soluble in the organic solvent and undergoes slow solidification to produce more porous microparticles. On the other hand, it can also be attributed to the smaller size of the microparticles, which provides more surface area for drug diffusion. With increase in wall material cross-linking, the release was slowed down which happen in complex coacervation where gelatin and Arabic gum were combined to develop coacervates polymeric wall. The release kinetic mechanism is study further by plotting Log-log graph of fraction release verses time as shows in Fig. 3.

![Figure 2. Percent release curve of encapsulants at specific times. (▲): simple coacervation. (●): complex coacervation.](image)

![Figure 3. Percent release curve of encapsulants at specific times. (▲): simple coacervation. (●): complex coacervation.](image)

The graph represents the plot of Log fraction release as function of Log release time for microcapsules from simple coacervation and complex coacervation process. The calculated values for k and n in the present study are given in Table 2. The release constant rate, k affecting by the characteristic of the polymeric wall of the microcapsules while the dimensionless exponent, n, represents the type of release mechanism of the active ingredient through the capsules wall.

**Table 2. Release Rate Constant and Diffusional Exponent of Coacervates**

| Process             | k (hour\(^{-1}\)) | n     |
|---------------------|-------------------|-------|
| Simple coacervation | 0.0617            | 0.4736|
| Complex coacervation| 0.0229            | 0.5454|

k is constant release in unit hour\(^{-1}\), while n is dimensionless exponent

Theoretically, the k constant decrease with increasing degree of cross-linking of the polymeric structure [9]. For dimensionless exponent, n, mathematical solutions of the model have been described in three cases to elaborate the types of release mechanism of the coacervates. First case is where n=0.5 indicates Fickian encapsulant diffusion. Second is non-Fickian or deviating from the regular form (anomalous) when 0.5<n<1.0. The third case with n<0.5 which is `Less Fickian` diffusion. Zero-order release kinetics is a constant rate of release for the encapsulant when n=1 [9, 13]. In this study, both...
coacervation methods reported dimensionless coefficient, n=0.5 which is Fickian diffusion mechanism where the release of core material is significant with time.

3.3 Capsule Morphology

The morphology of the capsules produced by simple coacervation and complex coacervation are presented in Fig. 4. Coacervates made from both process is spherical in shape. The capsules morphology gives further descriptions and correlation for the release mechanism of the encapsulant for both process. Capsules from simple coacervation have more porous surface compare to capsules made by complex coacervation which has smoother surface. Capsules with porous surface because higher initial burst effect resulted high release rate of the core material. Within 24 hours at 25°C temperature, the capsules already melt indicates fully oil release from the capsules. Capsules made by complex coacervation with lower release rate have more rigid shape and can sustained longer in room condition. The capsules still in the shape after 7 days. The initial burst of the capsules is directly affected by the molecular weight of the polymer. In general, low molecular weight polymer result in high burst release of encapsulate.

Figure 4. Capsules morphology under light microscope with 10x magnification objective. (a): Simple coacervation freshly after capsules harvesting. (b): Simple coacervation after 24 hours in room condition. (c): Complex coacervation freshly after capsules harvesting. (d): Complex coacervation after 7 days in room condition.

This is because the low molecular weight polymer is more soluble in the organic solvent and undergoes slow solidification to produce more porous microparticles thus provide higher surface area for core material release [15]. The capsules from simple coacervates made by Arabic gum only, have lower molecular weight compared to capsules made from complex coacervation with combination of gelatin and gum Arabic. The release mechanism of capsules from each process can be determined from Fig. 4b and Fig. 4d. Generally, different release mechanisms of encapsulated materials provide controlled, sustained, or target release of core material. From the figures, the capsules made by present simple coacervation possess dissolution or melting of capsules wall of release mechanism where water is absorbed into the microcapsule core through the pore and swelling the capsule’s wall. Subsequent swelling was ruptured the microcapsule shell thus change the capsule’s shape and release the core material to environment. The core materials release by diffusion through the wall for the capsules made by complex coacervation. Diffusion is wherein the core material leak out through the interstitial channels of capsule’s wall. The overall release depends on the rate at which the core material leaks out and disperse from the surface [16].

4. Conclusion

The simple coacervation and complex coacervation method in present study able to provide good encapsulation process with encapsulation efficiency around 94%. For release kinetic study, release constant of simple coacervation higher than complex coacervation indicates that the complex coacervation produce higher cross-linking of polymeric structure thus lower the release rate of the core material. Both coacervation methods reported dimensionless coefficient, n=0.5 which is Fickian diffusion mechanism. Coacervates made from both process is spherical in shape. Simple coacervation
possess dissolution or melting of capsules wall of release mechanism while the core materials release by diffusion through the wall for the capsules made by complex coacervation.

Acknowledgement
Deepest gratitude goes to Malaysia Ministry of Education and Universiti Teknologi MARA for the financial support for this project from the research grants of 600-RMI/RAGS 5/3(76/2013).

References
[1] Arie M, Linder C, and David S 2010 “Formulations containing microencapsulated essential oils,” 2010.
[2] Martin Á, Varona S, Navarrete A and Cocero M J 2010 Open Chem. Eng. J. Vol. 04, 3
[3] Solomon B, Sahle F F, Gebre-Mariam T, Asres K and Neubert R H H 2012 Eur. J. Pharm. Biopharm Vol. 80, no. 1, 61–66
[4] Umer H, Nigam H, Tamboli A M and Nair M S M 2011 Int. J. Res. Pharm. Biomed. Sci. Vol. 2 (2) 474–481
[5] Bansode S S, Banarjee S K, Gaikwad D D, Jadhav S L and Thorat R M 2010 Int. J. Pharm. Sci. Vol. 1, no. 2 38–44
[6] Dong Z, Ma Y, Hayat K, Jia C, Xia S and Zhang X 2011 J. Food Eng. Vol. 104 no. 3 455–460
[7] Park J K and Chang H N 2000 Biotechnol. Adv. Vol. 18 no. 4 303–19
[8] Karsa D R and Stephenson R A 1993 Encapsulation and Controlled Release. (United Kingdom: Royal Society of Chemistry)
[9] Vahabzadeh F, Zivdar M and Najafi A 2004 IJE Trans. Vol. 17 333–343
[10] Nguyen H X and Nguyen C N 2012 Pharm. J. Vol. 52 no 437 6–10
[11] Siow L and Ong C 2013 Vol. 4 no 1 1–5
[12] Jun-xia X, Hai-yi Y and Jian Y 2011 Food Chem. Vol. 125 no 4 1267–1272
[13] H. B. Bohidar, 2008 Vol. 24 no. 3 105–124
[14] Jyothi N V N, Prasanna P M, Sakarkar S N, Prabha K S, Ramaiah P S and Srawanl G Y 2010, J. Microencapsul. Vol. 2010 27 3,187–197
[15] Yeo Y and Park K 2004 Arch. Pharm. Res. Vol. 27 no. 1 1–12
[16] Singh M N, Hemant K S Y, Ram M and Shivakumar H G 2010 Vol. 5 no. 2 65–77.