Materials Today: Proceedings xxx (xxxx) xxx

Contents lists available at ScienceDirect

Materials Today: Proceedings

journal homepage: www.elsevier.com/locate/matpr

# Physical characterization of propolis encapsulated vitamin E TPGS as nanomedicine

Yee Tong Kong [a], Rajaletchumy Veloo Kutty [a,b,*]

[a] Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, 26300, Kuantan, Pahang, Malaysia
[b] Center of Excellence for Advanced Research in Fluid Flow, Universiti Malaysia Pahang, 26300, Kuantan, Pahang, Malaysia

ARTICLE INFO

*Article history:*
Available online xxxx

*Keywords:*
Propolis
Caffeic acid phenethyl ester
vitamin E TPGS
Nanomedicine
Drug delivery system

ABSTRACT

CAPE found in propolis has become the subject of research interest as it has various medicinal benefits such as antiviral, anti-inflammatory, antioxidant, anticancer and immunomodulatory properties [1]. In this study, propolis obtained in Kuantan, Pahang is encapsulated using biodegradable polymer, vitamin E TPGS as a drug delivery system whereas TPGS-CAPE is used as a control. The findings show the physical characterization of propolis loaded vitamin E TPGS polymeric micelles which includes size, polydispersity index, zeta potential, shape and morphology and encapsulation efficiency.

Selection and peer-review under responsibility of the scientific committee of the 2nd International Conference on Innovative Technology and Sciences (iCITES 2020).

## 1. Introduction

Propolis is a complex mixture that synthesized by honeybee from resin of a plant to build the hive and to keep moisture and temperature stable in the hive. The composition of propolis is varied by its botanical and geographical origin as well. Basically, raw propolis is mainly composed of plant resins (50–70%), oil and waxes (30–50%), 10% pollen essential (5%-10%) and other chemical compounds including amino acids, minerals, sugars, vitamin B, C and E, flavonoids, phenol and aromatic compound [2].

Since thousands years ago, ancient civilization utilized propolis for its versatile medicinal properties. For example, Egyptians used propolis which contains bee wax as material for mummification process [3]. Besides, Assyrian put propolis on wound and tumour to fight against bacterial infection and facilitate the healing process. In ancient Greece, propolis was also used to produce polyanthus perfume. Polyanthus is a kind of flower that emanates fragrance smell. According to Kuropatnicki *et al.*, Greeks introduced propolis as primary ingredient to mix with other materials such as olibanum, styrax, and aromatic herbs [4]. Nowadays, propolis has become the interest of study by researchers and chemist as more than 300 compounds has been identified in propolis such as phenolic compounds that include flavonoids, phenolic acid, tannins, quinines, stilbenes, coumarins and curcuminoids [2]. Hence, these bioactive compounds in propolis contribute many properties that are essential for pharmaceutical development. For instances, it has antibacterial, antifungal, antioxidants, anti-inflammatory, antidiabetic and antitumoral properties.

In this study, the target phenolic compound in propolis is caffeic acid phenethyl ester (CAPE). CAPE is one of the derivatives of caffeic acid. It is a hydrophobic, bioactive polyphenolic ester of propolis produced by honey bees and plants as well. Murtaza et al. emphasizes the biological and pharmacological activities of CAPE such as antimicrobial, anti-inflammatory, anticancer activities and diminishing chemotherapy-induced toxicities [1]. Furthermore, Indian stingless bee propolis was reported to have a potent anticancer activity on cell lines such as human breast adenocarcinoma (MCF-7), human colon adenocarcinoma (HT-29), human epithelial colorectal adenocarcinoma (Caco-2), and murine melanoma cell lines (B16F1). The propolis causes cell death due to induction of apoptosis [5].

Preparation of propolis into nanomedicine as targeted drug delivery system able to deliver the pharmacological active components to specific tissues. Nanocarriers help to improve drug properties by encapsulating hydrophilic or hydrophobic molecules in their core, hence controlling release and distribution, enhancing drug absorption by cells and protecting the drug from degradation before reaching the targeted site [6]. In this study, d-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS), also known as vitamin E TPGS is water soluble form of vitamin E. It possesses amphipathic properties is chosen as the nanocarrier to improve the solubility of

* Corresponding author.
*E-mail address:* vrajaletchumy@ump.edu.my (R. Veloo Kutty).

https://doi.org/10.1016/j.matpr.2021.02.320
2214-7853/
Selection and peer-review under responsibility of the scientific committee of the 2nd International Conference on Innovative Technology and Sciences (iCITES 2020).

propolis or CAPE. This is because propolis is less soluble in water and CAPE is hydrophobic in nature. Biodegradable nanocarrier is also one of the major concerns in pharmaceutical application as polymers is safe and it can be eliminated through natural pathways either by simple filtration of degradation by-products or metabolized products in body system.

Owning to its versatile medicinal properties, propolis has potential therapeutic in different diseases. It can be prepared into nanomedicine for targeted treatment and this also helps to improve bioavailability of the active components in propolis due to its water solubility issue.

## 2. Material and methods

### 2.1. Materials

Raw propolis was obtained from Q'Lulut Global in Kuantan, Malaysia. Caffeic acid phenethyl ester (CAPE), vitamin E TPGS, phosphate buffered saline (PBS) were all purchased from Sigma Aldrich whereas acetonitrile was purchased from Thermo Fisher.

### 2.2. Raw material preparation

Raw propolis was directly collected from hives of stingless bees (*Trigona thoracica*) in Q'lulut Global, Kuantan, Malaysia. Raw propolis was grounded into fine powder and kept in −80 °C freezer using for long-term storage.

### 2.3. Extraction of propolis

Propolis was extracted using 70% ethanol by maceration method [11]. The ratio of solvent and propolis was 10:1 (v/w). Firstly, 10 g of propolis was mixed vigorously with 100 mL of 70% ethanol for 3 min at room temperature, 25 °C. The mixture was left overnight at room temperature in the incubator shaker (Infors HT Ecotron, Switzerland) with rotation speed of 120 rpm for 24 h. Next, the suspension was filtered through Whatman No. 1 filter paper. The filtrate was then concentrated using a rotary evaporator at 40 °C with rotation speed of 120 rpm to eliminate the solvent. The concentrate was stored under −80 °C before freeze drying using VirTis Benchtop Pro freeze dryer (SP Scientific, New York, United States).

### 2.4. Preparation of TPGS-propolis micelles and CAPE micelles

TPGS-propolis micelles were prepared according to the solvent casting method [8]. A total of 3 mg of propolis and 50 mg of vitamin E TPGS were dissolved in 3 mL of chloroform. From this mixture, chloroform was evaporated using rotary evaporator at 40 °C and a thin film of drug dispersed TPGS was formed. The thin film was subjected to freeze drying for 2 h. Then, drug-loaded micelles was formed by resuspending the drug-loaded film in 1 mM PBS at pH 7.4. The mixture was incubated in incubator shaker at 37 °C for 48 h. The samples were centrifuged at 25000 rpm for 20 min to sediment the micelles for further characterization. All the steps was repeated to prepare CAPE micelles where 3 mg of propolis was replaced by 3 mg of CAPE.

### 2.5. Particle Size, polydispersity and zeta potential

The average particle size, zeta potential and polydispersity index (PDI) of the formulation was measured by dynamic light scattering analysis by photon correlation spectroscopy (Zetasizer Nano ZS, UK). Data are representative of at least three independent experiments.

### 2.6. Surface morphology

The shape and surface morphology of the micelles were visualized using transmission electron microscope (TEM). Samples were prepared by placing one drop of micelles solution on a copper grid and then dried under vacuum pressure.

### 2.7. Encapsulation efficiency

The encapsulation efficiency (EE) was determined by measuring the amount of free CAPE present in the formulation. The amount of CAPE in the formulations was determined by HPLC method (Agilent HPLC 1260 series, USA). Chromatographic separation was performed using an Eclipse Plus C18 column (250 mm × 4.6 mm, 5 μm) (Agilent, United State) by isocratic elution. The temperature of column was set at 35 °C and the injection volume was 1 μL. The mobile phases consist of ultra-purified water (A) and 95% acetonitrile (B) which were prepared respectively. Cleaning agent C and D are 20% acetonitrile and 100% acetonitrile respectively. Before the HPLC analysis, the mobile phase was filtered through a 0.45 μm membrane filter and samples were filtered through 0.2 μm polyethersulfone (PES) membrane filter. After that, mobile phase was sonicated for 30 min to degas before the HPLC analysis. The flow rate was set at 0.5 mL/min and the effluent was monitored using a DAD set at 245 nm for CAPE quantification. As shown in Table 1, a gradient elution mode is used for the analysis of CAPE. This is because the presence of related compounds, such as other caffeic acid esters have a close polarity, hence it does not allow for efficient determination of this component in the isocratic elution mode [7]. The amount of encapsulated propolis was calculated with the formula given below.

**Table 1**
The condition for the gradient elution of CAPE.

| Time (min) | Flow (mL/min) | A (%) | B (%) | C (%) | D (%) |
|---|---|---|---|---|---|
| 0 | 0.5 | 90 | 10 | 0 | 0 |
| 1 | 0.5 | 90 | 10 | 0 | 0 |
| 10 | 0.5 | 80 | 20 | 0 | 0 |
| 20 | 0.5 | 70 | 30 | 0 | 0 |
| 30 | 0.5 | 50 | 50 | 0 | 0 |
| 40 | 0.5 | 10 | 90 | 0 | 0 |
| 45 | 0.5 | 90 | 10 | 0 | 0 |

$$Encapsulationefficiency(\%) = \frac{TotalamountofCAPEinformualtion - freeCAPE}{TotalamountofCAPEusedinformulation} \times 100\%$$

### 2.8. Standard solution preparation and sample preparation

The stock solution was prepared by dissolving 32 mg of CAPE in 10 mL acetonitrile. Then, 200, 400, 800, 1600 and 3200 μg/mL of standard solution of CAPE were diluted from the stock solution by using mobile phase. For sample preparation, 0.1 g of propolis extracts in 10 mL of mobile phase. However, 2 mL of TPGS-propolis micelles and 2 mL of TPGS-CAPE micelles were freeze dried and then dissolved in 2 mL of mobile phase respectively. All the standard solution of CAPE and micelles samples were filtered through 0.2 μm PES membrane filter.

## 3. Results and discussion

### 3.1. Particle size, polydispersity index and zeta potential

The particle size, PDI and zeta potential of the micelles are measured by dynamic light scattering (DLS) analysis by photon

**Table 2**
Particle size, polydispersity index, zeta potential and encapsulation efficiency of TPGS-propolis and TPGS-CAPE micelles.

| Micelles | Size (nm) | Polydispersity index (PDI) | Zeta Potential (mV) | Encapsulation efficiency (%) |
|---|---|---|---|---|
| TPGS-propolis | 13.68 ± 0.13 | 0.24 ± 0.02 | −11.83 ± 1.56 | 97.19 |
| TPGS-CAPE | 15.56 ± 0.07 | 0.29 ± 0.05 | −13.73 ± 0.84 | 95.03 |

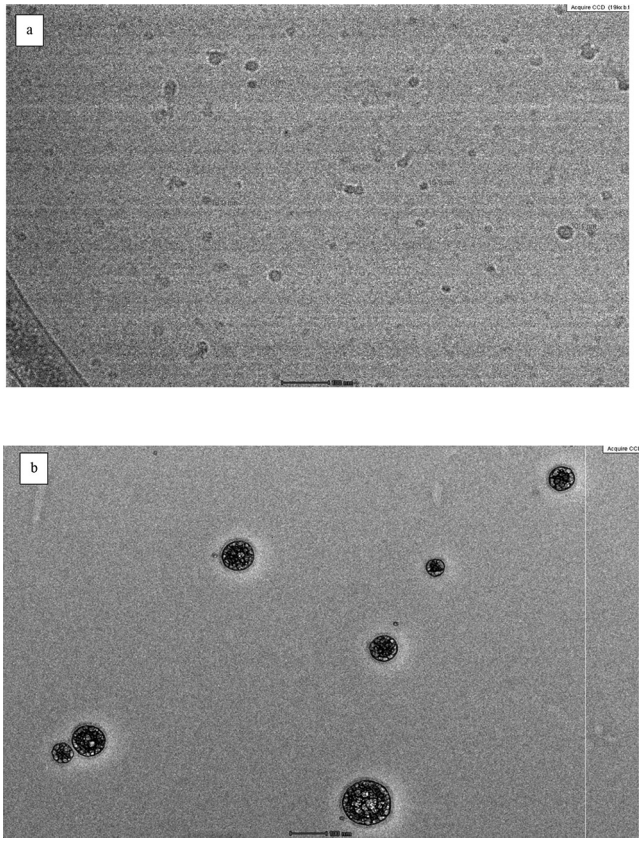


**Fig. 1.** Representative TEM images a) TPGS-propolis micelles; b) TPGS-CAPE micelles.

correlation spectroscopy are shown in Table 2. The mean sizes of TPGS-propolis and TPGS-CAPE micelles are distributed from 10 nm to 20 nm. This result can also be observed from TEM images as show in Fig. 1. The polydispersity index of all the micelles is less than 0.3 which indicates a narrow size distribution. It has been reported that typical micelles size is in the range of 10 nm to 100 nm which are suitable for drug delivery in cancer therapy [8]. These results are tallied with the docetaxel-loaded vitamin E TPGS micelles prepared by Muthu *et al.* [10].

Zeta potential indicates the surface charge of the nanoparticles which directly deals with the stability of the colloidal dispersion through electrostatic repulsion between the particles. Higher zeta potential able to prevents aggregation of the nanoparticles due to the Van der Walls force attraction between the nanoparticles. Zeta potential for both of the micelles maintains between −10 mV to −15 mV. The negative surface charge is caused by the presence of carboxyl end group located near the surface where the oxygen atom develops a partial negative charge (δ-) [9]. Besides, use of surfactant such as TPGS further reduces the surface charge. However, aggregation of nanoparticles can be prevented despite of lower value of zeta potential by the hydrophilic PEG chains of TPGS [9].

### *3.2. Surface morphology*

Transmission electron microscope (TEM) was used to reveal the surface morphology of the micelles. As shown in Fig. 1., the micelles were discrete, having a smooth surface and are spherical in shape. The particle size obtained in TEM image is well correlated with the measurement done by photon correlation spectroscopy.

### *3.3. Encapsulation efficiency*

In nanoparticle drug delivery system, the encapsulation efficiency is defined as the drug carrying capacity. Both of the samples had an encapsulation efficiency of 90% and above. For TPGS-propolis micelles, drug encapsulation efficiency was determined by CAPE present in the propolis extracts and micelles. The retention time of standard CAPE is 36.3 min.

## 4. Conclusion

This study shows the physical characterization of TPGS-propolis micelles as nanomedicine and its particle size is suitable to be used in targeted cancer treatment and other diseases. Encapsulate hydrophobic active component in nanocarrier can greatly improve water solubility of propolis. Hydrophilic surface possessed by TPGS micelles allows hydrophobic propolis to escape from reticuloendothelial system, therefore the micelles have a longer half-life in the systemic circulation [10].

## CRediT authorship contribution statement

**Yee Tong Kong:** Investigation, Visualization, Writing - review & editing. **Rajaletchumy Veloo Kutty:** Conceptualization, Supervision, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors would like to acknowledge Universiti Malaysia Pahang (www.ump.edu.my) for financially support this project under Research Grant (Project No. RDU1803181). Authors also would like to thank Fundamental Research Grant Scheme (FRGS/1/2017/TK05/UMP//1, RDU170130) from Ministry of Higher Education (MoHE), Malaysia for funding this work.

## References

[1] G. Murtaza, S. Karim, M.R. Akram, S.A. Khan, S. Azhar, A. Mumtaz, M.H.H. Bin Asad, Caffeic acid phenethyl ester and therapeutic potentials, BioMed Res Int. 2014 (2014) 1–9.
[2] Z. Ahangari, M. Naseri, F. Vatandoost, Propolis: Chemical composition and its applications in endodontics, Iran. Endod. J. 13 (2018) 285–292.
[3] G. Abdel-Maksoud, A.R. El-Amin, A review on the materials used during the mummification processes in ancient Egypt, Mediterr. Archaeol. Archaeom. 11 (2011) 129–150.

[4] A.K. Kuropatnicki, E. Szliszka, W. Krol, Historical aspects of propolis research in modern times, Evidence Based Complement. Alternat. Med. 2013 (2013) 1–11.
[5] M.K. Choudhari, R. Haghniaz, J.M. Rajwade, K.M. Paknikar, Anticancer activity of Indian stingless bee propolis: an in vitro study, Evidence Based Complement. Alternat. Med. 2013 (2013) 1–10.
[6] M.F. Attia, N. Anton, J. Wallyn, Z. Omran, T.F. Vandamme, An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites, J. Pharm. Pharmacol. 71 (2019) 1185–1198.
[7] D.I. Pisarev, O.O. Novikov, A.Y. Malyutina, E.V. Lupina, E.T. Zhilyakova, N.N. Boyko, Development of a technique for assessing the quality of propolis by the content of caffeic acid phenethyl ester by high-performance liquid chromatography with a diodematrix detector, Drug Invent. Today. 9 (2017) 53–58.
[8] R.V. Kutty, S.S. Feng, Cetuximab conjugated vitamin E TPGS micelles for targeted delivery of docetaxel for treatment of triple negative breast cancers, Biomaterials. 34 (2013) 10160–10171.
[9] B. Mishra, R. Padaliya, R.R. Patel, Exemestane encapsulated vitamin E-TPGS–polymeric nanoparticles: preparation, optimization, characterization, and in vitro cytotoxicity assessment, Artif. Cells Nanomed. Biotechnol. 45 (2017) 522–534.
[10] M.S. Muthu, S. Avinash Kulkarni, Y. Liu, S.S. Feng, Development of docetaxel-loaded vitamin E TPGS micelles: formulation optimization, effects on brain cancer cells and biodistribution in rats, Nanomed. 7 (2012) 353–364.
[11] Y.T. Kong, V. Abdullah, S.U. Mokhtar, R.V. Kutty, Preliminary studies on extraction of propolis using vitamin E d-α-Tocopheryl polyethylene glycol succinate (vitamin E TPGS) and compare their antimicrobial activities, J. Microbiol. 16 (2020) 346–352.