

Preparation and Characterisation of Carvacrol Encapsulated in Gellan Gum Hydrogel

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Abstract

Studies on plant materials as natural compound such as carvacrol (Carv) have gained much attention. Carv exhibits numerous potential as antimicrobial agent, food additives, antioxidant and etc. However, this free standing bioactive compound is unstable in the harsh environment conditions. Hence, the encapsulation technology provides protection to enhance the effectiveness in release manner. In this study, the preparation of Carv encapsulated in gellan gum hydrogel forming thin film (GG-Carv TF) was achieved by using 1.0 g of gellan gum at different concentrations of Carv (0.01-0.04 M). The FTIR spectra of GG-Carv TF revealed the combination of both functional groups from GG and Carv. The Carbon, Hydrogen and Nitrogen, CHN analysis further confirmed the encapsulation with the changes in the element percentage. Both swelling and degradation percentage increased with time and showed decreasing patterns in the range of 680.79-666.78 % and 26.83-19.15 % which can be observed as the concentration of Carv increased, respectively.

Keywords carvacrol, encapsulation, gellan gum hydrogel, thin film, natural compound

INTRODUCTION

Carvacrol (Carv) is found in the aromatic leaves and flowering plant of both thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*). Interestingly, Carv shown an effective antibacterial activity and has been proven to be potential agents in the treatment of infections and safe for human and animal consumption [1]. The world wide researchers have investigated the wide spectrum of antibacterial activity by Carv against various types of microorganisms such as *C. albicans* [2], *L. plantarum*, *S. cerevisiae*, *B. cinerea* [3], *S. aureus* [4], *Salmonella enterica* [5], *L. monocytogenes*, *E. coli* [6] and etc.

The host, hydrogel, is three-dimensional, hydrophilic, polymeric network that is capable of imbibing a large amount of water into its structure. It is highly permeable to various drug compounds, able to withstand acidic environments and high swelling properties which can release entrapped molecules through their web-like surfaces [7]. The component of hydrogel, gellan gum, is a microbial polysaccharide that is derived from *Sphingomonas elodea*, previously known as *Pseudomonas elodea*. Significantly, gellan gum is nontoxic, biocompatible, biodegradable and the resulting hydrogels is transparent and stable [8]. To date, this biopolymer based hydrogels has been gaining great attention as the potential carrier in controlled release studies.

Based on the foregoing, it is believed that the encapsulation technology provides stability and protection to enhance the effectiveness due to the facts that Carv is unstable in the harsh environment conditions. It is volatile, easily evaporates and prone to degradation during the process in growing to direct exposure of heat, pressure, light or oxygen [9]. To elucidate this matter, the Carvis encapsulated in biodegradable gellan gum hydrogel as an alternative way to extend its shelf life and to control the release manner, thereby the usage of the compound could be maximised. This study was carried out to prepare the Carv encapsulated in gellan gum hydrogel in the form of thin film and the physico-chemical properties were also investigated.

MATERIALS AND METHODS

The chemicals used in this study were glycerin (1,2,3-Propanetriol), gelzan (gellan gum), calcium chloride (CaCl_2) ($\geq 96\%$) and carvacrol (2-Methyl-5-(1-methylethyl)-phenol) which were obtained from Sigma-Aldrich ($\geq 98\%$), sodium dihydrogen orthophosphate (NaH_2PO_4) was purchased from BDH Chemicals Ltd Poole England ($\geq 98\%$), sodium hydrogen carbonate (NaHCO_3) was provided by Fisher Brand ($\geq 99.8\%$), sodium chloride (NaCl) was obtained from AnalaR ($\geq 99\%$), and potassium chloride (KCl) was purchased from HmbG Chemicals ($\geq 99.5\%$). All chemicals were used directly without any purification.

Preparation of Carvacrol Gellan Gum Thin Films (GG-Carv TF)

GG-Carv TF was synthesised via *in-situ* drug loading in which the Carv was first diluted in deionised water (18 M Ω cm) to the specific concentration accordingly and mixed with the dissolved 1 g of gellan gum before establishing the physical crosslinking protocol using CaCl_2 . The solution was stirred at 500 rpm using hotplate set at temperature of 80°C for a total mixing of 2 hours to ensure the homogeneity. 5 ml of glycerin was added as a plasticizer. The gellan gum hydrogel encapsulated with Carv with the concentration of 0.01, 0.02 and 0.04 M are hereon referred as GG-Carv 01, GG-Carv 02 and GG-Carv 04 respectively. The solution was poured into the petri dish and left in the oven for 48 hours at 35°C for drying before storing in dessicator for further characterisation.

Characterisations

FTIR spectra of the samples were recorded in the range of 400-4000 cm^{-1} on a Perkin-Elmer 1752X Spectrophotometer with KBr disc method. The elemental analysis was done using LECO CHNS-932 Analyser. The surface and cross section morphology of the sample analyses were observed with VPSEM (Variable Pressure Scanning Electron Microscopy) using LEO 1455.

The Study of Swelling Percentage

Water uptake of GG-Carv TF with the dimension of 2 cm x 2 cm was measured by weighing the dried films (W_d) prior to immersion into 20 ml of **Pseudo Extra Cellular Fluids, PECF** buffer solution with pH 5.5 at room temperature. The subsequent weight was recorded for every 24 hour. The films were removed after 72 hours, wiped gently with a tissue to expel the liquid from the surface, and were then weighed (W_w).

The percentage of water uptake was then determined from the equilibrium swelling ratio:

$$\text{Swelling Percentage (\%)} = (W_w - W_d) / W_d \times 100$$

Where; W_w = weight of wet sample

W_d = weight of dry sample

The Study of Degradation Percentage

Degradation of GG-Carv TF was measured by weighing the initial weight of 1.0g (W_i) and left on petri dish at the room temperature. The subsequent weight was recorded for every day until a constant weight (W_f) pattern was observed.

The percentage of degradation was then determined from the equilibrium degradation ratio:

$$\text{Degradation Percentage (\%)} = (W_f - W_i) / W_i \times 100$$

Where; W_f = final weight of sample

W_i = initial weight of dry sample

RESULT AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Chemical structures of the samples were characterized by FTIR (Figure 1). In general, Carv (Figure 1(a)) showed the characteristic peaks at 3360.88 cm^{-1} (phenolic-OH group), 2958.46 cm^{-1} (C-H stretching), 1583.49 and 1511.04 cm^{-1} (C-C ring stretching), 1421.54 cm^{-1} (O-H bending), 1359.10 cm^{-1} (isopropyl group), 1242.62 cm^{-1} (C-O stretching) and 864.50 cm^{-1} (aromatic ring). Meanwhile, the peaks of pure GG TF (Figure 1(e)) can be seen at 3273 cm^{-1} (O-H stretching), 2933.35 cm^{-1} (C-H stretching), 1625.45 cm^{-1} (C=C stretching), 1427.37 cm^{-1} (C-H bending), 1033.53 cm^{-1} (C-O stretching) and 919.62 cm^{-1} (C-H bending).

From the results obtained (Figure 1(b-d)), all of GG-Carv TF (s) showed the peak at the range of 3274.53-3290.70 cm^{-1} (O-H stretching) and 2890.57-2933.10 cm^{-1} (C-H stretching) which belonged to both gellan gum hydrogel and Carv. Furthermore, the peaks at 1638.52-1642.49 cm^{-1} and 1414.60-1415.16 cm^{-1} (C-C ring stretching), 1034.38-1035.99 cm^{-1} (C-O stretching) and 918.57-918.92 (aromatic ring) which belonged to Carv exist in all GG-Carv TF (s), reflecting the existence of Carv in the gellan gum hydrogel polymer.

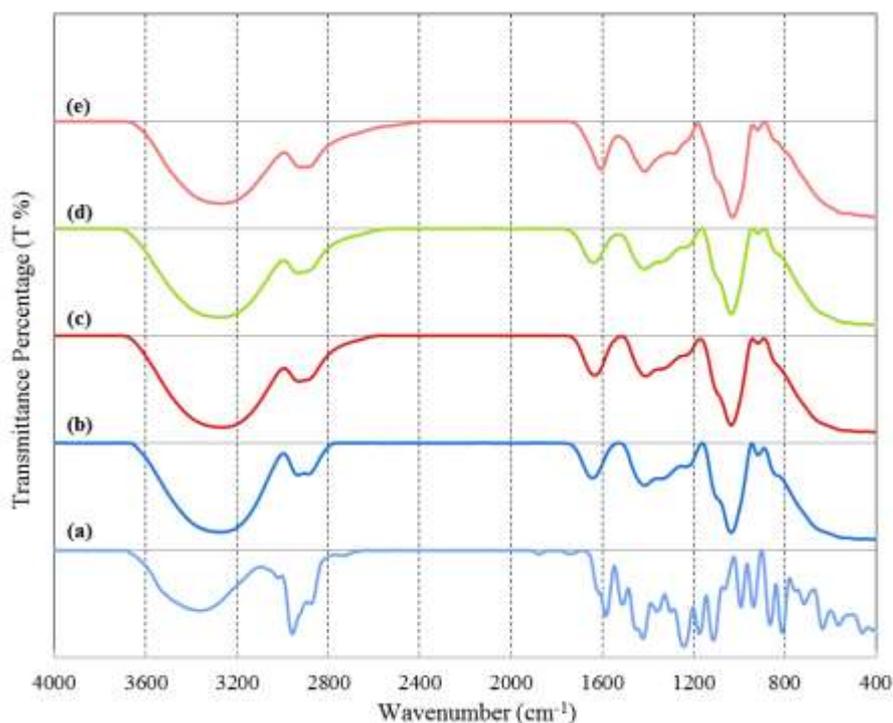


Figure 1 FTIR spectra of (a) Carvacrol (b) GG-Carv 01 (c) GG-Carv 02 (d) GG-Carv 04 (e) Pure GG TF

Elemental Analysis

Table 1 shows the weight percentage of carbon, C and hydrogen, H for pure GG TF and encapsulated GG-Carv TF with three different concentration of Carv. From Table 1, it could be observed in GG-Carv TF, that the content of C showed increasing pattern as the concentration of Carv increased. This inclined amount is due to the encapsulated Carv anion which caused the content of C to increase. Similarly, the H content in GG-Carv TF exhibited increasing pattern as the concentration of Carv increased. This analysis further confirmed the encapsulation with evidence of the changes in the element percentage.

Table 1 Weight percentage of carbon, C and hydrogen, H for pure GG TF and encapsulated GG-Carv TF with various concentration of Carv

Material	Weight Percentage (%)	
	C	H
Pure GG TF	20.33	8.97
GG-Carv 01 TF	22.52	9.08
GG-Carv 02 TF	23.76	9.29
GG-Carv 04 TF	26.77	9.52

Variable Pressure Scanning Electron Microscopy (VPSEM) Analysis

VPSEM micrographs were used to study the surface and cross sectional area of GG-Carv TF. The observation was made at 1000 times magnification. This technique is widely used to capture the characteristic ‘network’ structure in hydrogels [10].

Surface Morphology

Clear network structure can be observed on the surface morphology of pure GG TF (Figure 2(a)). Meanwhile, GG-Carv TF (Figure 2(b-d)) exhibited the round-shaped structure scattered evenly which is possibly due to the Carv binding to the surface of gellan gum hydrogel. The appearances of these structures were more abundant as the concentration of Carv increased with average diameter of 5 to 10 μm .

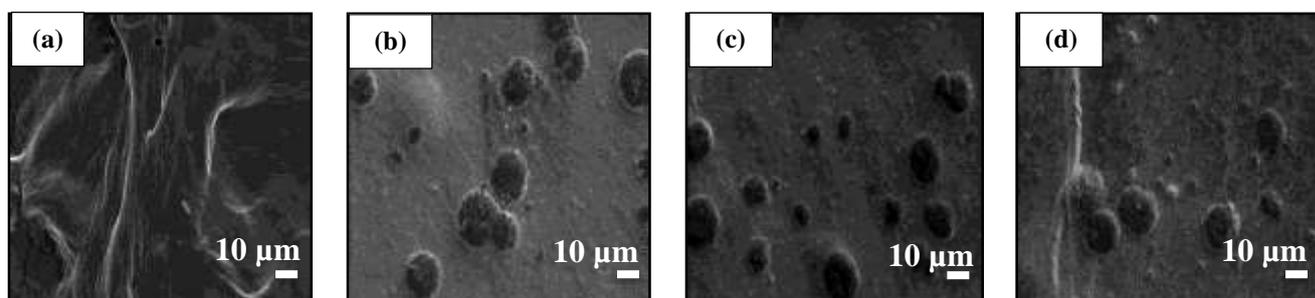


Figure 2 VPSEM surface micrograph at 1000x magnification (a) Pure GG TF (b) GG-Carv 01 (c) GG-Carv 02 (d) GG-Carv 04

Cross Sectional Morphology

Unpacked layers structure can be observed in the cross sectional morphology of pure GG TF (Figure 3(a)). Meanwhile, GG-Carv TF (Figure 3(b-d)) displayed a very compact layer as the concentration of Carv increased. This can be explained due to congestion of Carv molecules residing in the gellan gum hydrogel.

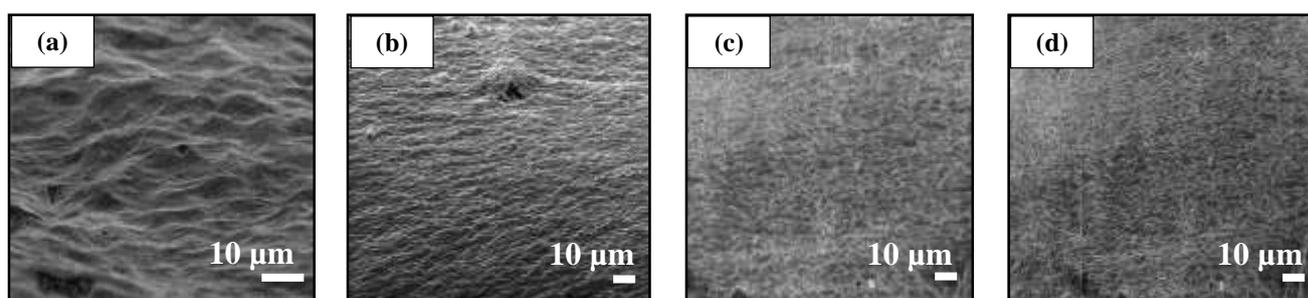


Figure 3 VPSEM cross section micrograph at 1000x magnification (a) Pure GG TF (b) GG-Carv 01 (c) GG-Carv 02 (d) GG-Carv 04

The result displayed the swelling percentage (Figure 4) increased with time. When higher concentration of Carv was used, the lesser the absorption of the solutions could be observed. This can be reflected as GG-Carv04 with the highest concentration of Carv had the lowest swelling percentage due to the formation of more rigid structure of gellan gum hydrogel. Besides that, Carv is known as the hydrophobic phenolic compound [11]. Thus, the resistance effect towards the solutions which account for the hydrophobicity of Carv also resulted in decreased swelling. Hence, the higher the concentration of Carv, the higher the water resistance of the film expected.

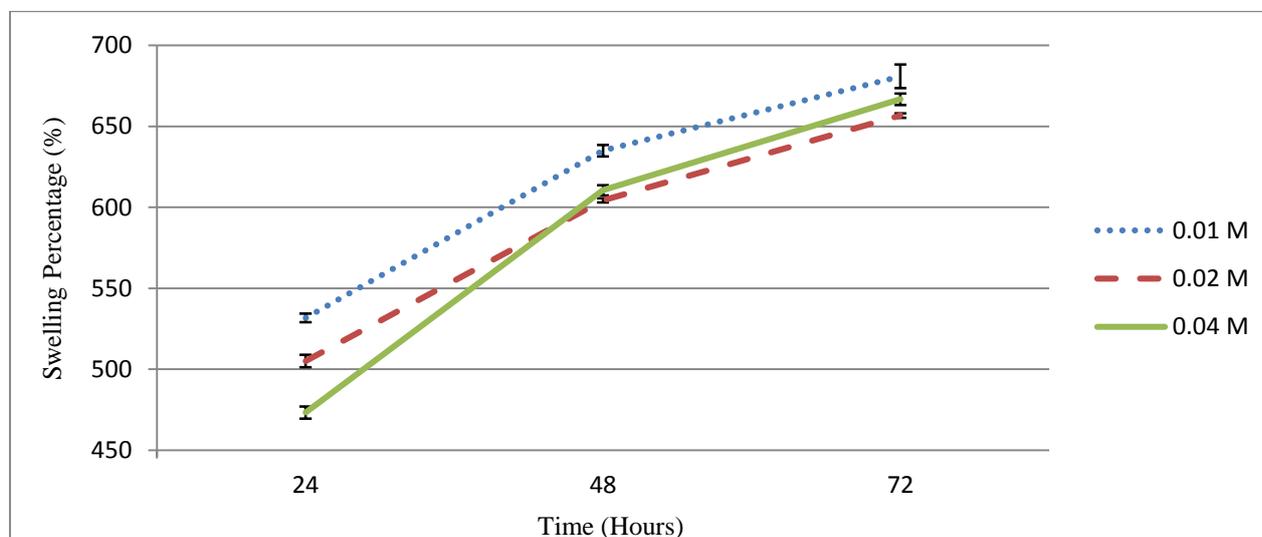


Figure 4 The Swelling Percentage

Degradation Percentage

Most of the degradation study of gellan gum was usually achieved in vivo through the action of enzymes and in vitro [12, 13] in accordance to their application in tissue engineering. However, to understand the degradation behaviour of polymers aimed to be used on the skin, it is important to predict and ultimately be tuned in to their condition at common room temperature for humans.

In Figure 5, the percentage of degradation was found to increase with the time. However, it was inversely proportional to the concentration. This can be seen as the concentration of Carv increased, the degradation percentage decreased. Similar to the swelling results, this might be explained by the formation of more rigid structure of gellan gum hydrogel occurring in GG-Carv TF at higher concentration. Hence, this stability resulted in more durable GG-Carv TF against the environment conditions as the concentration increased.

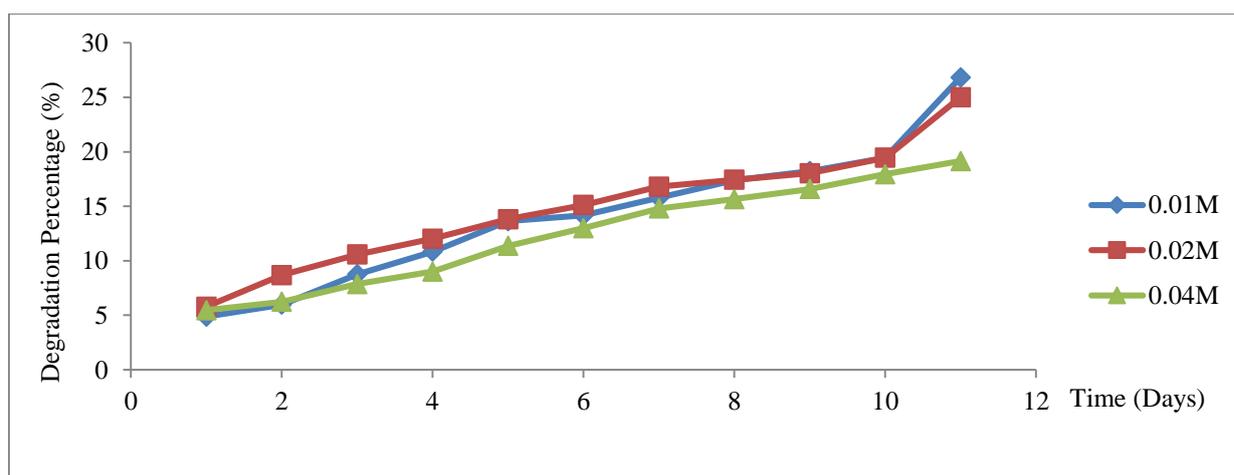


Figure 5 The Degradation Percentage

CONCLUSION

The preparation of carvacrol encapsulated in gellan gum hydrogel in the form of thin film (GG-Carv TF) was successfully achieved as confirmed by the FTIR spectrum of GG-Carv TF which showed the combination of both functional groups from the gellan gum hydrogel and Carv. The CHN analysis further confirmed the existence of the element with changes of the element percentage. The swelling and degradation percentage similarly increased with time and decreasing patterns can be observed as the concentration of Carv increased. This study has generated the fundamental knowledge of gellan gum hydrogel-Carv thin films which could be used for further studies in the development of antibacterial applications.

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