UNIVERSITI PUTRA MALAYSIA

PREPARATION, CHARACTERISATION AND RELEASE OF CARVACROL ENCAPSULATED IN GELLAN HYDROGEL AND CHITOSAN NANOPARTICLES FOR ANTIBACTERIAL APPLICATION

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By

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Studies of plant materials as natural compound such as carvacrol (Carv) for antibacterial agents have gained much attention in the scientific research. It has been proven to be the potential agent in the treatment of infections and is safe for human and animal consumption. However, this free standing bioactive compound is unstable in the harsh environment conditions which easily evaporates and prone to degradation due to volatilisation and chemical reaction. In this study, the encapsulation technology helps to provide protection in order to enhance the effectiveness and the release manner thereby improving cost effectiveness of the product. Thus, Carv was encapsulated in two types of host materials which are gellan gum hydrogel thin film (GG-Carv TF) and chitosan nanoparticles (CNP-Carv). Besides that, the release properties are studied for further antibacterial application against Gram-negative bacteria, *E. coli*. Based on the result, GG-Carv TF showed combination of both functional groups from GG and Carv in FTIR spectra. The CHN analysis further confirmed the encapsulation as evidence of the changes in the element percentage. The swelling and degradation percentage increased with time and the decreasing patterns can be observed as the concentration of Carv increased in the range of 680.94-424.20 % and 26.83-2.67 %, respectively. Highest accumulated release of Carv from GG-Carv TF was recorded with 97.6 %. From the kinetic fitting model, pseudo-second order was observed to fit the GG-Carv TF release profile with $r^2 > 0.9$. GG-Carv TF exhibited the significant antibacterial activity against *E. coli* with clear inhibition zone of 20 mm while the detection of the bacterial growth by optical density also displayed the continued decrease in sustained and controlled manner. Meanwhile, the encapsulation with the other host, CNP-Carv, the results showed the increment in the size distribution average to 139 nm prior to the blank

size of CNP with only 56 nm. This result was complementary with the size of nanoparticles on surface morphology observed using FESEM. The FTIR spectrum also revealed the combination of both functional groups from the CNP and Carv, proving that Carv was successfully encapsulated. Highest accumulated release of Carv from GG-Carv TF was recorded with 28.0 %. From the kinetic fitting model, pseudo-second order was observed to fit the GG-Carv TF release profile with $r^2 > 0.9$. CNP-Carv exhibited significant antibacterial activity against *E. coli* with the notable inhibition zone of 20 mm and the detection of bacterial growth by optical density also showed a continued decline of bacterial growth. Hence, the gellan gum hydrogel and chitosan nanoparticles are proven to be effective carrier of carvacrol for further antibacterial application.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Master Sains

PENYEDIAAN, PENCIRIAN DAN PELEPASAN CARVACROL TERKANDUNG DALAM GELLAN HIDROGEL DAN KITOSAN NANOPARTIKEL UNTUK APLIKASI ANTIBAKTERIA

Oleh

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Penyelidikan ke atas bahan-bahan tumbuhan sebagai sebatian semula jadi seperti Carvacrol (Carv) telah mendapat banyak perhatian dalam kajian saintifik. Carv telah terbukti sebagai agen yang berpotensi untuk merawat jangkitan dan selamat untuk penggunaan manusia dan haiwan. Walau bagaimanapun, sebatian bioaktif berdiri bebas ini adalah tidak stabil dalam keadaan persekitaran yang sukar di mana ianya mudah tersejat dan mudah terdedah kepada kerosakan akibat tindak balas kimia. Dalam kajian ini, teknologi pengkapsulan membantu memberi perlindungan untuk meningkatkan keberkesanan dan tatacara pembebasan serta meningkatkan penjimatan kos produk. Oleh itu, Carv telah dikapsulkan ke dalam dua jenis hos iaitu gellan gam hidrogel (GG-Carv TF) dan kitosan nanopartikel (CNP-Carv). Selain itu, kajian turut dijalankan terhadap cara pembebasan untuk tujuan aplikasi antibakteria terhadap gram-negatif bakteria, *E. coli*. Berdasarkan keputusan kajian, GG-Carv TF menunjukkan gabungan kedua-dua kumpulan berfungsi daripada GG dan Carv di dalam spektra FTIR. Analisis CHN seterusnya mengesahkan pengkapsulan dengan bukti perubahan dalam peratusan unsur. Peratusan pembesaran dan degradasi juga meningkat selaras dengan masa dan corak penurunan juga dapat dilihat dengan meningkatnya kepekatan dalam anggaran 680.94-424.20 % dan 26.83-2.67 % masing-masing. Perlepasan tertinggi Carv daripada GG-Carv TF didapati sebanyak 97.6 %. Model kinetic-pseudo kedua didapati sesuai dengan profil perlepasan dengan $r^2 > 0.9$. GG-Carv TF turut mempamerkan aktiviti antibakteria yang siknifikan terhadap *E. coli* dengan saiz 20 mm zon perencatan yang jelas serta pengesanan pembiakan bakteria melalui cara ketumpatan optik juga menunjukkan penurunan yang berterusan dan berkala. Selain itu, pengkapsulan menggunakan hos CNP-Carv pula menunjukkan peningkatan saiz purata 139 nm selepas pengkapsulan berbanding hanya 56 nm sebelumnya. Keputusan ini juga selaras dengan saiz yang dilihat melalui morfologi permukaan melalui FESEM. FTIR spektra turut mendedahkan gabungan kedua-dua kumpulan berfungsi daripada CNP dan Carv dan secara tidak langsung membuktikan kejayaan pengkapsulan. Perlepasan tertinggi Carv daripada CNP-Carv sebanyak 28.0 %. Daripada model kinetic-pseudo kedua didapati sesuai dengan profil perlepasan dengan $r^2 > 0.9$. CNP-Carv mempamerkan aktiviti antibakteria yang ketara terhadap *E. coli* dengan saiz 20 mm zon perencatan serta pengesanan pembiakan bakteria melalui cara ketumpatan optik juga menunjukkan penurunan. Oleh itu, gellan gam hidrogel dan kitosan nanopartikel telah terbukti untuk menjadi hos pembawa yang berkesan untuk aplikasi antibakteria selanjutnya.

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I certify that a Thesis Examination Committee has met on 9 November 2017 to conduct the final examination of Norafida bt Hasnu on her thesis entitled "Preparation, Characterization and Release of Carvacrol Encapsulated in Gellan Hydrogel and Chitosan Nanoparticles for Antibacterial Application" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

Carv	Carvacrol
GG TF	Gellan Gum Hydrogels Thin Films
GG-Carv TF	Gellan Gum Hydrogels-Carvacrol Thin Films
CNP	Chitosan Nanoparticles
CNP-Carv	Chitosan Nanoparticles-Carvacrol
PECF	Pseudo Extra Cellular Fluid
FTIR	Fourier Transform-Infrared
CHN	Carbon, Hydrogen and Nitrogen
VPSEM	Variable Pressure Scanning Electron Microscopy
FESEM	Field Emission Scanning Electron Microscopy
DLS	Dynamic Light Scattering
UV-Vis	Ultraviolet-visible Spectrophotometer
OD	Optical Density

CHAPTER 1

INTRODUCTION

1.1 Nanotechnology

Nanotechnology is the evolutionary technology of science. It is the study and application of extremely small things and can be used across all of other science fields, such as chemistry, biology, physics, materials science, engineering and etc. It also involves the manipulation of materials at the molecular level. The development of these nano materials is a result of fundamental research done by scientists and industries which have showed improvements to the existing products. It is commonly employed in several critical fields such as drug delivery, environmental science, catalysis, engineering and many other areas of society for better and advanced application.

Richard Feynman, a Nobel Prize winner and a physicist has become well known with his famous lecture entitled 'There's Plenty of Room at the Bottom' in 1959 (Feynman, 1965). He has played an important role in catalysing nanotechnology research area by inspiring others to indulge in this promising research area. Feynman describes nanotechnology as science of building things from the bottom up with atomic precision. Feynman's ideas have influenced Drexler in writing a book entitled 'Engines Creation, The Coming Era of Nanotechnology', which he forecasted that nanotechnology will sweep the world within ten to fifty years and explained that nanotechnology will definitely give an impact to human life in politics, information science, defences, human relations and etc (Drexler, 1986).

Nanotechnology has been leading the current research trend and become very popular in the development of the latest scientific technology which encompasses many disciplines. It is a field of applied science and technology covering a broad range of topics. Currently, nanotechnology considered as highly multidisciplinary field compromising knowledge from other fields. The main aim is to control matter between 1-100 nanometres in size. It covers the study of functional system in a much deeper and extensive research at the molecular scale. Commonly nanotechnology means the ability to construct different material starting from the very bottom, using the latest techniques and tools as to invent high performance and completely distinguished products.

In human daily life, research and technological development has a primary impact in boosting the efficiency of a product. Technology has enable people to create new things and make many scientific endeavours become possible in assisting human in life. Technology has such immense benefits in every phase of human lives, that it has not only made life easier but also raised the standard of living for every individual and society. The major advancement in technology is because of the increased of scientific research these days. Research in science has been the pillar of technology and therefore it is considered to be the most looked upon subject in the modern world due to its technological success.

1.2 Current Trend of Nanotechnology Research

The advantage of high surface-to-volume ratio in nano material has been manipulated by researchers worldwide to attain novel and useful application. Often in various cases, smaller nano materials are preferred, and it is generally found that specific applications require particle diameters of 30 nm or less to provide significant improvements over the use of 'conventional' nano or micron scale particles. Changes from bigger size material to nano structures involve the manipulation of structural, thermodynamic, electronic, spectroscopic, electromagnetic, dynamic and chemical features which could create potentials to various applications.

Coordination in multidisciplinary research of nanotechnology has enhanced various critical research fields such as drug delivery, tissue engineering, catalysis, filtration, efficiency of energy production and etc. Currently, the use of tailored nanoparticles has opened the avenue to optimize the capacity and transport beneficial material. Host material such as gellan gum hydrogel (GG) and chitosan nanoparticles (CNPs) have been modified to uptake the beneficial material, transport and finally release it at the targeted place. The development of nanotechnology has given the opportunities to synthesis, characterise, manipulate and organise the host material for various promising application.

Over the past decades, great attention has been focused on biopolymer-based hydrogels such as gellan gum hydrogel (GG) as the potential carriers in controlled drug delivery. Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amount of water or biological fluids, resembling biological tissues. Because of these properties, great interest was devoted to these systems for biomedical applications. Indeed, the physicochemical properties of the hydrogels can be tuned by varying the crosslinking degree (physical and/or chemical), thus making these networks suitable devices for a modulated drug delivery.

In conjunction to the increasing demand of this delivery system tremendously, CNPs polymers also concomitantly attract the attention of the researchers. They are capable of carrying and delivering a continuous supply of biologically active molecules into a specific environment. These systems are able to reduce the amount of active agent required for treatment by maintaining an effective concentration in the system applied over a certain period of time. Currently, most of the recently developed delivery systems consist of natural and synthetic polymers, polymer blends and composites of organic and inorganic materials that form membranes, capsules or micelles, depending on the application required. However, natural polymers such as CNPs are the most preferred.

The natural active molecule, carvacrol, which is the main constituents of oregano oil, has proven very usable in various applications, such as anti-microbial agents, antifungal, antiviral, antiparasitic, antioxidant, analgesic, anti-inflammatory and anti-cancer properties. It has a broad commercial interest because of the significant antimicrobial properties and able to inhibit or control the growth of pathogenic and/or spoiling bacteria. The previous reports have indicated that essential oils containing carvacrol (phenolic compounds) is one of the compounds showing the highest antibacterial performances (Meng *et al.,* 1998 and Dorman *et al.,* 2000). This finding directly highlighted the ability of carvacrol to inhibit bacterial growth and becomes the most promising idea for the development of natural antibacterial agent as the replacement of current used synthetic antibacterial agent.

Besides that, the existing hosts that aid to incorporate the active substance is tend to slowly degrade and lose their activity or become hazardous, by propagating a chain of oxidation reactions (Poshadri, 2010). Thus, there is a great need for the safe delivery systems. These two hosts, GG and CNPs, both are equally important for the encapsulation and delivery of natural active molecules such as carvacrol in diverse technological applications encompassing multidisciplinary areas. Their applications in human daily life bring the revival and new hopes in our technological system. Based on the foregoing, we can see that the combination of these natural polymers and natural active agents are widely used in various fields depending on the intended and specific application.

1.3 Nanotechnology Tools for Efficient Antibacterial Delivery

Malaysia is one of the countries that have high cases of bacterial outbreak of new and re-emerging infections due to the suitable temperature and condition for the growth of most bacteria. In recent years, an increasing number of bacterial outbreaks have been recorded and probably there should be more cases that were not detected or reported. There are many types of bacteria does exist, some of which cause illness through the infection and disease that has been associated with microorganisms like bacteria, fungi, viruses and parasites.

The infectious diseases are easily spread in various ways both direct and indirect contact. Most commonly, the outbreaks take place due to the ingestion of pathogenic bacteria like *Salmonella Typhi, Escherichia coli, Staphylococcus aureus, Vibrio cholera, Campylobacter jejuni,* and *Listeria monocytogenes* (Abdul-Mutalib, 2015). This has caused concerns for the wellbeing of Malaysians living locally and travelling abroad. The effort for prevention of diseases through the importance of hygiene has brought the matter to the forefront of public awareness.

Despite the discovery of new antibiotics, treatment of infections often fails to eradicate the pathogens completely. One major reason is that many antibacterial are difficult to be transported to the targeted area and the delivery time is short before the growth of bacteria can be completely inhibited due to the disadvantages of free standing antibacterial agent to the temperature, pressure, heat and environment.

Indeed, the challenge is to design the means of carrying this antibacterial agent direct to the targeted area such as human skin in order to prevent the infection and inhibit the bacterial growth. The crucial factor that should be taken into account is to provide the protection to this antibacterial agent by encapsulation technology with the intention to prolong and sustain the delivery time until the growth of the bacteria can be completely inhibited. This encapsulation technology is not merely enhances the efficiency of the release manner, but improving the cost effectiveness and the usage of the compound could also be maximised.

1.4 Problem Statement

Carvacrol is a natural compound and the main constituents of the oregano oil. It has been proven very usable and notorious for wide application including a useful source of antimicrobial compounds. Carvacrol has a significant antibacterial property to inhibit or control the growth of bacteria and has been recognised for its potential as antibacterial agent. The most compelling finding, carvacrol could be an alternative approach to control the spread of pathogenic organisms as well as the development of resistance to the conventional chemical antibiotics (Nostro *et al.,* 2012).

However, carvacrol is unstable in the harsh environment conditions. It is volatile, easily evaporates and prone to degradation during the processing owing to direct exposure of heat, pressure, light or oxygen (Charlier *et al.,* 2007). To elucidate this matter, carvacrol is encapsulated in gellan gum hydrogel and chitosan nanoparticles to provide stability and protection. This technology is hoped to extend the shelf life and sustain the release manner thereby the usage of the compound can be directly maximised. Therefore, in this study, the research was carried out to prepare carvacrol encapsulated in gellan gum hydrogel thin film (GG-Carv TF) and chitosan nanoparticles (CNP-Carv) and further characterised for antibacterial application.

1.5 Objectives of the Study

Carvacrol has been identified by previous researcher to have significant antibacterial properties and has been recognised for its potential as antibacterial agent. However, due to the disadvantages of this free standing carvacrol to the environment condition, the usage of this active compound is not fully maximised as it easily evaporated and degraded when directly exposed to the heat, oxygen, pressure or light (Charlier *et al.*, 2007). Thus, this study is attempt to bridge the gap between the issues of disadvantages of carvacrol and providing the protection from the host of gellan gum hydrogel and chitosan nanoparticles to ensure the function of carvacrol can be directly maximised.

Due to this concern, the major aim of this study is to encapsulate carvacrol in gellan gum hydrogel thin film and chitosan nanoparticles in order to enhance the effectiveness and release manner of carvacrol. The antibacterial properties were also studied for further application. Therefore, the specific objectives for this study are as follows :

1. To prepare and characterise the host of gellan gum hydrogel thin film (GG TF) and chitosan nanoparticles (CNP).
2. To prepare and characterise gellan gum hydrogel-Carv thin film (GG-Carv TF) and chitosan nanoparticles-Carv (CNP-Carv).
3. To study the release manner of both GG-Carv TF and CNP-Carv.
4. To study the antibacterial application of GG-Carv TF and CNP-Carv.

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LIST OF PUBLICATIONS

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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 (*Origanumvulgare*). 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 ($CaCl_2$) (≥96%) and carvacrol (2-Methyl-5-(1-methylethyl)-phenol) which were obtained from Sigma-Aldrich (≥98%), sodium dihydrogen orthophosphate (NaH_2PO_4) was purchased from BDH Chemicals Ltd Poole England (≥98%), sodium hydrogen carbonate ($NaHCO_3$) was provided by Fisher Brand (≥99.8%), sodium chloride (NaCl) was obtained from AnalaR (≥99%), and potassium chloride (KCl) was purchased from HmbG Chemicals (≥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 MΩ 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; Wf = final weight of sample
Wi = 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), 2933.35cm^{-1} (C-H stretching), 1625.45 cm^{-1} (C=C stretching), 1427.37cm^{-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 3274.53-3290.70cm^{-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 1414.60-1415.16cm^{-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.

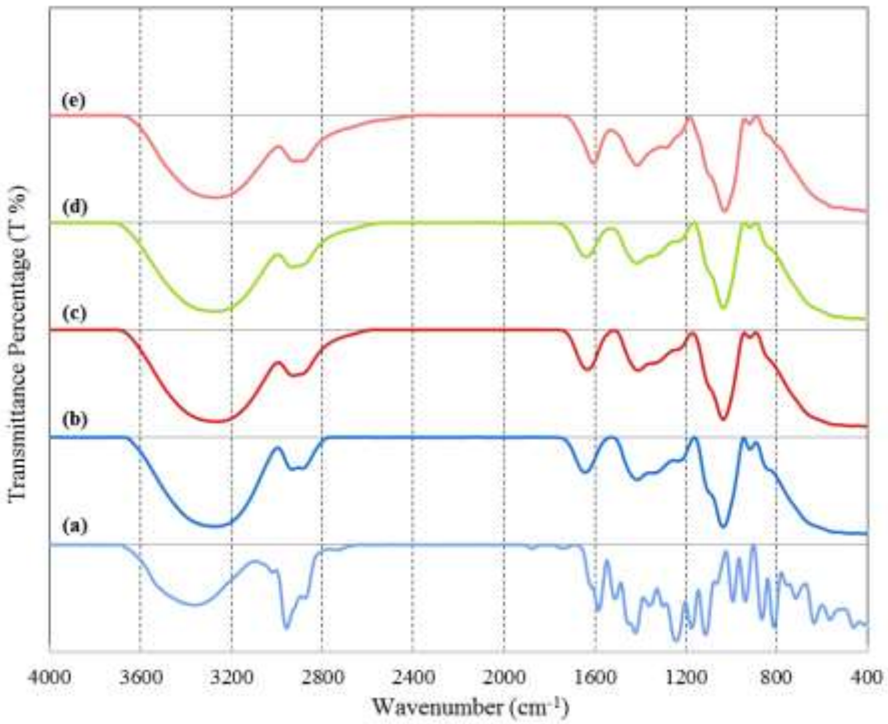

Figure 1 FTIR spectra of (a) Carvacrol (b) GG-Carv 01 (c) GG-Carv 02 (d) GG-Carv 04 (e) Pure GG TF

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 to10 µm.

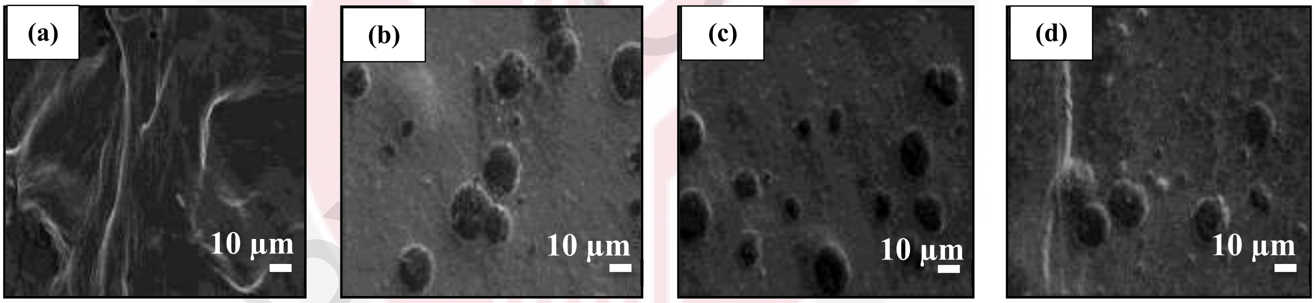

Figure 2 VPSEM surface micrograph at 1000x magnification (a) Pure GG TF (b) GG-Carv 01 (c) GG-Carv02 (d) GG-Carv 04

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.

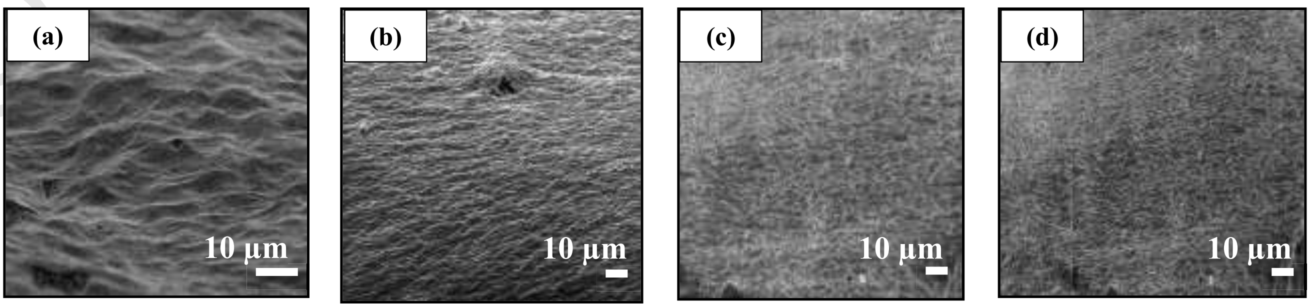

Figure 3 VPSEM cross section micrograph at 1000x magnification (a) Pure GG TF (b) GG-Carv 01 (c) GG-Carv 02 (d) GG-Carv 04

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.

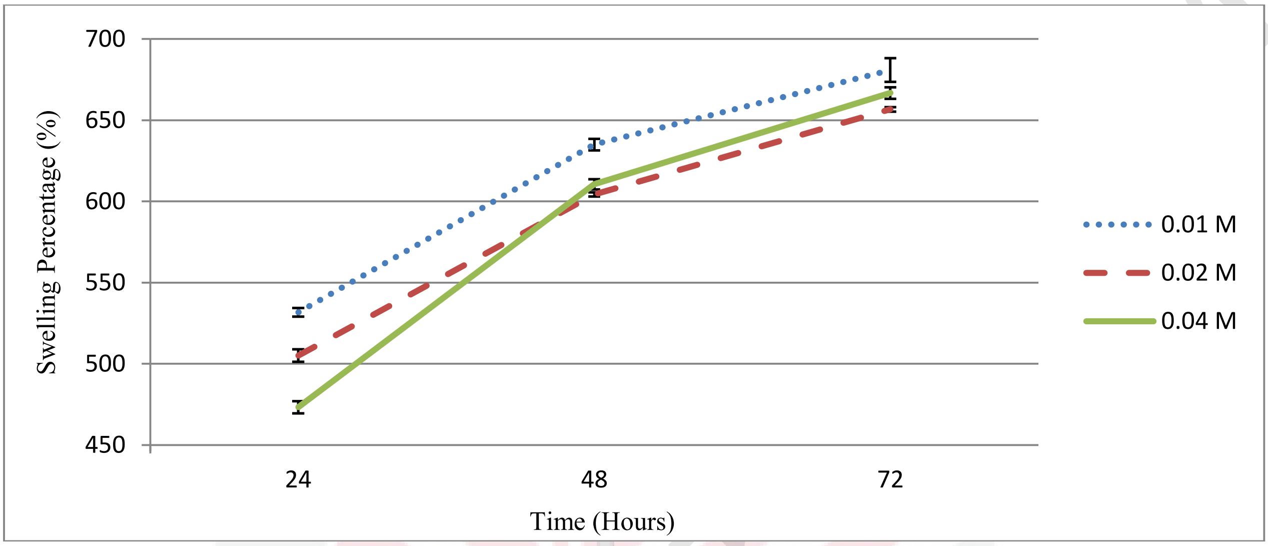

Figure 4 The Swelling Percentage

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.

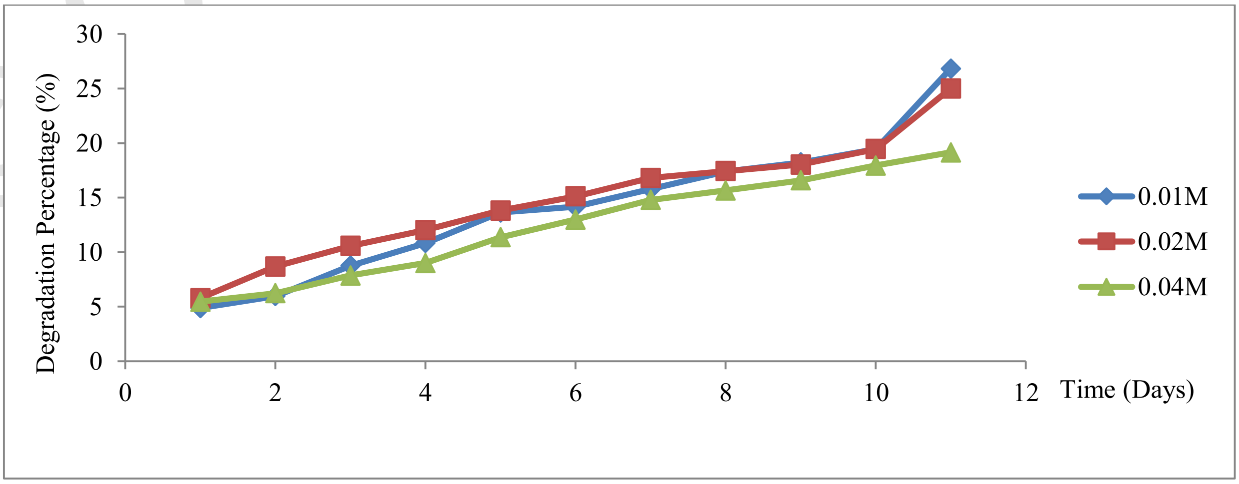

Figure 5 The Degradation Percentage

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-Carvthin films which could be used for further studies in the development of antibacterial applications.

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Preparation of Zinc Layered Hydroxide 2,4-dichlorophenoxyacetate (2,4-D) Nanocomposite

Penyediaan Nano Komposit Zink Hidrosida Berlapis 2,4-dichlorophenoxyacetate (2,4-D)

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Abstract

Recently, layered materials have gained great attention because they can be designed at the nanometer levels. Due to its advantages such as low cost material, easy to synthesize and excellent anion exchange properties, these material exhibit unlimited potential in catalysis, separation technology, medical science, nanocomposite material engineering, agriculture and etc. In this study, Zinc Layered Hydroxide (ZLH) has been intercalated with 2,4-Dichlorophenoxyacetic acid (2,4-D) anion by using ion exchange method. These nanocomposites were synthesized at a different mass of ZLH which are 1 g, 2 g and 3 g also with different concentration of guest anions (2,4-D) which are 0.01 M, 0.02 M, 0.04 M, 0.08 M, 0.16 M and 0.32 M. Powder X-ray Diffraction (PXRD) patterns showed an expansion of interlayer spacing with the value ranging from 26.8 Å to 39.6 Å, 29.4 Å to 37.3 Å, 28.7 Å to 31.6 Å for 1 g, 2 g and 3 g of ZLH at various concentration of 2,4-D respectively. This expansion of basal spacing implies that 2,4-D anion which is bigger size than the prior ZLH was successfully intercalated into the ZLHs. FTIR analysis further confirmed that 2,4-D were successfully intercalated between the interlayers of ZLH with evidence of functional groups of ZLH and 2,4-D in the ZLH-2,4-D nanocomposite (ZN) spectra.

Keywords Zinc Layered Hydroxide, 2,4-Dichlorophenoxyacetic acid (2,4-D), ion exchange, intercalation, nanocomposite

Abstrak

Pada masa kini, bahan berlapis telah mendapat perhatian kerana ia boleh direka bentuk pada skala nano. Kerana kelebihannya seperti murah, mudah disintesis dan sifat pertukaran anion yang baik, maka bahan-bahan ini menunjukkan potensi yang tinggi untuk digunakan bidang katalis, teknologi penapis, sains perubatan, bahan kejuruteraan nano komposit, pertanian dan berbagai kegunaan lagi. Dalam kajian ini, Zink Hidrosida Berlapis (ZLH) telah di sisipkan dengan anion asid 2,4-Dichlorophenoxyacetic (2,4-D) secara kaedah pertukaran ion. Komposit nano ini telah disistesis dengan berlainan jisim ZLH iaitu 1 g, 2 g dan 3 g serta masing-masing dengan berbagai kepekatan anion tumpangan (2,4-D) iaitu 0.01 M, 0.02 M, 0.04 M, 0.08 M, 0.16 M dan 0.32 M. Corak Pembelauan Sinar-X (PXRD) menunjukkan jarak antara lapisan mengembang dari 26.8 Å kepada 39.6 Å, 29.4 Å kepada

37.3 Å dan 28.7 Å kepada 31.6 Å masing-masing bagi 1 g, 2 g dan 3 g ZLH dengan berbagai kepekatan 2,4-D. Pengembangan jarak di antara lapisan ini menunjukkan bahawa anion 2,4-D adalah bersaiz lebih besar daripada saiz ZLH yang berjaya disisip ke dalam lapisan-lapisan ZLH. Analisis FTIR mengesahkan bahawa 2,4-D telah berjaya disisipkan di antara lapisan ZLH dengan bukti terdapatnya kumpulan berfungsi ZLH dan 2,4-D dalam spektrum FTIR komposit nano (ZN)

Kata kunci Lapisan Zink Hidroksida, asid 2,4-Dichlorophenoxyacetic (2,4-D), pertukaran ion, sisipan, komposit nano

INTRODUCTION

Layered single hydroxide salt (LSH) such as zinc layered hydroxide (ZLH) is a layered inorganic compound which has gained attention in wide range of applications, particularly due to its unique anion exchange properties (Abdul Latip *et al*., 2013). Recent studies reported that ZLH has high capacity to accommodate guest molecules and stronger host-guest interaction which leads to high stability; which makes it possible to be used as new host material and delivery system with controlled release rate of active agents (Kasai *et al*., 2006; Yang *et al*., 2007). ZLH structure is high potential host material in forming nanocomposites because it can expand or contract depending on nature of interlayer anions (Mohsin *et al*., 2013). Due to that, the ZLH particularly have been studied extensively and intercalated with various organic anions (Liang *et al*., 2004) mainly via ion exchange process, ranging from anionic dyes (Marangoni R *et al*., 2009), porphyrin sensitizers (Demel J *et al*., 2010) and an anti-corrosive compound (Rocca E *et al*., 2006).

Researches have proven this LSH is currently gaining attention due to its simple method of synthesis, as a precursor for a wide band gap of ZnO and its anion exchange properties (Thomas N *et al*., 2011). LSH also has demonstrated the ability to extend the release period of drug molecules and bioactive molecules (Hussein, M., *et al*., 2010) that prompting more investigations towards potential applications of LSH in drug delivery systems. LSH are thought to be ideal candidates for agricultural applications (Choy *et al.*, 2007). Others active agents such as drugs, vitamins and dyes need to be released in controlled manner to reduce their toxicity or other side effects to increase its durability and stability (Hwang *et al*., 2001).

Recently, the study on the intercalation of phenoxy herbicides into the interlayer of LDH (Layered Double Hydroxide) using various synthesis methods been reported. 2,4-D is highly selective herbicide which is toxic to broad leaved plants but less harmful to grasses. This chemical has complex mechanism of action against weeds, resembling those of auxins (growth hormones). Once adsorbed 2,4-D is translocate within the plant and accumulates at the growing points of roots and shoots where it inhibits growth (Kenneth, 1983). In this paper, we reported the preparation of Zinc Layered Hydroxide 2,4-Dichlorophenoxyacetate (2,4-D) nanocomposite.

MATERIALS AND METHODS

Synthesis of materials

The chemicals used in the synthesis were of analytical grade, obtained from various chemical suppliers and were used without any further purification. The chemicals are

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2,4-dichlorophenoxyacetic acid > 98% from Merck and Zinc Oxide > 99% from Acros Organics. All of the solutions were prepared using deionised water. The synthesis of ZN 1 was prepared by ion exchange method. 1 g Zinc Oxide, ZnO (which then referred as ZLH material) in 100 ml of water was mixed with 2,4-D solution at chosen concentrations (ranging from 0.01 M to 0.32 M). The solution mixture was stirred for 2 hours with magnetic stirring and was conducted under atmospheric conditions. Once stirring was completed, the precipitate was aged at 70 °C for 18 hours in an oil bath shaker, cooled, thoroughly washed and dried overnight in an electric oven at 70 °C. Finally, the dried sample was ground into fine powder by using mortar and pestle, then kept in bottle sample for further used and characterizations. Similar procedure was repeated for ZN 2 and ZN 3 with 2 g and 3 g of ZnO respectively.

Characterisation of material

Powder X-ray diffraction (PXRD) patterns of the samples were recorded between 2^{o} and 60^{o} on a Shimadzu 6000 model analytical powder diffractometer using Cu Kα radiation at 30 kV and 30 mA at the rate of 4^{o} min^{-1}. FTIR spectra of the materials were recorded over the range 400 - 4000 cm^{-1} on a Perkin-Elmer 1752X Spectrophotometer using KBr disc method.

RESULTS AND DISCUSSION

Powder X-ray Diffraction Analysis

Figure 1 show PXRD patterns of ZN 1 synthesised at different concentration of 2,4-D with 1 g of ZLH. The basal spacing showed increasing in the expansion from 26.75 Å to 31.75 Å, 33.08 Å, 35.57 Å, 37.10 Å, and 39.58 Å with the increasing concentration. Figure 2 also shows the same trends. ZN 2 gives the harmonic increasing in the expansion of basal spacing ranging from 29.43 Å to 37.25 Å. PXRD patterns of these two prepared samples display a high intensity diffraction peak indicating a pure phase material without any ZnO phase. This shows that a well-ordered nano-layered structure with good crystallinity was obtained at this optimum condition. The increase of the basal spacing is associated with the spatial orientation and revealed that the size of 2,4-D is bigger than nitrate in the interlayer region (Kuh and Huh, 1998). Meanwhile, in Figure 3, the poor trends detected with 3 g of ZLH. It shows the disorder expansion of basal spacing of 30.87 Å, 28.66 Å, 29.53 Å, 30.23 Å, 31.75 Å, and 27.76 Å due to poor crystallinity obtained.

According to all values of basal spacing, it is proven that 2,4-D was intercalated into ZLH interlayers. In Figure 4, comparison study on various masses of ZLH used in nanocomposite at 0.01 M of 2,4-D. It shows the increases of the basal spacing expansion is proportional with the increases mass of ZLH. The obtained basal spacing value is higher than those reported for the intercalation of other type of herbicides into the LDH interlayers (Cardoso *et al*., 2006; Sarijo *et al*., 2010). ZLH reportedly has larger interspacing than LDH to accommodate a greater number of incoming guest anions of varying sizes, due to its higher charge density (Kasai *et al*., 2006; Hwang *et al*., 2001; KoreYang *et al.*, 2007). Thus it is possible to simultaneously intercalate 2,4-D anions into the ZLH interlayers.

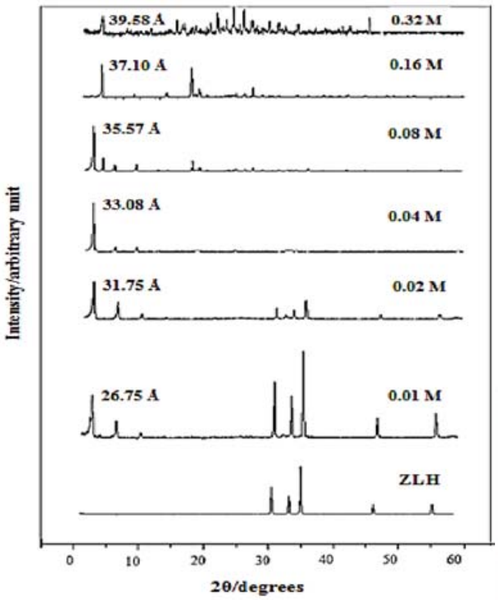

Figure 1 PXRD pattern for ZN 1

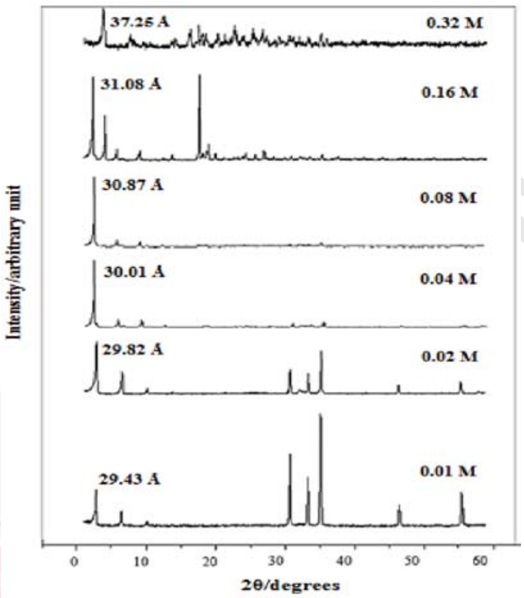

Figure 2 PXRD pattern for ZN 2

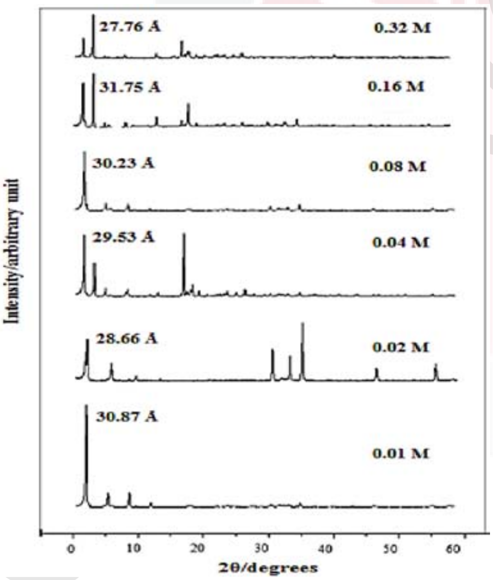

Figure 3 PXRD pattern for ZN 3

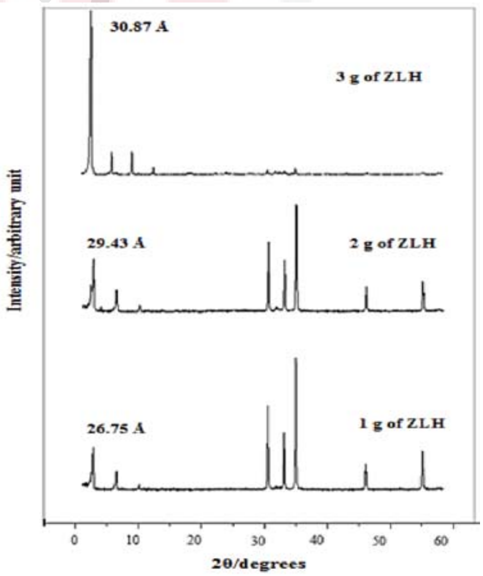

Figure 4 PXRD pattern for ZN 1, 2, 3 at constant 0.01 M of 2,4-D concentration

Fourier Transform Infrared Spectroscopy

Figure 5 shows the FTIR spectra of ZLH, pure 2,4-D and ZLHs. The insertion of 2,4-D into the interlayer ZLH was confirmed by the FTIR spectrum, which is complementary to that of PXRD results. All of the nanocomposites display similar absorption bands as

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the parent material, ZLH and guest ion, 2,4-D anion that are intercalated into the host interlayer galleries. The presence of 2,4-D anion are shown in the typical broad absorption bands of 2,4-D at 3078 cm^{-1} and 2969 cm^{-1}, corresponding to the O-H stretching vibration of the COOH, while a strong band at 1717 cm^{-1} corresponds to the stretching of C=O. The bands at 1471 cm^{-1} corresponds to the stretching that are attributed to C=C vibrations of the aromatic ring of phenoxy. Absorption band is observed at about 1230 cm^{-1} of FTIR spectra due to C-O-C symmetric stretching modes.

FTIR spectra of ZN 1, ZN 2 and ZN 3 at a constant 0.01 M concentration are observed. The same typical broad absorption bands were observed at 3388 cm^{-1} for ZN 2 and ZN 3, while ZN 1 showed broad absorption bands at 3542 cm^{-1}, 3437 cm^{-1}, and 3263 cm^{-1}. These absorption bands are corresponding to the vibrations of the hydroxyl groups at surface, interlayer water molecules and the water bending mode (Lakraimi *et al.,* 2000; Palmer *et al.,* 2009). Meanwhile, the bands observed at 1595 cm^{-1} for ZN 1, 1599 cm^{-1} for ZN 2, and 1604 cm^{-1} for ZN 3, corresponds to the stretching vibration of aromatic ring C=C. The disappearance of bands in the nanocomposites spectrum at 1717 cm^{-1} and 1230 cm^{-1} indicated C=O stretching vibration of the protonated carboxylic groups of the herbicides respectively (Cardoso *et al*., 2006), shows that anions in host material were completely exchanged with 2,4-D anions for the formation of the ZN.

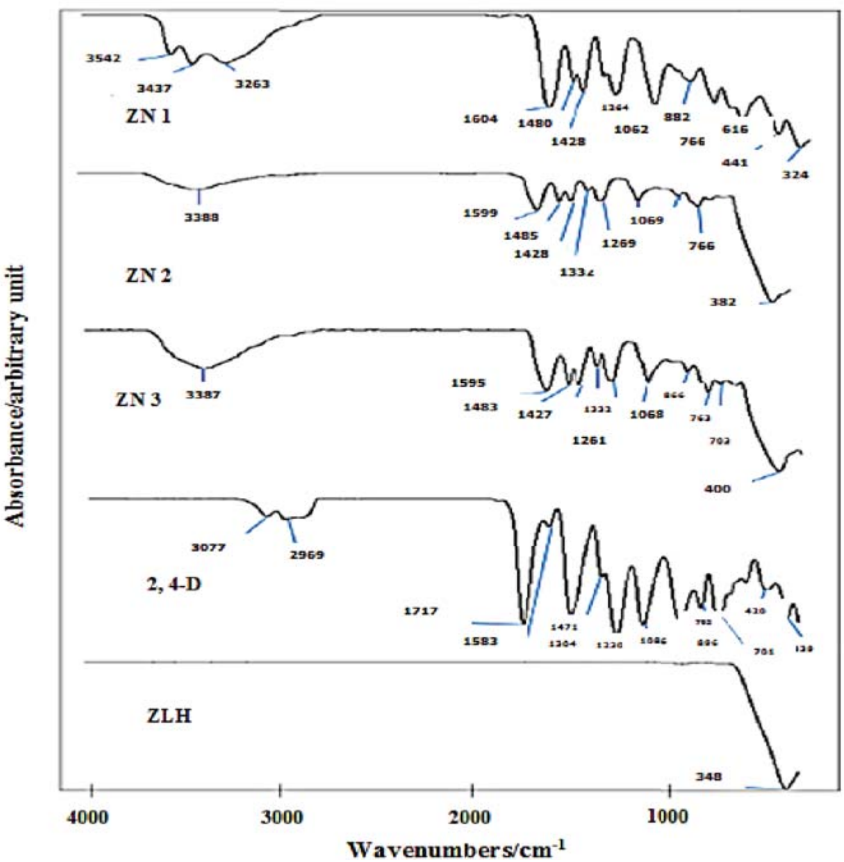

Figure 5 FTIR spectra for ZN 1, ZN 2 and ZN 3 at constant 0.01 M of 2,4-D concentration

CONCLUSION

A series of ZLH-2,4-D (ZN) nanocomposites prepared at different masses of ZLH (1 g to 3 g) and various concentration of 2,4-D (0.01M to 0.32M) have been successfully done via ion-exchange method. This study suggests that the layered hydroxide can be used as a carrier for the 2,4-dichlorophenoxyacetic acid (2,4-D) for further used as herbicide.

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