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CHARACTERIZATION OF CROSSLINKED ZERUMBONE LOADED GELATIN – ZEOLITE Y HYBRID COMPOSITES WITH GLUTARALDEHYDE FOR CONTROLLED RELEASE OF NATURAL ANTICANCER DRUG

(Pencirian Sambung Silang Zerumbon Dimuatkan dalam Gelatin-Zeolit Y Komposit Hibrid dengan Glutaraldehid untuk Pembebasan Terkawal Ubat Anti-Kanser Semulajadi)

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Abstract

Zeolite Y-gelatin hybrid spherical pellets were prepared by blending zerumbone loaded zeolite Y with gelatin solution and crosslinking achieved by glutaraldehyde. The pellets were characterized by using ATR-FTIR spectroscopy, Raman spectroscopy, thermogravimetric analysis (TGA) and swelling analysis. The zerumbone entrapment in zeolite Y was calculated via UV-VIS and results showed that zerumbone could be encapsulated to the maximum loading of porous zeolite Y. ATR-FTIR analysis showed the inert property of zeolite Y that only act as porous host carrier and crosslinking was achieved at 1650 cm^{-1}. Result from Raman spectra supported and proven that crosslinking occur at 1650 cm^{-1}. The swelling of crosslinked samples showed slower water penetration indicating that crosslinking had strengthened the spherical pellets. TGA results supported that after crosslinking took place, the thermal decomposition temperature for the composite increased indicating the increase of composite strength. This study showed promising results towards the composite development in controlled drug release area.

Keywords: controlled drug delivery, gelatin, zeolite Y, zerumbone, glutaraldehyde

Abstrak

Pellet sfera dari hybrid zeolit Y-gelatin telah disediakan dengan menggbungkan zerumbon yang dimuatkan di dalam zeolit Y ke dalam campuran gelatin dan sambung silang dicapai dengan menggunakan glutaraldehid. Pelet dicirikan dengan menggunakan ATR-FTIR spektroskopi, Raman spektroskopi, analisis termogravimetri (TGA) dan analisis penyerapan. Pemerangkapan zerumbon di dalam zeolit Y dianalisis melalui UV-VIS dan keputusan menunjukkan bahawa zerumbon boleh dimuatkan secara maksimum di dalam liang zeolit Y. ATR-FTIR menunjukkan sifat lengai zeolit Y yang hanya bertindak sebagai 'pembawa' berliang dan silang sambung dicapai pada 1650 cm^{-1}. Keputusan dari spectrum Raman menyokong dan turut membuktikan bahawa silang sambung berlaku pada 1650 cm^{-1}. Penyerapan sampel silang menunjukkan penembusan air secara perlahan-lahan lantas membuktikan bahawa silang sambung telah mengukuhkan pelet sfera. Keputusan TGA menyokong bahawa selepas silang sambung berlaku, suhu penguraian haba bagi komposit meningkat dan membuktikan peningkatan kekuatan komposit. Kajian ini menunjukkan keputusan yang memberangsangkan terhadap pembangunan komposit dalam pembebasan ubat secara terkawal.

Kata kunci: perlepasan ubat terkawal, gelatin, zeolite Y, zerumbon, glutaraldehid

Introduction

Biodegradable and non toxic drug delivery system was first formulated in 1978 by Marty [1]. Since then, various synthetic and natural polymers were adopted for production of biodegradable drug delivery such as polycaprolactane (PCL), polylactic acid (PLA), polyglycolic acid (PGA) and polylactide-co-glycolide (PLGA) [2]. Natural polymers used were proteins such as albumin, gelatin, and polysaccharides such as dextran, alginate and chitosan. Among the natural polymers, gelatin offers some advantages. Gelatin is available for chemical modification in bulk or finished product. It is also known for its good biodegradability and biocompatibility and also known as "Generally Recognized as Safe" (GRAS) by U.S Food and Drug Administration (FDA) [3]. Today, nanoparticle-mediated drug release and drug targeting are intensively studied. There are a range of engineered constructs, assemblies, and particulate systems to be used in the controlled release drug delivery. The application of nanoparticles to these fields obviously from the fundamental properties of nanoparticles which determined by its high surface area. The therapeutic agents are encapsulated, covalently attached, or adsorbed on to such nanoparticles that act as nanocarriers [4]. Chemical crosslinking is a highly versatile method to create and modify polymers, where properties can be improved such as mechanical, thermal and chemical stability [5]. In this study, glutaraldehyde has been chosen as gelatin crosslinker that used to strengthen and increase the thermal stability of the polymer. In the present research, we wish to report the preparation and characterization of crosslinked biodegradable gelatin-zeolite Y composite with glutaraldehyde, for oral chemotherapy by using zerumbone, a natural anticancer drug due to its excellent therapeutic effects. Studies have proven that zerumbone is one of the most gifted chemopreventive agents against colon, skin and breast cancer [6-7].

Materials and Methods

Extraction of Zerumbone

Zerumbone crystals were prepared from rhizomes of Zingiber Zerumbet according to method described earlier in previous study [8]. In brief, fresh rhizomes were cleaned, sliced, dried and then rotor evaporated to obtain the essential oil. The oil then was recrystallized using n-hexane to obtain pure zerumbone crystals.

Preparation of Crosslinked Composites

The preparation of composite pellets was described as in previous study [8]. The crosslinking of pellets with glutaraldehyde was done as follows. Zerumbone loaded gelatin-zeolite Y composite was pelletized by releasing the mixture drop wise from a syringe (60 °C) into a cooled mixture of sunflower oil and glutaraldehyde (25 % in water) at 10 °C with continous stirring and crosslinking for 24 hours. The resulting composites were decanted, freed of sunflower oil by repeated washings with 200 mL isopropyl alcohol and finally air dried over a period of 24 hours. The spherical pellets were stored in a refrigerator at temperature between 3 – 5 ºC until further evaluation.

Characterization: Entrapment Efficiency of Drug

The amount of zerumbone loaded into zeolite Y-was determined by soaking of 2 % w/v zeolite Y in a (5, 10 and 15 %) aqueous solution of 100 µM zerumbone. The loaded zeolites Y were then dried at room temperature for 24 h. Zerumbone loaded zeolite Y powder was then stirred for 24 h in 250 mL of pH 7.4 phosphate buffer. The amount of drug was analyzed by UV-VIS spectrophotometer (PERKIN ELMER Lambda 20) at wavelength 280 nm. Drug entrapment was calculated using Equation 1 (Jameela and Jayakrishnan, 1995).

$$\%\ \text{drug entrapment} = \left(\frac{\text{Actual drug loading}}{\text{Theoretical drug loading}}\right) \times 100 \quad (1)$$

ATR-FTIR Spectroscopy

ATR-FTIR spectroscopy was carried out on NICOLET 6700 model in order to determine the presence of specific chemical groups in the sample as well as to determine any interactions that might occur. The scan was done from 500 cm^{-1}-4000 cm^{-1}. All samples were transformed into thin film before being analyzed.

Raman Spectroscopy

Raman spectroscopy was carried out to confirm the presence of specific chemical groups in crosslinked sample using RAMAN DISPERSIVE PL (HORIBA). No sample treatment needed for this analysis. The scan was done in range of 1000 cm^{-1} – 4000 cm^{-1}.

Swelling Studies

Water absorption of the samples was measured following ASTM D 570-81 standard. The samples were preconditioned at 50 °C for 24 hours and then cooled in a desiccator before being weighed (W1). The preconditioned samples were submerged in distilled water at 25 °C for 24 hours. The samples were removed and dried with a paper towel before reweighing, (W2). Water absorption or swelling was calculated as a percentage of initial weight as shown in Equation 2

$$\text{swelling } \% = \frac{(W2-W1)}{W1} \text{x } 100 \quad (2)$$

Thermogravimetric Analysis

TGA has been used to investigate the thermal degradation, phase transition and crystallization of the polymers. TGA was used in the study to ascertain the thermal stability of the prepared samples using Perkin Elmer TGA7 Thermogravimetric Analyzer. All samples were cut into small pieces and 50 mg was taken for the analysis. The analysis was done from 50 ºC – 1000 ºC.

Results and Discussion

Zerumbone Entrapment Efficiency

The results in Table 1 revealed the zerumbone efficiency while loading in porous zeolite Y. Results obtained shows that almost 100 % of zerumbone can be loaded into porous zeolite Y. It shows that zeolite Y have the ability to encapsulate zerumbone to almost its maximum value.

Table 1. Zerumbone entrapment efficiency

Concentration of zerumbone, 100 µM	Actual drug loading (µg)	Theoretical drug loading (µg)	Encapsulation efficiency (%)
5 %	1.8195	1.942	93.7
10 %	3.639	3.741	97.3
15 %	5.45	5.492	99.2

ATR-FTIR Spectroscopy Analysis

The FTIR spectra of gelatin, zeolite Y, zerumbone, zerumbone loaded zeolite Y, zerumbone loaded gelatin, zerumbone loaded composite and crosslinked composite by glutraldehyde are shown in Figure1. FTIR analysis is very crucial to determine the interaction between zerumbone with gelatin and zeolite Y and also to determine whether crosslinking has taken place. It can be seen in Figure 1(a) that gelatin has an amide I peak (C=O stretch) at 1640 cm^{-1}, amide II peak (N-H bend and C-H stretch) at 1542-1450 cm^{-1}, amide III peak (C-N stretch plus N-H in phase bending) at 1242 cm^{-1} and amide A peak (N-H stretching vibration) at 3316 cm^{-1} which are the distinguishing features of gelatin. The result from this study is consistent with study done by Soliman and Furuta [9]. Organic matters have many types of bonds that are infrared active. Bonds such as those functional groups of OH, NH, CH and C = O. For inorganic substances, such as zeolite Y, it will only provide information of Si-O-Si and Si-O-Al bonds. In Fig 4.2(a), zeolite Y sample shows a strong peak at 1059 cm^{-1} that correspond to Si-O-Al and Si-O-Si bonds. A broad band at 3730-3500 cm^{-1} could be attributed to Si-OH, Si-OH-Al and –OH hydrogen group [10]. The zeolite Y incorporation in the gelatin was confirmed where zeolite Y related peaks at 1059 cm^{-1} which has reduced its peak intensity and shifted to 1076 cm^{-1}. Infra-red spectrum of gelatin-zeolite Y showed bands characteristic of

zeolite Y (Si-OH, Si-OH-Al and –OH) as well as gelatin (Amide A, N-H stretch) at 3400 cm^{-1} stretching. For zerumbone crystal, 3 peaks of alkyl group (C-H stretching) are observed at 2964 cm^{-1}, 2952 cm^{-1} and 2863 cm^{-1}. There is also a very strong peak of carbonyl group (C=O) at 1650 cm^{-1}, C=C peaks at 1457 cm^{-1} and C-H bending below 1000 cm^{-1} [11]. The previous peaks remained the same while C=O peak of carbonyl group disappeared from the spectrum. When the solution was diluted with an amount of isopropyl alcohol, the additional broad peak of O-H at 3700-3200 cm^{-1} indicate the presence of O-H functional group.

For zerumbone loaded zeolite Y, zerumbone incorporation in zeolite Y was confirmed with C=C bond of zerumbone at 1640 cm^{-1}. No additional band was observed in zerumbone loaded zeolite Y and this indicates that there is no interaction between zerumbone and zeolite Y, thus proving the inertness of zeolite Y which only behave as a host carrier. Similarly for zerumbone and gelatin, there is also no interaction between gelatin and zerumbone as no new bands are observed in the spectrum of the sample. In the FTIR spectrum of glutaraldehyde, $(CHO)CH_2CH_2CH_2(CHO)$, the broad peak at 3380 cm^{-1} is due to the stretching vibration of water. The CH_2 vibration of aldehyde occurs close to 2955 cm^{-1}. The presence of water is confirmed by its bending vibration at 1640 cm^{-1}. The CH_2 bending vibrations occur at 1442 and 1334 cm^{-1}. The group of peaks between 1200- 900 cm^{-1} are due to C-O vibrations which illustrate the hydration of glutaraldehyde. In addition to the previously mentioned peaks, a strong peak at 1650 cm^{-1} was observed in the crosslinked gelatin due to aldimine absorption thus confirming that crosslinking reaction between aldehyde group of glutaraldehyde with amine group (NH_2) group of gelatin [12]. The result from this study is consistent with many previous studies [13-16]. The uncrosslinked and crosslinked composites also can be discriminated by a slow change in color from light yellow to dark yellow or brownish. The color change occurred because the aldimine linkage (CH=N) reactions took place during the cross-linking process [16].

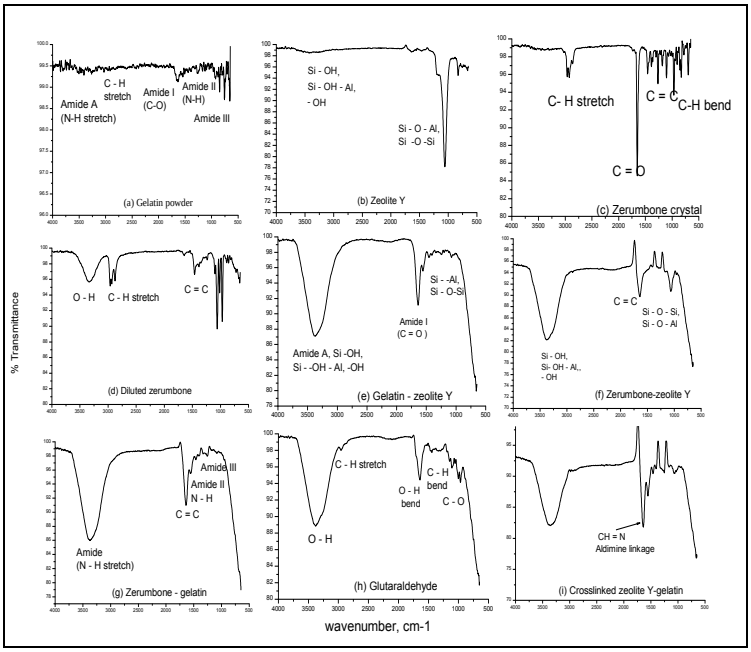

Figure 1. ATR-FTIR spectra of sample pellets

Raman Spectroscopy Analysis

Raman spectroscopy was used to compliment FTIR data and confirm the interaction between gelatin and croslinker (GTA). In Figure 2(a) shows a carbonyl group (C=O) at 1650cm^{-1}. In Figure 2(b), after crosslinking took place, a strong peak is observed at 1650 cm^{-1} in crosslinked gelatin. The increase of peak intensity was mainly contributed by the formation of the imine linkages (C=N) from gelatin-glutaraldehyde crosslinking chain overlapped with the C=O

stretching from the gelatin [13].The aldehyde group in glutaraldehyde reacts with the amino group of lysine (NH_2) consistent with previous studies [13-16]. Thus, confirming the crosslinking reaction.

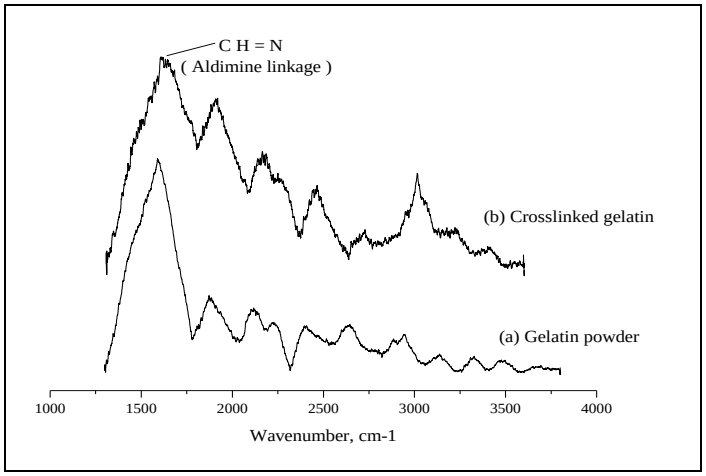

Figure 2. Raman spectra for (a) gelatin powder, (b) crosslinked sample with GTA

Swelling Analysis

Swelling studies of blended sphere composites were performed in distilled water. Table 2 shows swelling variations of the samples as a function of GTA concentrations at different time intervals. The swelling percentage of uncrosslinked gelatin is about 50% after 30 minutes. Swelling measurement at longer times were hindered due to gelatin microsphere dissolving in water. However, for crosslinked gelatin, swelling percentage decreased with increased in percentage of GTA, thus increased the time of sample solubility. Gelatin microspheres are known to swell in aqueous environments due to water adsorption. During crosslinking process, a new polymeric structure formed due to the introduction of bridges between polymeric chains where aldimine linkage (CH=N) reactions took place [12, 16]. The denser the crosslinking bridges, the more packed would be the structure. Such structure would slow down the solvent penetration. In this study glutaraldehyde is responsible for the formation of crosslinks, it can be concluded that increasing the amount of glutaraldehyde and crosslinking time would increase the crosslinking bridges. More crosslinking bridges formations would stabilize and strengthen the spherical pellets [16], resulting in less swelling and slower dissolution of pellets.

Table 2. Swelling analysis of pure and crosslinked gelatin

Time (hours)	**Pure gelatin**	**Crosslinked gelatin**			
	0	**0.1**	**0.2**	**0.3**	**0.4**
0.08	14.4	5.7	2.56	2.7	2.13
0.5	49.3	20	10.26	10.81	6.38
1	72.8	40	17.95	10.81	6.38
2	124	60	33.33	29.73	10.64
4	347	103	65.54	51.35	23.4
8	nd	163	92.31	75.68	21.28
13	nd	220	142.6	102.7	21.28
18	nd	309	235.9	125.4	53.19
24	cs	cs	274.7	210.8	10.43

nd: not defined, *cs*: completely soluble

Thermogravimetric Analysis

TGA was used in this study to determine the thermal degradation of composite samples subjected to crosslinking. The TGA thermograms of gelatin, zeolite Y, zerumbone crystal, gelatin- zeolite Y, zerumbone- zeolite Y and crosslinked gelatin are shown in Figure 3. Gelatin has two stages of degradation as shown in Figure 3(a). The first stage is around temperature 40 °C – 100 °C indicating the loss of water. The second stage at 135 °C- 250 °C indicates the decomposition of amino acid residues, as well as cleavage of the peptide bonds in the protein [17]. The TGA curve of zeolite Y in Figure 3(b) shows 2 steps from 25 – 200 ºC and 220 – 550 ºC. These weight losses correspond to loss of water and phase change in zeolite Y. And this temperature shows the retaining stability of zeolite Y where mass losses were slower which means that the zeolite Y was structurally stable [10]. For zerumbone crystal in Figure 3 (c), it starts to degrade from 57 ºC and completely degrade at 243 ºC. On comparing the TGA results of gelatin without the presence of glutaraldehye, the thermal stability of the crosslinked sample appears to increase compare to uncrosslinked gelatin where Figure 3(f) shows a high thermal stability when compared with uncrosslinked sample, thus confirmed that the thermal stability increased during in the presence of the crosslinking agent.

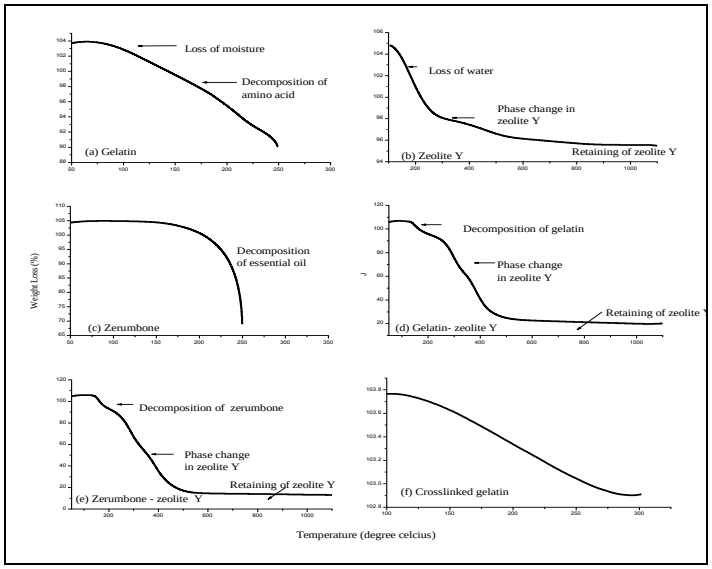

Figure 3. Thermogravimetric analysis curves of samples

Conclusion

The preparation of crosslinked zerumbone incorporated zeolite Y-gelatin composite with glutaraldehyde was successfully achieved. The used of glycerin is to induce homogenous mixture while the sunflower oil is to stimulate spherical shaped pellets. Results have proven that zerumbone solution can be encapsulated into porous zeolite Y. In order to determine interaction between zerumbone and host carrier, zeolite Y, ATR-FTIR spectroscopy has been done. Results showed that zeolite Y is inert due to no interaction has formed between zerumbone and zeolite Y. There is also no interactions occurred between zerumbone and gelatin. Due to the hygroscopic characteristic of gelatin as it is easily to swell and erode when immerse in water, crosslinking has been suggested. TGA analysis has supported that crosslinking increases the stability of the gelatin. Furthermore, the swelling test also proved that at an appropriate amount of glutaraldehyde, the swelling of gelatin could be slowed down. Swelling test showed that gelatin crosslinked with glutaraldehyde reduced water permeability of gelatin [18]. ATR-FTIR and Raman spectroscopy analysis had proven that crosslinking was successfully achieved where the aldehyde group in glutaraldehyde reacted with the amino group of lysine (NH_2) in gelatin into CH=N (aldimine linkage). From the

study, we suggested that zeolite Y-gelatin composite is a potentially useful composite for controlled release of zerumbone via crosslinking technique.

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