Sodium alginate-chitosan nanocomposite as a novel carrier agent for cinnamaldehyde: characterisation and release studies

S T S Wong*, A Kamari and J Jumadi

Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia.

Email: susanawongsiewtin@hotmail.com

Abstract. The ultimate aim of this study was to synthesize sodium alginate-chitosan (SA-Chi) nanocomposites for the first time in larvicide formulation. The physicochemical properties of the nanocomposites were characterized by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) spectroscopy, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The feasibility of SA-Chi nanocomposites to encapsulate and load cinnamaldehyde, a common essential oil used against mosquitoes larvae, was evaluated in terms of encapsulation efficiency (EE) and loading capacity (LC). There were three mass ratios of SA-Chi nanocomposites to cinnamaldehyde, namely 50:1, 100:1 and 125:1. Research findings suggested that the mass ratio of nanocomposites to drugs, significantly affected the functional groups availability, crystallinity property and thermal stability of nanocomposites. The EE and LC of cinnamaldehyde-loaded nanocomposites at 125:1 were determined as 91.33% and 75.25%, respectively. The release profile data of the nanocomposite were well fitted to Korsmeyer-Peppas kinetic model with correlation coefficient (R^2) of 0.9231 for the mass ratio of cinnamaldehyde-loaded nanocomposites at 125:1. The cinnamaldehyde-loaded nanocomposites at 125:1 had prolonged the drug release for 24 hours. Overall, results from this study highlight the potential of SA-Chi nanocomposites as novel nanocarriers to deliver cinnamaldehyde in mosquito larvicide formulations.

1. Introduction

When technology development induces a new innovation of materials, a drug carrier system is one of the most attentive products. Drug carrier systems are able to encapsulate drugs in order to enhance drug delivery process to targeted sites [1]. However, the amount of drug carrier systems has to be increased if the system is unable to encapsulate a fixed quantity of drugs. Therefore, the nanotechnology becomes a brand new innovative direction of the drug carrier system. The recent common materials for drug carrier systems are mostly from natural polymers, lipid, and polysaccharides, or synthetic polymers. A lot of studies have proved the unusual behaviour of atoms and molecules when the products are invented in nanoscale [2]. Large surface area to volume ratio, tuneable surface, better strength, and improved chemical reactivity are the benefits of using nanoscale in drug carrier systems [3-5]. Hence, the utilisation of nanosized drug carrier system is highly concerned by researchers and scientists in order to achieve effective drug delivery in the shortest time with the lowest possible drug dosage.

Hydrogel nanocomposites is the most effective drug carrier system in the water environment because of its good interaction with the environment and this could intensify the effectiveness of the
drug delivery process [6]. In this study, sodium alginate (SA) and chitosan (Chi) were chosen to be the drug carrier system for cinnamaldehyde in the water environment. SA is the sodium salt of alginic acid and it is mainly from the cell wall of brown algae which are from class Phaeophyceae. In a textile printing industry, SA has been used as a stabilizer or an emulsifier because it contains chains of mannuronic acid and guluronic acid [7]. Other than that, SA has been used widely in wound dressing and adsorbent materials because of its non-toxic and non-inflammatory [8]. The liquid-gel behaviour of SA allows more reactive sites for drugs to be delivered in the water environment. Chi is a linear polysaccharide which has deacetylated D-glucosamine and N-acetyl-D-glucosamine [9]. Chi can be obtained from hard shells of shellfish, and it has been an ideal material in drug delivery due to its properties of biodegradability, biocompatibility, and high membrane permeability [10]. This is because Chi has primary amino groups that enable controlled drug release, mucoadhesion, and in situ gelation [11]. Chi is also being applied widely in industry food, agriculture, textile, and paper.

There are various plant extracts have been studied few years back and essential oils (EO) is the topic the most studied by the researchers. EOs are the concentrated, volatile, aromatic compound of a plant and they have been extracted through steam distillation. There are a lot of studies related to applications of EO in various fields have been reported in recent years. According to Seenivasan et al., cinnamaldehyde has been applied against larvicidal activity of Culex mosquitoes [12]. However, most of the EOs has low water solubility. In this study, EO from bark of cinnamon tree has been chosen to be encapsulated in the carrier system. Cinnamon tree is from Lauraceae family and it is originated in Sri Lanka, India, and Myanmar then being cultivated in South America and the West Indies [13]. Cinnamaldehyde is the EO distilled from the bark fragments and it has been used in food, liquor, perfumes and drugs [14]. Not only those, cinnamaldehyde has been chosen as a natural larvicide formulation because it is effective against bacteria, microbes and fungi [15]. A drug carrier system is required for cinnamaldehyde to perform the larvicidal effects in water environment due to its low water solubility (1420 mg/L at 25 ºC). Knockdown effects of cinnamaldehyde could not reach mosquito larvae because they are living beneath water surface. Therefore, a drug carrier system is required to deliver cinnamaldehyde in water environment in order to reduce the number of larvae.

Drug delivery system is designed to carry drug in possible amount and deliver drug in the shortest period. In this study, attention is highly paid towards biodegradable polymer materials. Formation of SA-Chi is by ionic linkage between carboxyl groups of sodium alginate and amino groups of Chi after sodium alginate is induced gelation with calcium ions [16]. In this stage, formation of gel aggregates occurs and this interaction has been favoured effective drug encapsulation and drug delivery [17,18]. Thus, cinnamaldehyde can be encapsulated successfully and can be released in higher effective rate with SA-Chi nanocomposites compared with SA or Chi alone. The novelty of this study is the application of SA-Chi nanocomposites in larvicide formulations. In this system, the period of mosquito control could be extended and with high effectiveness.

2. Materials and methods

2.1 Materials
Chi (MW 600,000-800,000, degree of deacetylation ≥75%) and Tween 20 were purchased from Acros Organic. Food grade SA powders which are comprised of equal amount of mannuronic acid and guluronic acid units and calcium chloride were purchased online. Glacial acetic acid was purchased from ChemAR. Cinnamaldehyde (min. 99%) was purchased from ShangHai Kaiyi Chemical Co.

2.2 Synthesis
This method was proposed by Loquercio et al. [19] with some modifications. 4 µL of cinnamaldehyde was added in 20 mL of 20 ppm acetonitrile. 2.0 mL of the mixture solution was added into a beaker that contained 250 mL of 0.06 % (w/v) sodium alginate solution and 1.5 mL of Tween 20. Tween 20 was added as an emulsifier in order to disperse hydrophobic particles. The solution was stirred for 90 min and sonicated for 15 min (5 mins × 3 cycles). 15 mL of 0.2 % (w/v) calcium chloride was added to the mixture solution, stirred, and sonicated as described earlier. 0.5 g of Chi was dissolved in 200 mL of 1 % (v/v) acetic acid and then it was added to the mixture. The mixture solution was stirred and
sonicated to increase the uniformity and dispersity of particles. The nanoparticle solution was centrifuged at 4000 rpm for 15 min. A low speed filter paper was used to remove any excess polymer. Retentate was frozen and lyophilised. The nanocomposite obtained was kept in an air-tight container. The volume of cinnamaldehyde was calculated accordingly based on the ratio of nanocomposites:drugs, namely 50:1, 100:1 and 125:1.

2.3 Characterisation

FTIR spectra of precursors and the nanocomposites were recorded on a Shimadzu IRTracer-100 FTIR spectrophotometer in the wavenumber ranged between 4000 to 400 cm$^{-1}$ with over 32 cumulative scans to identify the functional groups and chemical bonds between molecules. XRD diffractograms of the samples were obtained using a Rigaku Miniflex II diffractometer equipped with a rotating anode and a CuK$\alpha$ source ($\lambda = 0.1504$ nm) for analyse the crystallinity of the nanocomposites. To evaluate the thermal properties of the nanocomposites, TGA and DSC analysis were performed under argon atmosphere at a heating rate of 10 °C/min and temperature range of 25-1000 °C using a TGA/DSC 1 Mettler Toledo analyser.

2.4 Encapsulation efficiency and loading capacity

5 mg of nanocomposite was dissolved in 5 mL of 95 % (w/v) acetonitrile and this was conducted in triplicates. The solution was left in the dark environment for 48 h for allowing cinnamaldehyde to be encapsulated into the nanocomposites. The solution was vortexed for 10 s and being filtered. The concentration of encapsulated cinnamaldehyde was measured using an Agilent Cary 60 Ultraviolet-Visible spectrophotometer at 280 nm. The encapsulation efficiencies and loading capacities were determined according to the following equations 1 and 2 [20,21].

Encapsulation efficiency = \( \frac{\text{Amount of encapsulated cinnamaldehyde}}{\text{Total amount of cinnamaldehyde}} \times 100 \% \) (1)

Loading capacity = \( \frac{\text{Amount of encapsulated cinnamaldehyde}}{\text{Mass of nanocomposite}} \times 100 \% \) (2)

2.5 In vitro release profile of the nanocomposite and fitted release kinetic models

The in vitro release of cinnamaldehyde from nanocomposite was conducted by separating cinnamaldehyde-loaded SA-Chi nanocomposite from the aqueous nanoparticulate suspension medium through ultra-centrifugation. Cinnamaldehyde-loaded nanocomposite (20 mg) was then placed in a dialysis membrane bag with a molecular cut-off of 5 kDa, tied and placed into 200 mL of deionised water at pH 7.0. The entire system was kept at 25 ± 0.5 °C with continuous magnetic stirring (100 rpm). At certain time intervals, 20 mL of the release medium was taken and 20 mL of deionised water was added into the system to maintain the sink conditions. The amount of cinnamaldehyde released from the nanocomposite was measured by using an Agilent Cary 60 Ultraviolet-Visible spectrophotometer.

The release mechanisms of cinnamaldehyde from the nanocomposites were studied using zero order, first order, Higuchi and Korsmeyer-Peppas kinetic models. The correlation coefficient ($R^2$) and release exponent ($n$) of Korsmeyer-Peppas model were also determined by regression analysis, as described by Ritger and Peppas [22].
3. Results and discussions

3.1 Characterisation

Figure 1. FTIR spectra of (a) cinnamaldehyde, (b) SA-Chi nanocomposite, and (c) SA-Chi nanocomposite loaded with cinnamaldehyde.

The spectra of cinnamaldehyde, SA-Chi nanocomposite, and SA-Chi loaded with cinnamaldehyde have been shown in figure 1. From figure 1(a), the absorption bands of cinnamaldehyde had been exhibited at 3273, 2914, 2351,1638 and 1050 cm\(^{-1}\) which corresponded to O-H stretching, C-H stretching, O=C=O stretching, C-H bending, and CO-O-CO stretching. Meanwhile, the broad and intense peak at 3325 cm\(^{-1}\) indicated that hydrogen bonds between –OH and –NH\(_2\) in Chi and –C=O and –OH of sodium alginate [23-25]. The intensity of C-H bending at 1390 cm\(^{-1}\) decreased and shifted to 1404 cm\(^{-1}\) due to strong interactions in the nanocomposite. There was a marginal reduction in the absorption intensity of –CO stretch band at 1290 and 1300 cm\(^{-1}\). Additionally, the band
represents C=O of sodium alginate at 1041 cm\(^{-1}\) became more prominent and shifted after interaction with NH\(^3\)\(^+\) of Chi. It is clear that the absorption bands at 3325, 2902, and 1604 cm\(^{-1}\) which represent the stretches of O–H, C–H, and C–O of nanocomposite (figure 1a) were shifted to 3323, 2899, and 1595 cm\(^{-1}\) (figure 1c), respectively following encapsulation of cinnamaldehyde in the nanocomposite. From figure 2(a), it is obvious that SA-Chi nanocomposite has a crystalline characteristic. There was no peak of any other phases was observed in the diffractogram indicating high purity of the nanocomposite. The broad peak of X-ray diffraction patterns indicated the significantly small size of the resulting crystallites [26]. The intensity of the reflection peak at 23.46° \(2\theta\) increased by two-folds following the encapsulation of cinnamaldehyde into nanocomposite (figure 2(b)). This increment could be related to additional reflection of X-ray caused by cinnamaldehyde molecules. A similar observation was reported by Lim and Ahmad whereby no peak being detected following encapsulation of imidacloprid into SA-Chi nanocomposite [27].

The TGA thermograms of nanocomposite and cinnamaldehyde-loaded nanocomposite are presented in figure 3(a). SA-Chi nanocomposite shows three thermal degradation steps. In the first step (108-275 °C), 26 % of weight loss occurred due to the elimination of loosely bonded water. The next degradation took place at 275 °C which could be due to nanocomposite decomposition and scission of the polymer chain. With increasing the temperature, the weight of nanocomposite decreased slowly in the last step. However, cinnamaldehyde-loaded nanocomposite exhibited four thermal degradation steps and this showed that the thermal stability of nanocomposite increased after encapsulation of cinnamaldehyde [27]. The first thermal degradation required a higher temperature for the release of absorbed water in the nanocomposite or vaporization of volatile components. Considering water molecules were bonded to carboxyl, amine, and hydroxyl groups of Chi, SA, and cinnamaldehyde, the degradation step at 345 °C involved 17 % of weight loss and this could be related to incomplete removal of water in the previous step. For the last step of degradation, the decomposition of polyelectrolyte composition might take place.

From figure 3(b), the nanocomposite had three endothermic peaks (60, 747, 934 °C) and two exothermic peaks (256, 367 °C), while two endothermic peaks (60, 747 °C) and four exothermic peaks (256, 367, 502, 876 °C) were seen for cinnamaldehyde-loaded nanocomposite. At 60 °C, a broad endothermic peak that could be related to water elimination was observed in nanocomposite and cinnamaldehyde-loaded nanocomposite. A higher enthalpy is required to overcome decomposition of amine unit by observing an increase in exothermic peaks at 256 and 367 °C. The second endothermic peaks represented at 747 °C could be ascribed to the dissociation of the nanocomposites. Heat energy is absorbed by the nanocomposite at 934 °C that may be due to bonds cleavage and this corroborates
with figure 3(b) which is about 10% of nanocomposite residue remained. In the context of cinnamaldehyde-loaded nanocomposite, an additional exothermic reaction occurred at 502 °C that could be associated with strong hydrogen bond formation after crosslinking. Furthermore, cinnamaldehyde-loaded nanocomposite altered the enthalpy of nanocomposite by releasing heat at 876 °C instead of absorbing heat.

![Figure 3](image_url)

Figure 3. (a) TGA thermograms and (b) DSC thermograms for SA-Chi nanocomposites before and after encapsulation of cinnamaldehyde.

3.2 Encapsulation efficiency and loading capacity

Table 1 reports the encapsulation efficiencies and loading capacities of the nanocomposites loaded cinnamaldehyde at different mass ratios. The encapsulation efficiency of the nanocomposites loaded cinnamaldehyde at 50:1, 100:1 and 125:1 was 37.07, 76.58 and 92.76%, respectively. When the mass ratio of nanocomposite/cinnamaldehyde had been increased, the encapsulation efficiency would be also increased. By increasing the amount of SA-Chi, there are more spaces were available to be fulfilled by cinnamaldehyde molecules. The cinnamaldehyde encapsulation efficiencies obtained from this study were higher than 27.42% for chitosan/tripolyphosphate nanocomposite at an initial cinnamaldehyde concentration of 0.4 ppm as reported by Joghataei et al. [28].

As shown in table 1, the loading capacity of the nanocomposite increased as the mass ratio was increased. The highest loading capacity was obtained for 125:1 ratio. Following cross-linking reaction between SA and Chi, the egg-box structure of polymer chains will be formed and agglomeration of the nanocomposite occurred due to adhesive properties of Chi[29]. Hence, more cinnamaldehyde will be encapsulated in the nanocomposite.

Table 1. Encapsulation efficiencies and loading capacities of cinnamaldehyde loaded SA-Chi nanocomposites in different mass ratios.

| Ratio of cinnamaldehyde loaded SA-Chi nanocomposite | Encapsulation efficiency (%) | Loading capacity (%) |
|-----------------------------------------------------|-----------------------------|----------------------|
| 50:1                                                | 30.02                       | 12.20                |
| 100:1                                               | 71.22                       | 13.90                |
| 125:1                                               | 91.18                       | 74.80                |
3.3 Release profiles of cinnamaldehyde from the nanocomposite

As depicted in figure 4, cinnamaldehyde exhibited three release stages while cinnamaldehyde loaded nanocomposites had four release stages. The total hours for cinnamaldehyde release were 48. The nanocomposites were able to prolong the release of cinnamaldehyde to 52, 60 and 70 hours for 50:1, 100:1, and 125:1 respectively.

The cinnamaldehyde showed a slow release rate for the first 21 h. Nearly 10 % of cinnamaldehyde was released in the next hour. A slow delayed release of cinnamaldehyde was observed until 100 % release has been reached. The results taken at specified intervals showed that cinnamaldehyde-loaded nanocomposites had burst releases of about 44 % for the first 20 h. The release of the cinnamaldehyde from the nanocomposites obtained a steady state from this hour and after about 24 h, about 58 % of cinnamaldehyde was released. A slow release trend was observed for 46 h duration (36th to 70th h). The decrease of cinnamaldehyde diffusion from the nanocomposites could be ascribed to the dense structure of polyelectrolyte complex. Lim and Ahmad also reported SA-Chi nanocomposite could release imidacloprid over 120 h [27]. Therefore, the release profile of the prepared SA-Chi nanocomposites showed that cinnamaldehyde could have a sustained release from synthesized nanocomposites.

![Cumulative release profile of cinnamaldehyde](image)

**Figure 4.** In vitro release of (a) cinnamaldehyde and cinnamaldehyde-loaded SA-Chi nanocomposite with different mass ratios of SA-Chi nanocomposites to cinnamaldehyde (b 50:1, c 100:1 and d 125:1) in deionized water at 25 ºC.

3.4 Best fitted release kinetic models

From table 2, it was found that Korsmeyer-Peppas model provided a higher correlation coefficient ($R^2 = 0.9814$) than other kinetic models. Therefore, the drug release profile of the nanocomposites followed non-Fickian mechanism/anomalous diffusion ($n$ is between 0.45 and 0.89) which indicated diffusion through the nanocomposite leads drug release process.

**Table 2.** Correlation coefficient ($R^2$) of the fitted kinetic models for the release of cinnamaldehyde.

| SA-Chi/cinnamaldehyde | Kinetic models |          |          |          |          |
|-----------------------|----------------|----------|----------|----------|----------|
|                       | Zero order     | First order | Higuchi  | Korsemeyer-Peppas |
|                       | $R^2$          | $R^2$    | $R^2$    | $R^2$    | $n$      |
| 50:1                  | 0.9633         | 0.7344   | 0.9247   | 0.9841   | 0.8252   |
| 100:1                 | 0.9412         | 0.8563   | 0.9456   | 0.9795   | 0.8378   |
| 125:1                 | 0.9158         | 0.9517   | 0.9153   | 0.9837   | 0.7250   |
4. Conclusion
In this study, the potential of SA-Chi nanocomposites as drug carriers for larvicide formulation was investigated by carrying out characterization studies. There were major changes to the properties of functional groups, thermal stability, and crystallinity of nanocomposites following interaction with cinnamaldehyde. The SA-Chi nanocomposites encapsulated cinnamaldehyde successfully at a different mass ratio of the nanocomposite to cinnamaldehyde. The kinetics profile of cinnamaldehyde was best described by Korsmeyer-Peppas model. These key properties enable SA-Chi nanocomposite as an ideal nanocarrier to carry hydrophobic larvicides. This research creates impacts on the environment because it is biodegradable and low-cost. It is worth to study on other water-insoluble larvicides.

Acknowledgments
We thank Ministry of Education Malaysia and Universiti Pendidikan Sultan Idris for providing financial support (FRGS 2019-0003-102-02) and Rising Star UPSI 2019-0121-103-01) to this project. We thank the laboratory assistants from the faculty for their technical support.

References
[1] Bruschi M L 2015 Strategies to modify the drug release from pharmaceutical systems (United Kingdom: Woodhead Publishing) chapter 2 15-28
[2] Simonazzi A, Cid G A, Villegas M, Romero Al, Palma S D, Bermudes J M. Nanotechnology applications in drug controlled release (William Andrew Publishing) chapter 3 81-116
[3] Ding C and Li Z 2017 Mater Sci Eng C Mater Biol Appl. 76 1440-53
[4] ud Din F, Aman W, Ullah I, Qureshi O S, Mustapha O, Shafique S and Zeb A 2017 Int. J Nanomedicine 12 7291
[5] Mukherjee B, Satapathy B S, Bhattacharya S, Chakraborty R and Mishra V P 2017 Pharmokinetic and Pharmacodynamic Modulations of Therapeutically Active Constituents From Orally Administered Nanocarriers Along with a Glimpse of Their Advantages and Limitations Nano-and Microscale Drug Delivery Systems ed A M Grumezescu (Asterdam: United Kingdom/United State Elsevier) chapter 19 357-375
[6] Patra J K, Das G, Fraceto L F, Campos E V, del Pillar Rodriguez-Torres M, Acosta-Torres L S, Diaz-Torres L A, Grillo R, Swamy M K, Sharma S and Habtemariam S 2018 16 71
[7] Rao S S, Rekha P D, Anil S, Lowe B and Venkatesan J 2019 Drug Delivery and Biomedical Applications (London: Academic Press) chapter 21 495-512
[8] Kim H J, Lee H C, Oh J S, Shin B A, Oh C S, Park R D, Yang K S and Cho C S 1999 J. of Biomater. Sci. 10 543-56
[9] Hamman J H 2010 Mar. Drugs 8 1305-22
[10] Kashyap P L, Xiang X and Heiden P 2015 Int. J. of Biol. Marcomol. 77 36-51
[11] Bernkop-Schnurch A and Dunnhaupt 2012 Eur J Pharm Biopharm 81 463-69
[12] Seenivasan P, Tennyson S, Jayakumar M and Prabu A 2019 Res J Life Sci Bioinform Pharm Chem Sci 5(3) 601-08
[13] Cardoso-Ugarte GA, Lopez-Malo A and Sosa-Morales M E 2016 Food Preservation, Flavor and Safety (London: Academic Press) chapter 38 339-47
[14] da Silva M L, Bernardo M A, Singh J and de Mesquita M F 2019 The Roles of Functional Food Security in Global Health (London: Academic Press) chapter 33 565-76
[15] Thomas J and Kuruvilla K M 2012 Cinnamon Handbook of herbs and spices ed K V Peter (United States of America: Woodhead Publishing Limited) pp 182-96
[16] Gombotz W R and Wee S 1998 Adv. Drug Delivery Rev. 31 267-85
[17] George M and Abraham T E 2006 J. Control. Release 114 1-14
[18] Hoare T R and Kohane D S 2008 Polymer 49 1993-2007
[19] Loquercio A, Castell-Perez E, Gomes C and Moreira R G 2015 J. Food Sci. 80 N2305-15
[20] Tu J, Boyle A L, Friedrich H, Bomans P H, Bussmann J, Sommerdijk N A 2016 ACS Appl. Mater. Interfaces 32211-19
[21] Guo J, Giusti M M and Kaletune G 2018. Int Food Res J 107 414-22
[22] Ritger P L, Peppas N A 1987 J Control Release 5 37-42
[23] Kulig D, Zimoč-Korzycka A, Jarmoluk A, Marycz, K 2016 Polymers 8 167
[24] Ji M, Sun X, Guo X, Zhu W, Wu J, Chen L, Wang J, Chen M, Cheng C and Zhang Q 2019 Food Hydrocoll 90 515-22
[25] Nalini T, Basha S K, Sadiq A M M, Kumari V S and Kaviyarasu K 2019 J Drug Deliv Sci Tec 52 65-72
[26] Trivedi M K, Branton A, Trivedi D, Nayak G 2015 Pharm Anal Acta 6
[27] Lim G P, Ahmad M S 2017 J Ind Eng Chem
[28] Joghataei M, Hosseini SF, Arab-Tehrany E 2019 J Food Process Preserv e13972
[29] González-Rodriguez ML, Holgado MA, Sanchez-Lafuente C et al 2002 Int J Pharm 232 225-34