ALGINATE/CHITOSAN FILM LOADING GOLDEN FLOWER TEA (CAMELLIA CHRYSANTHA) EXTRACT: PREPARATION AND CHARACTERIZATION

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ABSTRACT

Golden Flower Tea has the scientific name Camellia chrysantha. It can effectively enhance human immune function, prevent and fight cancer, be anti-aging and prolongs life, and be a powerful antioxidant. Polymer systems loading Golden Flower Tea extract can enhance their solubility and effectiveness in simulated body solutions such as stomach or intestine environments. In this paper, chitosan and sodium alginate, two natural polymers, were selected for the preparation of chitosan/alginate loading Golden Flower Tea extract thanks to their many advantages. The chitosan/alginate film loading Golden Flower Tea extract was prepared by solution method. The suitable ratio of alginate/chitosan obtained from previous studies is 7/3. The characteristics of these films are evaluated by Fourier Transform Infrared (FT-IR) spectroscopy, Scanning Electron Microscopy (SEM), and Differential Scanning Calorimetric (DSC) methods. The obtained results show that polyphenols interact and disperse regularly in polymer matrix.

Keywords: Golden Flower Tea extract, Camellia chrysantha, sodium alginate, chitosan, composite film.

1. INTRODUCTION

Nanoparticles based on biodegradable polymers, natural polymers, lipids, and polysaccharides have been focused on study and developed their applications in recent years. In particular, the polymer nanoparticles loading drug systems which can control the drug release have attracted much attention. The natural polymers can be biodegradable, safe and biocompatible with human body. Among them, sodium alginate (AGN) and chitosan (CS) are
potential candidates due to their great advantages. CS has the scientific name poly-(1,4)-2-amino-2-deoxy-β-D-glucose or poly-(1,4)-2-amino-2-deoxy-β-D-glucopyranose. It is a deacetylated derivative of chitin - a type of polysaccharide which is abundant in the shell of crustaceans such as crabs, shrimp, etc. [1]. AGN which has molecular weight in the range of 32000-200000 is synthesized from brown algae [2]. The combination of CS and AGN not only enhances surface activity of nanoparticles but also prolongs the drug active time and promote drug uptake thanks to the formation of polyelectrolyte complex of CS and AGN [2-3]. Manju Bernela et al. [3] prepared the AGN/CS/pluronic nanoparticles loading nisin and applied them in food preservation. The results showed that the antimicrobial activity of nisin was longer as it was introduced into polymer nanoparticles. Cui-Yun Yu et al. synthesized AGN/CS nanoparticles loading both 5-fluorouracil (5-FU) and tegafur with the average particle size in the range of 100-200 nm [4]. Some other publications also indicated that AGN/CS composites in both nanoparticle and film shape can also load drugs and improve drug active such as gatifloxacin, ginsenoside Rb1, ofloxacin and paclitaxel [5-8]. In our previous paper, AGN/CS films (the ratio of AG/CS: 8/2) loading lovastatine with the content of 10, 20 and 30 wt.% (in comparison with AG and CS weight) were prepared and their characteristics were investigated [9]. The obtained results confirmed that AGN/CS films can control the lovastatine release in pH 7.4 solution. The study on AGN/CS film loading the Golden Flower Tea extract has been limited.

In this work, the Golden Flower Tea extract (GFTE) which is extracted from the Golden Flower Tea was used as a model drug. It is one of the precious medicinal herbs because it contains over 400 chemical compositions (saponin, phenolic compounds, amino acids, folic acid, proteins, vitamins B1, B2, C, E, fatty acids, and many natural nutrients) and non-toxic. It plays roles of anti-cancer, anti-aging and anti-oxidant substances [10-13]. The GFTE has been loaded by AGN/CS composite according to solution method. The structure, morphology and thermal behavior of the AGN/CS composite films with and without GFTE are investigated and discussed.

2. MATERIALS AND METHODS

2.1. Materials

Sodium alginate (AGN, white powder, viscosity 300-500 mpa.s) and chitosan (CS, powder, deacetylation degree 75 % – 85 %, polymer density index 1.61 × 10^5 Da) were provided by Sigma Aldrich Co. Golden Flower Tea extract (GFTE) was extracted from the leaves of Golden Flower Tea which were collected at Tam Dao, Vinh Phuc province, Vietnam using ethanol solvent on the rotating machine. Ethanol, acetic acid 99.5 % are the commercial products of China using without purification.

2.2. Preparation of AGN/CS/GFTE composite films

AGN/CS/GFTE composite films (ACG) were prepared as follows: First, 0.07 g of AGN was dissolved in 25 mL of distilled water, then, 10 mL of ethanol solution containing GFTE was added into AGN solution. The mixture solution was stirred on the stirring machine and ultrasonic machine for 1 h to form a homogenous solution (solution 1). Next, a solution of 0.03 g CS dissolved into 20 mL CH3COOH 1 % was dropped into the solution 1. The mixture was stirring and ultrasonicing for 1 h to form a stable solution. Finally, the above mixture was poured to a petri dish and evaporated naturally until a thin film was obtained.
The other samples were prepared similarly with the AGN/CS ratio was fixed at 7/3. The composition and abbreviation of composite films were listed in Table 1.

Table 1. Composition and abbreviation of AGN/CS/GFTE composite films.

| Ratio weight of AGN: CS: GFTE (g/g/g) | Abbreviation |
|--------------------------------------|--------------|
| 0.070 : 0.030 : 0.005                 | AC73GE5      |
| 0.070 : 0.030 : 0.010                 | AC73GE10     |
| 0.070 : 0.030 : 0.015                 | AC73GE15     |
| 0.070 : 0.030 : 0.020                 | AC73GE20     |
| 0.070 : 0.030 : 0.000                 | AC73GE00     |
| 0.070 : 0.000 : 0.010                 | AC70GE10     |

2.3. Characterization of AGN/CS/GFTE composite films

- Fourier Transforms Infrared Spectroscopy (FTIR): FTIR spectra of AGN/CS/GFTE composite films were recorded on a Nicolet/Nexus 670 spectrometer (USA) at Institute for Tropical Technology, VAST at room temperature in air by 16 scans with 4 cm\(^{-1}\) resolution and wavenumber ranging from 400 to 4000 cm\(^{-1}\).

- Field Emission Scanning Electron Microscope (FESEM): Morphology of the composite films coated by platinum was conducted using a S-4800 FESEM instrument (Hitachi, Japan) at National Institute of Hygiene and Epidemiology.

- Differential scanning calorimetric (DSC): DSC diagrams of the composite films were carried out in N\(_2\) atmosphere on the Shimadzu DSC-50 machine at University of Natural Sciences – Hanoi National University with a heat speed of 10 °C.min\(^{-1}\) and temperature from room temperature to 400 °C.

3. RESULTS AND DISCUSSION

3.1. FTIR spectra of AGN/CS/GFTE composite films

FTIR spectra of AGN, CS and AGN/CS blend is shown in Fig. 1. Table 2 presents the position of some main groups in AGN, CS and AGN/CS blend. From Fig. 1 and Table 2, it can observe the stretching vibrations of NH\(_2\), OH groups of AGN and CS were assigned at 3453 cm\(^{-1}\) and 3447 cm\(^{-1}\). The alkyl group of AGN and CS was found at 2936 cm\(^{-1}\) and 2883 cm\(^{-1}\). The vibration of C=O group was seen at 1609 cm\(^{-1}\) (AGN) and 1654 cm\(^{-1}\) (CS). The peaks corresponding to symmetric and asymmetric stretching vibrations of C-O-C group was given at 1032 cm\(^{-1}\) and 1086 cm\(^{-1}\) (AGN) and 1082 cm\(^{-1}\) and 1154 cm\(^{-1}\) (CS). The band around 1598 cm\(^{-1}\) was attributed to the scissoring or bending vibration of N-H group in CS. Combining AGN and CS, the position of peaks characterized for vibrations of N-H, O-H, C=O, C=O, C-H groups in the FTIR spectrum of AGN/CS blend was shifted significantly as compared to corresponding peaks in the FTIR spectrum of AGN or CS. This can confirm that AGN and CS are interacted to each other through hydrogen bonding between N-H, O-H in CS and O-H, C=O in AGN [2-4].
Figure 1. FTIR spectra of AGN, CS, and AGN/CS blend.

Table 2. Wavenumbers of some main groups of AGN, CS, and AGN/CS blend.

| Vibrations          | AGN  | CS   | AGN/CS blend |
|---------------------|------|------|--------------|
| w \(-\text{NH}_2, -\text{OH}\) | 3453 | 3447 | 3380         |
| w \(\text{CH}\)       | 2936 | 2883 | 2928         |
| w \(\text{C=O}\)      | 1609 | 1654 | 1603         |
| \(\delta\)\text{-NH}_2|    - | 1598 | 1411         |
| w \(\text{C-O-C}\)    | 1032 | 1154 | 1036         |

As shown in FTIR spectrum of GFTE in Fig. 2, the stretching vibrations of O-H and C-H bonds of GFTE were recognized at 3437 cm\(^{-1}\) and 2920 cm\(^{-1}\), respectively. Peak at 1726 cm\(^{-1}\) was attributed to stretching vibration of C=O bond while peak at 1617 cm\(^{-1}\) was contributed to stretching vibration of C=C and bending vibration of O-H bond. The peak characterized for amine group appeared at 1516 cm\(^{-1}\). The bending vibrations of CH and CH\(_3\) group in GFTE were observed at 1447 cm\(^{-1}\) and 1383 cm\(^{-1}\). The bending vibration of C-OH and stretching vibration of C-O were also seen at 1252 cm\(^{-1}\) and 1044 cm\(^{-1}\). From FTIR spectrum of GFTE, it can be seen GFTE contains many substances such as saponin, phenolic compounds, amino acids, etc. [11-12]. Therefore, GFTE was used as a substance model in this study. It is necessary to purify GFTE to obtain the individual active compounds for further studies.
Figure 2. FTIR spectrum of GFTE.

Figure 3 and Table 3 present the FTIR spectra and wavenumbers of some main groups of AGN/CS/GFTE composite films. It can be seen that the characteristic peaks of AGN, CS and GFTE appeared in the FTIR spectra of these films and the peaks corresponding to NH, OH or C=O, C=C groups were resonated to form a larger peak. Although CS is absent in AC70GE10 sample, the FTIR spectrum of AC70GE10 (AGN/CS/GFTE = 70/0/10) is similar to the FTIR spectra of the AGN/CS/GFTE composite films due to the resonance of groups (Table 3). The absence of peak at 1726 cm\(^{-1}\) (C=O stretching vibration, in FTIR spectrum of GFTE) in the FTIR spectra of AGN/CS/GFTE composite films can be due to it was overlapped by peak of C=C vibration. The similarity in FTIR spectra of AGN/CS/GFTE composite films with different GFTE content showed that the GFTE content does not affect on the interaction between AGN and CS.

Figure 3. FTIR spectra of AGN/CS/GFTE composite films.
Table 3. Wavenumbers of some main groups of AGN/CS/GFTE composite films.

| Samples          | Wavenumbers (cm⁻¹) | AGN/CS blend | AC73GE5 | AC70GE10 | AC73GE10 | AC73GE15 | AC73GE20 |
|------------------|--------------------|--------------|---------|----------|----------|----------|----------|
| $\nu_{\text{NH}_2, \text{OH}}$ | 3380               | 3382         | 3392    | 3375     | 3376     | 3376     |
| $\nu_{\text{CH}}$      | 2928               | 2930         | 2926    | 2928     | 2928     | 2927     |
| $\nu_{\text{C}=\text{C}}$ | 1603               | 1602         | 1606    | 1605     | 1605     | 1605     |
| $\delta_{\text{NH}_2, \text{CH}}$ | 1411               | 1411         | 1414    | 1412     | 1411     | 1411     |
| $\nu_{\text{C}=\text{O}}$ | 1087-1036          | 1086-1037    | 1087-1036 | 1090-1036 | 1079-1036 | 1081-1036 |

3.2. Morphology of AGN/CS/GFTE composite films

From visual images of AGN/CS/GFTE composite films in Figure 4, it can be seen that the AGN/CS blend has white color while AGN/CS/GFTE composite films containing GFTE has yellow color. When increasing GFTE content in AGN/CS/GFTE composite films, the color of these films is dark-yellow and brown. However, all prepared films have smooth and non-crack surface.

![Figure 4](image)

Figure 4. Visual images of AGN/CS/GFTE composite films, (A): AC73GE00, (B) AC73GE5, (C) AC70GE10, (D) AC73GE10, (E) AC73GE15, and (F) AC73GE20.

The FESEM images of AGN/CS/GFTE composite films were expressed in Figure 5. The AGN/CS blend has quite homogeneous surface corresponding to the good dispersion and interaction between AGN and CS (Figure 5A). The size of CS phase is about 20 nm. The AGN/GFTE composite film (AC70GE10) has micro-pores on the surface (Figure 5C). It means...
that GFTE can interact poorly with AGN. These micro-pores can affect the thermal behavior and drug release from AGN/GFTE films. The FESEM images of AGN/CS/GFTE composite films containing different GFTE content indicate a more even structure without micro-pores (Figure 5 B, D, E, and F). The dispersion phase (both GFTE and CS) has size in the range of 20 – 100 nm. The AGN/CS/GFTE composite film loading 10 wt.% of GFTE (AC73GE10) has most regular structure.

![Figure 5. FESEM images of AGN/CS/GFTE composite films.](image)

3.3. Thermal behavior of AGN/CS/GFTE composite films

Thermal behavior of AGN/CS/GFTE composite films is evaluated by DSC method and shown in Fig. 6. The DSC parameters obtained from DSC diagrams are listed in Table 4. It can be seen the appearance of two peaks in DSC diagrams of all tested films, an endothermic peak around 133 °C and an exothermic peak around 249 °C [4].
Figure 6. DSC diagrams of AGN/CS/GFTE composite films.

Table 4. DSC parameters of AGN/CS/GFTE composite films, $T_{onset}$: onset melting temperature, $T_m$: melting temperature, $T_d$: degradation temperature, $\Delta H_m$: melting enthalpy.

| Samples   | $T_{onset}$ (°C) | $T_m$ (°C) | $T_d$ (°C) | $\Delta H_m$ (J/g) |
|-----------|-----------------|------------|------------|-------------------|
| AC73GE00  | 89.7            | 135.0      | 247.6      | 460               |
| AC73GE5   | 76.1            | 133.7      | 249.0      | 498               |
| AC70GE10  | 74.6            | 124.4      | 246.1      | 461               |
| AC73GE10  | 92.6            | 132.8      | 249.9      | 456               |
| AC73GE15  | 95.3            | 131.4      | 250.7      | 424               |
| AC73GE20  | 81.2            | 134.8      | 248.8      | 475               |

The melting temperature and degradation temperature values of AGN/GFTE composite films are lower than those of AGN/CS/GFTE composite films. It means that CS plays an important role in enhancing thermal stability of the composite films. The AGN/CS/GFTE composite films containing different GFTE content have lower melting temperature and higher degradation temperature as compared to the AGN/CS blend. In particular, the onset melting temperature of the AGN/CS/GFTE composite films containing 10 wt.% and 15 wt.% GFTE (AC73GE10 and AC73GE15) is higher than that of the AGN/CS blend and AGN/CS/GFTE composite films loading 5 wt.% and 20 wt.% GFTE (AC73GE5 and AC73GE20) as shown in Table 4). It can confirm that structure of the composite films with 10 and 15 wt.% GFTE are closer and more regular than that of the others. On the other side, the melting temperature and
melting enthalpy of the AGN/CS/GFTE composite films containing 10 wt.% and 15 wt.% GFTE are smaller than those of the others, corresponding to the crystal degree in these samples is lower than that of the others, leading to easier dissolution of these samples in solvents. As a result, the solubility of AGN/CS/GFTE composite films containing 10 wt.% and 15 wt.% of GFTE is better.

4. CONCLUSIONS

In this work, the AGN/CS/GFTE composite films containing different GFTE content are prepared by solution method. The FTIR spectra analysis shows that AGN interacted to CS and the GFTE content does not affect on the interaction between AGN and CS. The color of the AGN/CS/GFTE composite films changes from yellow to brown as increasing the GFTE content. The FESEM images indicate the AGN/CS/GFTE composite films have a regular structure. The FESEM and DSC analysis display that AGN/CS/GFTE composite films containing 10 wt.% and 15 wt.% of GFTE have closest and most regular structure.

REFERENCES

1. Gavhane Yogeshkumar N., Gurav Atul S and Yadav Adhikrao V. - Chitosan and its applications: A review of literature, International Journal of Research in Pharmaceutical and Biomedical Sciences 4 (1) (2013) 312-331.
2. Loreana L., Alexandre L.P., Valfredo F., Mauro C.M.L, Hellen K.S. - Development and evaluation of pH-sensitive sodium alginate/chitosan microparticles containing the antituberculosis drug rifampicin, Materials Science and Engineering C39 (2014) 161–167.
3. Manju B., Pawan K., Meenu C., Rajesh T. - Synthesis, characterization of nisin loaded alginate-chitosan-pluronic composite nanoparticles and evaluation against microbes, LWT - Food Science and Technology 59 (2014) 1093-1099.
4. Cui-Yun Y., Xi-Chen Z., Fang-Zhou Z., Xian-Zheng Z., Si-Xue C., Ren-Xi Z. - Sustained release of antineoplastic drugs from chitosan-reinforced alginate microparticle drug delivery systems, International Journal of Pharmaceutics 357 (2008) 15-21.
5. Miloslava R., Katefiina D., Lenka K. - Stability testing of alginate-chitosan films for colon drug delivery. Ceska a Slovenska Farmacie 61 (2012) 26–33.
6. Mohy Eldin M.S., Omer A.M., Wassel M.A., Tamer T.M., Abd Elmonem M.S., Ibrahim S.A. - Oval smart pH sensitive chitosan grafted alginate hydrogel microcapsules for oral protein delivery: I. preparation and characterization. International Journal of Pharmacy and Pharmaceutical Sciences 7 (10) (2015) 331-337.
7. Shinichi W., Katsuya S., Takumi Y., Noriaki H., Masahiro S., Hiroaki A. - Effect of oral mucosal adhesive films containing ginsenoside Rb1 on 5-fluorouracil-induced oral mucositis in hamsters. European Journal of Pharmacology 616 (2009) 281–286.
8. Vipin B., Pramod K. S., Nitin S., Om P. P. and Rishabha M. - Applications of chitosan and chitosan derivatives in drug delivery, Advances in Biological Research 5 (1) (2011) 28-37.
9. Chinh N. T., Ly N. T. H, Mai T. T., Trang N. T. T., Loc T. T., Giang L. D., Tung N. Q., Hoang T. - Characteristic and properties of chitosan/alginate polymer blend carrying lovastatin drug, Journal of Science and Technology 54 (2B) (2016) 118-124.
10. Lai W., Debmalya R., Sen S. L., Sheng T. Y. and Li S. - Hypoglycemic effect of Camellia chrysantha extract on type 2 diabetic mice model, Bangladesh J. Pharmacol 12 (2017) 359-363.

11. Jin-Bin W., Xiong L., Hui S., Yong-Hong L., Yu-Zheng P., Jun-Xiang R., Xia Q., Yong-Xin C., Cai-Li N., Zhi-Heng S. - Characterization and determination of antioxidant components in the leaves of Camellia chrysantha (Hu) Tuyama based on composition activity relationship approach. Journal of Food and Drug Analysis 23 (2015) 40-48.

12. Yokozawa K. - Antioxidative activity of green polyphenol in cholesterol-fed rats, National Institutes of Health 50 (12) (2002) 3549-3552.

13. Shirode A. B., Bharali D. J., Nallanthighal S., Coon J. K., Mousa S. A., Reliene R. - Nanoencapsulation of pomegranate bioactive compounds for breast cancer chemoprevention, Int J Nanomed 10 (2015) 475–484.