MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCES AND TECHNOLOGY

LUONG PHU HOANG

RESEARCH ON FABRICATION AND CHARACTERISTICS OF CHITOSAN/ALGINATE COMPOSITE MATERIALS CONTAINING POLYPHENOLS IN YELLOW TEA (CAMELLIA CHRYSANTHA)

***Specialization*: Organic Chemistry**

***Code*: 9440114**

SUMMARY OF THESIS DOCTOR OF ORGANIC CHEMISTRY

HA NOI – 2024

The project was completed at: Graduate University of Sciences and Technology - Vietnam Academy of Science and Technology

Science instructor 1: Prof. Dr. Thai Hoang

Science instructor 2: Assoc. Prof. Vu Quoc Trung

Reviewer1: …

Reviewer2: …

Reviewer3: ….

The thesis will be defended in front of the Doctoral Dissertation Judging Committee, meeting at the Academy of Science and Technology - Vietnam Academy of Science and Technology at … hour ..', date … month … 20… .

The thesis can be found at:

1. Library of graduate University of Sciences and Technology

2. National Library of Vietnam

LIST OF WORKS OF THE AUTHOR

1. Luong Phu Hoang, Nguyen Thuy Chinh, Vu Quoc Trung, Thai Hoang, Alginate/Chitosan film loading Golden Flower Tea (*Camellia Chrysantha*) Extract: Preparation and characterization, *Vietnam Journal of Science and Technology, 2018:56:209-218.*
2. Luong Phu Hoang, Nguyen Thuy Chinh, Vu Quoc Trung, Thai Hoang, Ly Thi Ngoc Lien, Tran Thi Kim Ngan, Tran Do Mai Trang, Pham The Dan, Preparation and Assessment of Some Characteristics of Nanoparticles Based on Sodium Alginate, Chitosan and Camellia chrysantha Polyphenols. *International Journal of Polymer Science, (SCIE) 2021 (3): 1-11.*
3. Luong Phu Hoang, Nguyen Thuy Chinh, Ly Thi Ngoc Lien, Thai Hoang, Vu QuocTrung, Characterization of alginate/chitosan film loading golden camellia extract (camellia chrysantha), *Analytical Journal of Chemistry, Physics and Biology, 2022: 27(1):250-260.*

INTRODUCTION

Camellia chrysantha is a species of angiosperm in the family Theaceae. The tree is found in Vietnam (Tam Dao, Quang Ninh, Lam Dong, Tuyen Quang, Yen Bai, Cuc Phuong) and China. There are more than 400 chemical ingredients in yellow flower tea, without toxicity or side effects, of which the main contents are phenolic compounds, amino acids, folic acid, proteins, vitamins B1, B2, C, E, fatty acids... and many natural nutritional ingredients. In addition, yellow flower tea also contains dozens of amino acids and many trace elements Ge, Se, Mo, Zn, V... that help protect health, improve resistance, and prevent diseases. . Recently, applying modern research methods, scientists have found that the biological effects of extracts from yellow camellia leaves and flowers are mainly due to polyphenols. Studies show that tea polyphenols have antioxidant, anti-inflammatory properties and support anti-cancer, anti-organ damage...

Chitosan (CS) and its derivatives have been applied in many different fields. In biomedicine and pharmaceutical chemistry, CS is used as a wound healing membrane, a substance that helps regenerate bone tissue, medicine... CS-based nanomaterials are also researched and applied in biomedicine due to their stability. relatively high concentration while still maintaining some properties of the original chitosan. Due to its small size and porous structure, CS has high adsorption capacity. Nano-CS is used as an adsorbent for various substances, especially drugs used in medicine.

Alginate (AG) is an abundant marine biopolymer in the world, first discovered by Stanford (1881). Common roles of alginate in pharmaceuticals are as thickeners, gelators, stabilizers, and in controlled-release medicinal products. Oral formulations using alginate are very common in pharmaceutical applications. Both CS and AG are natural, non-toxic, biodegradable, highly biocompatible and pH-sensitive polymers. They are widely used in the formation of microparticles through electrostatic attraction between amino and acid functional groups in the structure of CS and AG. Choosing to prepare drugs in the form of nano particles to control drug release is a potential research direction of the pharmaceutical chemistry industry. Based on advantages such as: more stable drug release control, minimizing the risk of overdose, and convenient preparation of different drug forms.

The disadvantage of polyphenols in tea is that they are less stable to temperature and light. Therefore, one of the new research directions is to combine natural polymers that carry medicinal substances and control the release of medicinal substances with yellow tea polyphenols for application in supporting the treatment of various diseases and anti-oxidation. chemistry, cancer. Among the

naturally occurring polymers used as carriers for camellia polyphenols, the most prominent are AG and CS thanks to their good properties: hydrogen bonds and dipole interactions between AG - CS contribute to controlling Control the drug release rate as well as promote the inherent biological activity of AG and CS. Published works show that the problem of researching and manufacturing AG/CS composite materials containing valuable compounds such as polyphenols in yellow flower tea is oriented to support the treatment of some diseases such as cancer prevention, inhibition of Growth inhibition of cancer cells, anti-oxidation and drug release research are just beginning. Therefore, the doctoral student chose the topic "Research on fabrication and characterization of chitosan/alginate composite materials containing polyphenols in camellia chrysantha (*Camellia chrysantha*)".

Objectives of the thesis topic:

1. Successfully extracted and manufactured the AG/CS combination containing yellow camellia extract using solution and microemulsion methods.

2. Evaluate the characteristics, properties and structural morphology of the AG/CS complex containing yellow camellia extract.

3. Develop an appropriate drug release kinetic model/equation, thereby exploring the cell inhibition, anti-oxidation, and cancer ability of the AG/CS combination containing yellow tea extract.

Main research contents of the thesis topic:

1. Fabrication of AG/CS/ combination containing yellow tea extract using solution and microemulsion methods.

2. Research on the characteristics and properties of the AG/CS combination containing yellow camellia extract.

3. Research on drug release from the AG/CS combination containing yellow camellia extract.

4. Research to explore the cell inhibition and antioxidant capacity of the natural polymer combination AG/CS containing yellow camellia extract.

CHAPTER 1. OVERVIEW

1.1. Introduction to chitosan

Chitosan (CS) is a deacetylated derivative of chitin, an abundant natural polysaccharide. CS is found in the shells of crustaceans, insects, molluscs and the cell membranes of some types of fungi... The output of waste separated from these insects and animals every year is estimated 109 - 1010 tons/year. One of the most important properties of CS is its antibacterial ability. The amine group with an

extra proton in CS has the ability to inhibit bacterial growth. CS has many unique properties such as non-toxic, biocompatible and biodegradable. CS has received much attention because of its biological activities such as antibacterial, anti-cancer and increased resistance properties. CS is widely used in fields such as biotechnology, pharmaceuticals, wastewater treatment, cosmetics...

1.2. Introduction to alginate

Alginate (AG) is an abundant marine biopolymer in the world. AG is the common name for salts of alginic acid, an anionic polysaccharide extracted from brown seaweed. The content and quality of AG varies according to species, season, age of seaweed, part of seaweed plant and living conditions.

AG is considered a rich and renewable source of polysaccharide, meeting the needs of strongly developing industrial production sectors in the future. In particular, AG is non-toxic, non-immunogenic, highly adaptable and biodegradable, so it is also called a "green compound" and is considered a new material serving the food industry. cosmetics, pharmaceuticals...

1.3. Drug-bearing natural polymer composite materials on the basis of alginate and chitosan

In recent years, natural polymer materials containing drugs based on alginate and chitosan have been widely researched in two popular forms: film and granule.

According to research by Mariana Altenhofen in 2012, AG membranes and AG/CS complexes containing natamycin (antibacterial agent) have a continuous structure and easily interact electrostatically with CS. An increase in CS content affects the morphology and properties of the membrane. Increasing the CS content leads to the breaking of the bond of the AG/CS membrane and increases the release rate of natamycin faster than with other membrane structures. The release kinetics of natamycin in water is very slow and is clearly hindered in the AG/CS composite membrane due to the electrostatic interaction between CS and natamycin. The natamycin release patterns from AG and AG/CS membranes in water showed that the membranes met antibacterial requirements and had high applicability in preserving dairy foods. In 2016, Bhunchu's research team created a nano-CS/AG composite as a drug carrier material for cancer treatment based on the ability to easily absorb intracellularly and increase the treatment effectiveness of CS and AG. The smaller size of nanoparticles allows penetration through blood capillaries and is easily absorbed into cancer cells with high efficiency. This combination of drug carrier materials has improved important parameters such as oral bioavailability of the drug, chemotherapeutic stability against enzymatic degradation, reduced drug toxicity and increased treatment effectiveness treat.

1.4. Introduction of yellow flower tea and tea polyphenols

1.4.1. Introduction to yellow flower tea

Camellia chrysantha (THV), also known as Camellia chrysantha, is a species of angiosperm in the Theaceae family. THV is often found in Vietnam (Tam Dao, Ba Che Quang Ninh, Lam Dong, Tuyen Quang, Yen Bai, Cuc Phuong) and China (Southwestern Guangxi province)

THV is widely used in biomedicine because it contains more than 400 active ingredients, including polyphenols and trace elements such as selenium (Se), germanium (Ge), potassium (K), vitamins B1, B2, C.. Compounds in THV have the ability to inhibit the growth of tumors by up to 33.8%, reduce cholesterol levels in the blood by up to 35%, reduce symptoms of atherosclerosis caused by fatty blood, and regulate blood pressure. , lower blood sugar, treat dysentery, bloody stools...

According to Chinese scientists, THV has 9 main effects:

– Tea leaves contain active ingredients that reduce total lipid content in blood serum, reduce low-density cholesterol (bad cholesterol) and increase high-density cholesterol (good cholesterol).

– Tea leaf decoction has a clear blood pressure lowering effect and the effect is maintained for a relatively long time.

– Tea leaf decoction has the effect of inhibiting the aggregation of platelets, preventing the formation of thrombi that cause blockage of blood vessels.

– Prevent cancer and inhibit the growth of other tumors.

– Nervous excitement.

– Strong diuretic.

– Detoxify the liver and kidneys, prevent atherosclerosis.

– Inhibits and destroys bacteria.

– In addition, tea leaves also have anti-inflammatory, anti-allergic effects and maintain the normal state of the thyroid gland.

1.5. Tea polymer/polyphenol composite materials

The important active ingredients in tea polyphenols are difficult to use because of their poor chemical stability and instability. Extensive research around the world focuses on manufacturing polymer complexes containing valuable active ingredients such as EGCG, EGC... in nano-sized THV to easily absorb and control the drug release process. and increase the ability to use precious medicinal herbs in THV. Including studies such as the use of polyphenols in green tea to inhibit and fight cancer are focused on research. In vitro tests show that polyphenols carried by chitosan, poly(caprolactone) and poly(lactide-co-glucoside) nanoparticles help enhance the dispersion of polyphenol active ingredients, have the ability to fight

breast cancer, necrosis... Synthesis, characterization and cytotoxicity studies of polyphenol/CS nanoparticles (polyphenol extracted from tea) on the liver and stomach also show that the polyphenol/CS nanoparticle combination is effective inhibit cancer cells.

Observe:

From domestic and foreign research projects, it has been shown that AG and CS are two naturally occurring polymers with potential for use as drug carriers. The issue of research and use of AG/CS with some anti-inflammatory, anti-bacterial and cancer treatment drugs has just begun. Through hydrogen bonds, dipole interactions between AG and CS, and AG/CS mixing ratio, the drug release rate can be controlled favorably.

Composite materials containing nano-sized tea polyphenols and AG/CS help drug molecules easily pass through cell walls, increasing drug absorption as well as overcoming some disadvantages of tea polyphenols such as poor stability. to heat and light sensitivity have not been focused on research. Therefore, composite materials containing tea polyphenols used for the following purposes: treatment of cardiovascular diseases, cancer, food preservation... will continue to be researched in this thesis topic.

CHAPTER 2. EXPERIENCE

2.1. Raw materials, chemicals and tools

2.1.1. Raw materials and chemicals

THV extract extracted from Tam Dao THV leaves of the tea species (Camellia tamdaoensis Ninh at Hakoda) was found and announced in 2010 by two scientists Tran Ninh and Hakoda Naotoshi. THV leaves were harvested in December 2017 in Tam Quan commune, Tam Dao District, Vinh Phuc province.

Alginate, chitosan, sodium tripolyphosphate, potassium chlorite, sodium hydroxide, calcium chloride, ... KB carcinoma cell lines and HepG2 human hepatocytes were sourced from the American Standardbred Museum (ATCC).

2.2. High extraction process from yellow flower tea leaves

2.2.1. High extraction process from yellow flower tea leaves

The process for high extraction from yellow flower tea leaves (CC) and enrichment of total polyphenol (CT) content from yellow flower tea leaves is shown in Figure 2.1 and Figure 2.2.

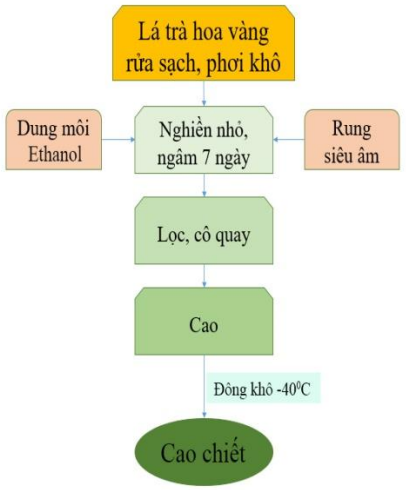

Figure 2.1. Diagram of high extraction from THV leaves

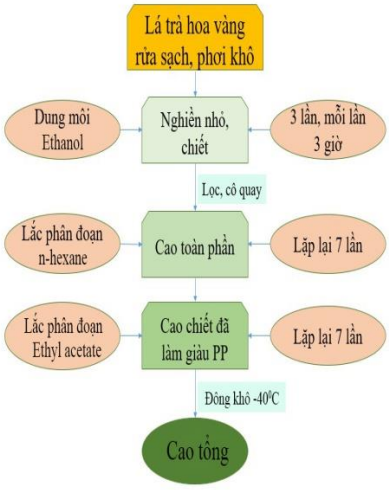

Figure 2.2. Diagram of high extraction from THV leaves and enrichment of total polyphenol content from THV leaves

2.3. Manufacturing alginat/chitosan composite materials bearing polyphenols from yellow tea leaf extract

2.3.1. Making alginat/chitosan/cao combination film extracted from yellow flower tea leaves by solution method

Table 2.1. Manufactured AG/CS/CC composite film models

Mass ratio AG/CS/CC	Mass of AG, CS and CC (gram)	Sample symbols
AG/CS/CC* = 7/3/5 %	0,07 : 0,03 : 0,005	AC73CC5
AG/CS/CC* = 7/3/10 %	0,07 : 0,03 : 0,01	AC73CC10
AG/CS/CC* = 7/3/15 %	0,07 : 0,03 : 0,015	AC73CC15
AG/CS/CC* = 7/3/20 %	0,07 : 0,03 : 0,02	AC73CC20
AG/CS/CC* = 7/3/0 %	0,07 : 0,03 : 0,00	AC73CC0
AG/CS/CC* = 7/0/10 %	0,07 : 0,00 : 0,01	AC70CC10

*: Calculated on the basis of the total volume of AG and CS

2.3.2. Manufacturing a combination of alginat/chitosan/high sum seeds from yellow flower tea by microemulsion method

Table 2.2. Manufactured AG/CS/CT particle samples

Mass ratio AG/CS/CT	Mass of AG, CS and CT* (grams)	Sample symbols
AG/CS/CT* = 8/4/10 %	0,08 : 0,04 : 0,012	AG/CS/CT10
AG/CS/CT * = 8/4/20 %	0,08 : 0,04 : 0,024	AG/CS/CT20
AG/CS/CT * = 8/4/30 %	0,08 : 0,04 : 0,036	AG/CS/CT30
AG/CS/CT * = 8/4/50 %	0,08 : 0,04 : 0,06	AG/CS/CT50

*: Calculated on the basis of the total volume of AG and CS

2.4. Research methods

Thin-layer chromatography (TLC).

Quantification of total polyphenols by Folin–Denis method.

Fourier transform infrared spectroscopic method (FT-IR).

Methods for determining the particle size distribution.

Scanning electron microscopy method.

Differential scanning calorimetry (DSC) method.

Ultraviolet-visible absorption spectrometric method (UV-Vis).

Methods of quantitative analysis.

2.5. Evaluation of biological activity of CT and AG/CS/CT combination

Methods for evaluating cancer cell inhibitory activity.

Antioxidant activity assessment method (reaction method via D, PP, H free radical scanning/capture).

Method for assessing anti-inflammatory activity (method for determining the ability to inhibit the production of NO).

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Characteristics and properties of yellow flower tea extract and total

3.1.1. Results of quantification of total polyphenols according to Folin – Denis method

In order to contribute to the assessment of the composition of basic natural compounds contained in the extract (CC) and total extract (CT) of yellow flower tea. The researcher conducted qualitative reactions, thin layer chromatography and quantified total polyphenols according to the Folin - Denis method.

Qualitative results show that the composition of typical compounds in CC and CT is quite rich, containing all common natural compound groups such as: alkaloids, saponins, flavonoids, tannins and total polyphenols. All samples tested positive for the reagent.

Chromatogram of total yellow tea in different solvent systems. In the CT sample, there are many polyphenol groups separated with different colors. Where the red and purple-red bands correspond to anthocyanins, the blue light region may correspond to some simple phenols. The yellow band corresponds to polyphenols belonging to the flavonoid group. For example: rutin, catechin, quercetin, luteolin or myricetin. The brown band observed during separation corresponds to flavones. From the analysis results of polyphenol compounds in CT and CC samples, it shows that CT contains many rich polyphenol components and quite high polyphenol content.

Total polyphenol content in CC and CT extract samples was determined according to the Folin - Denis method according to TCVN: 9745 - 1: 2013.

The total polyphenol content in the extract sample (CC) and the extract sample enriched in total polyphenol content (CT) were determined according to the Folin - Denis method according to TCVN: 9745 - 1: 2013. As a result, the CC sample contained The total polyphenol content reached a value of 47.63 mg/g (GAE/CC) or equivalent to 4.763 % in % units and the enriched sample total polyphenol content (CT) reached a value of 300.25 mg/g (GAE/CC) or 30.25 % in %. The process of enriching total polyphenol content increased the CT content in yellow flower tea by 6.35 times.

3.1.2. Morphological and structural features of CC and CT

3.1.2.1. Fourier transform infrared (FTIR) spectroscopy of *CC*

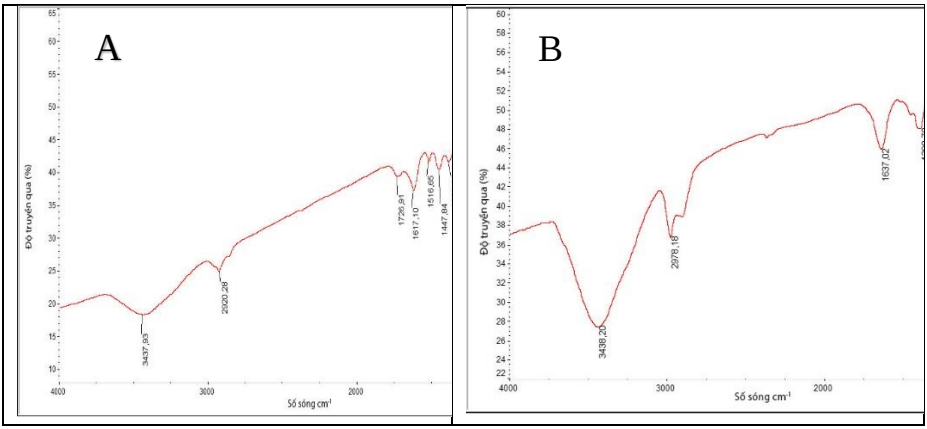

Figure 3.1. FTIR spectrum of CC(A) and CT(B)

Basically, the spectral fringes on the FTIR spectrum of CT still have similarities in position with the spectral fringes on the FTIR spectrum of CC. However, the intensity of the spectral fringes in the CT sample is stronger. Among them, the most obvious difference is shown in the two spectral fringes at positions 1726 cm–1 and 1516 cm–1, which characterize the valence vibrations of the C=O group and the amine group: on the FTIR spectrum of CT, there is no longer any visible difference appearance of these two spectral fringes. That may be because CT enrichment has eliminated compounds containing carbonyl, amine and amino acid functional groups in CC or the content of groups containing these functional groups is low, so the characteristic fluctuation peaks do not appear on the spectrum.

3.1.2.3. Field Emission Scanning Electron Microscopy (FESEM) of CC and CT

Figure 3.2 is a FESEM image of a CT and CC sample with magnification levels of 10,000, 20,000, and 25,000 times. Observing the overall samples, it can be seen that the samples all have a particle structure with uneven size, the diameter of the particles ranges from 100 – 200 nm. The particles all tend to bind, agglomerate into clumps together to form concentrated structural blocks and voids. The cause of agglomeration may be the formation of interactions between hydrogen bonds in polyphenols.

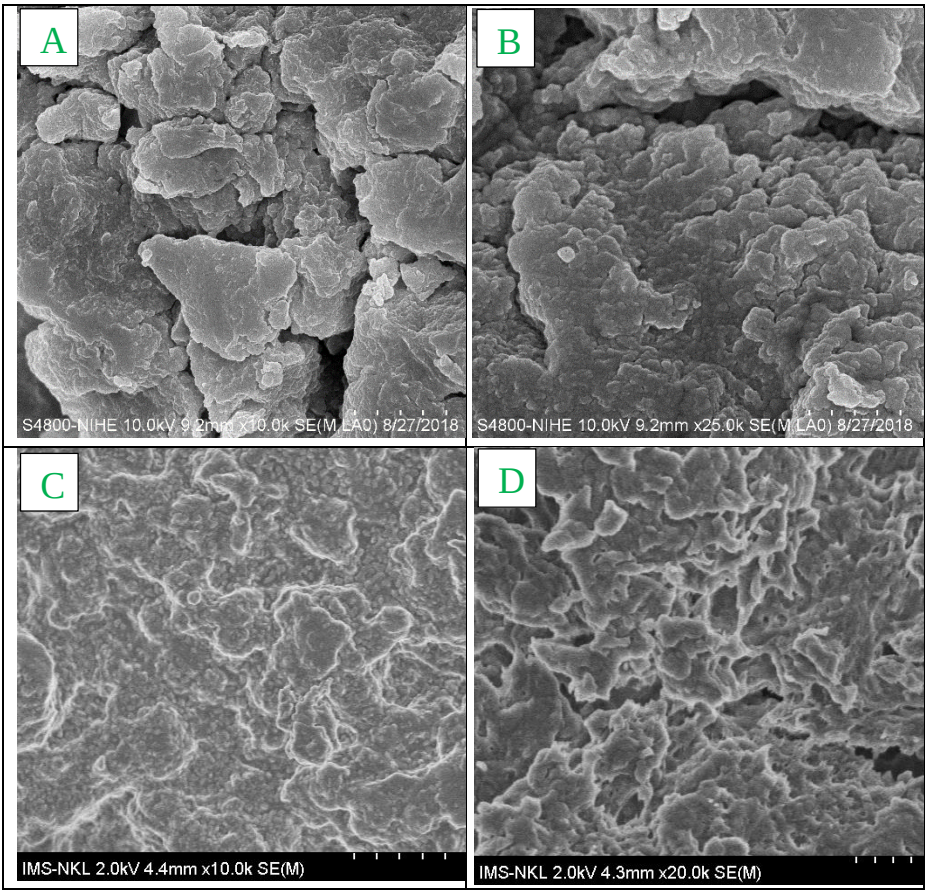

Figure 3.2. FESEM photos of CT (A, B) and CC (C, D)

3.1.2.4. UV spectrum – Vis of CT

CT is a mixture of many components including polyphenols such as EGCG, ECG, EG... Therefore, in the UV-Vis spectrum of CT in ethanol solvent, many absorption peaks appear at different wavelengths (Figure 3.3). On the UV-Vis spectrum of CT, a strong absorption peak appears at wavelengths from 240 - 300 nm. Among them, there are 2 maximum adsorption peaks in the range of 243 - 246 nm and 270 - 280 nm.

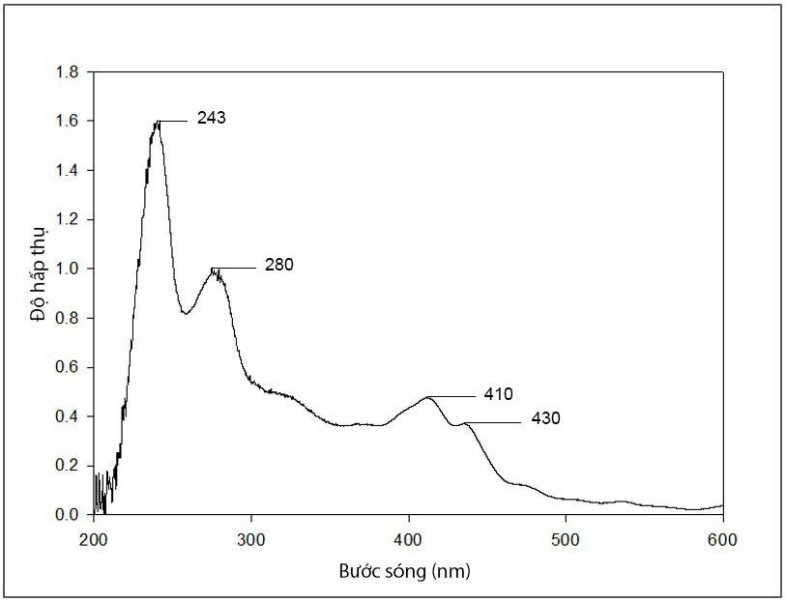

Figure 3. 3. UV spectrum – Vis of CT

3.2. CC-bearing AG/CS composite membrane

3.2.2. FTIR spectrum of CC-bearing AG/CS composite membranes

FTIR spectrum of AG/CS composite membrane carrying CC with AG/CS ratios (7/0; 7/3) and CC content added in the following ratios: 0%, 5%, 10%, 15%, 20% is presented in Figure 3.4. It can be seen: the characteristic spectral fringes of AG, CS and CC still appear in the FTIR spectra of these membranes and the peaks correspond to NH_2, OH, CH, C=O or C=C groups. Although CS is not present in sample AC70CC10, the FTIR spectrum analysis results of this sample are still similar to the AG/CS/CC samples due to the resonance of specific functional groups. The disappearance of the peak at 1726 cm^{-1} (C=O stretching vibration, in the FTIR spectrum of CC) in the composite membranes may be because it was covered by the peak of the C-C vibration peak or the content of substances containing The C=O group in the complex is quite small so no signal appears on the spectrum. The similarity in shape between the spectra proves that the CC content included in the samples does not greatly affect the interaction of AG and CS.

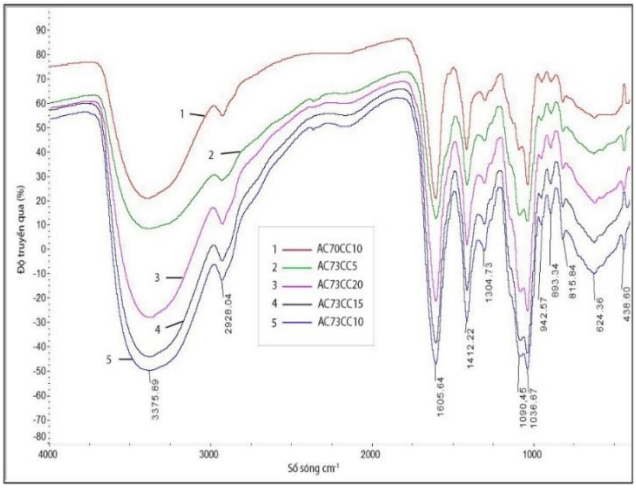

Figure 3.4. FTIR spectroscopy of CC-bearing AG/CS composite membranes

3.2.2. Thermal characteristics of CC-bearing AG/CS composite membranes

Thermal characteristics of 5 composite membrane samples AC73CC with different CC content (Figure 3.5) and membrane without CS (AC70CC10) were evaluated by differential scanning calorimetry (DSC). It can be seen that in all analyzed samples there are two peaks, including the endothermic peak typical for Tnc in the range of 124.4 - 135.0 oC with ΔHm = 424 - 498 (J/g). and the typical exothermic peak for Tph is in the range of 246.1 - 250.7 ^{o}C.

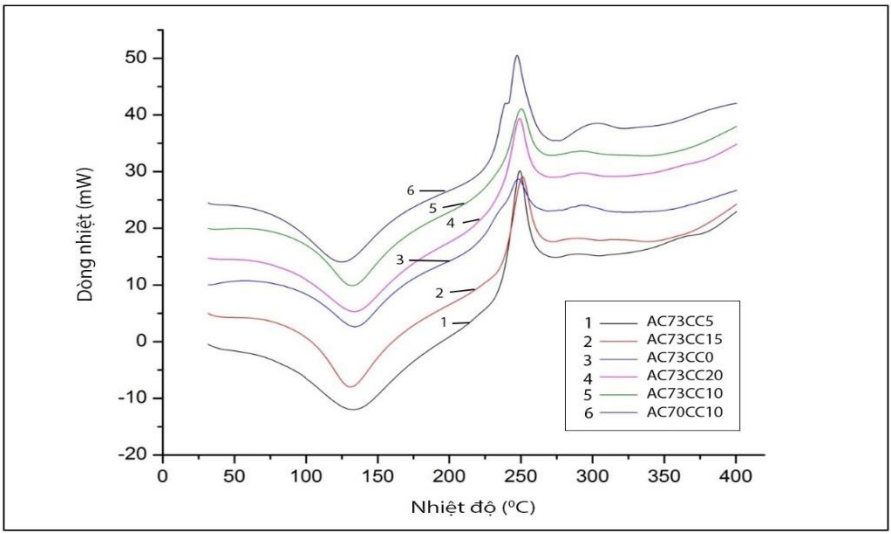

Figure 3.5. DSC diagram of CC-bearing composite membranes

3.2.4. CC bearing efficiency of AG/CS composite membranes

Compared with the composite membrane samples containing CS (AC73CC), the AC70CC10 sample without CS has the lowest CC carrying percentage of 64.32%. It can be seen that the presence of CS increased the CC carrying efficiency due to the improvement of the interaction between AG-CS and AG-CS-CC components. AG/CS composite membrane samples containing CC, CC carrying efficiency AC73CC5, AC73CC10, AC73CC15 and AC73CC20 are in the range of 70.72 - 82.44%. When the CC content in the composite membrane is 5%, the CC carrying efficiency is 70.72%, when the CC content increases in the range of 10 - 15%, the CC carrying efficiency increases to a maximum of 82.44%. However, when the CC content increased to 20%, the high carrying efficiency decreased. This can be explained because the CC - CC interaction is more dominant than the CC - AG/CS combination interaction, so the CC carrying efficiency of the composite membrane decreases. In general, the CC carrying efficiency by the AG/CS composite membrane is most suitable when the CC content in the composite membrane is 15%.

Table 3.1. CC bearing performance of AG/CS composite membranes

Sample	Initial mass of CC(% by volume)	Mass is carried of CC (% by volume)	CC bearing efficiency (%)
AC73CC5	5	3,54	70,72
AC70CC10	10	6,43	64,32
AC73CC10	10	7,75	77,51
AC73CC15	*15*	*12,37*	*82,44*
AC73CC20	20	17,75	76,73

3.2.5. Study of CC release from CC-bearing AG/CS composite membranes in different pH solutions

3.2.5.1. Effect of pH on CC release

Observing Figures 3.6a, Figure 3.6b depicting the CC content released from the combination membrane AC73CC10 and AC73CC20 in different pH environments, it can be observed:

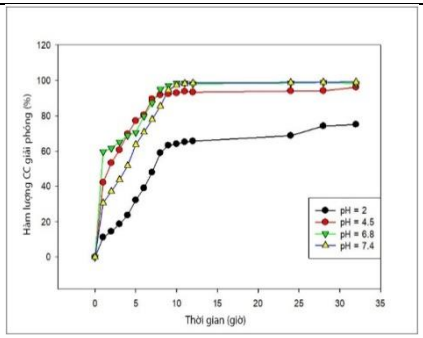

Figure 3.6a. CC content released from AC73CC10 composite film in various pH solutions

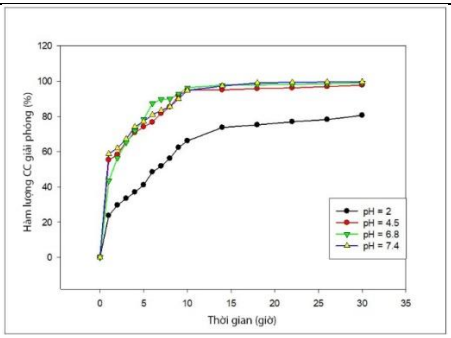

Figure 3.6b. CC content released from AC73CC20 composite membrane in various pH solutions

The process of releasing CC from the AC73CC composite membrane is highly dependent on the pH value of the solution. The CC content released in a slightly alkaline environment is better than in an acidic environment, specifically pH 7.4 ≈ pH 6.8 > pH 4.5 > pH 2.

3.2.5.2. Effect of time on CC release

The CC content is released the most in the first 3 hours, because the CC content sticks to the surface and near the membrane surface, so it is easily released. Then, the release gradually decreased and stabilized over the next 7 hours with the total released content ranging from 56.74 - 66.08 % (pH 2) and 92.80 - 98.49 % (pH 4, 5; 6.8 and 7.4). After 10 hours, CC content began to be released in a controlled manner from inside the composite membrane. At the end of the 30-hour survey, CC was almost completely released with a content of 96.05 - 99.59% for composite membranes conducted at pH 4.5 solutions; 6.8 and 7.4. Particularly for the composite membranes surveyed at pH 2, the threshold was only 61.23 - 80.54%.

3.2.5.3. Effect of CC content in combinatorial membrane on CC release

In pH 2 solutions; 4.5 and 7.4 in the first 1 hour of the survey, the CC content released from the composite membranes increased with increasing CC content. For example, at pH 2 solution, the CC content released from samples AC73CC5, AC73CC10, AC73CC15 and AC73CC20 were 8.60 %, 11.20 %, 15.98 % and 23.70 %, respectively. The increase in CC content released from the composite membrane can be explained as follows: when increasing the CC content introduced, the amount of CC concentrated on the membrane surface increases, so the CC content diffused and released in the early stages increases. fast. Besides, when increasing the CC content in the membrane, the hydrogen bond between CC is more dominant than the hydrogen bond between CC - AG/CS, causing the CC to

clump together, leading to a less tight membrane structure. , therefore, CC release from composite membranes with large CC content will be easier at the early stage.

3.2.6. CC release kinetics from CC-bearing AG/CS composite membranes

At pH = 2 solution, the CC release kinetics according to the FO, HG, and KMP models all have regression coefficient values ^{R2} > 0.900. A KMP kinetic model with a diffusion constant (n) of 0.001 - 0.004 indicates that the combinatorial magnetic release mechanism obeys Fick's law type 1, which means that the release of CC follows only the normal diffusion mechanism. The remaining pH samples in the controlled release phase all followed the HCW model with R^2 values both greater than 0.93 with samples released at pH = 4.5, greater than 0.96 with pH = 6.8, and greater than 0.97 with pH = 7.4.

Summary of results section 3.2

1. The results of infrared spectrum analysis show that CC was carried by AG and CS polymers through the shift of peaks in the spectrum, due to interactions between components in the AC73CC composite membrane (hydrogen bonds, interactions bipolar).

2. FESEM images of the samples show that the composite particles are relatively small in size and unevenly dispersed in size. The size of AG/CS complexes ranges from 20 to 100 nm on average.

3. The CC carrying efficiency of the combination ranges from 64.32 - 88.73%. The CC content released from the AG/CS composite membrane in pH 4.5, pH 6.8 and 7.4 takes place faster and more stably than in pH 2. CC content and release time are affected. greatly affects the CC release process. The process of releasing CC from the AG/CS composite membrane consists of 2 stages, a fast stage in the first 10 hours and a slow stage during the remaining investigation period.

3.3. Alginat/chitosan seed combination brings high total yellow flower tea

In this project, researchers have manufactured the AG/CS/CT particle combination using the microemulsion method with the CT sample enriched in total polyphenol content (30.25%) 6.35 times higher than the total polyphenol content. Total polyphenols in CC (4.763 %). Aims to remove impurities contained in the CC sample and promote the effectiveness of polyphenols in THV when carried by the AG/CS particle combination. Then determine the properties and structural morphology of 4 particle combination samples AG/CS/CT10, AG/CS/CT20, AG/CS/CT30 and AG/CS/CT50 with CT content of 10%, 20%. %, 30 % and 50 % calculated according to the total mass of AG and CS. In particular, the total PP content achieved is about 3%, 6%, 9% and 15% respectively calculated according to the total mass of AG and CS.

3.3.1. Particle size distribution of CT-bearing AG/CS particles

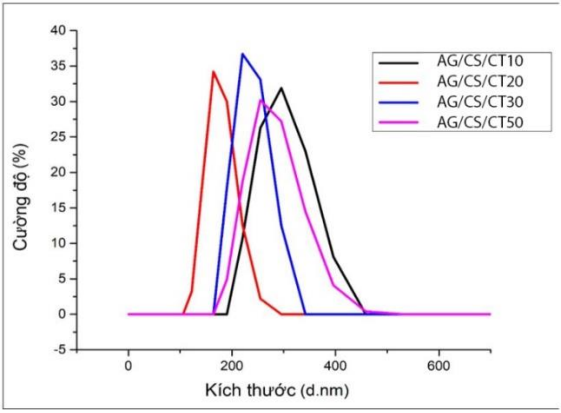

Figure 3.7. Particle size distribution diagram of AG/CS/CT nanoparticle combinations with different CT content

The average particle size of AG/CS/CT composite particle samples ranges from 141.8 to 396.1 nm. The average particle sizes of AG/CS/CT10, AG/CS/CT20, AG/CS/CT30 and AG/CS/CT50 are 295.6 nm, 182.3 nm, 235.8 nm and 284.3 nm, respectively. nm. AG/CS/CT20 composite particle sample contains particles with the smallest average size (182.3 nm), AG/CS/CT10 composite sample contains particles with the largest average size (295.6 nm). In general, the size of CT-bearing composite particles varies unevenly and does not follow certain rules. This difference may be because the composite particles are influenced by dipole interactions, hydrogen bonds between water and CT, water and polymer AG, CS.

3.3.2. FTIR spectroscopy of CT-bearing AG/CS particle complex

Observe in Figure 3.8, on the AG/CS/CT composite particle spectrum, the peak intensities of these characteristic functional groups can resonate with each other. Therefore, the peak intensities of these characteristic groups in the FTIR spectrum of AG/CS/CT composite particles are larger than the characteristic groups in the FTIR spectra of CS, AG or CT separately. The wavenumbers of characteristic groups in CT are reduced in AG/CS/CT composite particles. Specifically, the characteristic wave number for the –OH group of CT decreased from 3438 cm-1 to 3288.83 - 3299.09 cm-1 in the composite particle samples. The AG/CS/CT composite particle samples have a small shift in the characteristic wave numbers of the characteristic groups –NH2, –OH... This may mean that AG, CS and CT interacted with each other by (hydrogen bonding, electrostatic interactions) through hydroxyl, carbonyl and amine bonds.

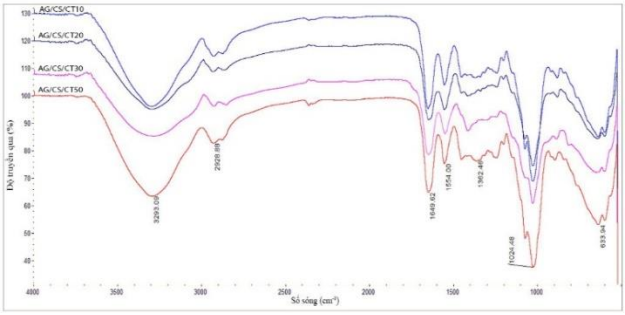

Figure 3.8. FTIR spectroscopy of AG/CS/CT particles with different CT ratios

3.3.3. Structural morphology of CT-bearing AG/CS combinatorial particles

The greater the CT content introduced into the combinatorial particle, the clearer the agglomeration of the particles. Samples of composite particles AG/CS/CT20, AG/CS/CT30, AG/CS/CT50 tend to coagulate together, resulting in larger average sizes of these particles compared to composite particles Ag/CS/CT10. Comparing the CT sample SEM with other AG/CS/CT combinatorial samples, the combinatorial particle samples were more uniformly sized.

3.3.4. Thermal characteristics of CT-bearing AG/CS particle complexes

The DSC schema and DSC characteristics of AG/CS/CT composite particle samples with different CT content are shown in Figure 3.9.

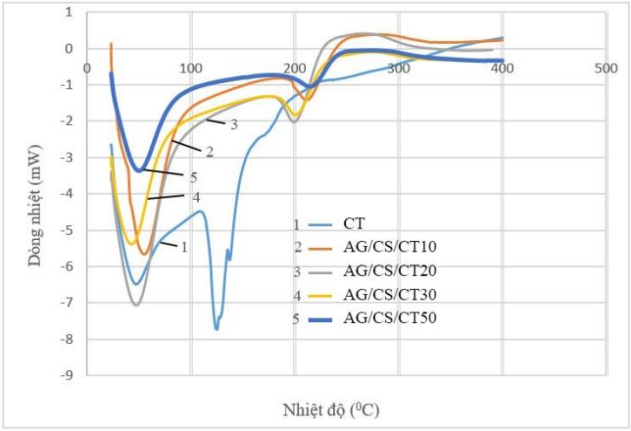

Figure 3.9. Thermal stability analysis schema of combinatorial particles AG/CS/CT with different CT content

Comparing with the AC73CC composite membranes with different CC contents in Table 3.5, it can be seen that the Tnc of the particle composite samples is lower than that of the composite membranes. The composite particle samples melt at a temperature of about 50 oC. Specifically, samples with similar CC and CT mass compositions such as (AC73CT10 with AG/CS/CT10) and (AC73CT20 with AG/CS/CT20) both give high Tnc and Tph results of composite membrane samples. much better than composite particles. This can be explained by the fact that the interactions between the components in the process of creating composite membranes using the solution method are stronger than when creating composite particles using the microemulsion method, leading to a tight structure of the composite membranes. than the composite particle sample. The ΔHm of the composite membrane sample is higher than the composite particle samples with similar comparative composition.

3.3.5. CT-bearing performance in CT-bearing AG/CS composite particles

CT-containing composite particle samples all had a fairly high percentage of CT, ranging from 78.43 % – 89.55 %.

Table 3.2. CT bearing efficiency of AG/CS/CT particle combinations

Sample symbols	Initial volume of CT (% mass)	Actual volume of CT (% by mass)	CT bearing efficiency (%)
AG/CS/CT10	10	7,84	78,43
AG/CS/CT20	20	16,93	84,64
AG/CS/CT30	30	26,87	*89,55*
AG/CS/CT50	50	43,16	86,31

3.3.6. Study of CT release from CT-carrying AG/CS particle assemblages in different pH solution environments

3.3.6.1. Effect of time on CT release kinetics

The CT content released from the combinatorial particles increased over the study period. CT content released from AG/CS/CT10 - AG/CS/CT50 composite granules occurs in 2 phases similar to CC release from AC73CT composite membranes: rapid release in the first 10 hours and controlled release in the next 20 hours. Initially, the release occurs immediately on the surface of the combinatorial granule. Then the release process is controlled by diffusion from within the combinatorial particle.

3.3.6.2. Effects of pH solution

The pH environment has a great influence on the release of CT from the AG/CS/CT complex. The CT release process at different pH solutions is arranged in the order of pH 2.0 < pH 4.5 < pH 6.8 ≈ pH 7. Specifically, during the first hour, the CT content released corresponded to the AG/CS/CT30 sample in pH 2.0; pH 4.5; pH 6.8; and pH 7.4 are: 24.78 percent, respectively; 32,35 %; 53.83 % and 53.51 %. After 10 hours, the CT content released corresponding to pH from low to high was: 89.40 %; 90,04 %; 91,09 %; 93.82 %, resulting in slow and controlled CT release.

3.3.6.3. Effects of CT content

It can be seen that in all pH solutions, CT samples not carried by the Ag/CS complex are released faster than CT samples carried by Ag/CS, since the CT sample is released directly without release from the surface of the AG/CS/CT complex or under the influence of intermolecular interactions, group organized in the Ag/CS complex, even in pH solutions (4.5; 6.8; 7.4). However, at pH 2, % CT is slowly released, and after 10 hours onwards, CT is even slower released than CT carried by the Ag/CS combination. This result suggests that CT release is also significantly influenced by the strongly acidic pH.

3.3.7. CT release kinetics from AG/CS/CT

In solutions of pH 2.0 and pH 6.8 the CT content released depends on the CT concentration, corresponding to the grade 0 kinetics, in pH 4.5 solutions the CT release mechanism follows the KMP kinetic model and in order 0, in pH 7.4 solution the CT release follows the HG kinetic model.

3.3.8. Results of assessment of biological activity of* CT *and* composite *granular materials AG/CS/CT

3.3.8.1. Test results of cytotoxic activity

Potential for oral carcinoma cytotoxicity (KB)

The samples demonstrated activity with IC_{50} ranging from 14.63 – 43.17 μg/ml on KB cell lines. Notably, according to the standards of the US National Cancer Institute (NIC), the extracted residue is considered to have good activity when the $IC_{50} \leq 20$ μg/ml. So, it can be confirmed that CT values with IC values of IC_{50}= 14.63 ± 1.56 exhibit good activity against human oral carcinoma (KB) cell lines. Composite AG/CS/CT samples with different ratios also showed activity on KB cell lines ranging from 25.08 ± 1.35 to 43.17 ± 3.11. In general, activity increases as CT content increases. The composite sample for best activity is Ag/CS/CT30 with IC_{50} = 25.08 ± 1.35.

In the AG/CS/CT10 sample, the CT content included is only 10 % of the total mass of AG and CS, equivalent to the CT content in the sample is only about 1/10 of the CT content not carried by AG and CS. However, the IC 50 of the AG/CS/CT10 sample gives an IC_{50} = 43.17 ± 3.11, which is about 2.95 times that of the CT sample, similar to AG/CS/CT20, AG/CS/CT30, AG/CS/CT50, models, also have IC_{50} times 2.16; 1.71; 2.02 times that of CT samples. This suggests that the activity of the combinations was increased compared to the CT sample not carried by the combination (based on % of CT mass introduced into the combination). This suggests factors such as: nanoparticle size, the reciprocal role of AG and CS in the combination have increased the activity of CT in the complex, in which the main component of CT is PP accounting for 30.25 % CT exhibiting quite effective KB cancer cytotoxic activity.

Table 3.3. Cytotoxic results on the human carcinoma line (KB) from CT and combinatorial particle samples

µg/ml concentration	% inhibition					
	CT	AG/CS/CT10	AG/CS/CT20	AG/CS/CT30	AG/CS/CT50	Ellipticine
100	97,83	101,81	103,05	101,69	101,15	98,72
20	66,71	24,28	37,31	52,40	42,47	89,31
4	11,08	4,22	13,95	24,24	10,31	50,91
0,8	-1,12	-4,08	4,63	14,11	8,26	24,16
IC_{50}	14,63 ±1,56	43,17 ± 3,11	31,61 ± 1,98	25,08 ± 1,35	29,49 ± 1,46	0,34 ±0,04

Potential for cytotoxic human hepatocellular cancer (HepG2)

Results demonstrating cytotoxic activity of liver cancer in humans are shown in Table 3.4. The results showed that HepG2 cytotoxic CT with IC value 50 = 17.31 ± 0.27 and HepG2 cytotoxic AG/CS/CT50 with IC_{50} = 16.36 ± 0.37 both showed good activity against HepG2 cells (according to US National Cancer Institute (NIC) standards). Furthermore, IC_{50} = 16.36 ± 0.37 of AG/CS/CT50 was lower than that of CT samples not carried by combinations, suggesting that CT bearing by AG and CS was effective in enhancing the solubility and dispersion of CT in the combination compared to CT not carried by the combination.

Table 3.4. Cytotoxic outcomes on human liver cancer cell lines (HepG2) from CT and combinatorial particle samples

TT	Template name	IC value$_{50}$ (µg/ml)
1	CT	17.31 ± 0.27
2	AG/CS/CT10	>100
3	AG/CS/CT20	>100
4	AG/CS/CT30	48.89 ± 3.08
5	AG/CS/CT50	16.36 ± 0.67
6	Ellipticine	3.5 ± 0.30

Potential for human embryonic stem renal cytotoxicity (HEK-293A):

Within the framework of the thesis, 4 samples of AG/CS/CT particles contained different levels of CT and CT were sent to investigate the potential for embryonic renal cytotoxicity in humans (HEK-293A). The results in Table 3.5 show that samples studied at different test concentrations from 200 µg/ml to 1.6 µg/ml did not show activity on benign cell lines HEK-293A at test concentrations with IC values of 50 > 100 (µg/ml) at all concentrations. Ellipticine positive control agent acts stably in vitro. These results suggest that CT and combinations of AG/CS/CT contain different levels of CT that are not cytotoxic to benign human embryonic renal stem cells (HEK-293A). This has significant implications for the evaluation of the biological activity of CT and the AG/CS/CT complex.

Table 3.5. Results of human embryonic stem renal cytotoxicity (HEK-293A) from CT and combinatorial granular samples

TT	Template name	IC_{50} value (µg/ml)
1	CT	>100
2	AG/CS/CT10	>100
3	AG/CS/CT20	>100
4	AG/CS/CT30	>100
5	AG/CS/CT50	>100
	Ellipticine	0.04 ± 0.03

3.3.8.2. Results of antioxidant activity evaluation

The antioxidant capacity of CTs is of particular interest to food and pharmaceutical scientists. Currently, antioxidant activity is often evaluated based on its ability to discolor stable free radicals such as 2,2-diphenyl-1-picrylhydraxyl

(DPPH). Because the reactions of DPPH are very sensitive to experimental conditions, e.g. the concentration of antioxidants, the nature of the solvent, temperature, time and pH of the solution.

Table 3.6. Antioxidant activity test results on DPPH system of CT and combinatorial particle samples

TT	Template name	EC value$_{50}$ (µg/ml)
1	CT	13.99 ± 0.26
2	AG/CS/CT10	31.16 ± 2.31
3	AG/CS/CT20	14.57 ± 0.41
4	AG/CS/CT30	14.37 ± 0.17
5	AG/CS/CT50	13.99 ± 0.26
	Quercetin	9.97 ± 0.25

The oxidant resistance of the AG/CS/CT particle complex increases with increasing concentration of test specimens. The oxidation resistance is directly proportional to the CT content of the AG/CS/CT combination particle. The most obvious is that the AG/CS/CT50 combination gives EC 50 = 13.99 ± 0.26 equal to the EC_{50} of CT not carried by the combinatorial particle. The AG/CS/CT20, AG/CS/CT30 samples also showed the ability to effectively scan/capture DPPH radicals withvalues of 14.57 ± 0.41 and 14.37 ± 0.17, respectively, for CT samples not carried by the complex. This once again demonstrates the compatible role of AG and CS in promoting the bioavailability of PP in high extraction when carried by the complex.

3.3.8.3. Test results of nitric oxide inhibition activity (Nos Inhibition)

Table 3.7. The ability to inhibit NO production of CT and combinatorial particle samples

TT	Template name	IC value$_{50}$ (µg/ml)
1	CT	20.14 ± 1.46
2	AG/CS/CT10	>100
3	AG/CS/CT20	61.23 ± 4.19
4	AG/CS/CT30	66.53 ± 3.76
5	AG/CS/CT50	47.93± 0.15
6	Dexamethasome controls	14.20 ± 0.54

CT's ability to inhibit NO production has an IC value of 50 = 20.14 ± 1.46, Ag/CS/CT10 samples have an IC value of 50 > 100 (µg/ml) have not shown the ability to inhibit NO production, the remaining AG/CS/CT composite particle samples have the ability to inhibit NO production from 47.93 ± 0.15 to 66.53 ± 3.76. The dexamethasome positive control IC 50 = 14.20 $\pm_{0.54}$ was stable in the experiment. In general, CT and AG/CS/CT combinatorial samples have shown the ability to inhibit NO but have not reached the level of efficacy

Summary of results section 3.3

The AG/CS/CT particle combination has been successfully manufactured using the microemulsion method with CT contents of 10%, 20%, 30%, 50% calculated according to the total mass of AG and CS, respectively.

1. AG/CS/CT particle combination has high crystallinity, diameter ranges from 182 nm - 295 nm. Particles tend to agglomerate when the added CT content increases. The AG/CS/CT combination has quite low melting and decomposition temperatures.

2. CT carrying efficiency of AG/CS/CT composite particles is in the range of 78.43 - 89.55%. CT is released from AG/CS/CT composite particles in two phases: rapid in the first 10 hours and controlled in the next 20 hours. Factors such as pH, time and drug content greatly affect the amount of CT released. In the rapid release phase, the released CT content does not follow a certain kinetic model, in the controlled CT release phase, all samples follow the Korsmeyer - Peppas kinetic model with a value of $n < 0.5$.

3. Results of biological activity research from CT and combined samples show that both CT and AG/CS/CT samples demonstrate KB cancer cell cytotoxicity and antioxidant activity on the immune system. DPPH, the ability to inhibit NO production gives quite good results. All research samples were not toxic to benign cells (human embryonic kidney stem cells HEK-293A).

CONCLUDE

1. Successfully extracted yellow flower tea extract (THV) with a total polyphenol content of 4.763% (CC) and enriched total polyphenol content of 30.25% (CT). From there, the AC73CC membrane combination containing different CC contents was fabricated using the solution method and the AG/CS/CT particle combination using the microemulsion method.

2. CC carrying efficiency from AC73CC composite membranes ranges from 64.32 - 88.73%, with CT in AG/CS/CT composite particles fluctuating from 78.43 - 89.55%.

3. With AC73CC membrane samples, in the rapid CC release phase, following the Higuchi model. With the controlled release phase in pH 2 solution, the FO, HG and KMP models all have regression coefficients $R2 > 0.900$.

With AG/CS/CT composite particle samples, during the rapid release phase, the CT content released from the AG/CS/CT composite particles does not follow a certain kinetic model, the controlled release phase, CT released from the samples all follow the Korsmeyer - Peppas kinetic model with a value of $n < 0.5$.

4. CT and AG/CS/CT composite particle samples both show cytotoxic activity against KB cancer cells and HepG2 liver cancer cells. Antioxidant activity on DPPH system of CT with EC50 = 13.99 ± 0.26 μg/ml and magnetic combination sample with EC50 from 13.99 ± 0.26 μg/ml – 31.16 ± 2.31 μg /ml. All research samples were not toxic to benign cells (human embryonic kidney stem cells (HEK-293A). CT samples and AG/CS/CT30, AG/CS/CT50 demonstrated the ability to inhibit NO production mechanism.

NEW CONTRIBUTIONS OF THE THESIS

1. Successfully fabricated AG and CS composite membranes containing CC by solution method and AG and CS composite particles containing CT by microemulsion method. It was determined that the CC carrying efficiency from the composite carrier ranged from 64.32 - 88.73%, CT from the composite particles reached 78.43 - 89.55%.

2. Build appropriate kinetic models for CC and CT in different pH solutions. Factors: time, pH, CC and CT content clearly affect the CC and CT content released from the AC73CT composite membrane and AG/CS/CT composite particles.

3. AG/CS/CT, CT composite particles all show good activity against KB cancer cells, HepG2 liver cancer cells, antioxidant activity on the DPPH system and are not toxic to benign HEK- cells. 293A.