MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

ACADEMY OF SCIENCE AND TECHNOLOGY

DINH VAN THOAI

SYNTHESIZE AND EVALUATE ACTIVITY OF GELATIN PLURONIC SYSTEMS ENCAPSULATE QUERCETIN CO-ORDINATING ANTI-CANCER DRUG

Speccialty: Organic chemistry

Code: 9 44 01 14

SUMMARY OF DOCTORIAL THESIS IN CHEMISTRY

TP. HO CHI MINH – 2022

The thesis was completed at: Academy of Science and Technology - Vietnam Academy of Science and Technology

Science instructor 1: TS. Le Van Thu
Science instructor 2: PGS. TS. Tran Ngoc Quyen

Reviewer 1: …
Reviewer 2: …
Reviewer 3: ….

The thesis will be defensed at the Academy of Doctoral thesis evaluation council, meeting at the Academy of Science and Technology - Vietnam Academy of Science and Technology at … o'clock ..', date … month … year 20….

Can find out the thesis at:
- Library of the Academy of Science and Technology.
- Vietnam National Library.

INTRODUCTION

1. Need of the thesis:

Cancer treatment drugs have drawbacks bring to reduction of effective treatment such as poor solubility in water, concentration of drugs to be drop quickly after entering the body, they can cause unwanted chemical effects or to be distributed through the body, lack of selectivity with diseased tissues and lead to normal ones to be negatively affected as well. It is these limitations which have motivated a lot of number of scientists interested in for solutions.

There are some research directions to improve the effectiveness of cancer treatment, such as studying for new drugs which are more effective, or combination of multiple treatments, for example, chemotherapy and radiation therapy. Interesting research direction is synthesis of materials which have capable of delivering drugs. These new ones play the role of storing and transporting drugs to the right target for treatment, have ability slowly releasing and the correct dose of medications to aim maintaining bioavailability, minimizing drug toxicity to healthy cells in the body. Experiencing the process of studying documents anh realizing the potential of this research one, I choice the research way of synthesizing gelatin derivative as materials to create nanogels and carried out the thesis: *"Synthesis and evaluation of activity of Gelatin Pluronic Nanogel systems carrying Quercetin in combination with anticancer drugs"*.

Gelatin, pluronic are safe materials, have special structural properties which can form nanoparticles with core – shell structure and carry opposite charge, in which the core has positive charge that is favorable

factor for the application of materials to carry poor water solubility drugs and has negative charge like quercetin.

2. Research objectives of the thesis:

Synthesis copolymers base on grafting gelatin with various pluronics such as P123, F127, F87 and F68 in different ratios. Finding out the optimal pluronic type and coupling ratio in term of particle size and one surface charge (Zeta potential). The preparation nanogel gelatin-pluronic for attached folic acid as a target ligand for cancer cells to apply for carrying quercetin and paclitaxel, aim increasing their therapeutic effect.

3. The main research contents of the thesis:

- Synthesis copolymers base on grafting gelatin with various pluronics such as P123, F127, F87 and F68 in different ratios. Finding out the optimal pluronic type and coupling ratio, attaching folic acid as a target ligand for cancer cells.

- Evaluation of structure, particle size, micelle and gel formation concentration (CMC and CGC), Zeta potential of particle surface, productivity of graft reaction to create synthetizing copolymer.

- Preparation and investigation of quercetin/paclitaxel encapsulation ability of synthetized nanogel.

- Evaluation of release ability of QU and PTX of QU/PTX-loaded nanogels under medium conditions pH = 7,4 and pH = 5,5, degree of heat 37 ^{o}C in buffer solution PBS (Phosphate Buffered Saline).

- Evaluation *in vitro* for effective in killing cancer cells of QU/PTX-loaded nanogels.

- Evaluation *in vivo* for effective in killing cancer cells of QU+PTX-loaded nanogels.

The layout of the thesis: The thesis has introduction, chapter 1: Overview, chapter 2: Research, chapter 3: Results and discussion and conclusions and recommendations. Thesis has 19 tables of data, 74 pictures and figures and 2 published-related works.

CHAPTER 1. OVERVIEW

1.1. Introduction for nanogel

1.1.1. Concept and application of nanogel

Nanogel is particle has nano size and gel structure which are formed by network of polymers which linked together through chemical bonds or physicochemical interactions. The strong development in the field of nanotechnology has led to the development of nanogel systems with potential applications to deliver therapeutic drugs with ability to target and maintain drug concentration.

1.1.2. Nanogel synthesis method

Due to the wide range of applications in gene and drug delivery systems, nanoparticles with nanogel structure have been published by a lot of studies, from 1987, there have been the first studies on methods of synthesizing nanosystems base on gelatin such as the desolvation technique, fractional coagulation method, solvent evaporation method to form emulsions, reverse phase emulsification method, or nanoprecipitation method.

1.2. Gelatin material

1.2.1. Overview of gelatin

Gelatin is a typical protein, capable of acting as an acid or a base. This amphoteric property is due to the carboxyl groups (-COOH) represents acidity and amine group ($-NH_2$) represents the basicity produced during

hydrolysis. In general, gelatin exists in solution as a dipole $^{+}NH_3–CH_2–COO^{-}$, commonly known as ion "Zwitter".

1.2.2. Application of gelatin in drug delivery system synthesis

Drugs and genes when carried by nanoparticles are absorbed in the matrix or on the surface of the particles, this absorption occurs during nanoparticle formation. Hydrophilic drugs can be successfully introduced into the internal structure of nanoparticles by mixing them with an aqueous solution of gelatin before nanoparticle formation occurs, by electrostatic interactions, or by covalent binding. In addition, hydrogen interactions, hydrophobic tail interactions between drugs and gelatin were also investigated.

Gelatin has attracted much attention because of its high biocompatibility and biodegradability. Because of the Arg-Gly-Asp peptide chains in the molecule, gelatin can promote cell adhesion. On the other hand, gelatin has a positive charge state in the physiological environment of the human body. Gelatin can help create a high interaction with cancer cells as well as normal cells in the body.

1.3. Pluronic

1.3.1. Overview of pluronic

The pluronics have the same chemical structure, differing only in the relative amount of poly (ethylene oxide)-poly (propylene oxide), and is usually symboled as (PEO-PPO-PEO), The properties, molecular weight, surface activity characteristics of each type are different, the pluronics are commonly applied such as F127, F68, F87, P123.

Some pluronics have the characteristic property of changing state by temperature. At low temperature (0 – 4 °C), the pluronic solution with appropriate concentration will exist in the liquid state, but when the

temperature is raised to room temperature (20 ~ 25 °C) it turns into a solid gel. The gelation temperature is the critical temperature, when the temperature is higher than the critical temperature, the polymer solution will solidify into a gel. This is explained by the hydrophilic PEO group, the hydrophobic PPO group, so when dissolved in water, the micelle structure is formed with the nucleus being PPO and the outer shell being PEO. Increasing temperature will lead to dehydration and change the conformation in the region containing hydrophobic groups, as a result the PPO groups tend to lose water forming a core with an outer shell of hydrated PEO chains forming a micelle shape that is thermodynamically stable and gel structure is formed.

1.3.2. Application of pluronic in drug delivery system synthesis

Some pluronics are nontoxic, hydrophilic copolymers which are widely used as excipients to increase stability and improve the poor water solubility of drugs. Loading of poorly soluble drugs into pluronic micelles can improve drug pharmacokinetics and increase biodispersion. Pluronic has great potential in carrying drugs that are difficult to dissolve in water, especially cancer drugs, and it can easily be combined with another substance to create a more effective drug-carrying nanosystem (due to the functional group -OH at the end of the chain).

1.4. Cancer and anti-cancer drug

1.4.1. Overview of cancer

Cancer occurs due to mutations in DNA, leading to disorganized cell proliferation that does not follow the control mechanisms of body development. Through recent studies, cancer has been shown to be related to genetic factors. Variations in genes lead to changes in cell function and division. These changes are the result of interactions

between genetic factors and external agents, including: physical agents such as ultraviolet light, ionizing radiation; chemical agents such as arsenic, aflatoxin and especially cigarette smoke, biological agents such as infections by viruses, bacteria, parasites.

1.4.2. Active agent quercetin (QU)

Quercetin (3,3',4',5,7-pentahydroxyflavone) belongs to the polyphenolic group. Flavonoid compounds are almost universally found in plants and plant-based foods. QU can be used as a functional food against certain diseases because it can act on glutathione, the enzymes. The application of QU in the pharmaceutical field is limited because of its low absorption in the body due to its poor solubility in water, low bioavailability, and poor stability.

1.4.3. Paclitaxel (PTX)

Paclitaxel (PTX) – one of the most effective chemical compounds in the treatment of many types of cancer, including ovarian, breast, colon, bladder, esophageal, lung, multiple myeloma and Kaposi cancers. Patients after using PTX all have hair loss, more than 90% of patients got myelosuppression. Some patients had side effects such as congestion, rash (39%), anorexia (25%), peripheral edema (10%). The problem is to be overcome the water solubility, improve the therapeutic effect of PTX, and reduce the side effects caused by this drug.

1.5. Folic Acid Targeting Agent (FA)

Studying found that FA receptors are overexpressed in many types of cancer cells, especially ovarian cancer. Therefore, FA has been used in the design of targeted drug delivery systems based on surface receptor proteins to deliver drugs to the right tumors need treatment.

1.6. Previous studies

There have been scientific publications on the preparation of drug carrier materials such as quercetin, paclitaxel based on gelatin, pluronic in order to increase the therapeutic effect of the drug. As the authors are Jan Zillies and Conrad Coester (2004), author Z.Lu et al conducted research to develop nanoparticles from gelatin carrying the drug Paclitaxel for use in the treatment of bladder cancer, Kumari et al. the successful synthesis of nanoparticles from synthetic polymers (2011), The research group of Dr. Tran Ngoc Quyen at Ajou University, Korea successfully synthesized a heat-sensitive, electronegative nanogel carrier based on heparin that carries and slows down electropositive proteins (2011), and so on.

The results have not yet met expectations in terms of nanoparticle size, drug delivery and delivery efficiency, and cancer is still a danger disease of mankind. And at present, there are no domestic and foreign scientific works on the preparation and application of nanogels from pluronic grafted gelatin with the cancer cell-targeting agent folic acid with two combination drugs, quercetin and paclitaxel, published.

CHAPTER 2. RESEARCHING METHOD

2.1. Chemmicals

Hóa chất	Xuất xứ
Gelatin	Merck, Germany
Pluronic P123 (MW= 5.800)	Sigma–Aldrich, America
Pluronic F127 (MW= 12.600)	Sigma–Aldrich, America
Pluronic F68 (MW= 8.400)	Sigma–Aldrich, America
Pluronic F87 (MW= 7.700)	Sigma–Aldrich, America
Folic acid	Merck, Germany

Quercetin ($C_{15}H_{10}O_7$, KLPT=302 g/mol)	Sigma–Aldrich, America
Paclitaxel	Sigma–Aldrich, America
p-nitrophenyl chlorofomate (p-NPC) (MW=201,56 g/mol)	Acros organics, America
Tetrahydrofuran (THF)	Merck, Germany
Diethyl ether	RCI Labscan, Thailand
3-amino-1-propanol	Acros organics, America
Chloroform	Fisher Chemical, America
Methanol	Merck, Germany
Ethanol	Fisher Chemical, America
Dichloromethane	RCI Labscan, Thailand
n-hexane (99%)	Acros Organics, America
1,4 - Diaminobutane, 99%	Acros Organics, America
N-Hydroxysuccinimide - BASF Corp	Sigma–Aldrich, America
1-Ethyl-3-(3-Dimethylaminopropyl) Carbodimide	Acros Organics, America
Khí Nitrogen	Viet Nam

2.2. Tools and equipments

Laboratory equipments and high-tech analytical equipment are used such as electronic balance, 4 odd numbers (A&D company-Japan), magnetic stirrer with thermal correction (A&D company-Japan), magnetic stirrer with temperature correction (VELP Scientifica - Italy), lyophilizer (FDU-1200 EYELA - Japan), centrifuge (Hermle Labortechnik GmbH - Germany), calorimetric analyzer TGA - DSC (Mettler Toledo - Switzerland), and so on.

2.3. Research Methods

- The nanogel systems were synthesized on the basis of pluronic grafted gelatin (F127, P123, F87, F68).

- The product structure was determined by ^{1}H-NMR and FT-IR spectroscopy. The TGA method to determine the percentage of gelatin grafted into the pluronic.

- The morphology, size, and Zeta potential of the products were determined by TEM, DLS technique.

- Using iodine and UV-Vis spectroscopy to determine the CGC value of nanogels.

- Investigation of drug-carrying and release ability of synthetic nanogels by UV-Vis spectroscopy.

- Folic acid ligand grafted with the new gelatin-pluronic nanogel which has the most optimal values in terms of graft ratio, particle size, zeta potential, quercetin, paclitaxel carrying efficiency.

- Evaluating *in vitro* biocompatibility of gelatin-pluronic nanogels which synthesized by SRB method (Sulforhodamine B). And evaluating *in vitro* the effect of killing MCF-7, HeLa cancer cells of the drug encapsulated on gelatin-pluronic nanogels by the SRB test method.

- Evaluating *in vivo* the efficacy killing MCF-7 cancer cells of quercetin, paclitaxel which loaded on gelatin-pluronic nanogel system through a mouse model of tumor with Xenograft method.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Synthesis and investigation results of GP-F127 nanogel

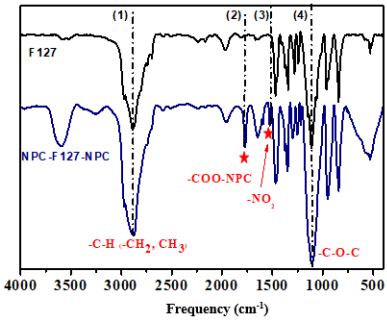
3.1.1. Results of determination of composition and structure of GP-F127 copolymers

3.1.1.1. Results of FT-IR and ^{1}H-NMR spectral analysis of intermediate products NPC-F127-NPC and NPC- F127-OH

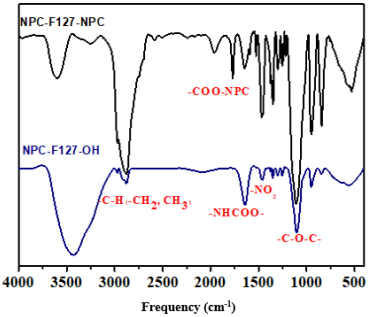
a. FT-IR analysizing results of NPC-F127-NPC and NPC-F127-OH

Absorption band of $–CH_2$ group, $-CH_3$ 2883.43 cm^{-1} (1) of F127. The $–NO_2$ group is directly associated with the aromatic nucleus on the NPC molecule appearing at 1593.77 cm^{-1} (3), an oscillating shift appears peak of 1769.58 cm^{-1} (2), the oscillation of the -C=O bond of the ester group due to the formation of an ester bond between the pluronic F127 and the NPC creating the product NPC-F127-NPC (Figure 3.1).

The FT-IR spectrum of NPC-F127-OH shows a new signal at 1643.35 cm^{-1}, due to partial substitution of the p-nitrophenyl chloroformate moiety by 3-amino-1-propanol via ureathan (-NHCOO-) linkage, proves that the product NPC-F127-OH is synthesized.

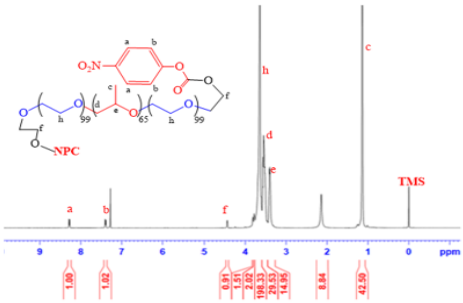


Figue 3.1. FT-IR spectrum of F127 and NPC-F127-NPC



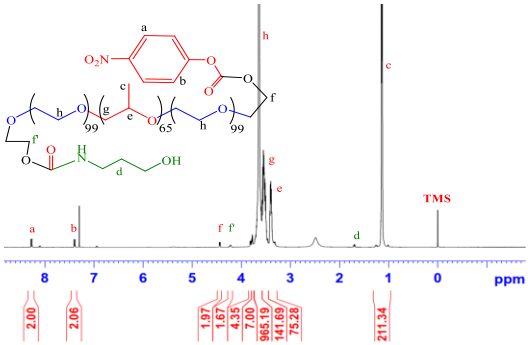
Figue 3.2. FT-IR spectrum of NPC-F127-NPC and NPC-F127-OH

b. ^{1}H-NMR analysizing results of NPC-F127-NPC and NPC-F127-OH



Figue 3.3. ^{1}H-NMR spectrum of NPC-F127-NPC

Peaks δ = 7.39 ppm and δ = 8.28 ppm demonstrate the presence of proton H at the binding site (-CH) on NPC's benzene ring. A multi-peak δ = 4.44 ppm is the proton H on the PEO chain that bound directly to the NPC part (-CH2-O-NPC), this signal only appears when the pluronic is activated by NPC. Peak at δ = 3.62 ppm due to the presence of proton H on the PPO chain at the group binding site (-CH_2-CH-). Peak at δ = 3.2 – 3.5 demonstrates the presence of proton H on PPO at (-CH_2, -CH). Peak δ = 1.14 ppm shows the presence of proton H on the PPO chain at the binding site of the (-CH_3) group, NPC-F127-NPC is synthesized.



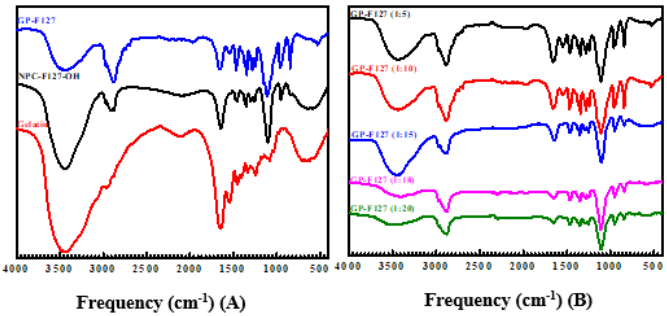
Figue 3.4. ^{1}H-NMR spectrum of NPC-F127-OH

The protons on the NPC-F127-NPC also have resonance signals at δ = 4.44 ppm show proton H on the PEO chain at the binding site with the (–CH_2-O-NPC) group to move a substantial part of the region δ = 4.22 ppm due to the substitution of NPC head with 3-amino-1-propanol, shows proton H on PEO chain directly bonded with the (-O-NH-) group. The higher the 3-amino-1-propanol replace, signal intensity at δ = 4.22 ppm will increase more. Peak at position δ = 1.70 – 2.2 ppm is that of proton H on saturated C that not directly link with N inside 3-amino-1-

propanol (-CO-NH-CH_2-CH_2-) group, confirmed 1 NPC head is replaced by 3-amino-1-propanol.

3.1.1.2. Analysis results of FT-IR and ^{1}H-NMR spectrum of GP-F127

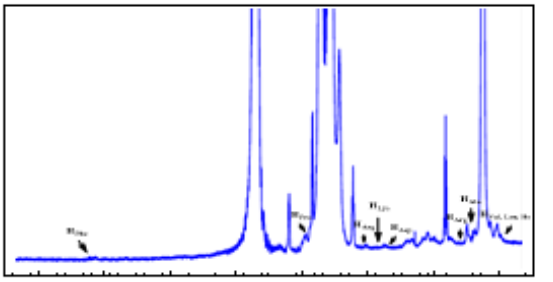
a. Analysis results of FT-IR spectrum of GP-F127



Figue 3.5. FT-IR spectrum of Gelatin, GP-F127 (A) and ratios of GP-F127 (B)

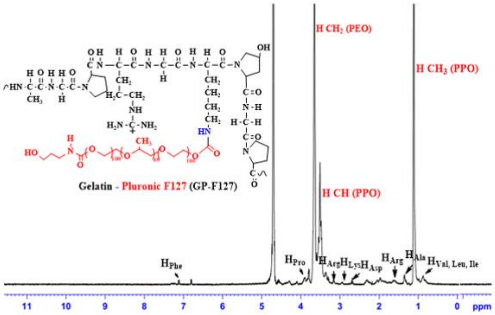
The spectrum of gelatin (Figure 3.5) has characteristic peaks such as peak at 3567 cm^{-1} is the -OH valence vibration on gelatin; HNC=O at 1690 cm^{-1}, stretching vibration of C-O bond and bending vibration of aromatic ring C-H at 1233 cm^{-1} and 1030 cm^{-1}, C-H aromatic ring out-of-plane bending vibration at 876 cm^{-1} and 712 cm^{-1}. GP-F127 has absorption noses in the 1297.96 – 1659,05 cm^{-1} region that characterizes the vibrations of primary, secondary, and tertiary amides on the gelatin chain, these signals do not appear in the FT-IR spectrum of NPC-F127-OH, due to the reaction formed from the ($–NH_2$) group on gelatin chain and (-C=O) group of Intermediate product NPC-F127-OH when synthesizing GP-F127. Confirmed that pluronic F127 has been successfully incorporated into the gelatin chain.

b. Analysis results ^{1}H-NMR spectrum of GP-F127



Figue 3.6: ^{1}H-NMR spectrum of gelatin

Characteristic signals of amino acids in gelatin: at 4.58 ppm of (-CH-, proline); 4.27 ppm of (-CH-, hydroxyproline); 3.88 ppm of (-CH-, alanine); 1.34 ppm of (-CH_3, alanine); 3.57 ppm of (-CH_2-, glycine); 2.23 ppm of (-CH_2-, glutamic acid); 1.60 ppm of (-CH_2-, arginine); 3.14 ppm of (-CH_2-, phenylalanine); 7.20 ppm, 7.23 ppm and 7.29 ppm of (-CH-, phenylalanine).

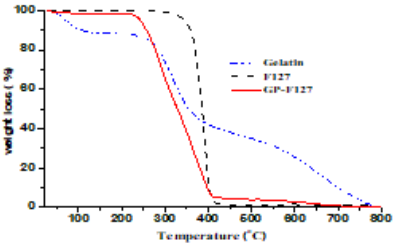


Figue 3.7. ^{1}H-NMR spectrum of GP-F127

Proton signals of gelatin: at 4.8 ppm (proton of aromeric carbon in gelatin), other signals at 0.8 – 4.6 ppm (proton of alkyl of gelatin), signals of 7.23 – 7.29 ppm showed that there is a proton bonded to the benzene ring carbon of phenylalanine and some other protons of amino acids in gelatin (Figure 3.7). Protons in PPO of F127 (–CH_3) at 1.08 ppm and (–CH_2) in PEO at 3.6 ppm also appear in the spectrum. Besides, suppression of the signal noses of p-NPC at 7.39 and 8.28 ppm (figue

3.4), ^{1}H-NMR of NPC-F127-OH) showed the substitution of the NPC head by the primary amine group of gelatin to form the GP copolymer.

3.1.1.3. TGA analysis results of GP-F127



Figue 3.8. Result TGA of F127, gelatin and GP-F127 copolymers

Mass loss of pure gelatin in three stages, temperature range 120 – 140 ^{o}C, explained by the loss of water (free form) because gelatin structure contains many –OH, =O groups of acid cacboxylic which are hydrophilic ones. The other temperature ranges at 200 – 420 ^{o}C and 500 – 800 ^{o}C, because of three-dimensional (3D) structure is broken and combined sample decomposition by heat. The TGA curve of the grafted copolymers is higher than that of pure pluronic one, which suggests that gelatin contributes to the increased stability of the copolymer structure. Gelatin-pluronic coupling efficiency calculated as percentage of weight loss of synthetic copolymer sample (GP-F127) at 420 ^{o}C.

3.1.1.4. Analysis results CMC value of GP-F127

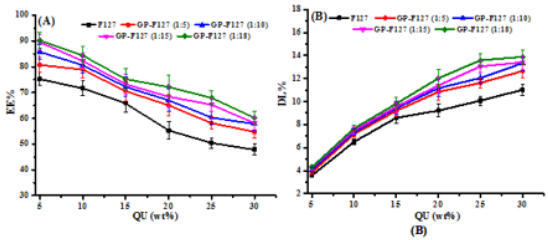
CMC value determined using iodine as a hydrophobic probe. Iodine dissolved in the solution will participate in the hydrophobic environment of pluronic F127 to cause displacement I_3^- to I_2 from KI odd in solution. CMC calculated by plotting the absorption intensity of I_2 in comparision with log function of polymer concentration (% wt).

3.1.1.5. Zeta potential and particle size analysis results of GP-F127

TEM result of micelle pluronic F127 distribute around 24,5 ± 7,82 nm, and has a spherical shape, medium size of nanogel GP-F127 around 58-206 nm (by TEM), bigger than size of micelle pluronic F127. DLS result of nanogel GP-F127 distribute around 68-285 nm, much smaller in micelle pluronic F127 size. The nanogel size measured by DLS is larger than TEM because the hydration of PEO (F127) in water increases the nanogel structure significantly.

3.1.2. Synthesis results of nanogel GP-F127 load QU and PTX

3.1.2.1. Synthesis results of nanogel GP-F127 load QU

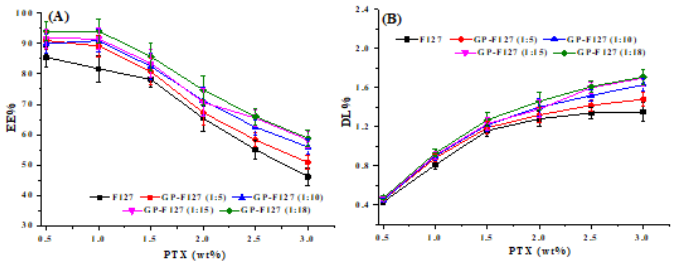


Figue 3.14. Result loading QU into F127 and GP-F127

(EE: Carrying efficiency QU (A) and DL: QU loading efficiency (B))

Pluronic F127 or copolymers GP-F127 at the different ratios all show a decrease in EE and an increase in DL when increasing the concentration of QU, EE and DL of GP-F127 (1:18) always are the biggest, for example, EE obtain more than 85%, DL get close to 8% at concentration of QU to be 10% wt.

3.1.2.2. Synthesis results of nanogel GP-F127 load PTX



Figue 3.15. Result loading PTX into F127 and GP-F127

(EE: Carrying efficiency QU (A) and DL: QU loading efficiency (B))

The EE value decreased gradually and the DL increased gradually with increasing the concentration of PTX in the range of values 0,5 – 3%. The greater the amount of pluronic F127 grafted into the gelatin, the larger the hydrophobic interaction on the GP-F127 molecule, the results of carrying PTX higher than that of pluronic F127, detail: GP-F127 (1:18) > GP-F127 (1:15) > GP-F127 (1:10) > GP-F127 (1:5) > F127.

3.2. Synthesis and investigation of nanogels GP-P123, GP-F87, GP-F68 compare with GP-F127

3.2.1. The results of determining the composition and structure of the copolymers GP-P123, GP-F87 and GP-F68

3.2.1.1. Analysis results FT-IR and ^{1}H-NMR spectrum of intermediate products NPC-P123-NPC, NPC- P123-OH, NPC-F87-NPC, NPC-F87-OH, NPC-F68-NPC, NPC-F68-OH

Analysis results FT-IR, ^{1}H-NMR spectrum of products NPC-P123-NPC, NPC-P123-OH, NPC-F87-NPC, NPC-F87-OH, NPC-F68-NPC, NPC-F68-OH are similar results of NPC-F127-NPC and NPC-F127-OH.

3.2.1.2. FT-IR and ^{1}H-NMR results of GP-P123, GP-F87 and GP-F68

FT-IR spectrum of GP-P123, GP-F87 and GP-F68, ^{1}H-NMR (GP-P123) are similar results FT-IR spectrum of GP-F127.

3.2.1.3. TGA analysis results of GP-P123, GP-F87 and GP-F68

No difference between the pure pluronics in TGA thermal analysis. The presence of Ge increases thermal stability of pluronic, the GP systems have a higher decomposition temperature than pure pluronic. The TGA results indicate decomposition temperature of the pluronics is about below 420 °C, while GP and Ge are about above 550 – 800 °C.

3.2.1.4.Analysis results CMC/CGC value of GP-P123, GP-F127, GP-F87 and GP-F68

The ratio of hydrophilic and hydrophobic chain lengths in the structure of grafted copolymers related to CGC value, structure of copolymer GP-P123 has the largest length of the hydrophobic PPO chain of pluronic P123 leading to an increase in hydrophobic interactions in the structure of the copolymer, and as a result, the CMC value decreases.

3.2.2. Synthesis results of nanogel GP-P123, GP-F127, GP-F87 and GP-F68 loading QU

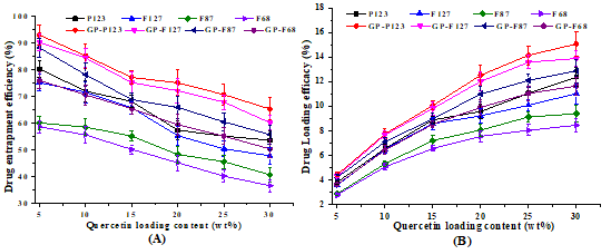


Figure 3.21. Result of loading QU into pluronic and GP

(EE: Loading efficiency QU (A) và DL: Loading productivity QU (B))

F68 nanogels increased proportionally with increasing pluronic grafting rate. Smaller HLB corresponds to an increase in the hydrophobic interaction on the carrier GP-P123 > GP-F127 > GP-F87 > GP-F68.

3.2.3. Comparison of analytical results of nanogels GP-P123, GP-F127, GP-F87 and GP-F68 loading QU

The GP-QU particle size increased gradually with the increase of the CGC value of the GPs, increasing from 40 nm to 120 nm. GP-P123 has the smallest TEM size, most distributed at 42 ± 2.51 nm, followed by GP-F127 with distribution value at 64 ± 3.72 nm, GP-F87 has size 87 ± 5.29 nm and GP-F68 for the largest size with the largest distribution value at 122 ± 2.93 nm. Nanogels with sizes below 100 nm have potential applications for drug delivery in vivo as they can avoid renal elimination (below 10 nm), avoid capture in the liver (greater than 100 nm).

3.2.4. Investigation of slow release of QU from nanogels GP-P123, GP-F127, GP-F87 and GP-F68

3.2.4.1. Slow release of QU from GP nanogels

After 12 hours, the amount of QU used in the free form was released up to 75.65% and completely released after only 24 hours. Meanwhile, the pluronic and GP samples surveyed at the same time showed that the QU release rate was much slower than that of the free QU sample.

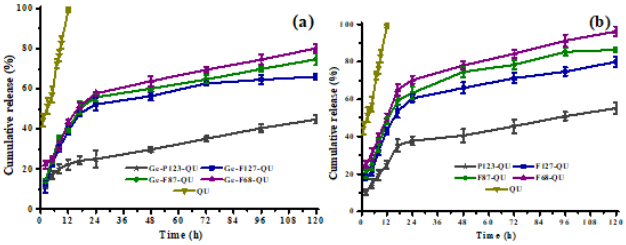


Figure 3.23. Releasing result of QU from nanogel GP-QU

The sample GP-P123 has the slowest QU release rate and the fastest is GP-F68, this result is argument about HLB and CMC values of pluronics, P123 has the lowest HLB and CMCs is the smallest, so the encapsulation capacity is high and the release rate is slowest.

3.2.4.2. Kinetics of QU release from nanogels GP

The GP samples all have the same QU release pattern as P123, spherical release related to Fickian. From the kinetics of QU release, the mechanism of QU release from nanogel systems is shown. This is an important premise for further preclinical and *in vivo* animal studies.

3.2.5. Comparison of the ability to inhibit cancer cells of materials nanogel GP-P123 and GP-F127 loading QU

The results show that QU carried by GP-P123 material always has stronger inhibitory ability than GP-F127. IC_{50} value decreased when QU was encapsulated in nanogel GP-P123, IC_{50} for MCF7 cells to be 34,08

± 2,62 µM and HeLa cells to be là 45,83 ± 1,13 µM. Therefore, it can be confirmed that there is a positive influence of quercetin on the ability of quercetin to inhibit cancer cells. This is explained because gelatin with amine structure has a good interaction with the cell membrane, helping to better protect the nanogel particles through the barrier of the cell membrane, penetrating into the cell.

3.3. Investigation result of GP-P123 nanogels at different grafting rates

3.3.1. Analysis results of Zeta potential and particle size of nanogel GP-P123 at different grafting ratios

The particles are spherical in shape with diameters arranged in the range of 40 – 55 nm (by TEM) and mean particle size distribution of 50 – 79 nm measured by DLS at 25 ^{o}C. The size of the GP-P123 nanogel is larger than that of the pure pluronic P123 micelle (8,34 ± 3,67 nm).

3.3.2. Synthesis results of nanogel GP-P123 loading PTX and QU

3.3.2.1. Synthesis result nanogel GP-P123 loading QU

EE and DL value of loading material pluronic P123 pure form and its copolymer with gelatin encapsulation QU. GP-P123, grafting ratio (1:4) gives the most optimal value, this is completely consistent with the experiment to investigate the particle size, Zeta potential and is explained by the structure of P123.

3.3.2.2. Synthesis result nanogel GP-P123 loading PTX

The results of PTX also have similar rules with the results of carrying QU, this contributes to more firmly strengthen the conclusions about GP materials synthesized according to the process of the thesis. And ratio GP-P123 (1:4) is the most optimal of the survey ratios of P123.

3.4. Synthesis results and evaluation of nanogel FA-GP-P123

3.4.1. FT-IR and ^{1}HNMR analyzing results of FA-GP-P123

FT-IR and ^{1}HNMR spectrum affirmatively synthesized successfully FA-GP-P123.

3.4.2. The results of characterization of nanogel FA-GP-P123

3.4.2.1. TGA result of FA-GP-P123

Comparing the TGA curve of FA-GP-P123 and that of GP-P123 is the basis for confirming that folic acid is successfully attached to the copolymer GP-P123.

3.4.2.2. CMC value of FA-GP-P123

CGC value of FA-GP-P123 is 0,0046 %wt, larger than the CMC value of pure P123 that is 0,0028 %wt, especially the CGC value of FA-GP-P123 is lower than that of GP-P123. This result indicates that FA when incorporated into GP-P123 will increase the hydrophobic part of the polymer leading to a decrease in the CMC value of the sample solution. A low CGC value also means a high hydrophobic drug carrying capacity.

3.4.2.3. Synthesis results of nanogel FA-GP-P123 loading QU

EE and DL value of nanogel GP-P123 with added folic acid (FA-GP-P123) QU encapsulation also follows the same transformation rule as the GP-P123 nanogel, the optimal value is the ratio FA-GP-P123 (1:4). The binding of FA only plays a role in targeting the material on cancer cells.

3.4.2.4. Synthesis results of nanogel FA-GP-P123 loading PTX

The results are similar to the PTX carrying results of the GP-P123 nanogel. And the ratio Ge:P123 at the value (1:4) gives the best results.

3.5. Synthesis results and evaluation of nanogel FA-GP-P123 loading PTX combination QU (FA-GP-P123/PTX/QU) on tumor-bearing mice

3.5.1. Carrying capacity PTX combination QU of nanogel FA-GP-P123

FA-GP-P123 (1:4) was used with two types of combined active ingredients capable of treating cancer, PTX and QU. The nanogel system carrying PTX and combining QU (FA-GP-P123/PTX/QU) encapsulated 88.57% QU and 98.67% PTX.

3.5.2. Survey results on the ability to release drugs of FA-GP-P123/PTX/QU

3.5.2.1. Slow release of drugs from nanogels FA-GP-P123

QU present in the FA-GP-P123/QU nanogel was released in a larger amount than that of FA-GP-P123/PTX/QU. The PTX contained in the FA-GP-P123/PTX/Q nanogel released very quickly while the QU released slowly, only about 60% at pH = 5.5 and 50% at pH = 7.4. The results show the potential for application of FA-GP-P123 material in biomedical field.

3.5.2.2. Kinetics of drug release from nanogel FA-GP-P123

FA-GP-P123/PTX/QU (pH = 5.5 and pH = 7.4) with n from 0.2188 to 0.3462 indicates that the release mechanism is Fickian diffusion.

3.5.3. In vitro evaluation results Effective in killing cancer cells of nanogel FA-GP-P123

3.5.3.1. In vitro evaluation results biocompatibility of nanogel GP-P123

The results of cell viability tests and morphometry-based staining showed that GP-P123 is a potential drug carrier without any side effects.

3.5.3.2. In vitro evaluation results Effective in killing MCF-7 cancer cells of nanogel FA-GP-P123 loading PTX and QU

The survey value shows a statistical, IC_{50} value of GP-P123/PTX/QU is 1.22293 ± 0,12684 µM While IC_{50} value of FA-GP-P123/PTX/QU is

0.72783 ±0.11647 µM (P=0.0078 <0.01), This confirms the positive role of the FA target orientation agent in the material structure FA-GP-P123.

3.5.3.3. In vitro evaluation results effectively destroy HeLa cancer cells of nanogel FA-GP-P123 loading PTX and QU

When there is a factor of FA target, IC_{50} value of materials carrying two drugs FA-GP-P123/PTX/QU decreased to the value 38,0 nM in comparision with GP-P123/PTX/QU, around 47.0 nM. This proves the effectiveness of the FA in the carrier material.

3.5.4. In vivo evaluation result Effect of destroying cancer cells of nanogel FA-GP-P123/PTX/QU

Nanogel FA-GP-P123 loading two combined drugs PTX and QU, tumor volume decreases to 94,20 ± 5,9% after 14 experiment days, there is one of the groups with tumors that are eliminated after 14 days. The tail area of the mouse does not show signs of inflammation or necrosis such as 2 free medicine solutions. Experimental results show that nanogel FA-GP123/PTX/QU material can contribute to increasing the effectiveness of destroying tumors and significantly reducing the toxic side effects of the drug.

CONCLUSION

Through the research, thesis achieved the following results:

1. Successfully synthetized nanoparticles that conduct nanoparticles from Pluronic Gelatin Gelatin to combine the characteristics of these two ingredients. The Nanogel GP-P123 and FA-G123 materials are new materials, successfully attached folic acid has not been studied before. Synthetic nanogel products are assessed structure by methods ^{1}H-NMR, FT-IR, TGA, CMC/CGC, DLS, TEM.

2. Nanogel GP materials (Ge:P123, ratio 1:4, Ge:F127, ratio 1:18) carry QU obtain from 2.85 to 13.29% wt, bring PTX obtain from 0.5 to 2%wt. Nanogel GP-P123 at the 1:4 ratio of Gelatin-Pluronic P123 has the highest ability to carry QU and PTX, In accordance with the statement of the characteristics of the material such as the smaller the value of CMC/CGC, the zeta, these favorable properties are created when combining gelatin combination with pluronic.

3. Successfully conducted capsule concomitantly two types of drugs PTX and QU into nanogel material FA-GP-P123. When comparing the two nanogel materials GP-P123 and FA-GP-P123 with the same GE transplant ratio : P123 is 1:4, showing that FA-GP-P123 materials are better capable of carrying PTX.

4. Nanogel materials GP-P123 and FA-GP-P123 (The same grafting rate Ge:P123 is 1:4) loading QU and PTX have the speed of releasing the drugs much slower than the sample QU and PTX which using free form (control) due to the hydrophobic drug molecules are loaded in the core – The hydrophobic part of nanoparticles, concomitantly, it is surrounded and interacted to lead to the stability of the particle structure of the gelatin shell. In addition, the comparison between the two pH values shows that the percentage of release of QU and PTX in the medium pH = 5.5 - similar to the extracellular one pH value of cancer cells) with medium pH = 7.4 - similar to the pH value of the human body. This contributes to improving the ability to release drugs towards cancer cells.

5. Nanogel FA-GP-P123 (FA attached) loading QU and PTX is capable of inhibiting the growth of the MCF-7/HELA cell line higher than that of the QU and PTX carried by nanogel GP-P123 respectively because the surface of cancer cells contains many folate receptors

nanoparticles bring medicine that FA grafted will easily be absorbed into cancer cells. Nanogel FA-GP-P123 material when applied with two drugs simultaneously PTX and QU has been highly effective with the test on the white mouse model carrying the MCF-7 cell tumor heterogeneous transplant from humans. Detail, The tumor has a decrease to 94,20 ± 5,9% after 14 days of testing. In addition, PTX drug has a much more toxicity decrease than the free form of PTX (control), rats do not appear symptoms of inflammation and necrosis in the tail area, the injecting site. Nanogel FA-GP-P123 carries two drugs simultaneously PTX, QU which not yet studied and tested on animals before.

PETITION

After the thesis is approved, I will continue this research with experiments investigating of the ability to carry other anti-cancer drugs of synthetic nanogel materials to achieve better efficiency.

Continue to study and evaluate on nude mice mice implanted with immunodeficiency gene (the most stable and accurate assessment model) combining multi -anti -cancer drugs on synthetic nanogel materials, in order to increase the effectiveness of treatment as well as reduce the toxicity of the drug.

The corresponding study of copolymer assembled with targeted agents, in order to increase the effectiveness of tumor treatment anti - cancer drugs which are poorly soluble in water.

LIST OF PUBLISHED-RELATED WORKS

1. **Van Thoai, D**., Nguyen, D. T., Nguyen, N. H., Nguyen, V. T., Doan, P., Nguyen, B. T., Le, V. T., Nguyen, N. T. & Quyen, T. N. Lipophilic effect of various pluronic-grafted gelatin copolymers on the quercetin delivery efficiency in these self-assembly nanogels. Journal of Polymer Research, 27(12), 1-12.,2020. (**IF: 3.097, Q2**)

2. Trung Nguyen Dinh, **Thoai Dinh Van**, Hang Dang Le, Nam Nguyen Dang, Bach, Long Giang, Truc Nguyen Cong, Bich Tram Nguyen Thi, Van Thu Le, Ngoc Quyen Tran, Dual interactions of amphiphilic gelatin copolymer and nanocurcumin improving the delivery efficiency of the nanogels, Polymers. 11(5), 814 (2019) (**IF 4.329, Q1**).