MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty of Chemical and Biopharmaceutical Technologies
Department of Industrial Pharmacy

Master's thesis

RESEARCH AND DEVELOPMENT OF NANO MICROGEL FOR ANTICANCER DRUG CARRIERS

Completed: student of the group MPhch-20 of the specialty 226 Pharmacy, industrial pharmacy

Li Cheng
(student's first name, last name)

Supervisor Roman KACHAN
(first name, last name)

Reviewer Galyna KUZMINA
(first name, last name)

Kyiv 2021

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Institute, faculty Chemical and Biopharmaceutical Technologies

Department Industrial Pharmacy

Speciality 226 Pharmacy, industrial pharmacy

(code and title)

Approve
Head of Department Industrial Pharmacy,
Professor, Doctor of Pharmaceutical Science
Vladyslav STRASHNYI
"14" December 2021

ASSIGNMENTS
FOR THE MASTER'S THESIS
Li Cheng

1. Thesis topic Research and development of nano microgel for anticancer drug carriers

2. Scientific supervisor Roman KACHAN, Associated Professor, PhD
(last name, first name, patronymic, academic degree, academic title)
approved by the order of the higher educational institution on 4th October 2021, N 286

3. Deadline for student submission of work 14th December 2021

4. Initial data to work scientific and scientific-practical sources, patents, articles, legislative, normative and normative-legal acts and documents, overview types of wastewater in Pharmaceutical Industry and methods of their treatment, analysis of pharmaceutical wastewater treatment technologies, desin new materials and development technology of photoelectrochemical catalysis of pharmaceutical wastewater in China and all around the world

5. Content of the thesis *(For example: a review of the literature on antibacterial hydrogels, the development of methods for the manufacture of antibacterial hydrogels, the study of the properties of antibacterial hydrogels, development of a technological scheme..).*

6. Consultants of the master's thesis sections

Section	Surname, initials and position of the consultant	Signature, date	
		the task was issued	the task accepted

7. Date of issuance of the assignments _____

8. Execution schedule

No	The name of the stages of the master's thesis	Terms of performance of stages	Note on performance
1	Introduction		Signed by the supervisor, the student
2	Section 1		Signed by the supervisor, the student
3	Section 2		Signed by the supervisor, the student
4	Section 3		Signed by the supervisor, the student
5	Conclusions		Signed by the supervisor, the student
6	Draw up a master's thesis (final version)		Signed by the supervisor, the student
7	Submission of master's thesis to the department for review (14 days before the defence)		Signed by the supervisor, student and consultants
8	Checking the master's thesis for signs of plagiarism (10 days before the defence)		Signed by the supervisor, student and consultants
9	Submission of master's thesis to the master's department to check the implementation of the appendix to the individual curriculum (10 days before the defence)		Signed by the supervisor, student and consultants
10	Submission of master's thesis for approval by the head of the department (from 7 days before the defence)		Signed by the head of the department, supervisor, student, consultants, head of the monitoring department and head of the master's department

Student _____

_____

(surname and initials)

(signature)

Scientific supervisor _____ _____

(signature)

(surname and initials)

Head of the magistracy department _____ _____

(signature)

(surname and initials)

Summary

Li Cheng. Research and development of nano microgel for anticancer drug carriers- Manuscript

Master's thesis on the specialty 226 Pharmacy, industrial pharmacy. - Kyiv National University of Technology and Design, Kyiv, 2021.

Drug carrier can improve drug utilization, safety and timeliness, reduce drug administration frequency, improve drug odor, improve dose accuracy and accurate drug release to targeted tissues and organs, so it has been widely concerned by people. The development of nanotechnology has promoted the research of drug carrier, and the research and application of nanoscale drug carrier has made great contributions in the field of medicine.

In this paper, leucine (Leu) and hydroxyethyl starch (HES) were used to prepare nano-hydrogels, and Lau-Leu-HES nano-hydrogels were prepared to be used as drug carriers. The reaction method of 1- (3-dimethylaminopropyl) -3-ethylcarbondiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) was used to promote condensation. We studied and characterized the appearance, molecular weight, particle size, pH response and other characteristics of the prepared nano-hydrogels. The performance of the two kinds of nano-hydrogels was studied by in vitro degradation experiment, drug-loading and drug-release experiment, and cytotoxic MTT experiment. The results showed that the prepared nano-hydrogels were suitable for drug carrier. In addition, we designed the factory for the production of the drug capsules and evaluated the entire process of quality control, risk assessment and solution for the conditions required to build the factory.

***Key words**: drug carrier, Nano-hydrogel, PH responsiveness, Drug delivery, release, cytotoxicity quality management.*

.

Анотація

Лі Ченг. Дослідження і розробка нано-мікрогелю як носіїв протипухлинних препаратів - Рукопис

Магістерська робота за спеціальністю 226 Фармація, промислова фармація. - Київський національний університет технологій та дизайну, Київ, 2021.

Носій активного фармацевтичного інгредієнту може поліпшити використання ліків, безпеку і своєчасність, зменшити частоту прийому препарату, поліпшити запах препарату, підвищити точність доз і точний викид препарату в цільові тканини і органи, тому це дуже актуальна тема. Розвиток нанотехнологій сприяє дослідженню носія лікарського засобу, а дослідження та застосування нанорозмірного носія лікарського засобу зробили великий внесок у галузі медицини.

У цій роботі для приготування наногідрогелів використовувався лейцин (Лей) і гідроксиетиловий крохмаль (HES), а наногідрогелі Lau-Leu-HES були готові до використання в якості носіїв ліків. Метод реакції 1- (3-диметиламінопропіл) -3-етилкарбондіаміду гідрохлориду (ЕДК) і N-гідроксисучиніміду (NHS) був використаний для сприяння конденсації. Ми вивчили і охарактеризували зовнішній вигляд гелю, молекулярну масу, розмір частинок, реакцію на pH та інші характеристики підготовлених наногідрогелів. Продуктивність двох видів наногідрогелів була вивчена експериментом з деградації in vitro, експериментом з

завантаженням та вивільнення лікарського засобу, а також дослідженням цитотоксичності МТТ. Результати показали, що підготовлені наногідрогелі підходять чк носії лікарських засобів. Крім того, ми розробили технологічний процес виробництва капсул препарату і оцінили весь процес контролю якості, оцінки ризиків і рішення для умов, необхідних для технологічного виробництва.

Ключові слова: носій лікарського засобу, наногідгель, ph чутливість, доставка ліків, управління якістю цитотоксичність.

CONTENT

Introduction

Drug carriers can improve the utilization rate and safety of the drug, reduce the frequency of administration, improve the unpleasant odor of the drug. It also can improve the accuracy of the administration dosage and the accurate release of the drug to the targeted tissues and organs. Therefore, drug carriers have received extensive attention. The development of nanotechnology has promoted the research of drug carriers. The research and application of nano-scale drug carriers have made great contributions in the medicine field.

1. 1. Overview of drug carriers

In pharmacy, pharmaceutical preparations in the system theory, technology and application of research and other aspects are gradually mature, slow control agent has become a hot spot in the field of pharmaceutical preparations research. In the field of pharmaceutical production and application, many drugs have many unstable properties. Different APIs have different structures of physical and chemical properties, and APIs have certain problems such as toxicity, instability, low solubility, low absorption efficiency, unstable release rate and poor water solubility [1]. In the use of drugs, the use of large amounts of drugs does not mean that the use of drugs is good, but may be harmful to the body, such as drug-induced diarrhea, causing accidental injury to normal tissues. Therefore, in the process of drug use, drug dosage and release rate should be strictly controlled [2].

As a slow control system, drug carrier can change the route of drug delivery, control the release rate of drugs, and realize the targeting function of drug delivery process. The research of drug carrier has attracted the attention of researchers.

Drug carrier can improve drug safety, improve drug utilization, cover up unpleasant drug odor, accurately deliver drug dose to targeted tissues and organs, thus attracting people's attention [3,4].

There are many kinds of drug carriers, including microcapsules, microspheres, nanoparticles and liposomes [5].

The outer layer of the microcapsule is a polymer membrane, and the core of the capsule wraps drugs, forming a tiny capsule, which releases the drugs in the capsule under specific conditions. The particle size is generally 1-250 μm.

Microsphere is a kind of micron spherical particles, generally formed by polymer, microsphere can absorb and disperse drugs, through the nature of the microsphere itself to control the drug type, adsorption capacity, release speed, its particle size is generally 1-250 μm.

Nanoparticles are usually made of natural or synthetic polymer materials, on the one hand, through static adsorption, covalent connection combination of drugs on its surface, on the other hand through the pore structure technical package, the use of its own have targeted molecular structure of the nature of the functional groups or with receptors of cells and tissues, using nanoscale particle’s structure slow controlled release drugs, its particle size is generally 10-1000 nm [6].

Liposomes are usually used as artificial membranes to prepare bimolecular microvesicles, which encapsulate the original drug in the structure. Bimolecular layers are generally composed of amphoteric molecules, making it easy to prepare microvesicles, and their particle size is generally 10-1000 nm.

Different delivery methods also have high requirements on drug carriers, and

the main delivery methods include oral, transdermal, mucosal and targeted delivery [7,8].

(1) Oral administration of drugs, through swallowing drugs, absorbing drugs in the stomach and intestines of the human body, to achieve the digestive tract site administration or systemic administration after absorption.

Oral drug delivery accounts for a large proportion in the way of drug delivery, is a common way of drug delivery, has a wide range of applications in the pharmaceutical field.

(2) Transdermal drug delivery, which enables the drug in the carrier to reach the subcutaneous tissue through the skin to achieve pathological treatment.

Transdermal drug delivery can improve the efficiency and utilization of drugs, and the drug delivery process is efficient and simple. Usually, the drug carrier does not penetrate the skin during transdermal drug delivery.

(3) Mucosal drug delivery system, the drug directly contact with the mucosa, through the role of mucosal cells to achieve drug absorption.

Common mucous membrane has mucous membrane of oral cavity, eye, apply to protein of a few polypeptide

Drugs with complex molecular structure and large molecular weight [9].

(4) Targeted drug delivery: Generally, drugs are adsorbed and wrapped by drug carriers, and the environmental responsiveness of drug carriers is used to transport drugs to specific parts of the body for controlled release of drugs, so as to achieve precise site drug release [10].

Targeted drug delivery is a hot research topic with high efficiency and little

harm to the body.

The materials used to prepare drug carriers mainly fall into two categories: one is natural polymer materials, usually including animal lipids, biological peptide sugars, amino acids, peptides and proteins. The other is a synthetic polymer material, usually alkyl acrylate, polylactic acid (PLA) and polylactic acid-polyglycolic acid copolymer (PLGA).

The natural polymer materials used in drug carriers are derived from living organisms and obtained by direct adoption and separation and extraction.

The main characteristics of biopolymers are: good biocompatibility, small toxic and side effects on the body, easy degradation in vivo, simple material acquisition and controlled release mechanisms.

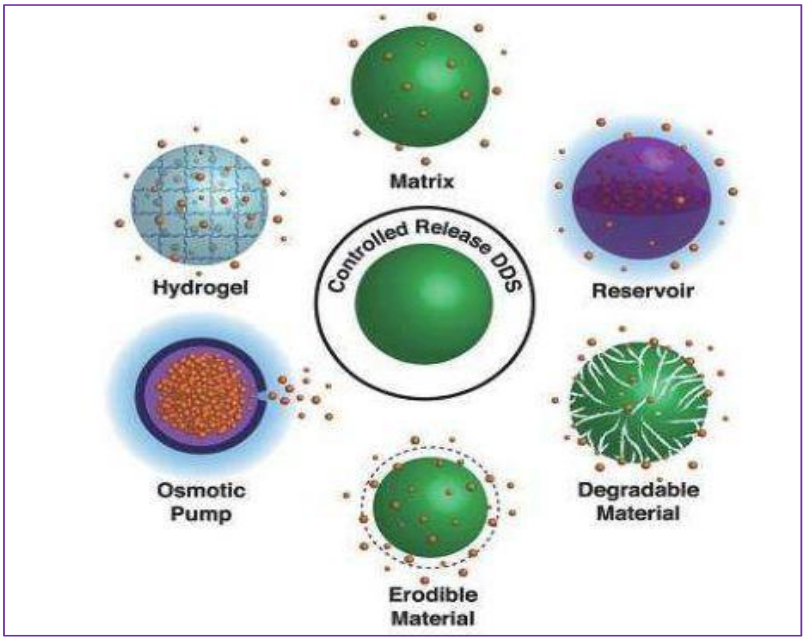


Figure 1.1 Some typical polymer nanodrug carriers and their forms.

In the research of drug carrier, the recent hot spot is the addition of nanotechnology. The properties of nano drug carrier are more in line with the

requirements of clinical pharmacy. The main reasons are as follows :

(1) nano drug carrier has smaller particle size and shape, easy to absorb;

(2) better sustained-release drugs and increased blood concentration can reduce drug degradation;

(3) it has a high drug load, the concentration of drug treatment at the disease location is increased, the dosage of drugs is reduced, and the toxic and side effects of drugs are reduced;

(4) The release rate can be easily controlled and predictable;

(5) the carrier is easy to degrade in vivo and be absorbed and discharged from the body [12,13].

Although drug carrier has been discovered and applied by more and more researchers, its development and research technology is not mature enough.

Most of the experiments were not actually produced in factories, and most of them are still in the laboratory.

There are few products on the market due to the lack of research theories in the selection of carrier materials and preparation process, but the unique properties of drug carrier will certainly make it widely used in the medical field [14].

Camptothecin (CPT) is a plant anticancer drug, chemical formula is $C_{20}H_{16}N_2O_4$, is light yellow needle-like crystal. It was extracted from Camptotheca acuminata distributed in south-central and south-western China.

In 1976, Chinese chemist Gao Yisheng and others successfully synthesized racemate camptothecin. In 1966, Wall et al. first extracted camptothecin from the seed or root bark of Camptotheca davidia, a davidia species endemic to China, and identified

its structure. Camptothecin has a good effect on gastrointestinal and head and neck cancer, and there are many derivatives, but its disadvantages are poor solubility and high level of adverse drug reaction characteristics.

The physical and chemical properties and structures of different apis differ greatly, and some drugs have certain toxicity, instability, low solubility, low absorption efficiency, unstable release rate, poor water solubility and other problems. One drawback of camptothecin is its poor water solubility.

In recent years, the rapid development of nanotechnology has promoted the research of drug carrier, and the research and application of nanoscale drug carrier are increasing day by day.

In order to overcome these shortcomings, researchers mostly use the method of making the insoluble drugs such as camptothecin and natural polymer materials with good biological compatibility into nano preparations, such as nanoparticles, microcapsules and microspheres.

Hydroxyethyl starch (HES) is a semi-synthetic polysaccharide, is corn or potato amylopectin glucose ring hydroxyethyl formation of polymer complex.

It is often used as plasma dilatant in clinic.

With other than expansion agent such as dextran and plasma albumin, hydroxyethyl starch has a lower incidence of allergic reactions, does not cause infusion caused by bacterial and viral infections, and no drug interactions, and more cost-effective, has been used in drug carrier mainly has the following advantages:

(1) Hydroxyethyl starch has good solubility in water;

(2) Hydroxyethyl starch has good biocompatibility and biodegradability;

(3) Hydroxyethyl starch is widely used as plasma dilatant in clinic, which is clinically acceptable.

(4) Hydroxyethyl starch has been mass-produced;

(5) Hydroxyethyl starch has structural prunability;

(6)Hydroxyethyl starch contains a large number of hydroxyl groups which can be modified by multi-functionalization.

However, because hydroxyethyl starch only contains a large number of hydrophilic groups -- OH, it cannot achieve self-assembly in the preparation of nano preparations, which is not conducive to drug loading.

Leucine is an essential amino acid that cannot be synthesized by itself. Chemically named L-2-amino-4-methylvalerate, leucine is an effective branch chain amino acid. Leucine's functions include working with isoleucine and valine to repair muscle, control blood sugar, and provide energy to body tissues.

Leucine, isoleucine, and valine are branched-chain amino acids that help promote post-training muscle recovery.

Leucine is one of the most effective branched amino acids, preventing muscle loss because it is faster broken down into glucose to control blood sugar and provide energy to body tissues.

In addition, it helps increase the production of growth hormones and helps burn visceral fat that, because it's inside the body, can't be effectively affected by diet and exercise alone.

But its solubility is very poor, which is a big disadvantage.

Therefore, its application can be further developed by modifying it.

In order to overcome the above shortcomings, this paper introduces the preparation of a leucine-hydroxyethyl starch composite nanospheres. Firstly, leucine is chemically modified by lauric acid and purified to obtain lauric acid-modified leucine. The reaction mechanism is shown in Figure 2:

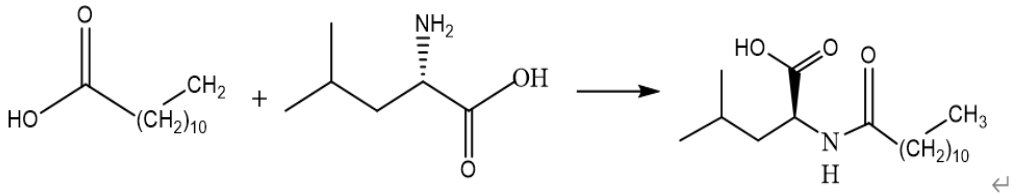


Figure. 2. Lauric acid modified leucine

The polymer was then prepared by grafting the modified leucine onto hydroxyethyl starch using a condensation reaction promoted by N,N' -diisopropyl carbodiimide (DIC), 1- (3-dimethylaminopropyl) -3-ethylcarbdiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS).The modification and grafting of leucine were verified by ir and NMR.Nano microspheres were obtained and characterized. Sem and particle size test were carried out. It was proved that the obtained nano hydrogels had good morphology, uniform dispersion and uniform particle size in aqueous solution, about 120 nm. The cytotoxicity of nano-hydrogels was tested by MTT method, and the survival rate of cells was detected. Cytotoxicity experiments showed that the prepared nanospheres had no toxic and side effects on cell growth, had good biocompatibility and promoted cell growth. Therefore, these nanospheres could be used as safe and efficient drug carriers.

1.2. Current Status and Time Trends of Cancer Incidence and Mortality Worldwide

As the second most common cause of death in the world, cancer has become a persistent public health challenge. The incidence and mortality in different countries and regions or of multiple cancer types are significantly different, which is closely related to economic development level, lifestyle and environmental factors. A large number of epidemiological studies have focused on cancer burden, epidemic pattern, etiology and prevention, which is very important for the government to formulate cancer prevention policies based on medical evidence and protect population health.

Cancer is a continuing global public health challenge. As the second most common cause of death worldwide, cancer is expected to be the most important obstacle to life expectancy growth in the 21st century. The burden of cancer is growing globally. It is projected that the greatest impact and fastest increase in the burden of cancer in the coming decades will continue to be in low - and middle-income countries, many of which are already facing unmanageable difficulties [15].

1.2.1. Global cancer epidemic trends

In February 2020, the International Agency for Research on Cancer (IARC) released the latest Edition of the World Cancer Report, a systematic summary of 134 countries, Cancer is the first or second leading cause of premature death (i.e., death between 30 and 69 years of age), and the third or fourth in the other 45 countries [16]. There are significant differences in cancer incidence and mortality in different countries, which are closely related to the inherent gaps in medical technology and health infrastructure [17]. Cancer mortality rates are declining in most countries with high HDI,

mainly due to effective prevention, early detection and treatment. In contrast, mortality rates for many cancers, including breast, lung and colorectal cancers, are still increasing or stabilizing in countries in transition [18-19].

1.2.2 Changes in the overall level of cancer worldwide

In 2008, the number of new cancer cases in the global population was about 12.7 million. The top 10 cancers were lung cancer, breast cancer, colorectal cancer, stomach cancer, prostate cancer, liver cancer, cervical cancer, esophageal cancer, bladder cancer and non-Hodgkin's lymphoma, accounting for the total number of cancer cases 67.9% [20]. In 2018, the number of new cancer cases in the global population was about 18.1 million, an increase of 42.5% compared with 2008. The top 10 cancers were lung cancer, breast cancer, prostate cancer, colorectal cancer, stomach cancer, liver cancer, esophageal cancer, cervical cancer, thyroid cancer and bladder cancer, accounting for 60.8% of the total number of cancers [21]. From 2008 to 2018, the global incidence spectrum of cancer has changed to some extent.

Table 1 Comparison of worldwide top 10 new cancer cases between 2008 and 2018

Ranking	2008		2018		Growth rate (%)
	Tumor sites	New cases (10^3)	Tumor sites	New cases (10^3)	
1	Lung	1608	Lung	2094	30.2
2	Breast	1383	Breast	2089	51.0
3	Colorectum	1233	Prostate	1276	39.8
4	Stomach	989	Colorectum	1414	14.7
5	Prostate	913	Stomach	1034	4.6
6	Liver	748	Liver	841	12.4
7	Cervix uteri	529	Esophagus	572	18.7
8	Esophagus	482	Cervix uteri	570	7.8
9	Bladder	386	Thyroid	567	167.5
10	Non-Hodgkin lymphoma	355	Bladder	549	42.2

The most significant is that thyroid cancer has rapidly increased from 212,000 cases in 2008 to 567,000 cases in 2018, with a growth rate of 167.5%, replacing non-Hodgkin lymphoma as the top 10 new most common cancers. Breast cancer, bladder cancer, prostate cancer and lung cancer all maintained high growth rates of over 30%, while gastric cancer and cervical cancer kept growth rates below 10%, as shown in Table 1.

1.2.3 Changes in the overall level of cancer deaths worldwide

In 2008, there were about 7.6 million cancer deaths worldwide and in 2018, there were about 9.6 million cancer deaths worldwide, an increase of 26.3% [22-23]. Globally, the top 10 cancer causes of death for both men and women remained unchanged in 2018 compared to 2008, but demographically defined death rates for most cancers declined or remained flat.

The United States

According to the recent cancer statistics report in the United States [24], it is estimated that there will be 1.807 million new cancer cases and 607,000 deaths in 2020. Prostate, lung and colorectal cancers, the most common, account for 43 percent of all cases in men, and prostate cancer alone accounts for more than a fifth of new cases. The three most common cancers in women are breast, lung and colorectal, accounting for more than 50 percent of all new cases, with breast cancer alone accounting for 30 percent. In terms of causes of death from cancer, prostate cancer, lung cancer and colorectal cancer are the main causes of death for men; Lung, breast and colorectal cancer are the leading causes of death among women.

Historical statistics show that the death rate from cancer in the U.S. population

has increased since

After reaching 215.1/100,000 in 1991, the mortality rate gradually decreased, and by 2017, the overall mortality rate decreased by 29% (152.4/100,000). This progress is caused by the long-term decline in the mortality rate of four types of cancer with high incidence (lung, colorectal, breast and prostate) [25]. The decline rate of lung cancer mortality has been accelerating, with lung cancer mortality in men decreasing by 5% annually from 2013 to 2017 and in women by nearly 4% annually, resulting in an overall decline of 2.2% from 2016 to 2017, the largest single-year decline ever recorded in the United States [26]. The slowing down of lung cancer mortality in the United States may be related to the promotion of anti-smoking measures, the change of smoking rates in both men and women in the United States, and the promotion of new lung cancer screening technologies [27-28]. However, lung cancer still caused more deaths in 2017 than breast, prostate, colorectal and brain cancer combined [29]. In addition, with the approval of a series of innovative therapies by the US FDA, the mortality rate of cutaneous melanoma has also decreased significantly, and its incidence has been on the rise until 2013 [30]. It is noteworthy that the death rate of liver cancer, which has been increasing rapidly for a long time, has slowed down in women and stabilized in men [31]. The report is about the incidence and treatment of cancer in adolescents and children. The situation deserves attention. Since 1975, the incidence of cancer among children and adolescents in the United States has increased slightly (0.7 percent per year) for reasons that are not yet clear, while child and adolescent mortality rates have continued to decline, from 6.3 per 100,000 children and 7.1 per 100,000 adolescents in 1970 to 2.0 per 100,000 and 2.7 per 100,000 adolescents in 2017, respectively. Cancer deaths in children and

adolescents were reduced by 68 percent and 63 percent, respectively. This progress is mainly due to the sharp decline in leukemia mortality in children and adolescents, which decreased by 83% in children and 68% in adolescents, and the optimization of existing chemotherapy drugs may be an important reason [32]. In addition, mortality from lymphoma, brain and other neurological tumors has been significantly reduced in children and adolescents [23-33-34].

The European

The Population of Europe (referring to 40 countries in four regions of Europe identified by the United Nations) accounts for 9% of the world's total population, but 25% of the global cancer burden [35]. In 2018, there were about 3.9 million new cases of cancer in Europe, of which 53 percent (2.05 million) were men and women

47 percent (1.85 million). The most common primary tumors in men were prostate cancer (450,000, 21.8% of the total), lung cancer (312,000, 15.1%), colorectal cancer (272,000, 13.2%) and bladder cancer (154,000, 7.5%). Among women, breast cancer was the most common tumor (523,000, 28.2% of the total), followed by colorectal cancer (228,000, 12.3%), lung cancer (158,000, 8.5%) and endometrial cancer (122,000, 6.6%). Of the estimated 1.93 million cancer deaths in Europe in 2018, men accounted for 56% (1.08 million) and women for 44% (850,000). Lung cancer remained the most common cause of cancer deaths in men (267,000, 24.8%), followed by colorectal cancer (130,000, 12.0%) and prostate cancer (107,000, 10.0%). Breast cancer was the leading cause of death among women (138,000, 16.2%), followed by lung cancer (121,000, 14.2%) and colorectal cancer (113,000, 13.2%). Taking the UK as an example, the incidence rate of male cancer in the UK is 455.9/100,000, and the mortality rate is

185.7/100,000. Among them, the top three types of new cancer cases are prostate cancer, lung cancer and colorectal cancer respectively. Lung cancer is the most common cause of cancer death, followed by prostate cancer and colorectal cancer. The incidence of cancer in British women is 398.5 per 100,000, and the mortality rate is 138.7 per 100,000. Among them, the top three types of new cancer cases are breast cancer, lung cancer and colorectal cancer. Lung cancer is the most common cause of cancer death, followed by breast cancer and colorectal cancer.

Australia

According to the Report of the Australian Institute of Health and Welfare [36], In 2017, 134,000 new cancer cases were diagnosed in Australia, with 72,000 (54%) in men and 62,000 (46%) in women. The incidence in men was 1.2 times higher than in women. Age-specific rates for all cancers increased with age in 2017, with the highest rates for men and women aged 85 and older. Age-specific rates were similar for men and women under 30. The incidence was slightly higher in women than in men between the ages of 25 and 54, but lower in women than in men aged 55 and older. Lung cancer was the leading cause of cancer deaths in Australian men and women in 2017, with 5 179 and 3 842 deaths respectively. Prostate cancer was the second leading cause of cancer deaths in men (3,452 cases), followed by colorectal cancer (2,136 cases). These three leading causes of cancer deaths account for about 40 percent of all cancer deaths in men. Breast cancer was the second leading cause of cancer deaths in women (3,087), followed by colorectal cancer (1,978). These three leading causes of cancer deaths account for about 43 percent of all cancer deaths in women.

Latin America

By searching cancer death certificates and population data in the databases of WHO and Pan American Health Organization, a study obtained the statistical data of cancer mortality in seven Latin American countries from 1970 to 2015: Argentina, Brazil, Chile, Colombia, Cuba, Mexico and Venezuela [19]. It is estimated that the total death rate from cancer has declined in all countries except Argentina, where women are affected by breast cancer; Cuba had the highest incidence of all cancers in Latin America in 2019, 136.9 per 100,000 men and 90.4 per 100,000 women; Mexico had the lowest at 63.8 per 100,000 men and 61.9 per 100,000 women. From 1990 to 2019, cancer death rates will drop by about 18 percent in Argentina, 26 percent in Chile, 14 percent in Colombia, 17 percent in Mexico and 13 percent in Venezuela, equivalent to nearly half a million cancer deaths averted. Brazil and Cuba showed no declines.

The African

In 2018, there were 1,049,000 new cancer cases and 700,000 deaths in Africa, an increase of 23.8% (847,000 cases) and 15.6% (591,000 cases), respectively, compared with 2012 [37]. The most common type of cancer in women was breast cancer (1.34 million cases), followed by cervical cancer (99,000 cases). Prostate cancer was the most common among men (60, 000), followed by liver cancer (39, 000) and Kaposi's sarcoma (24, 000). Concord-3 (Global Cancer Survival Analysis) updated data from 322 cancer registries in 71 countries worldwide in 2014, but for Africa, only six countries were included Eight registries. In 2014, 322 registries around the world covered nearly 1 billion people, while cancer registries in Africa covered only 3.5% of the population [38]. This is due to the fact that almost all of the 54 countries in sub-Saharan Africa have low HDI values and high HDI [39].

Asia

According to IARC's GLOBOCAN2018 Statistical Report, 2018

There were 8.76 million new cancer cases and 5.5 million deaths in Asia, accounting for 48.4% and 57.3% of new cancer cases and deaths in the world, respectively [31]. Geographically, east Asia has the highest cancer incidence and death rates, south-central Asia has the lowest, and South-east Asia and West Asia are close. Compared with 2008, the incidence of cancer in both men and women in east Asia, southeast Asia and west Asia increased, with the most significant increase in west Asia, from 152.8/100,000 in men in 2008 to 190.1/100,000 in 2018, and from 119.5/100,000 in women to 154.6/100,000[40]. According to the international Center for Research on Cancer (IARC), The Incidence of cancer in Five Continents volume 11 (2008-2012) [41], the incidence of cancer in men in Israel, Turkey and the Philippines is gradually decreasing, while that in Japan and South Korea is still on the rise. Women in Japan, South Korea and China are all on the rise. In terms of cancer spectrum, the incidence of lung cancer and breast cancer ranked first in Asian men and women respectively, while other common tumors showed significant differences in different regions. The incidence of esophageal cancer in men of central and South Asia, prostate cancer in men of west Asia and cervical cancer in women of southeast Asia and central and South Asia is higher [3,7]. In addition, the incidence of colorectal cancer has been increasing in Asia since 2008, and it is now the third most common cancer in Asian men.

Cancer prevalence in China

As the most populous country in the world, China accounts for about 23% of new cancer cases and 30% of cancer deaths globally [42]. In addition, China accounts for

about 50 percent of the world's new cases of liver cancer, esophageal cancer, stomach cancer and more than one-third of new cases of lung cancer. China's cancer burden has been increasing in recent decades, posing a serious threat to public health and a heavy economic burden. According to the survey, the direct economic burden caused by cancer in 2015 was us $221.4 billion, accounting for 5.4% of the total health expenditure and 17.7% of the government's public health expenditure [43].

The country's 368 cancer registries reported registration data.

In 2015, there were about 3.929 million new cancer cases in China, and the incidence rate was about 285.83/100,000 [44], among which the incidence rate of males and females was 305.47/100,000 and 265.21/100,000 respectively, and the standardized incidence rate of Chinese population was 190.64/100,000. The world population standard incidence rate is 186.39 per 100 000. In 2015, there were about 2.338 million cancer deaths in China, of which the mortality rates of males and females were 210.10/100,000 and 128.00/100,000, respectively. The standardized mortality rates for China and the world were 106.72/100,000 and 105.84/100,000 respectively. China's population has the highest incidence of cancer types mainly for lung cancer, gastric cancer, colorectal cancer, liver cancer and breast cancer in women, the top ten cancer accounts for about 76.7% of all new cases, and lung cancer, liver cancer, gastric cancer, esophageal cancer and colorectal cancer is the leading cause of cancer, the top ten types of cancer deaths accounted for about 83.0% of all deaths.

Over the past 40 years, the death rate from lung cancer in China has increased fourfold to 45.87 per 100,000. Therefore, lung cancer has replaced gastric cancer as the main cause of cancer death in China [35]. Although the smoking rate in China is slowly

decreasing, the overall reversal of lung cancer development trend may take several decades. Therefore, the current increase in new cases of lung cancer may be the result of the past smoking epidemic, and the impact of the current anti-smoking campaign on the incidence of lung cancer will be felt in the future. Breast and colorectal cancer rates have risen rapidly over the past 20 years, especially in urban areas. From 1970s to 1990s, liver cancer, stomach cancer and esophageal cancer were the most common cancers in urban and rural areas, and are still the main cancer types in rural residents [45-46]. From 2000 to 2015, age-standardized morbidity and mortality rates for all three types of cancer showed a downward trend, as a result of the country's socio-economic development and a series of cancer prevention and control programs, as well as comprehensive prevention and control of infections such as hepatitis B and C viruses and Helicobacter pylori. It also plays a role in reducing the incidence of cancer [47-48].

In recent decades, the annual incidence of malignant tumors has remained about the same A 3.9 percent increase, and a 2.5 percent annual increase in death rates. From 2003 -- 2005 to 2012 -- 2015, age-standardized five-year relative survival rates for all cancers analyzed increased significantly, from 30.9% to 40.5%; Age-standardized five-year relative survival rates also increased for most cancer types, including esophageal, gastric, laryngeal, bone, cervical, bladder, and thyroid cancers [49]. This reflects the overall improvement in the quality of cancer treatment in China and the growth in medical resources, including the increase in the number of beds and registered doctors as well as the increase in medical expenditure.

According to the above data shows, the past decades, cancer has become a threat to human health and life of one of the major diseases, the morbidity and mortality

rate is very high, to China and countries in the world caused a huge loss, especially the impact to the whole human society is very large, so to explore one of the difficulties has become a hot spot in the field of cancer.

1.3 Common cancer treatments

In addition to the surgical resection methods commonly used in hospitals, there are also chemotherapy, radiotherapy, photothermal therapy, photodynamic therapy, gene therapy, immunotherapy and so on. Some of these technologies have already begun trial operation for clinical application, and are still under continuous research and improvement. In recent years, we can still see them in the top journals, and we can really understand the pros and cons of these treatments

1.3.1 Treatment methods

1.ChemotherapyTreatment

Principle: the use of chemical drugs to treat malignant tumors, intravenous injection, oral or other forms of chemotherapy drugs into the body to kill tumors.Analysis of advantages and disadvantages: Suitable for systemic tumors, such as hematologic tumors. But there are many side effects, such as the toxicity of anti-cancer drugs and the resistance of cancer cells to drugs, which will greatly reduce the efficacy of drugs, and cause systemic side effects such as nausea and loss of appetite.

2. Radiotherapy

Treatment principle: namely radiation therapy, with a variety of different energy rays (X ray, γ line, electron line) irradiation tumor, thereby inhibiting, killing cancer cells of a treatment method.

Analysis of advantages and disadvantages: The local treatment method can

assist surgical treatment and improve the effect. Like chemotherapy, it has the problem of "dividing the enemy and me", that is, cancer cells and normal cells are killed together, and there are shortcomings such as the effective dose and toxic dose are very close, and there are relatively large toxic and side effects.

3. Photodynamic therapy

Treatment principle: Similar to photothermal therapy, it is also used to irradiate the tumor site with a specific wavelength to activate the photosensitizer selectively gathered in the tumor tissue and trigger the photochemical reaction to destroy the tumor. However, photothermal therapy uses photo heat to kill cells, while photodynamic therapy uses photoinduced generation of reactive oxygen species and singlet oxygen to kill cells. Singlet oxygen and reactive oxygen species both have cytotoxic effects, especially singlet oxygen is the main damage form of tumor necrosis induced by photodynamic action. Singlet oxygen can destroy the microvessels in cancer tumor, resulting in local ischemia and cell death. Several days later, the tissue in this site will be necrotic and fall off, so as to achieve the purpose of local cancer treatment.

Pros and cons: Compared with traditional cancer therapy, photodynamic therapy has the advantage of being able to perform effective treatment precisely and with minimal side effects. But there are two headaches. Generally speaking, photosensitizer, light source and tissue oxygen are the most important components of PDT. However, due to the hypoxia of tumor site and the limitation of tissue penetration by excitation light, photodynamic therapy is not effective.

4. Gene therapy

Treatment principle: Normal gene is introduced into target cells to correct or

compensate the disease caused by defective/abnormal gene, so as to achieve the therapeutic purpose.

Gene therapy is a treatment that can treat many gene-related diseases. Gene therapy is used in clinical research of cancer treatment, accounting for about 65% of the total number of cancer clinical trials. This method does not take drugs, effective, non-toxic and good tolerance for many patients to bring good news, not like radiotherapy, chemotherapy, there are a lot of additional harm and adverse reactions to patients. But this method still exists some problems, such as safety, because is the introduction of foreign genes, can cause cell mutations situation, lead to serious immune response, long-term stability and exogenous genes in the body difficult to express, used in many genes and many factors such as hypertension, diabetes disease, caused by complexity is greatly increased.

5. Immunotherapy

Treatment principle: in view of the body's low or hyperactive immune state, artificially enhance or inhibit the body's immune function to achieve the purpose of treating diseases.

Analysis of advantages and disadvantages: Tumor immunotherapy aims to activate the human immune system and kill cancer cells and tumor tissues by its own immune function. Compared with traditional treatment, immunotherapy has obvious advantages of quick response, small side effects and lasting curative effect. However, there are still some shortcomings, such as the body's extremely fast immune response, so the intensity of tumor immune response needs to be well controlled.

1.4 Progress in nano-antitumor

1.4.1 Application of nano drugs in clinic

Cancer is one of the leading causes of human death. At present, cancer treatment still faces many challenges, among which, how to accurately deliver drugs to tumor lesions to achieve targeted therapy is a major scientific problem that needs to be solved urgently. With the rapid development of nanobiotics, anti-tumor nanomedicine is expected to improve cancer treatment strategies and improve therapeutic effects. Studies have shown that nanomedicine can not only improve the specificity of tumor tissue and inhibit tumor growth by changing the pharmacokinetics and tissue distribution, but also reduce the toxicity of drugs to normal tissues. In recent years, nano drugs have shown great application value and development prospect in anti-tumor drug delivery and improvement of cancer treatment effect. Scientists used the latest nanotechnology on the shape, size and function of nano drug rational design and control, to extend the time of the role of nanodrugs, nano drug outside surface can also be a target molecule modification, etc., increase the amount of drug concentration in the tumor tissue, achieved the integration of effective treatment of tumor and tumor diagnosis and treatment. Nanodrugs can achieve targeted delivery and intratumoral transport of drugs, avoid systemic non-specific release of drugs, improve pharmacokinetic and pharmacodynamic properties of drugs, and help drugs overcome the mechanism of drug resistance in tumor cells. In addition to conventional chemotherapeutic drugs, nanodrugs can also deliver polypeptide drugs, proteins, nucleic acids and other substances because they can protect the integrity and biological activity of bioactive substances during drug delivery.

The development of nanomedicine began in the 1960s, when scientists proposed the application of nano-lipid vesicles (i.e., liposomes) for drug delivery. Since then, a large number of nanodrug delivery systems have been developed in subsequent research. The main development history of nanomaterials can be summarized as follows: In 1976, Langer first proposed the delivery system of sustainable-release drugs. In 1980, Yatvin designed liposomes with ph-responsive drug delivery and active targeting for drug delivery. In 1986, Matsumura and Maeda proposed the enhanced permeability and Retention effect (EPR effect), that is, based on the pathological features of tumor vascular discontinuity and tumor lymphatic system unsound, Nanodrugs can accumulate at the tumor site and remain in the tumor tissue. In 1987, Allen proposed that surface modification of liposomes with polyethylene glycol (PEG) could reduce the clearance of liposomes by phagocytes in vivo, extend the blood circulation time of liposomes and provide more opportunities for liposomes to accumulate in tumor tissues. In 1994, Langer prepared the first long-cycle polyethylene glycol - poly (lactic acid - glycolic acid) nanoparticles. In 1995, Doxil®, a pegylated liposome loaded with the anti-tumor drug Doxorubicin (Dox), was approved by the U.S. Food and Drug Administration as the first nanodrug for clinical use in ovarian cancer, metastatic breast cancer, and HIV-related Kaposi's sarcoma. Since then, a variety of nanodrugs have entered clinical trials, some of which have been approved for the clinical treatment of tumors.

1.4.2 liposomes

Liposomes are bilayer spherical vesicles formed by lipid molecules (such as phospholipids and cholesterol, etc.) containing water-soluble chambers and vesicle shells. They have characteristics of plasticized particle size, good biocompatibility, low toxicity

and low immunogenicity, and can load different hydrophilic and hydrophobic drugs at the same time. Liposomes are one of the main types of anti-tumor nanomaterials and the most successful ones in clinical transformation. They are widely used in the treatment of cancer, inflammation and skin diseases. At present, liposomal coated doxorubicin has been applied in the clinical treatment of ovarian cancer, metastatic breast cancer, Kaposi's sarcoma and other tumors in many countries including China, and its commercial name is Doxil®. Adriamycin is a kind of chemotherapy drug, but there is cardiotoxicity, which affects its use in the treatment of tumors. Adriamycin wrapped by liposome can effectively reduce the toxicity of adriamycin, and achieve the safety of clinical use. In addition to Doxil®, a variety of liposomal nanomedicine drugs are also approved for clinical use. These nanomedicine drugs mainly include: 1) DaunoXome®, which is a 50 nm nM-sized liposome for the treatment of AIDS-related Kaposi's sarcoma; 2) Thioocyte ®, a non-pegylated adriamycin liposome with a grain size of 150 nm, used in the treatment of metastatic breast cancer (Europe and Canada); 3) MM-398®, a 100 nm irinotican liposome, is administered in conjunction with 5-fluorouracil and folate and is primarily used in the treatment of pancreatic cancer. In addition, a variety of liposome nanodrugs are in clinical trials, such as Themodox® and Lipolatin in clinical phase III trials, Lipoxal and Endotag-1 in clinical phase II trials, etc. Phase I liposomes include IHL-305 and LiPlaCis. Themodox® is a doxorubicin thermosensitive liposome for the treatment of hepatocellular carcinoma and breast cancer. In combination with radiofrequency ablation, Themodox® can rapidly change liposome structure to form a "small opening" to release doxorubicin at ambient temperatures ranging from 40 ℃ to 45℃. Lipolatin is a liposome preparation of the anti-tumor drug cisplatin, which is

suitable for the treatment of non-small cell lung cancer. Studies have shown that the composition of cisplatin liposomes contains anionic lipid molecules dipalmitoylphosphatidyl glycerol, which can assist cisplatin liposomes to achieve trans-membrane transport. In addition, Lipolatin remained in tumor tissue for a long time, and there were no adverse reactions during treatment. Lipoxal is a liposome preparation of oxaliplatin, which is suitable for the treatment of advanced malignant tumors. Phase I clinical results showed that oxaliplatin liposomes showed high therapeutic activity against a variety of tumors, such as gastric cancer and pancreatic cancer, and only when the dose reached 300-350 mg·m-2, oxaliplatin liposomes showed certain body toxicity, such as peripheral neurotoxicity. Endotag-1 is a cationic paclitaxel liposome. Its cancer treatment strategies include anti-tumor angiogenesis and anti-tumor cell growth, and it is suitable for breast cancer, liver cancer and pancreatic cancer. Ihl-305 is a pegylated irinotecan liposome suitable for the treatment of advanced solid tumors. Preclinical studies showed that IHL-305 showed antitumor activity in a variety of tumor models, such as colon cancer, non-small cell lung cancer, small cell lung cancer and prostate cancer, and significantly improved survival in tumor-bearing mice. LiPlaCis is a cisplatin liposome with phosphatase A2 (PLA2) responsive release agent and is indicated for advanced solid tumors. However, phase I results indicated that LiPlaCis had significant side effects, such as nephrotoxicity, and the clinical trial was discontinued. At present, a variety of nanodrugs based on liposome have been approved and entered clinical trials, because liposome is a relatively mature clinical drug model with low risk, successful marketing of liposome dosage forms as a reference and relatively mature preparation technology.

1.4.3 Nano micelle

Nanomicelles are core-shell polymer aggregates with particle size ranging from 10 to 200 nm formed by self-assembly of amphiphilic block polymers in aqueous solution. In the process of micelle formation, the hydrophobic drug molecule and the polymer segment have a synergistic effect, and are wrapped into the hydrophobic core to form drug-loaded nano-micelles. PEG is the most commonly used hydrophilic polymer for nano-micellar hydrophilic shells. The dense polyethylene glycol shell layer on micellar surface can effectively prevent non-specific adsorption or recognition of nano-micelles by plasma proteins or macrophages, and prolong the blood circulation time of nano-micelles. Currently, clinically approved nanomicelles include Genexol-PM® and Paclical®, both of which are nanomicellar pharmaceutical preparations of paclitaxel. Genexol-pm ®, with an average particle size of about 20~50 nm, is self-assembled from polyethylene glycol - polylactic acid block polymers and is suitable for the treatment of metastatic breast cancer (Korea). Paclical® has an average particle size of about 20~60 nm. The micelle structure of Paclical® introduces the amphiphilic surfactant XR-17 (A vitamin A analogue), which can be metabolized by the body, and is suitable for the treatment of ovarian cancer (Russia). Paclical® has been shown to provide significantly higher drug load than both Taxol® and Abraxane®, thus supporting the use of high dose therapy in patients with cancer. The pharmacokinetic behavior of Abraxane® is almost identical to that of Abraxane®, ensuring the safety of patients during treatment. In addition, a variety of novel nano-micelles are in clinical trials, most of which are prepared from biocompatible peg - poly amino acid block polymers, such as PEG - poly aspartic acid and peg - poly glutamate. The polymer nanomicelles in clinical trials mainly include:

1) NK105 (Clinical Phase III), peG-POLY (aspartic acid) nanomicelles loaded with paclitaxel (85 nm), for breast cancer and gastric cancer. Clinical trial results showed that NK105 showed good antitumor activity against breast cancer, and could significantly reduce the toxic and side effects caused by paclitaxel (such as neutropenia, etc.), with an overall response rate (ORR) of 25% (phase II clinical data). 2) NC-6004 (Clinical Phase III), pegyl-polyamino acid nanomicelle loaded with cisplatin (20 nm), suitable for the treatment of pancreatic cancer. The study showed that THE disease control rate of NC-6004 against pancreatic cancer was 64.7% (phase II clinical data), and it had a high tolerance dose. Only when the dose of NC-6004 reached 120 mg·m-2, cisplatin-related toxicity appeared. 3) NK012 (Clinical Phase II), pegylene-polyamino acid nanomicelles (20 nm) loaded with 7-ethyl-10-hydroxycamptothecin (SN38) for triple negative breast cancer, etc. Phase II clinical study showed that NK102 had positive efficacy in patients with recurrent small cell lung cancer, with an ORR of 22%. 4) NK911 (Clinical Phase II), doxorubicin-loaded pegylated poly (aspartic acid) nanomicelles (40nm) for a variety of solid tumors. Studies have shown that the plasma area under pharmacodynamic curve (AUC) of NK911 is twice that of free adriamycin, and NK911 only causes mild nausea and vomiting, but does not cause serious side effects such as bone marrow suppression. 5) NC-4016 (Clinical Phase I), pegyl-polyamino acid nanomicelles loaded with cisplatin (30nm), suitable for a variety of solid tumors. Preclinical studies showed that the plasma AUC of NC-4016 was about 1000 times higher than that of oxaliplatin, and showed high anticancer activity against mouse colon cancer, human pancreatic cancer, gastric cancer and melanoma in animal models, without significant systemic neurotoxicity in mice during treatment.

1.4.4 Nanoparticles

Nanoparticles refer to nanoparticles whose size is within the scope of nanometer scale. Their structure is mainly composed of shell, core and active substances (drugs, etc.). The properties of nanoparticles (such as stability, blood half-life, etc.) are mainly determined by the physical and chemical properties of the shell material and nanoparticles, while the core often determines the type of drug active substance loaded by nanoparticles. Nanoparticles can be loaded with a wide range of active substances, such as antitumor drugs, siRNA, proteins and contrast agents. Abraxane® and Transdrug® are currently clinically approved nanoparticle nanomedicines. With annual revenue of approximately $967 million, Abraxane® is one of the major success stories in nanodrug development. Abraxane® is a paclitaxel-binding albumin nanoparticles with an average particle size of approximately 130 nm. Abraxane® is indicated for pancreatic and metastatic breast cancer. In clinical trials, Abraxane® not only maintained the antitumor efficacy of paclitaxel, but also eliminated the toxicity associated with the emulsifier Cremophor®EL in Taxol®, a commercial paclitaxel formulation. Pharmacokinetic studies have shown that Abraxane® has a higher paclitaxel clearance rate and tumor distribution volume than Taxol®, based on "ligand receptor" targeting mediated by Abraxane® active albumin transport pathway. Abraxane® has a maximum tolerated dose (MTD) approximately 50% higher than Taxol®. Transdrug® is BioAlliance's Transdrug based doxorubicin nanoparticles, which are supported by polyisocyanylacrylate and are suitable for the treatment of hepatocellular carcinoma. In addition, a variety of nanoparticles are also in clinical trials, among which dhaD-PBCA-NPS and CRLX101 are mainly in clinical phase II trials, and Nanoxel® and DoceTaxel-

PNP are in clinical Phase I trials. DHAD- PBCA-NPS is a mitoxantrone nanoparticle suitable for hepatocellular carcinoma. CRLX101 is a kind of nanoparticle formed by wrapping camptothecin with polyethylene glycol and polylactic acid. CRLX101 can be used in combination with bevacizumab, which has been shown to exhibit high antitumor activity and be well tolerated against metastatic renal cell carcinoma. In addition, CRLX101 is also suitable for the treatment of non-small cell lung cancer. Nanoxel® is a non-albumin-bound paclitaxel nanoparticle with a size of approximately 10 to 50 nm and is suitable for advanced breast cancer. Nanoxel® significantly improves the pharmacokinetic behavior of paclitaxel, reducing side effects such as allergic reactions and fluid retention while maintaining its anti-tumor efficacy. Docetaxel-pnp is a polyene paclitaxel nanoparticles suitable for a variety of solid tumors. The results showed that the blood clearance half-life of Docetaxel-PNP was 1.5 to 2 times that of the anti-tumor drug Paclitaxel, which was more conducive to the accumulation of docetaxel-PNP in tumor tissues. Therefore, Docetaxel-PNP had better therapeutic effect and lower clinical toxicity. China has also made progress in the development of nano drugs such as nanoparticles. So far, a total of five pharmaceutical enterprises in China (ct, nolato, the weather is fine, jiangsu hengrui, qilu pharmaceutical and hunan koren) has been approved by the variety of paclitaxel combined with albumin type injection for clinical, and gradually into the clinical trials, among them, the 1.6 class 5 kinds of new drugs, 2.4 class one kind of new drugs, new drugs have seven kinds of 3.4.

1.4.5 Polymer-drug conjugates

(PDCs) are drug carriers that conjugate active drug molecules to polymers through chemical covalent bonds. The polymer materials used for polymer-drug coupling

should be highly soluble, non-toxic and non-immunogenicity in aqueous solution, mainly including poly [N-(2-hydroxypropyl) methyl acrylamide] (PHPMA), polyethylene glycol, polysaccharide polymers (such as hyaluronic acid, glucan, etc.) and polyglutamic acid. Currently, no PDCs nanomedicine has been clinically approved, but several PDCs nanomedicine are in clinical trials. PK1 (FCE28068, PHPMA-DOX conjugate) is the first N-(2-hydroxypropyl) methyl acrylamide polymer-drug conjugate to enter phase I clinical evaluation. Phase I clinical studies showed that PK1 showed anti-tumor activity against non-small cell lung cancer, colorectal cancer and drug-resistant breast cancer, but phase II clinical studies showed that PK1 only showed anti-tumor activity against breast cancer and non-small cell lung cancer patients. Other polymer-drug conjugals based on N-(2-hydroxypropyl) methyl acrylamide entering clinical evaluation include PK2 (PHPMA-DOx-galactoamine, Clinical Phase I/II), PNU-166945 (PHPMA-PTX, clinical Phase I) and PNU-166148 (PHPMA-CPT, Clinical Phase I). Clinical phase I) and so on. Among them, galactosamine structure targeting asialoglycoprotein receptor (ASGR) has been introduced into PK2 structure, which is suitable for the treatment of primary liver cancer. However, galactosamine can cause the accumulation of PK2 in normal liver cells. Lead to greater toxicity of its body. In addition, the clinical trial results of PNU-166945 and PNU-166148 were also unsatisfactory, and both showed obvious toxicity in the treatment of cancer, such as cystitis. Peg-based polymer-drug conjugants in clinical trials include Prothecan (PEG-CPT, Clinical Phase I). Prothecan contains about 1.7% camptothecin and has a plasma half-life of 72h, during which it maintains anticancer activity against a variety of solid tumors. The dose limiting toxicity was mainly neutrophils and thrombocytopenia. Polysaccharide polymer-drug conjugates in clinical

trials include AD-70 (clinical phase I) and so on. Among them, AD-70 is the first glucan-drug conjugate to enter clinical trials. The molecular weight of glucan carrier is about 70,000 g mol-1, and the drug loaded is doxorubicin. However, due to the easy uptake of dextran by the endothelial reticular system, AD-70 exhibits significant toxic and side effects, such as hepatotoxicity and thrombocytopenia. Clinical trials of polyglutamate polymer-drug conjugated compounds include Xyotax® (Clinical Phase III). Xyotax® is a conjugated compound of polyglutamic acid (Mw=17000 g/mol) and paclitaxel, and its drug loading capacity is up to 37%. Studies have shown that Xyotax® has good pharmacokinetic performance and has shown high antitumor activity in multiple tumor models, such as non-small cell lung cancer and ovarian cancer.

1.4.6 Inorganic nanoparticles

Inorganic nanoparticles have been widely concerned by researchers in the field of nanomedicine. Compared with organic nanomaterials, inorganic nanoparticles have better size and morphology controllability and larger specific surface area. In addition, due to the inherent properties of inorganic nanoparticles, such as surface plasmon resonance and magnetic response, inorganic nanoparticles are widely used in thermal therapy, thermal imaging, magnetic resonance imaging and other fields, and have great application prospects for the integration of nanomaterials in diagnosis and treatment. Common inorganic nanoparticles include magnetic nanoparticles, silica nanoparticles, carbon nanoparticles and quantum dots. Feraheme is a semi-synthetic superparamagnetic iron oxide nanoparticle that has received clinical approval for the treatment of iron deficiency anemia in patients with chronic kidney disease. Feraheme can also be used for magnetic resonance imaging. A variety of inorganic nanoparticles are also in clinical

trials, with applications ranging from tumor imaging to thermotherapy. Cornell Dots is a 124I radiolabeled silica nanoparticle modified with polyethylene glycol and cRGDY peptides suitable for imaging melanoma and brain tumors. AuroLase is a peG-modified silica - gold nanoparticles suitable for the photothermal treatment of metastatic lung cancer. Although inorganic nanoparticles have made great progress in the field of nanomedicine in recent years, their biosafety has always been a potential problem and needs to be evaluated.

1.4.7 Actively targeting nanomedicine

Active targeting refers to the introduction of active targeting molecules to increase the targeted enrichment of nano-drugs at the focal site and the internalization of tumor cells. The identification of tumor biomarkers is the basis for the selection and design of actively targeted nanodrug ligands. Common target molecules include antibody, non-antibody target molecules and aptamers. Antibody target molecules mainly include monoclonal antibodies and antigen-binding fragments, while non-antibody target molecules mainly include vitamins, polysaccharides and peptides. Tumor tissue targets can be divided into tumor cell targets and tumor endothelial cell targets. Tumor cell targets are transferrin receptors, folic acid receptors and glycoprotein receptors overexpressed by tumor cells. Tumor endothelial cell targets are vascular endothelial growth factor (VEGF), α V β3 integrin and vascular cell adhesion factor-1 (VCAM-1) overexpressed by tumor endothelial cells. At present, most of the nanomedicines are non-active targeting nanomedicines, whose medicinal properties have been confirmed by clinical trials. However, there is still a lack of active targeting nanomedicines on the market, mainly due to the lack of significant differences in clinical trial results. Compared

with active targeting, the accumulation of passive targeting nanomaterials in tumor tissues is largely determined by the physicochemical properties of nanomaterials. Therefore, even if the targeted ligand is missing, the targeting of nano-drugs to tumor tissues can be realized by optimizing the physical and chemical properties of nano-drugs, or non-specific uptake by tumor cells. On the other hand, active targeting can promote specific uptake of nanodrugs by cancer cells. Therefore, the development of active targeted nanomedicine is still a hot research topic in the field of nanomedicine.

At present, only a few active targeted nanomedicines have entered clinical trials. These nanomedicines mainly include:

1) McC-465 (Phase I), doxorubicin immunoliposomes modified with F(AB ')2 fragment of human monoclonal antibody GAH, which can specifically bind to gastric and intestinal tumor tissues. The results showed that McC-465 had no obvious tumor suppressive effect on CACO-2 tumor with GAH negative expression, but had obvious tumor suppressive effect on WIDR-TC and SW837 tumor with GAH positive expression.

2) SGT-53 (Phase I), liposome nanocomplex for p53 gene delivery modified by fragment of single chain antibody against transferrin receptor (TfRscFv), in which TfRscFv actively targets transferrin receptors in tumor cells. Studies have shown that SGT-53 can significantly enhance the sensitivity of tumor cells to radiotherapy/chemotherapy.

3) Bind-014 (Clinical Phase II), nanoparticles loaded with polyenetaxel, specifically targeting prostate specific membrane antigen (PSMA). Studies have shown that BINd-014 has a good inhibitory effect on a variety of tumor models, such as prostate cancer and non-small cell lung cancer, and its pharmacokinetic behavior is significantly

better than that of free dotenaxel, with lower systemic toxicity.

4) CALAA-01 (Phase I, terminated), a cyclodextrin and adamantane - polyethylene glycol coated nanoparticle, is the first active siRNA targeting nanodrug to enter clinical trials. Human transferrin (hTf) modified on the surface of CALAA-01 specifically targets the transferrin receptor, but unfortunately the clinical trials related to CALAA-01 have been discontinued due to cost and safety concerns. Although the progress of clinical transformation of nanodrugs with active targeting function is slow, the development and exploration of active targeted drug delivery system will be an important direction of nanodrug research and development in the future. Because of the specific recognition of tumor cells by actively targeted nanodrugs, precision in tumor treatment can be improved and unnecessary side effects to normal tissues and organs can be reduced. At the same time, different target molecules can be modified on the surface of ordinary nanomedicine, which can broaden the application range of nanomedicine and target different tumors according to the different target molecules, thus improving the targeting and effectiveness of nanomedicine on a single tumor.

1.4.8 Advantages and limitations of nanomedicine

Nanomedicine has many advantages in cancer treatment, including :

(1) the shape, size and function of nanomedicine can be rationally designed and regulated by using nanotechnology to achieve optimal performance;

(2) Nano-drugs can realize the integration of diagnosis and treatment;

(3) Nano-drugs tend to be preferentially enriched in tumor tissues;

(4) Nano drugs can reduce the toxic and side effects of drugs;

(5) Nanomaterials can make bioactive substances complete and bioactive during

drug delivery without being degraded by enzymes.

This paper reviews the current status of clinical transformation of antitumor nanomedicine, introduces the clinical application of antitumor nanomedicine and some candidates in clinical trials, as well as some challenges and opportunities of antitumor nanomedicine for tumor therapy.

Nano treat cancer although it has been developing rapidly, but there are still many problems need to continue to delve into, such as looking for small side effects, good biocompatibility, biodegradation, the load of high rate of drug carrier material, how to guarantee the accuracy of the carrier into the tumor cells, clear drug release of various physical and chemical conditions are yet to be further explored.

The development and clinical transformation of nanomedicine are both challenges and opportunities. In order to promote the clinical transformation of nanomedicine, the mechanism of action of nanomedicine in human body should be further explored. The key to efficient and targeted delivery of nanomedicine to tumor tissue lies in the concentration of nanomedicine in tumor site and the interaction between nanomedicine and tumor cells and tumor microenvironment. How to rationalize the design of nanomaterials to increase system stability and improve tissue distribution, how to overcome a series of in vivo biological barriers of nanomaterials, and improve the ability of tumor tissue targeting, tumor penetration and tumor cell internalization. How to overcome genetic diversity, heterogeneity and drug resistance of tumor cells. How to overcome the off-target effect of nano-drugs. Key scientific issues in the field of nanomedicine, such as how to conduct large-scale reproducible preparation and screening of nanomedicine, need to be solved. In addition, most of the clinically

approved nanodrugs are supported by existing anti-tumor active small molecule drugs, while new therapeutic agents (such as siRNA, mRNA and gene editing, etc.) and new molecular entities (such as kinase inhibitors, etc.) are expected to be included in the development of a new generation of nanodrugs. In addition, due to the lack of evidence for EPR effect in human tumors, a better understanding of tumor heterogeneity and EPR markers is needed to maximize the therapeutic effect of nanomedicines. Therefore, clinically relevant tumors and tumors with EPR response can be preferred as tumor models for nanomedicine research. At the same time, nanomedicine can also be developed and applied to intratumoral delivery of nanomedicine or imaging of tumor microenvironment, which is not based on EPR effect. As the research progresses, more nanodrugs will enter clinical trials and be approved for the treatment of cancer patients, improving the efficacy of cancer treatment, reducing the side effects of treatment, and improving public health.

There are many kinds of controlled drug release systems, which can be divided into diffusion mechanism, chemical reaction mechanism and solvent activation system according to the release mechanism.

1. Proliferation control drug release system

Diffusion control drug release system can be divided into storage type and matrix type. The former involves embedding the drug in a polymer carrier and then diffusing from the polymer system to release it into the environment. This kind of controlled release system is usually made of polymer materials into planar, spherical, cylindrical carrier forms, drugs embedded in them, and with time changes in a constant speed of release. In matrix release systems, the drug is bound to the polymer carrier in

the form of dissolution or dispersion. For the controlled drug release system using non-biodegradable polymer material as carrier, the drug solubility in the system is the controlling factor of its release rate. For biodegradable polymer materials, the drug release rate is controlled by the solubility of the drug in the system and the degradation rate of the polymer carrier. If the degradation rate is much lower than the diffusion rate, the diffusion becomes the controlling factor of drug release. Conversely, if the drug is difficult to move in the carrier, degradation becomes the controlling factor for release.

1.5 Chemical control of drug release system

Chemical control drug release system can be divided into two drug systems, that is, hybrid drug film biodegradable system and biodegradable macromolecule drug system. In the mixed drug film system, drugs are dispersed in biodegradable polymer materials, and it is difficult to diffuse in the polymer carrier. Only after the degradation of the outer polymer, drugs can be released from the carrier. In the biodegradable macromolecule drug system, the drug is linked with the macromolecule carrier or molecule in the form of chemical bond, and the drug release must be carried out by hydrolysis or enzymatic hydrolysis.

Solvent activation control drug release system

As a controlled drug release carrier, polymer can control drug release at a certain rate through osmosis and swelling mechanism. The former is based on the osmosis principle of semi-permeable membrane. The rate of drug release is related to the solubility of the drug, but has nothing to do with other properties of the drug. The latter is controlled by the swelling of the polymer to control the release rate of the drug. The drug is usually dissolved or dispersed in the polymer carrier. The drug does not diffuse

at first, but when the solvent penetrates into the polymer, the polymer begins to swell, the polymer chain relaxes, and the drug diffuses out of the polymer carrier. Therefore, the carrier of this drug controlled release system requires a polymer material that can be swollen. Such as EVA, PVA, etc

1.6 Overview of nano-hydrogels

Over the past few decades, nanotechnology has emerged as a powerful tool for studying processes involving biological systems. In particular, in the field of controlled drug release, nanocomposite hydrogel molecular networks or nanoparticles overcome some of the current challenges and are considered to be innovative materials. In the synthesis of drug carriers and natural polymers, hydrogels show great advantages. Previous articles have described a variety of novel and intelligent biocompatible hydrogels, including supramolecular and low molecular gels. In recent years, the development trend is to integrate nanoparticles such as polymers, metals and carbon-based nanomaterials into hydrogel networks, and prepare nano-composite hydrogels with high strength properties, which are used in various biomedical applications [50,51,52].

Research on hydrogels began in the early 1940s, methods for synthesizing hydrogels emerged in the 1950s, and Wich and Lim developed a hydrogel for biological mechanisms in the early 1960s. Due to the unique properties of hydrogels, in order to improve and expand the role and potential of hydrogels, people have paid a lot of efforts in the research and synthesis of hydrogels. In recent years, the continuously developing hydrogel technology has been increasingly mature in the field of medicine and biomedicine, showing its great potential and achievements [53].

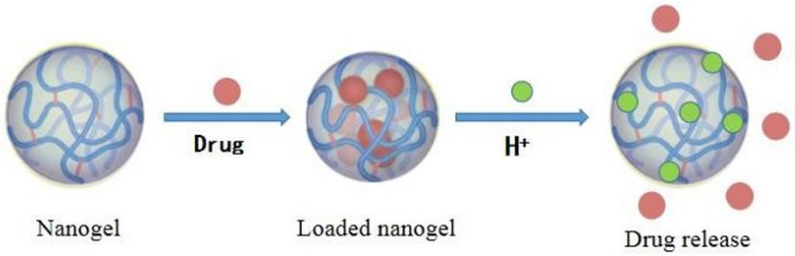
A nanohydrogel is usually defined as a watery dispersion of hydrogel particles

made by physically or chemically crosslinked nanoscale polymers. Broadly speaking, hydrogels have a 3D spatial network structure, which can absorb water hundreds or even thousands of times its own weight and only swell in water without dissolution. Hydrogels have a strong affinity for water, mainly due to the existence of hydrophilic groups, such as -oh, -so3h, -nh2 [54,55]. Hydrogels have high water content, but different hydrogels have different water content, mainly because different materials contain different functional groups, hydrogels synthesized from different materials show different properties. It is found that hydrogel has some physical and chemical properties similar to living tissue, which is incomparable to other biological synthetic materials. Molecular chain crosslinking in hydrogels can be divided into two main ways. One interacts with each other in physical ways, through electrostatic, van der Waals force and hydrogen bond interaction, which can provide force in polymer network. There is also a chemical reaction [56].

Drug loading is usually accomplished spontaneously by electrostatic, intermolecular force and hydrophobic interaction between drug and polymer matrix. After drug loading, the nanogel forms stable nanoparticles and the drug is trapped in them. Generally, a dispersed hydrophilic polymer, polyethylene glycol, is introduced into the structure of nanogels to prevent the aggregation of nanogels after preparation and drug loading [57][58]. Nanogels are outstanding for their high load capacity, high stability and responsiveness to environmental factors.

Way of drug carrier. Since the first review of the synthesis and application of nanogels in 2002, this novel nanomaterial has attracted more and more attention in the delivery of drugs, biomolecules and developer. The following figure shows drug loading

and drug release mechanism of nano-hydrogel.



drug loading and drug release mechanism of nano-hydrogel

1.7 Feasibility analysis

After reviewing these reviews and documents, we decided to continue our research on one of the current hot topics - the design of nano anticancer drugs. However, I want to add some biological macromolecules that are necessary and beneficial to human body but cannot be synthesized by ourselves into the raw materials. After screening, I finally decide to use leucine to carry out the experiment. Here's why:

Leucine, isoleucine, and valine are branched-chain amino acids that help promote post-training muscle recovery. Leucine is one of the most effective branched amino acids, preventing muscle loss because it is faster broken down into glucose. However, the solubility of leucine itself is very poor, and the amino and hydroxyl groups on the molecule are prone to hydroxylamine reaction, resulting in more difficult synthesis. In the end, it is proposed that leucine is first modified with long chain acid to protect the amino group on the molecule, and then grafted to hydroxyethyl starch. Finally, it is mixed with camptothecin and self-assembled into drug-loaded nanospheres. First refer to the physical and chemical properties of the raw materials involved. After simple chromatography column and steam operation, I extracted lauric acid modified leucine,

and then measured by Fourier infrared spectrometer results show that lauric acid successfully modified leucine, laying a foundation for the next experiment.Hydroxyethyl starch (HES) is a semi-synthetic polysaccharide, is corn or potato amylopectin glucose ring hydroxyethyl formation of polymer complex. It is often used as plasma dilatant in clinic. With other than expansion agent such as dextran and plasma albumin, hydroxyethyl starch has a lower incidence of allergic reactions, does not cause infusion caused by bacterial and viral infections, and no drug interactions, and more cost-effective, has been used in drug carrier mainly has the following advantages: (1) hydroxyethyl starch has good solubility in water; ② Hydroxyethyl starch has good biocompatibility and biodegradability; (3) Hydroxyethyl starch is widely used as plasma dilatant in clinic, which is clinically acceptable. (4) Hydroxyethyl starch has been mass-produced; (5) Hydroxyethyl starch has structural prunability; ⑥ Hydroxyethyl starch contains a large number of hydroxyl groups which can be modified by multi-functionalization. This provides a basis for further design of nanospheres.

Conclusion to section 1

In the first part, we discuss cancer incidence statistics for major countries in the world, such as Australia, The United Kingdom, the United States and China. We also describe the advantages and disadvantages of the current mainstream anticancer methods, as well as the main preparation methods of nano drugs in clinical anticancer. Finally, the feasibility analysis of this experiment is discussed.

2 Preparation and characterization of nanospheres

This part involves the synthesis and characterization of the experiment. We first modified leucine, then grafted it to hydroxyethyl starch, and also synthesized a part of serine. The instruments and reagents used in the experiment, the synthesis steps, and the characterization of each part will be described below.

2.1 Laboratory instruments and reagents

2.1.1 Laboratory instruments

Experimental instrument	model	manufacturer
Rotary evaporator	QSB-2100	TOKYO RPKAKAI
Vacuum drying oven	ZKT-50E	Nanjing Baifunuo Co
Circulating water vacuum pump	SHK- iii	Zhengzhou Ketai Experimental Equipment Co
Electric blower drying oven	DHG-9055	Shanghai Yiheng instrument
Heat collecting magnetic stirrer	DF-101Z	zhengzhou Ketai Experimental Equipment Co., LTD
Vacuum freeze dryer	ScientzS CIENT-20ND	Ningbo Xinzhi Biotechnology Co

GPC	G2500H XL	Shimazu, Japan
Transmission electron microscope	Quanta 200	Japan Electronics Co., LTD
Atomic Force microscope	Mutimo de 8 Nanoscope V	Bruck，Germany
Fourier Transform infrared spectrometer	IRPresti ge-21	Shimadzu, Japan
Uv-uv visible light calorimeter	UV-1700	Shanghai Shupei Experimental Equipment Co., LTD
Nuclear magnetic resonance Spectrometer	AVAN CE II 400	Bruck，Germany
Zeta potentiometer	ZS-90	British Malvern Instrument Co
Cytological centrifuge	Cyto-Tek 2500	Netherlands

2.1.2 Experimental drugs and reagents

Experimental drugs and reagents	**specifications**	**manufacturer**

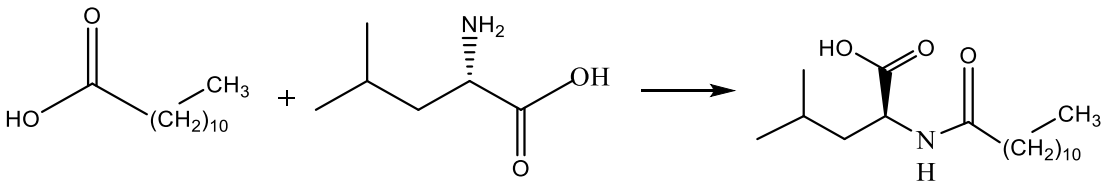
L-Leucine	Analysis of pure（MW131.7)	Macklin
Lauric acid	Analysis of pure（MW200.32)	Macklin
Hydroxyethyl starch	Analysis of pure（MW200.33)	Shanghai Yuanye Biological Co., Ltd
serine	Analysis of pure	Macklin
Dichloromethane	Analysis of pure	Tianjin Fuyu fine Chemical Co. Ltd
methanol	Analysis of pure	Tianjin Fuyu fine Chemical Co. Ltd
DMSO	Analysis of pure	Macklin
DIC	Analysis of pure	Macklin
DCC	Analysis of pure	Macklin
NHS	Analysis of pure	Macklin

DAMP	Analysis of pure	Macklin
HCL	Analysis of pure	Macklin
NAOH	Analysis of pure	Macklin
Dialysis bag	MD44-5M（3500Da）	Its group
camptotHESin	Analysis of pure	Macklin
PBS	Cell level	Beijing solarbio science technology Co.,Ltd

2.2 Modification of Leucine

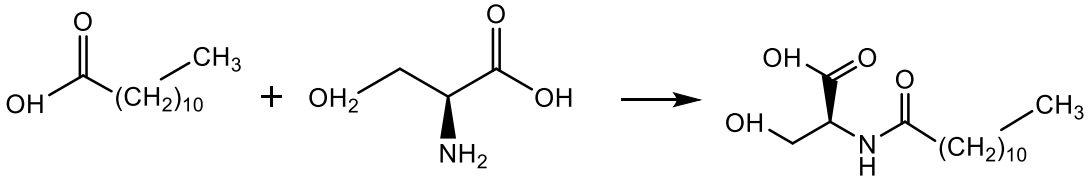
1g Leucine and 1.216g lauric acid were accurately taken. 1.253 gDCC; 0.699gNHS, then 15mL DMSO was added to the reaction bottle, and after its dissolution, it was stirred at 550r/min at room temperature for 12h (note: quantitative methylene chloride can be added). Filtrate, add 300ml dichloromethane to wash three times, discard white solid, preserve liquid. Add an appropriate amount of distilled water to extract several times until the white turbidity of the upper layer is reduced, then remove the clear liquid, and finally take the wet chromatography column to obtain the sample solution, and finally steam until the solid appears, to obtain Lauric acid modified Leu sample, stored in the -20℃ freezer. The reaction mechanism of lauric acid modified Leucine is

shown below



lauric acid modified Leucine

1g serine and 1.112g lauric acid were accurately taken. 1.153 gDCC; 0.499gNHS, and then 15mL DMSO was added to the reaction bottle. After its dissolution, it was stirred at room temperature at 550r/min for 12h (note: quantitative methylene chloride can be added). Filtrate, add 300ml dichloromethane to wash three times, discard white solid, preserve liquid. Add an appropriate amount of distilled water to extract several times until the white turbidity of the upper layer is reduced, then remove the clear liquid, and finally take the wet chromatography column to obtain the sample solution, and finally steam until the solid appears, then obtain Lauric acid modified Serine sample , the sample stored in the -20℃ freezer.



lauric acid modified serine

2.2.1 Characterization of lauric acid modified Leucine

(1) Lauric acid -Leu infrared spectrum test

Lauric acid-Leu of a certain quality was taken and dried in an electric drying oven for 8 h at 75 ℃. Take proper amount of dry KBr and grind it in agate mortar, grind

it to no particle feeling, and press blank KBr sample. A certain amount of dried Lauric acid-Leu was added into an agate mortar and ground for another 10 min to make the Lauric acid -Leu and KBr fully mixed, and the sample was pressed using a tablet press. Fourier transform infrared spectroscopy (FTIR) was used for analysis.

At the same time, the infrared spectrum of Leucine was used as the control to determine whether the reaction occurred.

(2) Lauric acid -Leu NUCLEAR magnetic hydrogen spectrum test

In order to further verify the success of Lauric acid -Leu grafting, 1h NMR test was carried out. Lauric acid-Leu with a certain quality was dried in a vacuum drying oven at 50 ℃ for 12 h. A clean nuclear tube was taken, 20 mg Lauric acid-LEU was added, and DMSO was added to make the sample completely dissolved. The signal was collected by NMR, and the Lauric acid-Leu hydrogen spectrum was obtained. At the same time, the nuclear magnetic hydrogen spectrum of Leucine was detected as the control, and the success of the reaction was judged by comparison.

2.3 Preparation and characterization of hydroxyethyl starch grafted with modified Leucine

1g hydroxyethyl starch (hydroxyethyl starch repeat unit 0.4EQ) was precisely weighed and added into the reaction flask for drying and dehydrating, followed by 25mL DMSO, which was thoroughly stirred at room temperature until dissolved, followed by Lauric acid-Leu, followed by DIC after 5 minutes, and stirred continuously for 15 minutes. Add DAMP 40mg of catalytic capacity at room temperature, stir for 24h, and dialysis for 72h with a dialysis bag of 3500Da of molecular retention. Change water for two hours in the early stage and 8h in the later stage, and prepare Leu-modified

hydroxyethyl starch by freeze-drying. (Hydroxyethyl starch is slightly soluble in DMSO at room temperature, can be heated to 70-80℃, stir to dissolve)

The gradient of this group of experimental samples is set as follows:

HES/g	DMSO/ ML	Lauric acid -Leu/ mg	DIC/mg	DAMPmg
1	25	200	119	40
1	25	300	181	40
1	25	400	241	40
1	25	500	302	40
1	25	600	362	40

2.3.1 Characterization of Lauric acid- Leu-HES

(1) Lauric acid-LEU-HES infrared spectrum test

Lauric acid-LEU-HES of a certain quality was taken and dried in an electric drying oven for 8 h at 75 ℃. Take proper amount of dry KBr and grind it in agate mortar, grind it to no particle feeling, and press blank KBr sample. A certain amount of dried

Lauric acid-Leu-HES was added to the agate mortar for further grinding for 10 min to make Lauric acid-Leu-HES and KBr fully mixed, and the sample was pressed by a tablet press. Fourier transform infrared spectroscopy (FTIR) was used for analysis.

At the same time, the infrared spectrum of Lauric acid- Leu was used as the control to determine whether the reaction occurred.

(2) Lauric acid-Leu-HES NUCLEAR magnetic hydrogen spectrum test

In order to further verify the grafting success of Lauric acid-Leu-HES, 1h NMR test was carried out. Lauric acid-LEU-HES was dried in a vacuum drying oven at 50 ℃ for 12 h. Clean nuclear magnetic tube was taken, 20mg Luric acid-LEU-HES was added, and DMSO was added to make the sample completely dissolved. The signal was collected by NMR, and the hydrogen spectrum of Lauric acid-Leu-HES was obtained. At the same time, the nuclear magnetic hydrogen spectrum of Lauric acid- Leu was detected as the control, and the success of the reaction was judged by comparison.

2.4 Preparation of Lauric acid-Leu-HES nanospheres

The preparation of nanospheres was carried out by hydrophilic and hydrophobic self-assembly principle. 50mg Lauric acid-LEU-HES and 25mg camptotHESin were accurately weighed, and then 50mL DMSO was added (at a speed of 1080r/min, 50mL deionized water was slowly dropped into a drip hopper, and the dripping was completed in half an hour. The reaction was continued to be stirred for 6h, and then transferred to a 3500Da dialysis bag for dialysis for 48h. Deionized water was changed once every 8 hours. After that, the liquid in the dialysis bag was transferred to a glass petri dish and freeze-dried in a vacuum freeze-drying oven for 24 h, and Lauric Acid-Leu-HES nanosphere samples were obtained.

2.4.1 Characterization of Lauric acid-Leu-HES nanospheres

(1) Transmission electron microscopy and scanning electron microscopy of Lauric acid-LEU-HES nanospheres

Lauric Acid- LEU-HES was observed by TEM. Sample preparation: Lauric acid-LEU-HES nanospheres were obtained and dissolved for dialysis. The post-dialysis solution was dipped in copper mesh and dried in a vacuum drying oven at 70 ℃ for 12 h. Then, 2% of the dye phosphotungstic Acid solution was taken with a rubber dropett for staining and dried in a vacuum drying oven at 70 ℃ for 12 h. The microscopic morphology was observed by TEM.

SEM was used to observe the appearance of the nanospheres. Sample preparation: Paste a conductive tape of appropriate size on a clean and tidy SEM sample holder, prepare post-dialysis Lauric acid-LEU-HES freeze-dried powder, dip a small amount of the powder with a cotton swab, and apply it evenly on the surface of the conductive tape. The sample pedestal containing samples was sprayed with gold and put into scanning electron microscope to observe the appearance and morphology characteristics.

(2) Atomic force electron microscopy test of Lauric acid-LEU-HES

Atomic force microscopy (AFM) was used to analyze the surface appearance of samples. A small amount of dialyzed Lauric acid-LEU-HES was taken through a straw and dropped on a 5 mm×5 mm quartz plate, placed in a vacuum drying oven, set at 50 ℃, and dried for 10 h. The sample image information measured by AFM is processed by NanoScopeAnalysis software, and the atomic force microscope image is output.

(3) Lauric acid-Leu-HES particle size test and Zeta potential test

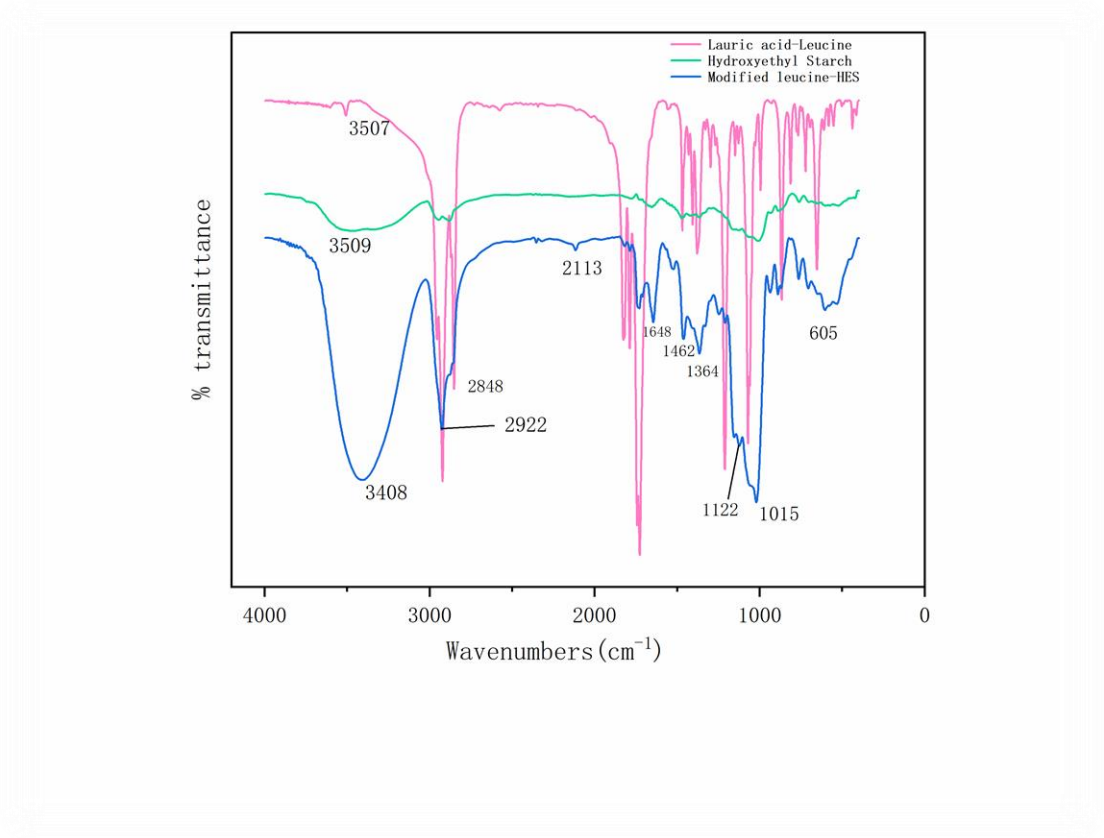
The sem data can describe the morphological characteristics of Lauric acid-LEU-HES, but the particle size analysis is only at a relatively simple stage, and the particle size analysis needs to be more accurate. Lauric acid-LEU-HES was dissolved in ultrapure water and configured to a sample solution of 0.3%. Then, the samples were filtered with 0.22μm filter membrane, and the cuvette was washed with sample solution twice. The cuvette was loaded to two-thirds of the surface, and the data were scanned three times by Zeta laser particle size analyzer. The average value of the three results was taken as the reference particle size.

As Lauric acid-Leu-HES nano-hydrogel, pH is an important factor affecting Zeta potential. In order to explore the Zeta potential of Lauric acid-Leu-HES nano-hydrogel at different pH values, We adjusted the pH of the nano-hydrogel solution to 3, 4, 5, 6, 7, 8, 9, 10. Aqueous solution of HCl and NaOH was used as the regulator of pH value, and the pH value was adjusted to the set value. Then the modified nano-hydrogel solution was transferred to a 5 mL sample tube. Zs-90 Zeta potentiometer was used to measure the Zeta potential of nano-hydrogels at different pH values.

2.5 Results and discussion

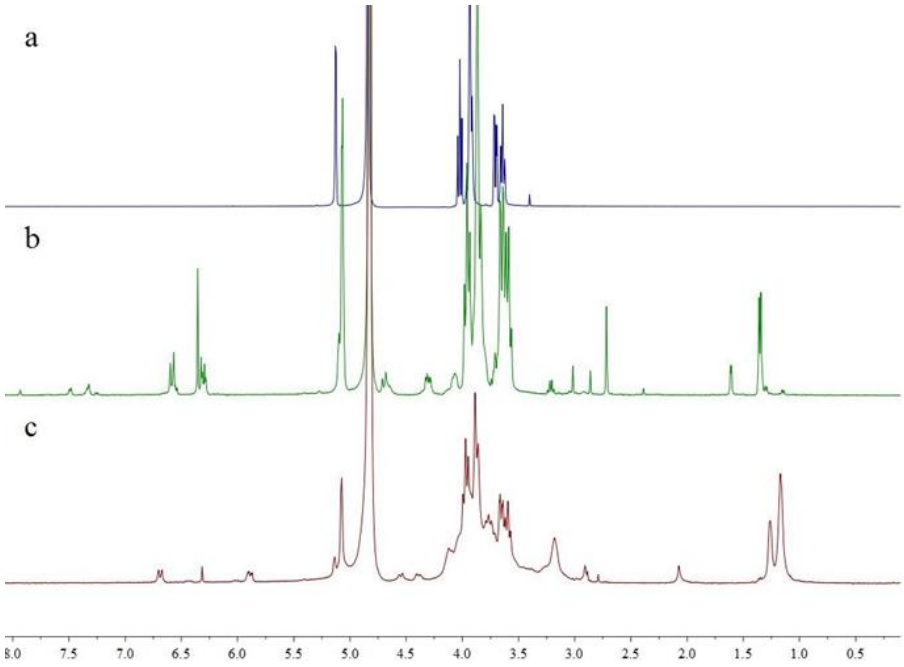
We have designed the optimal reaction conditions through many experiments. The ideal experimental product was obtained.

2.5.1 Fourier infrared spectroscopy analysis



The structure of Lauric acid-Leu-HES copolymer was confirmed by FTIR spectroscopy, as shown in the figure above. In the infrared spectra of HES, Lauric acid-Leu and Lauric acid-Leu-HES, the characteristic peak of -- OH tensile vibration is 3507 cm-1. Lauric acid-Leu-Hes is connected by amide bond. The characteristic peaks of -- CH tensile vibration are 2922 cm-1 and 1015 cm-1, respectively. In contrast, there were two newly formed peaks at 1648 and 1015cm-1 in Lauric acid-Leu-HES, which could be attributed to C = O and C = C tensile vibrations, respectively, indicating successful grafting of Lauric acid-modified leucine to hydroxyethyl starch.

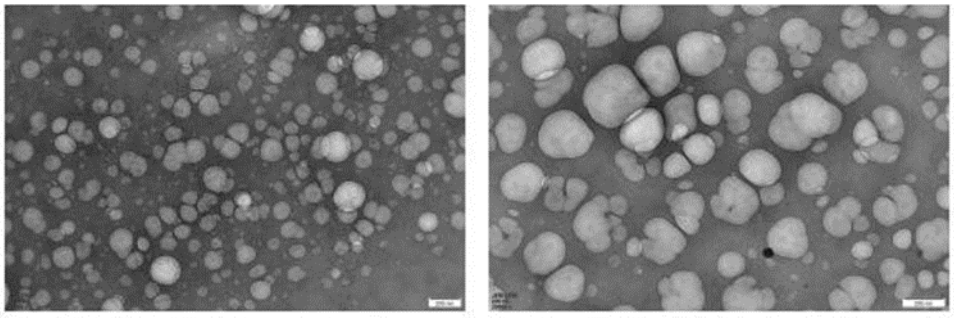
2.5.2 NUCLEAR magnetic hydrogen spectrum analysis



(a) Lauric acid-Leu,(b) HES, (c) Lauric acid-Leu-HES nanohydrogels

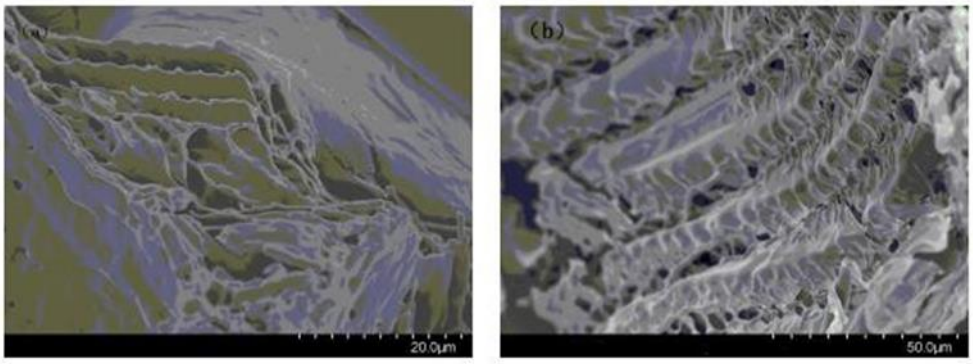
The figure above shows the 1H NMR spectra of (a) Lauric acid-Leu,(b) HES, and (c) Lauric acid-Leu-HES nanohydrogels. Obviously, the peaks at 5.08, 3.98, 3.67, and 3.60 PPM, FIG. (a) can be attributed to the H-1, H-3, H-2, and H-4 protons of Lauric Acid-Leu respectively, and to H-5 and H-6 at 3.92 -- 3.87 PPM. The new peaks at 6.53 and 6.25 PPM in Figure (b) correspond to the proton of an olefin in HES, confirming the successful introduction of Lauric acid-LEu into HES. In Figure (c), a proton of Lauric acid-LEu migrates from 6.25ppm to 5.86ppm, which can be explained by the chemical reaction of the carboxyl group in Lauric acid-LEu with -NH2 in Lauric acid to form an amide bond. In addition, peaks at 1.22-1.13 PPM were attributed to methyl groups of Lauric acid-leu, and all results clearly showed that Lauric acid-leu was successfully grafted to HES under mild conditions.

2.5.3 TEM and SEM analysis



Lauric acid-LEU-HES- Scale of 200nm

The TEM diagram of Lauric acid-Leu-HES is shown in the figure above. The water nanospheres obtained in our experiment are smaller in size and more evenly distributed, which is an ideal situation.

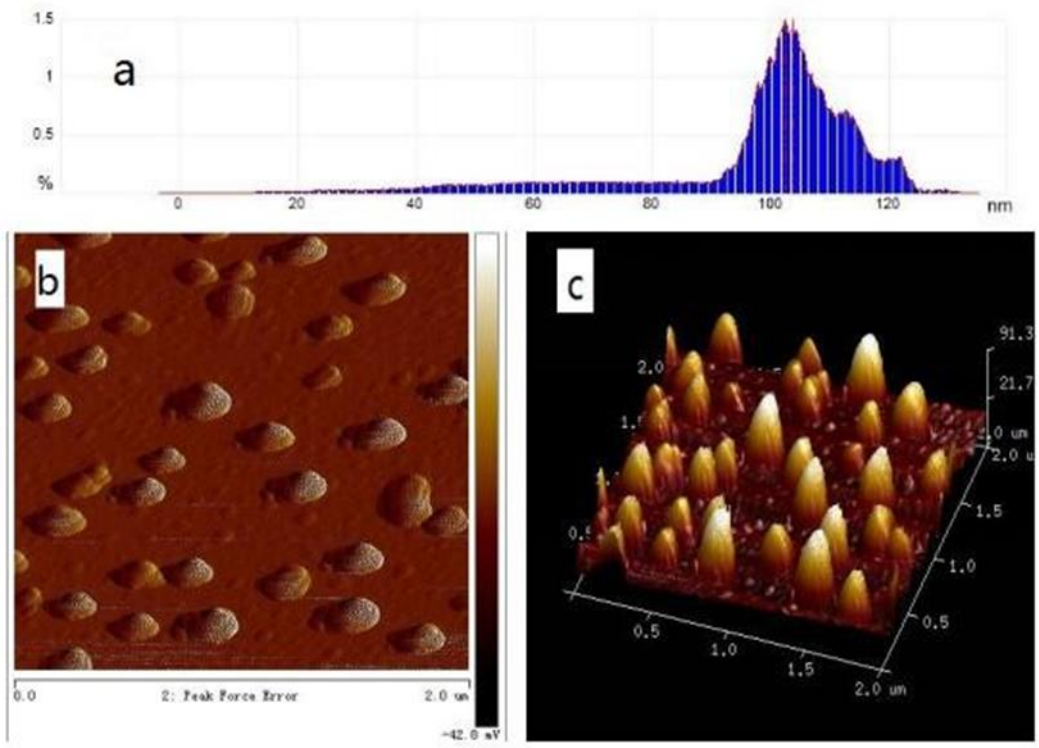


Lauric acid-Leu-HES SEM-Scale of 200nm

The microstructure (including porosity, density and surface area) of Lauric acid-Leu-HES nano-hydrogel can directly affect its performance as drug carrier and delivery system. Therefore, the surface morphological characteristics of our synthetic copolymer were observed by SEM, and the results were shown in Figure 3.8. The copolymer shows a wrinkled surface structure and a variety of uniformly distributed pits, and some randomly distributed holes can be observed on its surface. This unique structure observed

in Lauric acid-Leu-HES suggests that the copolymer can be used as a drug carrier.

2.5.4 Atomic force electron microscopy analysis



AFM images of Lauric acid-Leu-HES :(a) size distribution, (b) plane graphics, (c) three-dimensional image, scale 2 μm

Atomic force microscopy (AFM) images can provide surface morphological characteristics of nanoparticles, such as surface roughness, which is crucial for studying the relationship between the surface of nanoparticles and their biological properties. In order to obtain more information about the surface morphological characteristics of our synthesized copolymer, AFM images of Lauric acid-Leu-HES were measured and the results are shown in the figure above.

The size of copolymer is distributed in the range of 95-120 nm. Figure A is the particle size analysis diagram. The size is small enough, indicating that its specific surface area is large, which can achieve high drug loading efficiency. Figure B is the

plane plan of AFM, and Figure C is the three-dimensional image, indicating that the size of Lauric acid-Leu-HES is uniformly distributed and the particle size is nanometer. AFM images directly show the overall morphology of Lauric acid-Leu-HES, which can be used for drug loading.

2.5.5 Particle size test and Zeta potential test analysis

mL auric-acid-Leu:mHES	Average size	PDI
200 :1	255nm	0.47
300 :1	134.3nm	0.38
400 :1	140.6nm	0.45
500 :1	125.1nm	0.44
600 :1	166.4nm	0.44

The particle sizes of Lauric acid-Leu-HES and PDI were further analyzed by dynamic light scattering (DLS), as shown in the figure above. All measurements were made in triplicate at 25 ℃, pH=7.4. The average particle size of Lauric acid-Leu-HES

was different with different molar ratios, and the ratio particle size of 500:1 was the best. The PDI of Lauric acid-Leu-HES is less than 0.5, indicating that the dispersion and stability of hPC-G-HP -β -Cd are good. The successful preparation of Lauric acid-Leu-HES not only improves the stability of Lauric acid-Leu-HES, but also reduces the particle size of Lauric acid-Leu. The prepared nano-hydrogel meets our expectations.

We also studied Lauric acid-Leu-HES particle sizes under different pH conditions, ranging from 2 to 10, and obtained the following figure by DLS particle size test. It can be seen from the figure that with the increasing pH, the particle size of the nano-hydrogel first decreased to the minimum of 125.1 nm, and then the pH value was around 7.0 and pH > 8, and the particle size kept increasing. When the pH is between 7 and 8, the particle size is in the minimum state. We believe that the pH of the prepared nano-hydrogel is 7.5, which is suitable for human tissues.

Zeta potential of Lauric acid-Leu-HES nano-hydrogel at different pH values

pH								0	1
Zeta	6.1	3.0	3.2	.9	.6	0.2	0.5	1.2	1.7
（mV）									

The Zeta potential of Lauric acid-Leu-Hes copolymer was measured at 25 °C in a wide pH range, and the results are shown in Table 3.5. It was found that the synthesized nano-hydrogel appeared isoelectric point in the pH range of 7-8, at which time the nano-particles had no charge, while the normal pH of human body was 7.4. It was used in

human body without blood or tissue electrolyte disorder, indicating its potential application in physiological pH.

Conclusion to 2 part

This chapter introduces the preparation of Lauric acid-LEu by dehydration condensation reaction promoted by DCC and NHS, and the grafting of HES with Lauric acid-Leu by DIC and DAMp-mediated amide reaction. Finally, Lauric acid- LEU-HES nanospheres were prepared by hydrophilic self-assembly.

In the first part of this chapter, leucine was modified with lauric acid, and characterized by Fourier infrared spectroscopy and NUCLEAR magnetic hydrogen spectroscopy, indicating the successful modification of leucine. The modified Lauric acid-Leu was grafted onto HES. The successful grafting of Lauric acid-Leu onto HES was confirmed by ir, NMR and characterization. The morphology of the copolymer was analyzed by SEM, TEM and AFM.

We used Zeta analyzer to analyze the particle size and Zeta potential of nano-hydrogels, and analyzed the particle size of nano-hydrogels at different pH values. The results showed that the particle size of the prepared aqueous nanocapsules was 125nm, and the size changed in different pH environments. The particle size was the smallest at the pH of 7-8, which was 119.2nm. The Zeta potential was the largest and the solution was the most stable. Nano hydrogels have uniform and stable particle size distribution and good particle strength. Hydroxyl and amide groups exist on the surface of the prepared nano hydrogels, providing excellent properties for drug loading.

In this chapter, in the process of grafting hydroxyethyl starch, we designed an orthogonal test to investigate the degree of grafting Lauric acid-Leu and HES at different

reaction molar ratio, reaction time and reaction temperature to determine the best reaction conditions. The optimal reaction conditions were Lauric acid-Leu and HES molar ratio of 1:2. Since the preparation of nano-hydrogels is not good for dispersing after freeze-drying, we added the nano-hydrogels after preparation

Nano-hydrogel freeze-dried powder with good redispersibility was obtained by selecting 8% mannitol solution protector and using 1/2 of the volume of dialysis liquid.

After the above preparation experiments, we can basically judge that Lauric acid-LeU-HES nanohydrogel has been successfully prepared.

2.6 Properties of Lauric-Acid-Leu-HES and Lauric-Acid-Serine-HES nanospheres

2.6.1The introduction

Among known anticancer therapies, chemotherapeutic drugs are often used to increase the lethality of cancer cells while minimizing the toxicity to normal cells. However, no matter what methods are adopted to reduce the side effects of chemotherapy drugs, they are limited. Scientists are constantly looking for ways to selectively combine with chemotherapy drugs with the help of nanocarriers, so as to achieve the target efficacy while reducing the dose of chemotherapy drugs. Chawla and Amiji have provided insights into the development of nanocarriers for cancer drug delivery through the use of liposomes as drug carriers. Nanoparticles, as a characteristic nanotechnology, can adsorb drugs or bind them to their surfaces in different ways, thus improving the targeting ability of drugs. Drug targeting using nanoparticles shows many advantages over other delivery systems, such as their small size and ease of penetration

Tumor permeators accumulate in the site, thus achieving the role of tumor treatment, and due to its unique nano size, easy to intravenous, intramuscular and subcutaneous administration of drugs. Currently, nanoparticles have been used to treat a variety of cancers, such as lung cancer, where they are a useful and safer tool.

In the previous chapter, we observed the particle size and dispersion of lauric-acid-LeU-HES nanoparticles by the morphology characterization of the previously prepared nanospheres. In this chapter, the binding degree of Lauric-acid-Leu-HES and actothecin was calculated by ULTRAVIOLET spectrophotometry. That is, the drug loading rate and release rate of camptothecin. In order to determine the effective storage life and stability of lauric-acid-LEU-HES nanoparticles, the storage stability of the prepared lauric-acid-LeU-HES nanoparticles was tested. Finally, in order to determine the uptake capacity of cancer cells to the Lauric-acid-LeU-HES nanoparticles prepared by us, the Lauric-acid-Leu-HES nanoparticles were labeled with fluorescence and observed under fluorescence microscope. Determining that lauric-acid-LEU-HES nanoparticles can be taken up by cancer cells and selected for different types of cancer cells,

The cell inhibition rate was tested to further confirm that the Lauric-acid-LEU-HES nanoparticles prepared by us did have an inhibitory effect on cancer cells

The nanoparticle prepared in this paper is selected as the final dosage form, not only based on the unique advantages of nanoparticle, but more importantly, the properties of biological materials such as amino acids and starch. In addition, due to the needs of cancer patients due to the proliferation of cancer cells in the process

Leucine is a kind of amino acid that can not be synthesized by itself, which can

repair muscle damage. Therefore, choosing amino acid as raw material can also improve this phenomenon.

Drug carriers must have excellent characteristics [88], including high drug load, strong biocompatibility, small particle size, no cytotoxicity, good biodegradation and low cost [81,82]. Natural materials have excellent degradability, non-toxic and easy to be absorbed, and are often used to prepare drug carriers. We prepared two kinds of nano-hydrogels, lauric-acid-LeU-HES nanospheres and Lauric-acid-Serine-HES nanospheres. These two hydrogels use hydroxyethyl starch and two amino acids, bright amino Acid and Serine. It has good performance in cost, price and biocompatibility [83].

The mechanical properties and plasticity of synthetic polymer materials are much higher than those of natural materials, and they also have the characteristics of environmental responsiveness. Since the beginning of this century, people have no longer simply studied the drug carrier system, but focused on the slow-controlled release of drugs and environmental responsiveness [84]. However, in the preparation process, the continuous innovation of research technology may lead to the change of toxicity and performance of the drug carrier itself. Therefore, in the preparation of drug carrier, the degradation, drug loading rate and cytotoxicity of the product have always been the focus of research.

The selection of drug carrier type is the key to judge the performance of drug carrier. The nanohydrogels studied by us have the presence of starch derivatives, which can improve the loading rate of water-insoluble drugs [87]. There are a variety of commonly used insoluble drugs in anticancer drugs. In this chapter, camptothecin, a drug extracted from southwest China, was used to test the drug loading performance of nano-

hydrogels. The isoelectric point of Lauric-acid-LeU-HES nanohydrogel is between pH6 and 7, and the particle size reaches the minimum in this pH range. Camptothecin is suitable for drug release in cancer cells, and the pH inside cancer cells is about 6.85-6.95. Therefore, lauric-acid-LEU-HES nanohydrogel was used for drug loading and drug release of camptothecin. The isoelectric point of Lauric-acid-LeU-HES nanohydrogel is between pH7 and 8, and the particle size reaches the minimum in this pH range.

In this chapter, lauric-acid-Leu-HES and Lauric-acid-serine-HES nanohydrogels were respectively tested for degradation, drug delivery and cytotoxicity.

2.7 Laboratory instruments and reagents

2.7.1 Laboratory instruments

Experimental instrument	**model**	**manufacturer**
Constant temperature Water Bath	SHA-B	Changzhou Runhua Electric Appliance Co., LTD
Uv-visible Spectrophotometer	TU-1900	Beijing General Analysis Instrument Company
Dialysis bag	MD 44	Sinopharm Group
Centrifuge	TD L-80-2B	Shanghai Anting Instrument Factory
Elisa analyzer	DNM-9602	Beijing Pulang Technology Co., LTD

2.7.2 Experimental drugs and reagents

Experimental drugs and reagents	specifications	manufacturer
camptothecin	Analysis of pure	Aladdin
DMSO	Analysis of pure	Its group
DMEM	Analysis of pure	Aladdin
PBS	Analysis of pure	Its group
Acetic acid	Analysis of pure	Aladdin

2.8 In vitro degradation experiments of nanospheres

In vitro degradation experiments were performed by weighing method. The specific steps of the experiment are lauric-acid-LEU-HES nanospheres as an example. A group of lauric-acid-LeU-HES of the same weight was weighed to prepare PBS buffer solution with pH=7.4, and Lauric-Acid-Leu-HES was dissolved in 10 mL PBS buffer solution to make it fully dissolved. The dissolved solution was put into a dialysis bag with a molecular retention capacity of 30000 Da, and the dialysis bag was soaked in a large beaker with 500 mL PBS buffer solution. Put the beaker into a thermostatic water bath, set the temperature at 37 ℃, and replace the dialysate every 18h. After the dialysis time was set, the solution in the dialysis bag was taken out for freeze-drying and weighed. The remaining weight percentage of nanogel (nanogel weight, %) is calculated by the following formula:

Nanogel weight = *100%

Wd=Weight of nanohydrogel after freeze-drying, g;

W1= Weight of nano-hydrogel before degradation, g.

The in vitro degradation experiments of Lauric-acid-serine-HES nanohydrogels were the same as those of Lauric-acid-LeU-HES nanohydrogels.

2.9 Drug loading and drug release experiments of nanospheres

2.9.1 Lauric-acid-LEU-HES drug delivery and release

The lauric-acid-LeU-HES nanohydrogel was loaded with camptothecin to simulate the intestinal environment for drug release experiment.

(1) Spectral curve of camptothecin

Take a 100mL volumetric bottle, weigh 0.001g camptothecin, add into the volumetric bottle, methanol constant volume, ultrasound at room temperature until completely dissolved. Then zero was corrected with methanol solution, and the characteristic absorption wavelength of camptothecin was determined by full wavelength scanning at 800-200nm on ultraviolet spectrophotometer.

(2) Standard curve of camptothecin

Take a 50 mL volumetric bottle, weigh 0.001g camptothecin, add into the volumetric bottle, methanol constant volume, ultrasound at room temperature until completely dissolved. Add 1 mL, 2 mL, 3 mL, 4 mL, 5 mL and 6mL into 6 10mL volumetric bottles to the calibration line. The absorbance was measured by uv spectrophotometer, and the standard curve of camptothecin was prepared by selecting appropriate absorption peak. Standard curve drawing: Take the absorbance at 360 nm of the prepared solution of five concentrations. Concentration (C, μg/mL) as the abscissa,

absorbance (Abs, abbreviated as A) as the ordinate, Origin as the standard curve.

(3) Lauric Acid- LEU-HES nanoparticles loading experiment

The Lauric Acid-Leu-HES nanospheres were loaded with camptothecin. Specific steps:

The prepared Lauric-acid-Leu-HES 0.1000 g was weighed and dissolved in a 250 mL three-way flask containing 100 mL pure water, and stirred at high speed in a constant temperature water bath at 25 ℃. 0.0800g drug was weighed and added slowly in three times, and stirred under dark conditions for 48 h, so that the nano-hydrogel could fully absorb the drug. The drug was loaded, centrifuged in a high-speed centrifuge, and the supernatant was saved. The precipitate is dried by freeze-drying machine, the freeze-drying powder is preserved and weighed. The drug concentration of supernatant was calculated, and the absorbance of camptothecin in supernatant was measured by ultraviolet spectrophotometer, and the drug content in supernatant was obtained by standard curve. The drug loading rate and encapsulation rate of the nanospheres were calculated.

(4) Lauric-acid-Leu-HES lauric-acid-Serine-HES nanospheres for drug release

Na_2HPO_4 buffer solution with pH=7.4 was prepared for later use, and 0.0100 g of camptothecin loaded nanospheres were dissolved in 5 mL ultrapure water for full dissolution. Immediately after completion, the solution was transferred into a 5000Da dialysis bag and immersed in 100 mL of Na_2HPO_4 buffer solution with pH=7.4. In vitro drug release experiment was carried out under 37 ℃ constant temperature water bath and shock. The drug release capacity of the nano-hydrogel was detected within 24 h. The buffer solution was removed 2 mL every 1, 2, 4, 6, 8, 12, 16 and 24 h, and then the same

volume of buffer solution was added to continue dialysis. The concentration of ibuprofen in dialysate was determined by uv spectrophotometer and the cumulative release of drug was calculated.

2.10 Cytotoxicity test

MTT method test principle

MTT can be reduced by some dehydrogenases in the mitochondria to forma crystalline dark purple product, formazan, which can be completely dissolved in the presence of a specific solvent and then measured by a microplate at wavelengths around 490nm. The faster the cell proliferation, the higher the absorbance; The higher the cytotoxicity, the lower the absorbance.

Human laryngeal squamous cell carcinoma cell line (Hep2) was selected to further determine the inhibitory rate of Lauric-acid-leu-HES on different cancer cells. Lung cancer cells (A549), hepatoma cells (Hepg2) and malignant melanoma cells (A375) were selected as cancer cell samples, and endothelial cells (HFF) were selected as normal cells for the following MTT test.

The cells were first subcultured in the early stage, the cells were removed, all the medium in the petri dish was removed, and PBS buffer was added for three times to clean the inner wall of the petri dish, and the added PBS buffer was completely removed to avoid incomplete digestion of cells in the next step. Appropriate amount of trypsin was added to the petri dish and digested in the CO2 incubator for 1 min. After taking it out, the cells were observed under the microscope whether they were digested completely. Appropriate amount of medium was added to the petri dish and the cells on the inner wall of the petri dish were blown up to suspend them. Cell SAP from 200 μL medium was

placed in A cell counting tank under A microscope and observed by direct counting method (average number of cells in 16 squares A×10^4/mL). The 96-well plates were taken out, and the number of cells added to each well was about 5000-10000 by dilution after counting. The cells were cultured in a CO2 incubator for 24 h. Camptothecin and Lauric-acid-LEU-HES samples were taken, six concentration gradients were set, three parallel tests were performed, and two PBS contrast groups were used for different cancer cells. Set the concentration gradient of 100200300400500700900 (including g/mL, in CO2 incubator culture after 48 h, after adding 0.5% determined by MTT reagent, continue to develop in O2 incubator 4 h, take out, mixture poured out from the 96 - well plates, 110 microliter DMSO was added to each hole, and the constant temperature shaking shaker was used to avoid light for 10 min. The absorbance value of the corresponding sample in each well was measured at 490nm using a microplate reader, and the cell inhibition rate was calculated as follows:

Cell viability =(Experimental group OD - Blank group OD)/(Control group OD- Blank group OD) *100%

2.10.1 Observation of cell uptake

In order to determine whether the prepared Lauric-acid-Leu-Hes and Lauric-acid-serine-Hes can enter cancer cells, cell uptake experiments of the product can be performed by modifying fluorescein groups on proteins [97]. Cell uptake of free FITC and Lauric-acid-LEU-HES was studied using fluorescence microscopy.

An appropriate amount of Lauric-acid-LeU-Hes was dissolved in a minimum volume of 50 mM NaHCO3 solution, and 10 times the molar amount of FITC was added and stirred at 25 ℃ for 3 h. After the unreacted FITC was inactivated by adding excessive

ethanolamine to the reaction volume, the mixed solution was dialyzed in PBS buffer solution with a dialysis bag of molecular weight 500. After 72 h, the dialysate was replaced every 8 h. The resulting solution was freeze-dried to obtain LAURic-acid-LEu-FITC-Hes labeled by FITC. At a density of 1×105 cells per well, Hela cells were inoculated on the prepared six-well plates. After 24 h culture, the same amount of PBS buffer was added into the 1-2 well as the control, and 50μg/mL FTIC was added into the 3-4 well. 50 μg/mL Lauric-acid-LEU-FITC-HES was added to the 5-6 well and cultured in a CO2 incubator at 37 ℃ for 24 h. After the sample solution was removed from the cells, the cell plate was cleaned 5 times with PBS buffer solution. Fixed with 4% paraformaldehyde for 30 min, washed with PBS buffer solution for three times, added DAPI (1μg/mL, PBS buffer solution) for 3 min, washed with PBS buffer solution again, and placed the six-well plate under fluorescence microscope for focused observation.

Cell viability = *100%

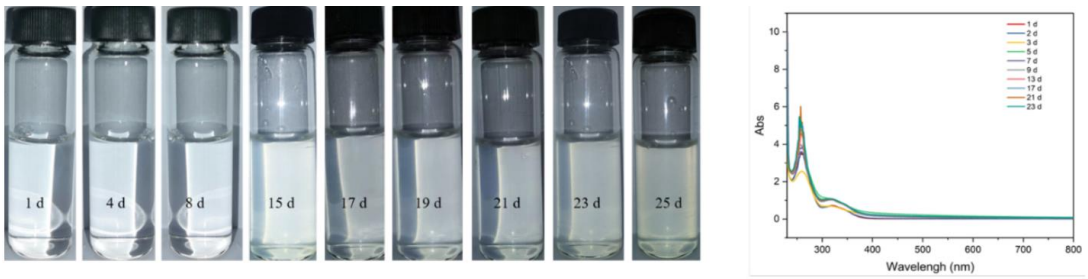
2.10.2 Observation of cell uptake

In order to determine whether the prepared Lauric-acid-Leu-Hes and Lauric-acid-serine-Hes can enter cancer cells, cell uptake experiments of the product can be performed by modifying fluorescein groups on proteins [60,61,62]. Cell uptake of free FITC and Lauric-acid-LEU-HES was studied using fluorescence microscopy.

An appropriate amount of Lauric-acid-LeU-Hes was dissolved in a minimum volume of 50 mM $NaHCO_3$ solution, and 10 times the molar amount of FITC was added and stirred at 25 ℃ for 3 h. After the unreacted FITC was inactivated by adding excessive ethanolamine to the reaction volume, the mixed solution was dialyzed in PBS buffer solution with a dialysis bag of molecular weight 500. After 72 h, the dialysate was

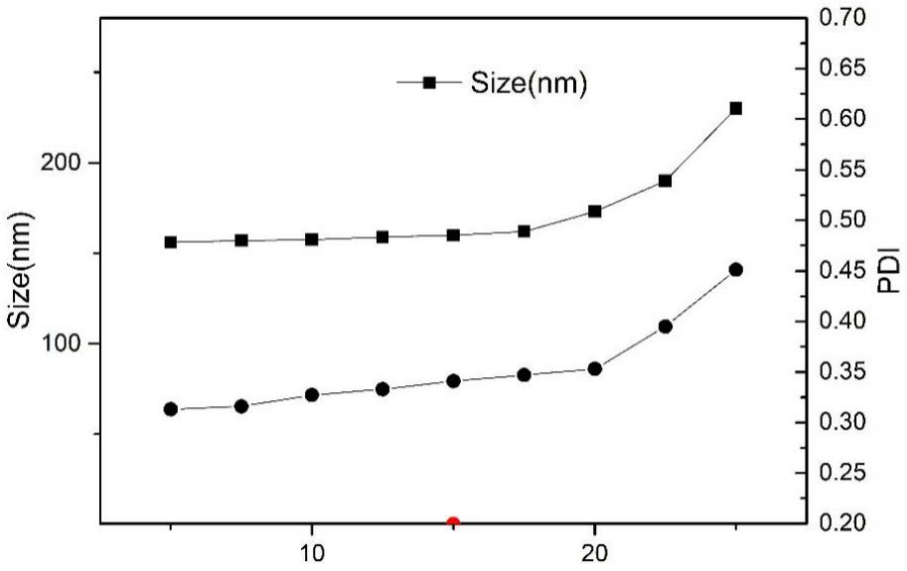
replaced every 8 h. The resulting solution was freeze-dried to obtain LAURic-acid-LEu-FITC-Hes labeled by FITC. At a density of 1×10^5 cells per well, Hela cells were inoculated on the prepared six-well plates. After 24 h culture, the same amount of PBS buffer was added into the 1-2 well as the control, and 50μg/mL FTIC was added into the 3-4 well. 50 μg/mL Lauric-acid-LEU-FITC-HES was added to the 5-6 well and cultured in a CO^2 incubator at 37 ℃ for 24 h. After the sample solution was removed from the cells, the cell plate was cleaned 5 times with PBS buffer solution. Fixed with 4% paraformaldehyde for 30 min, washed with PBS buffer solution for three times, added DAPI (1μg/mL, PBS buffer solution) for 3 min, washed with PBS buffer solution again, and placed the six-well plate under fluorescence microscope for focused observation.

2.10.3 Storage stability test



a b

Lauric-acid-Leu-Hes storage stability test Figure: A turbidity contrast figure B ULTRAVIOLET spectrum figure



C．Particle size and PDI value of Lauric-acid-Leu-Hes at different time

The Lauric-acid-Leu-Hes solution was left to rest at room temperature for 1 month (pH = 7.4). FIG. 3.3.5(a) shows the changes in transparency of solutions at different placement times. After standing for 20 days, a certain degree of flocculation was observed and the original solution changed from clear to light yellow. The absorbance of the filtered solution was detected by uv analysis, and the stability of the sample solution was further determined. Figure 3.2(b) shows the UV of Lauric-acid-Leu-Hes solution after standing for 20 days.

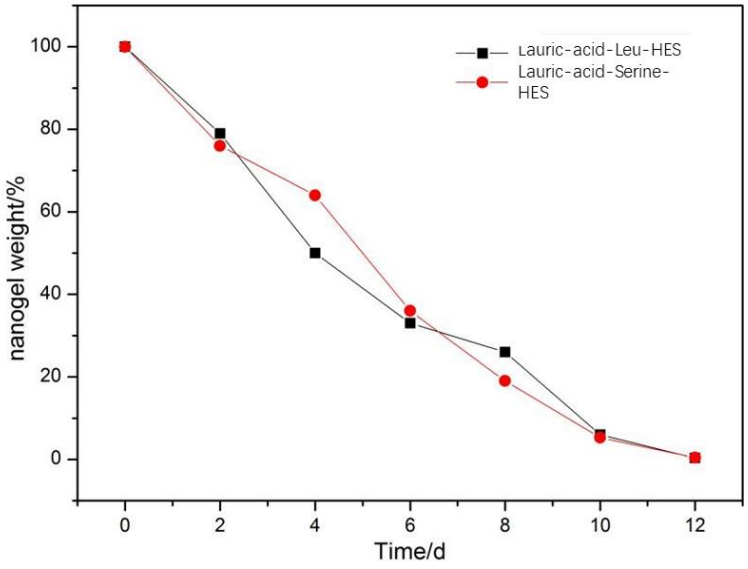
The absorption changes significantly. The change of particle size and PDI value of the original solution of Lauric-acid-Leu-Hes solution under different storage time was measured by laser particle size analyzer, as shown in Figure 3.3.5C. After 20 days, the particle size and PDI value of the nanoparticles gradually increased significantly, but remained stable for 20 days. This is mainly caused by the unique particle size of the nanoparticles (< 200 nm), according to Stokes' law, smaller particle size leads to slower

Brownian motion, which

As a result, Lauric-acid-Leu-Hes solution is relatively stable in the corresponding solvent, and its freeze-dried powder can be preserved for a longer time

2.10.4 Results and discussion

2.10.5 Degradation performance analysis of nanospheres in vitro

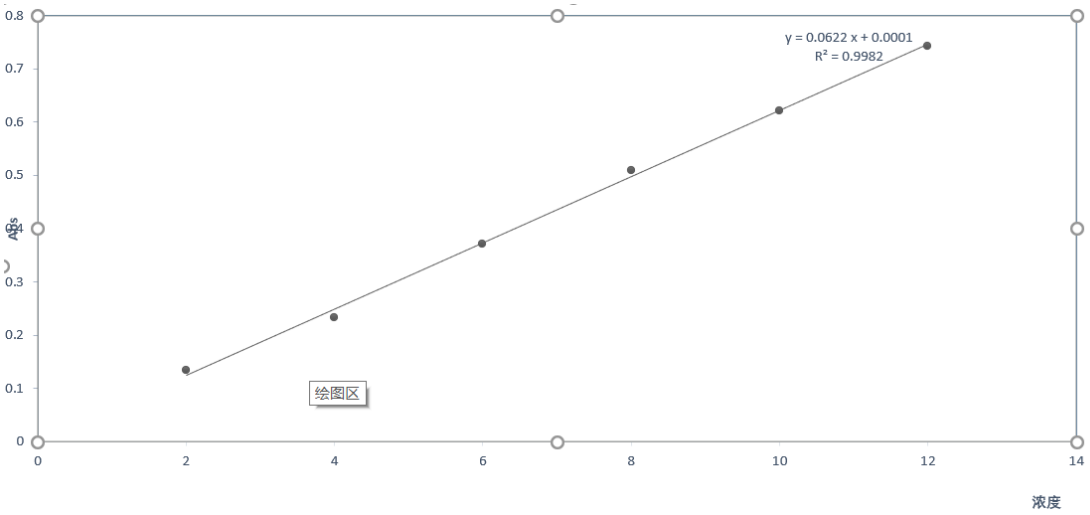


Degradation curves of Lauric-acid-Leu-Hes and Lauric-acid-serine-Hes nanohydrogels in vitro

In vitro kinetic degradation curves of Lauric-acid-Leu-HES and Lauric-acid-Serine-HES nanospheres are shown in the figure above. For Lauric-acid-LeU-HES nano-water microspheres, hydrolysis can be divided into three stages, 0-4 days, and the weight of the nano-hydrogel decreases rapidly. We believe that amino acids and part of Lauric-acid-LeU fall off from Hes molecular chain and dissolve in dialysate. The degradation rate slows down after 4-6 days. We believe that the main reason is the hydrolysis of modified Lauric-acid-LeU and HES linked amide bond, and the modified Leu is dialysed

externally. After 8-12 days, the degradation rate was relatively fast, and we believed that part of hydroxyethyl starch was aminohydrolyzed. The in vitro degradation trend of Lauric-acid-Serine-HES nanospheres was similar to that of Lauric-acid-Leu-HES.

2.15 Drug loading analysis of nanometer hydrogel



The Lauric-acid-Leu-HES standard curve

The standard curve of camptothecin was calculated by Lambert Beer's law, and it could be seen that the concentration of camptothecin was positively correlated with the absorbance at 360nm. Use Origin to get the standard curve equation: Y =0.0622x+0.0001 (R2=0.9982), good linear relationship, can be used for concentration calculation.

The drug loading rate and encapsulation rate can be calculated by the following formula:

Drug-loading rate ==*100%

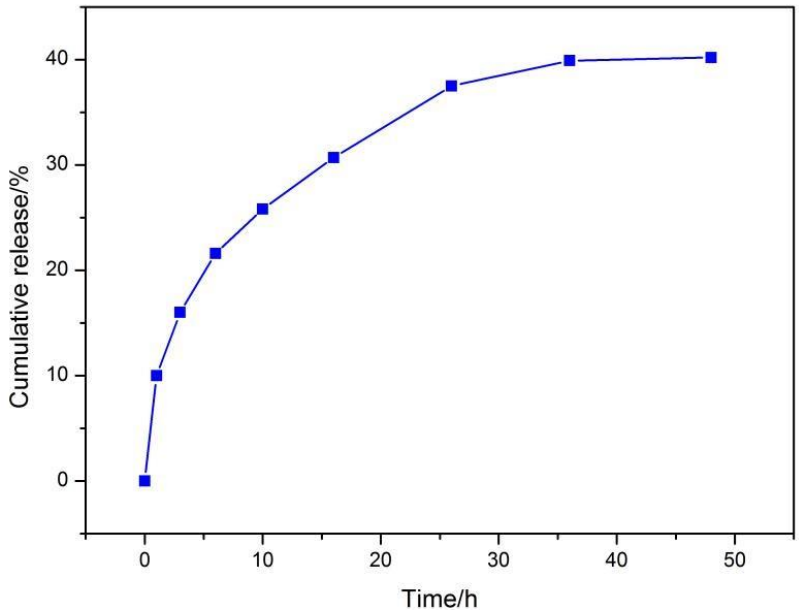
The encapsulation rate =□100%

m_0= Nano-hydrogel quality； m_1= In dose ；m_2= No drug loadings

Calculation of Lauric-acid-LEu-HES drug loading capacity: The content of

camptothecin in the supernatant solution was 0.0391 g according to the standard curve, and the encapsulation rate of camptothecin was 60% and drug loading rate was 37.4%.

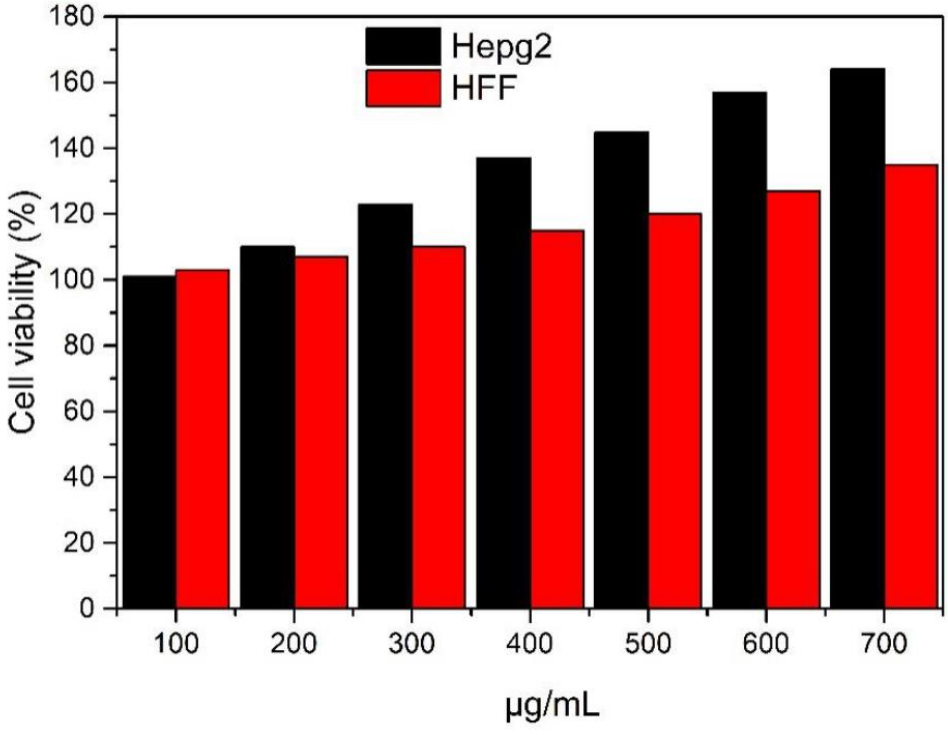
2.10.6 Drug release analysis of nanospheres



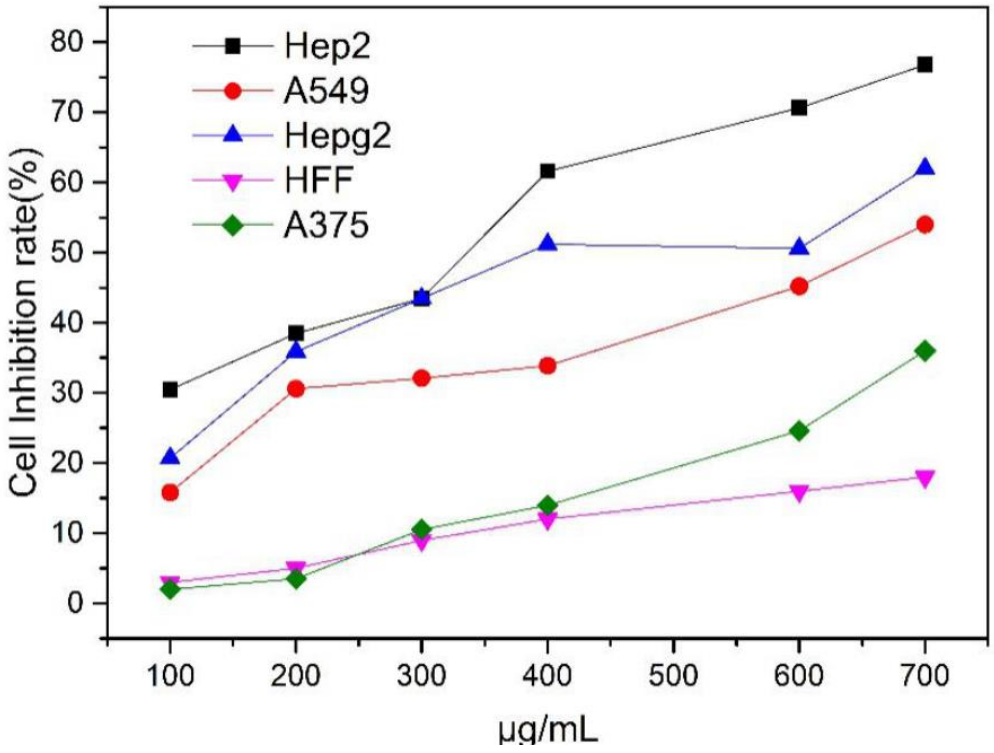
Cumulative release curve of Lauric-acid-Leu-HES to camptothecin

The figure above shows the cumulative release amount of Camptothecin from 0-48 h by Lauric-acid-LeU-HES nanohydrogel. We chose 48 hours as the observation time, mainly because camptothecin needs a long time for slow and controlled release to enter cancer cells for treatment. The cumulative release amount of camptothecin was 40.2%, and the release rate was relatively fast within 24 h, but the release rate was slow after 24 h. The results showed that the lauric-acid-Leu-HES hydrogel camptothecin had obvious slow-release effect and was suitable for drug application in human body.

2.10.7 MTT Test



Graph of Leu-HES inhibition rate on normal cells and hepatoma cells



Graph of Lauric-acid-Leu-HES inhibition rate on different cancer cells

MTT assay was used to determine the different inhibition rates of Lauric-acid-Leu-HES on different cancer cells. As can be seen from the figure above, after 48 h of sample treatment, BSA has almost no obvious cytotoxicity to both cancer cells and normal cells, and can even promote the growth of cells. When the concentration increases, the survival rate of cells increases. In the figure, the inhibition rates of Lauric-acid-Leu-HES on Hepg2, A549 and Hep2 cells were all above 50%, among which, Hep2 was inhibited

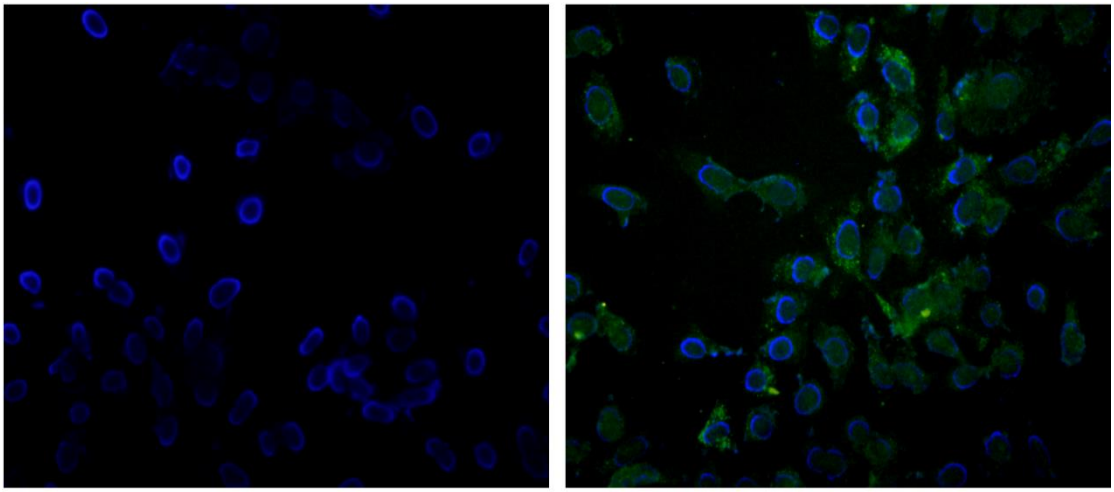
The inhibition rate of normal cells was less than 30%. Conclusion is drawn, although the preparation is obtained

Lauric-acid-Leu-HES nanoparticles have a certain inhibitory rate on different cancer cells, but the inhibition of different cancer cells

There are also great differences in the rate, and the effect on some cancer cells is not strong, but the inhibition ability on Hep2 cells is high, which can be used for targeted cancer treatment of human laryngeal squamous cell carcinoma. In addition, although the prepared albumin nanoparticles are obviously cytotoxic to cancer cells, they are non-toxic or slightly toxic to normal cells, which greatly expands the application range of albumin nanoparticles.

2.10.8 Observation of cell uptake

Cell uptake of Lauric-acid-Leu-HES and FITC-Lauric-acid-Leu-HES (green



a b

and blue represent FITC fluorescence and DAPI stained nuclei, respectively)

Cell absorption studies showed that Hela cells had taken up Lauric-acid-Leu-HES, as shown in the figure above. The figure on the left shows the uptake of free FITC by cells. It can be seen that FITC can enter cells through diffusion, but at a relatively slow speed and in a small amount. When loaded with FITC through Lauric-acid-Leu-HES, as shown in the right image, compared with the left image, green fluorescence is more obvious and has a wider range, and Hela cells absorb more FITC molecules through

endocytosis. This phenomenon indicates that the prepared Lauric-acid-Leu-HES can enter cancer cells smoothly and have certain ability to load small molecules, which provides theoretical evidence for future drug delivery.

Summary of section 2

In this chapter, lauric-acid-LEU-HES nanospheres were subjected to in vitro degradation experiments, drug delivery and release experiments, cytotoxicity and cell uptake tests, and the properties of the two prepared nanoparticles were studied.

The first part of this chapter is the in vitro degradation experiment of nano-hydrogel. The experiment adopts weighing method to study the in vitro degradation kinetics of nano-hydrogel. The nano-hydrogels were placed in dialysis bags and degraded in vitro. The results showed that both kinds of nano-hydrogels could be degraded successfully within 12 days. It can be judged that the nano-hydrogels prepared by us can be degraded in vitro and are suitable drug carriers for human body.

The second part of this chapter is drug loading and drug release experiment. The lauric-acid-LEU-HES nanohydrogel was used for drug loading of camptothecin, and the encapsulation rate and drug loading rate of camptothecin were 51.13% and 40.90%, respectively, by simulating the standard curve of methanol solution. For ibuprofen loading by Lauric-acid-LEu-HES nano-hydrogel, the standard curve of ibuprofen aqueous solution was simulated, and the encapsulation rate and loading rate of Ibuprofen were calculated to be 60.00% and 37.40% respectively. We studied the cumulative release of camptothecin 0-48 h, and the cumulative release of berberine hydrochloride reached 40.2%. The results showed that Lauric-acid-LEu-HES hydrogel had a significant sustained release effect on berberine hydrochloride, which was suitable for drug

application within 24 hours. We investigated the cumulative release of ibuprofen from 0 to 24 h, and the cumulative release of Ibuprofen was 52.0%, indicating that Lauric-acid-LEU-HES is suitable for anticancer therapy.

The third part of this chapter is the cytotoxic MTT test of the nano-hydrogel to detect the survival rate of cells. The cytotoxicity test showed that the two nano-hydrogels prepared by us had no toxic and side effects on cell growth, had good biocompatibility and promoted cell growth. The lauric-acid-LEU-HES nano-hydrogels prepared by us could be used as drug carriers.

In the fourth part of this chapter, the cell uptake effect of the prepared nanospheres in vitro was studied. The results showed that compared with camptothecin, the nanospheres prepared by us could enter cells more quickly and in larger quantities, and had a wider distribution in cells, with a very good effect.

In this chapter, degradation experiments, drug loading and release experiments and cytotoxicity experiments were carried out on lauric-acid-LEU-HES nanospheres, and the properties of the two hydrogels were studied. The results showed that the nano-hydrogels prepared by us had good degradation effect and strong drug loading and release ability, which could improve the drug loading ability and slow and controlled drug release effect of berberine hydrochloride and ibuprofen. It is not toxic to cells and is beneficial to cell growth. It can be determined that the Lauric-acid-LEU-HES nanospheres prepared by us can be used as drug carriers.

3 Industrial production of products

After our above laboratory level design experiment, we obtained a relatively ideal nanosphere complex containing camptothecin. However, the ultimate goal of our basic experiments is to scale up the small laboratory experiments from the reaction bottle to the reaction kettle and promote large-scale industrial production. This is what the vast number of scholars, researchers and human society are eagerly looking forward to. Therefore, I will design a more scientific production plant.

3.1 Scale up production research

The intermediate experimental stage is to further study the changing rules of chemical reaction conditions at each step in a certain scale device and solve the problems that cannot be solved or discovered in the laboratory. Although the nature of chemical reaction does not change with different experimental production, the optimal reaction conditions for each step of chemical reaction may change with the external conditions such as experimental scale and equipment. Therefore, pilot scale up is very important.

The importance of pilot tests

When drug research and development laboratory process is completed, the pharmaceutical process route is determined, the argument will generally need to pass a small laboratory scale enlarge the pilot 50 ~ 100 times, in order to further study in a certain scale of each step reaction conditions, and solve the laboratory failed to solve the problem of or has not been found.

To put it simply, the pilot test is the simulation test of small scale production, which is an essential link from small scale to industrial production. Medium test is a feasible plan to study industrialization according to small scale experiment. It further

studies the variation rule of chemical reaction conditions at each step in a certain scale device, and solves the problems that cannot be solved or discovered in the laboratory, providing design basis for industrial production. Although the nature of chemical reaction does not change with different experimental production, the optimal reaction conditions for each step of chemical reaction may change with the external conditions such as experimental scale and equipment. Generally speaking, pilot scale test is an important transition stage from rapid, high-level to industrial production, and its level represents the level of industrialization.

Research institutions generally focus on pilot research, while enterprises focus on industrial production. However, due to the relationship of manpower, material resources and capital, intermediate experiments are often ignored by research institutions and enterprises. We should realize that the preparation of API should follow the r&d rules of API, that is, scientifically follow the rules of small trial - pilot test - industrial production. The general steps of API and intermediates development are: literature review - pilot exploration - pilot study - industrial production.

The purpose of the pilot test

First of all, the purpose of the pilot test. Pilot test is a necessary transitional link from small scale experiment to industrial production. Basically complete the transition from pilot test to production operation process on the model production equipment, to ensure that products with predetermined quality standards can always be produced according to the operation procedures; Is the use of small production equipment in the production process, its equipment design requirements, selection and working principle and large production basically the same; After the pilot test is mature, the pilot test is

carried out to study the industrialization feasible technology and equipment selection and provide basis for the industrialization design. Therefore, the purpose of pilot scale is to verify, review and improve the synthetic process route studied and determined by the laboratory process, whether it is mature and reasonable, and whether the main economic and technical indicators are close to the production requirements; Study selected industrial production equipment structure, material, installation and workshop layout to provide data and optimal material quantity and material consumption for formal production. In short, the pilot scale should prove that the technological conditions and operation process of each chemical unit reaction can produce products with predetermined quality indexes on the model equipment with good reproducibility and reliability under the condition of using specified raw materials. Economic and technical indicators such as raw material consumption can be accepted by the market; The formulation of treatment plans and measures for waste three can be accepted by the environmental protection department; Safety, fire prevention, explosion-proof measures can be accepted by the fire protection and public security departments; The occupational safety measures provided can be accepted by the occupational-disease-prevention authorities.

Pilot scale up the content of the study

1. Review of production process route

In general, the cell reaction method and production process route should be determined at the laboratory stage. In the pilot scale up stage, only the specific process operation and conditions are determined to adapt to industrial production. However, the selected process route and process, in the pilot scale test exposed difficult to overcome

major problems, it is necessary to review the laboratory process route, modify its process.

2. Selection of equipment material and type

At the beginning of pilot scale, we should consider the material and type of the required equipment, and check whether it is suitable, especially pay attention to the choice of equipment material that contacts corrosive materials.

3, the agitator type and stirring speed examination

Most of the reactions in drug synthesis are heterogeneous reactions and their thermal effects are large. In the laboratory, due to the small volume of the material, good mixing effect, heat transfer, mass transfer problems show obvious steps, but in the pilot scale, due to the effect of mixing efficiency, heat transfer, mass transfer problems are prominently exposed. Therefore, the type of stirrer must be studied according to the properties of materials and reaction characteristics in pilot scale, and the influence of stirring speed on reaction law must be investigated, especially in the case of solid-liquid heterogeneous reaction, the stirrer type and the appropriate stirring speed should be selected.

4. Further study of reaction conditions

The optimal reaction conditions obtained in the laboratory may not meet the requirements of pilot scale. The main influencing factors, such as the feeding rate in thermal reaction, the heat transfer area and coefficient of the reaction tank, and the refrigerant, should be deeply studied in order to master their change rules in the pilot plant, so as to obtain more suitable reaction conditions.

5. Determination of process flow and operation method

In the pilot scale stage, it is necessary to consider how to adapt the reaction and

post-treatment methods to the requirements of industrial production, especially to shorten the process and simplify the operation.

6. Quality control of raw materials and intermediates

Determination of physical properties and chemical parameters of raw materials and intermediates. Formulation of quality standards for raw materials and intermediates.

Conditions for conducting pilot tests

At what stage will the pilot test be conducted? To put it simply, the pilot test is the combination of small test technology and equipment. Therefore, the pilot test should at least have the following conditions:

1, the pilot synthesis route has been determined, the pilot process has been mature, the product yield is stable and the quality is reliable.

Mature pilot process should have the following conditions: synthesis route is determined; Clear operation steps; Reaction condition determination; Purification method is reliable and so on.

2. The process investigation of the pilot test has been completed. Many batches of stable and detailed experimental data of the pilot process have been obtained. The stability test of 3 ~ 5 batches shows that the process is stable and feasible.

3. Methods and requirements for refining, crystallization, separation and drying of finished products have been determined.

4. Established quality standards and testing and analysis methods have been mature and determined. Includes testing and analysis methods for final products, intermediates and raw materials.

5, some equipment, pipe material corrosion test has been carried out.

6. Material balance was carried out.

7, the three wastes problem has a preliminary treatment method.

8. Raw material specifications and unit consumption quantity have been proposed.

9. Production safety requirements have been put forward.

Tasks in pilot scale up phase

There are mainly the following ten points, according to different situations in practice, prioritize, planned and organized.

1. Final determination of process route and unit reaction operation method. Especially when the original route and unit reaction method are difficult to solve the major problems in the pilot scale, it is necessary to choose another route and then follow the new route for pilot scale scale.

2. Selection of equipment material and model. Special attention should be paid to the selection of equipment materials that contact corrosive materials.

3. Investigation of stirrer type and stirring speed. Many reactions are heterogeneous and the thermal effect is large. In the pilot scale test, due to the small volume of the material and good mixing effect, the heat and mass transfer problem is not obvious. However, in the pilot scale test, according to the properties of the material and reaction characteristics, attention must be paid to the effect of mixing type and mixing speed on the reaction, so as to select the required agitator and determine the suitable mixing speed.

4. Further study of reaction conditions. Laboratory stage to obtain the optimum reaction conditions may not completely accord with the requirement of pilot

magnification, therefore, should be the main influence factors, such as feed rate, stirring effect, heat transfer area of the reactor and factors such as refrigerant and heat transfer coefficient, in-depth study, in order to grasp the change rule of the device in the middle. Get more suitable reaction conditions.

5. Determine the process flow and operation method. Consideration should be given to adapting reaction and post-treatment operations to industrial requirements. Pay special attention to shorten working procedure, simplify operation, improve labor productivity. Finally determine the production process and operation methods.

6. Material balance. When the reaction conditions and operation methods are determined, the material balance should be carried out for some reactions with low yield, high by-products and high waste. The total weight of the reaction product and other products is equal to the sum of the amount of materials before the reaction is the precision of the material balance must be achieved. In order to address the weak links. Tap potential energy saving, improve efficiency, recovery of by-products and comprehensive utilization and prevention of three wastes to provide data. The chemical composition without analytical method should be studied by analytical method.

7. Determination of physical properties and chemical constants of raw materials and intermediates. In order to solve the problems in production process and safety measures, it is necessary to measure the properties and chemical constants of some materials, such as specific heat, viscosity, explosion limit, etc.

8. Formulation of raw material intermediate quality standards. If the quality standard is not perfect in the pilot test, it should be revised and improved according to the pilot test.

9. Determine consumption quota, raw material cost, operator and production cycle, etc. On the basis of the summary report of pilot study, infrastructure design can be carried out and the purchase plan of model equipment can be made. Design and manufacture of amorphous equipment, workshop building and equipment installation according to the construction drawings. After all production equipment and auxiliary equipment are installed. If the trial production is qualified and the short-term trial production is stable, the process can be formulated and delivered to production.

Industrial amplification is the most important and perhaps the most difficult problem in the development of chemical process. There are many factors to consider, and there is a lack of effective theory to guide. Most industrial development still depends on experience and step by step amplification, especially in China. The following is the survey:

1) General methods and procedures of industrial amplification

General methods: experience amplification method, mathematical model method, combination of the two methods:

Test in amplification is standard procedure: - (concept design) - patterns - (pilot) - the pilot - (industrial design) - industrialization; But in many cases, this process will not be completely followed, and there may be only a few steps missing

2) Criteria and main parameters for reactor amplification in industrial amplification. This should be discussed on a case-by-case basis. Generally, the reaction process is amplified. The most important factors are temperature effect and concentration effect, and other factors generally affect the chemical process through these two factors.

3) The difficulties that may occur in the process of industrial amplification

(interpreted as the process difficulties in the control of supersaturation in crystalology) are determined according to the complexity of the process to be amplified. The more complex the process, the more problems may be encountered. Therefore, some chemical processes have taken decades to develop. For example, the small test may be very easy to heat transfer, but after industrialization, the cooling is not good, there is material sintering, agglomerate. In general terms, the difficulty of this kind is that various factors affect temperature and concentration by means of mass and heat transfer, and thus determine the realization of the chemical process.

4) which links (parameters) in industrial amplification can be determined unchanged through the accurate system of small test, pilot test, after reasonable and accurate analysis of test results and data, the comparative nature and basic understanding of the process, it is possible to know which several factors are the basic influence factors.

For example, in a reaction process, the intrinsic kinetic rate is constant. If the small test can basically eliminate the effect of transfer resistance, get the intrinsic kinetics of the reaction process, then the industrialization can be directly used. Similarly, for the crystallization process, after the material embodiment decision, the crystallization rate of the small test and the factory production itself is unchanged, but the mass and heat transfer situation is completely changed.

Organic synthesis magnifies matters needing attention synthetic products need to be considered in the final production.

1. Temperature gradient and concentration gradient are different between laboratory and scale-up experiments

The temperature and concentration of small experiments are better controlled,

but the amplification experiment is different. First of all, the temperature because of the increase in volume, it takes a long time to reach a certain temperature, and the uneven temperature leads to the uneven reaction. Sometimes if the exothermic reaction is strong, it is easy to lead to the local temperature too high and accelerate the side reaction. The drip is not easy to uniform. There are measures to reduce the occurrence of this situation: for heating reaction (and low temperature side reaction is serious) can be the material separately heated to the required temperature, and then add. In order to control the local concentration is too large, the liquid adding point can be set near the maximum linear speed of agitation, or change the drip adding to spray adding

2. Deviation of temperature indication

Laboratory thermometers can be inserted directly into the reaction liquid to reflect the reaction temperature in a timely and rapid manner, while the amplification experiment is not possible, the temperature has to go through a long conduction process to the thermometer, reflecting the actual temperature will be delayed, and the fluctuation will be smaller. For simple reactions, all reaction, the above effect is not big, amplification effect is mainly for complex reactions, exothermic reactions. Heterogeneous reaction is generally diffusion control: it is necessary to stir vigorously to make the dispersed phase small and accelerate the reaction. It also has the effect of reducing the interface temperature and temperature gradient for exothermic reaction. Local temperature and concentration gradients are the most critical, directly related to the failure and success of amplification, addressed above. The only way to control the endothermic reaction is to control the heating medium of the jacket. The temperature

control of exothermic reaction is as follows:

1. Stir well to evenly distribute the concentration and temperature

2. Direct the liquid flow to the point where the stirring line speed is maximum

3. Reduce droplets to achieve good distribution, such as injection

4. Reduce droplet temperature to reduce local overheating

5. The reaction temperature is controlled by low limit

In the production process, the order of chemical synthesis reaction or biosynthesis pathway is directly related to the conditions (including the ratio of ingredients, temperature, reaction time, agitation, post-treatment method and refining conditions, etc.) is called the technological conditions. Other processes become auxiliary processes.

Enlarge experiment

3.2 Department establishment of the factory

I plan to set up 7 departments in the factory, including human resources administration Department, Safety and environmental protection Department, Production Department, quality Management Department, packaging Department, engineering department and Finance Department. The human resources and administration department is responsible for daily staffing and attendance, which will meet staffing requirements. The production department is mainly responsible for the scheduling and storage of API entering the factory and the orderly implementation of the whole production process, which is a key step. The quality management department is responsible for quality control, quality measurement in strict accordance with SOP

manual, sampling and testing from incoming raw materials to some key nodes in the production process on time to ensure quality and safety. The engineering department should arrange personnel to repair the machine in time. Accidents often occur when the machine runs continuously in industrial production. It is necessary to repair the machine in time to ensure the smooth progress of production and not delay the construction period. The finance department is responsible for the payment of employees' salaries and the statistics of daily expenses and income. Safe environmental protection, as the name suggests, is responsible for the safety and environmental protection of two parts, in large industrial production safety accidents, safety of life is greater than all, safe environmental protection should formulate relevant rules and regulations to ensure staff safety, environmental protection is the human are now facing a big challenge, is our factory should be the target of energy saving and emission reduction, Sewage treatment stations should be set up to deal with standard discharge. These are the preliminary arrangements for the establishment of the department.

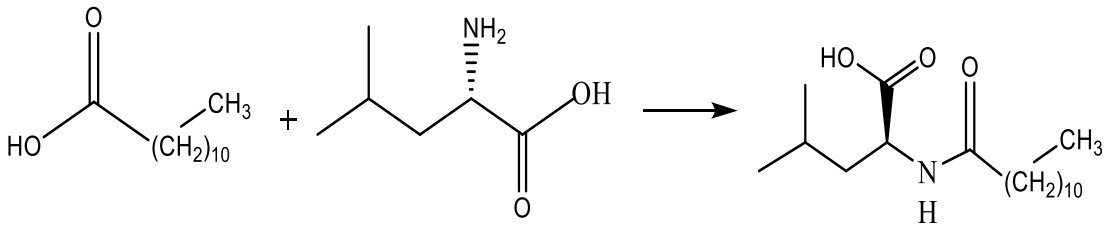
3.3 Process route

Design and selection of process Flow The general pharmaceutical manufacturing process consists of three stages: Raw materials to chemical reaction, separation and purification products to agents, medicine production the same product can be used in most cases a number of different production line, even if the same raw material route, the specific process arrangement or operating indicators also has difference, what kind of production line, must be economic evaluation analysis was carried out on the line, find the advanced technology, the product cost is low, the yield is high, With low investment, low energy consumption, and complete environmental protection process,

our drugs do not have the principle of pretreatment step, because we use non-toxic biological macromolecules, buy back and use directly.

3.3.1 Modification and purification of leucine

I plan to use a 10-ton reaction kettle to modify leucine, feeding in the ratio of Lauric acid to leucine 1.1:1. The reaction principle and reaction device are as follows:



The upper part of the device with manhole, convenient observation and feeding.

The purification equipment uses the following equipment with multiple glass observation holes for real-time observation of the extraction status, which is connected to the leucine-modified equipment through the main pipe.

Extraction equipm

3.3.2 Hydroxyethyl starch was grafted with modified leucine

The equipment used in this process is approximately twice the size of 3.3.1, and it is planned that all the two POTS of modified leucine end products are put into this step in order to allow time for washing equipment and piping in 3.3.1. This is enough to ensure that sanitary conditions are up to standard.

The equipment used is shown as follows:

This equipment with heat preservation function, but also can withstand a certain pressure, because hydroxyethyl starch at room temperature dissolve slowly, at 60-70℃ dissolve significantly faster, this equipment can play a certain purpose of optimization process.

The above two steps require dialysis to further remove impurities. The principle and influencing factors of dialysis are as follows: Dialysis is an important means to separate small molecules by virtue of the principle that small molecules can pass through while large molecules cannot pass through a semi-permeable membrane. The usual approach is to put a mixture of small and large molecules into a dialysis bag made of a semi-permeable membrane and sink it in a lot of water. The small molecules in the bag continue to pass through the membrane into the external solvent until equilibrium is reached. If the fluid dialysis or constantly change the solvent of the dialysis bag can achieve the mixture in the bag contains almost no small molecules. The rate of dialysis is affected by a number of factors. These factors are briefly discussed below.

A, membrane

1, material fire cotton gum is the most commonly used dialysis bag material. Various specifications of fire cotton plastic bags have been made into commodities for sale. Cellophane can also be used instead of fire glue.

2. Preparation First, a dialysis tube of an appropriate size and length is placed in alkaline EDTA solution and boiled for 30 minutes to avoid the loss of activity of molecules to be dialyzed. The dialysis tube is then washed with distilled water. Ligate one end of the tube and fill the dialysis tube (bag) with the studied material, then ligate the top. Dialysis is best performed in freshly prepared tubes because wet dialysis bags are highly susceptible to microbiological infection. If the dialysis bag must be preserved, trace amounts of benzoic acid should be added to the solution.

3. Permeability The permeability of dialysis bag varies with the size of the bag and the pretreatment method. However, during overnight dialysis, the semi-permeable membrane can generally allow the passage of compounds with relative molecular weight (Mr) less than 30000. In fact, there is no strict boundary, and larger molecules can penetrate the membrane during prolonged dialysis. A range of commercial materials with higher dialysis speed and finer permeability range can be used for fine separation.

Because of the scale of our industrial production and the comprehensive consideration of many factors, we decided to choose ceramic membrane filter, the instrument picture is as follows:

此设备能将残留有机溶剂和分子量小的物质过滤去除，保证产品中有机物质及副产物去除干净。

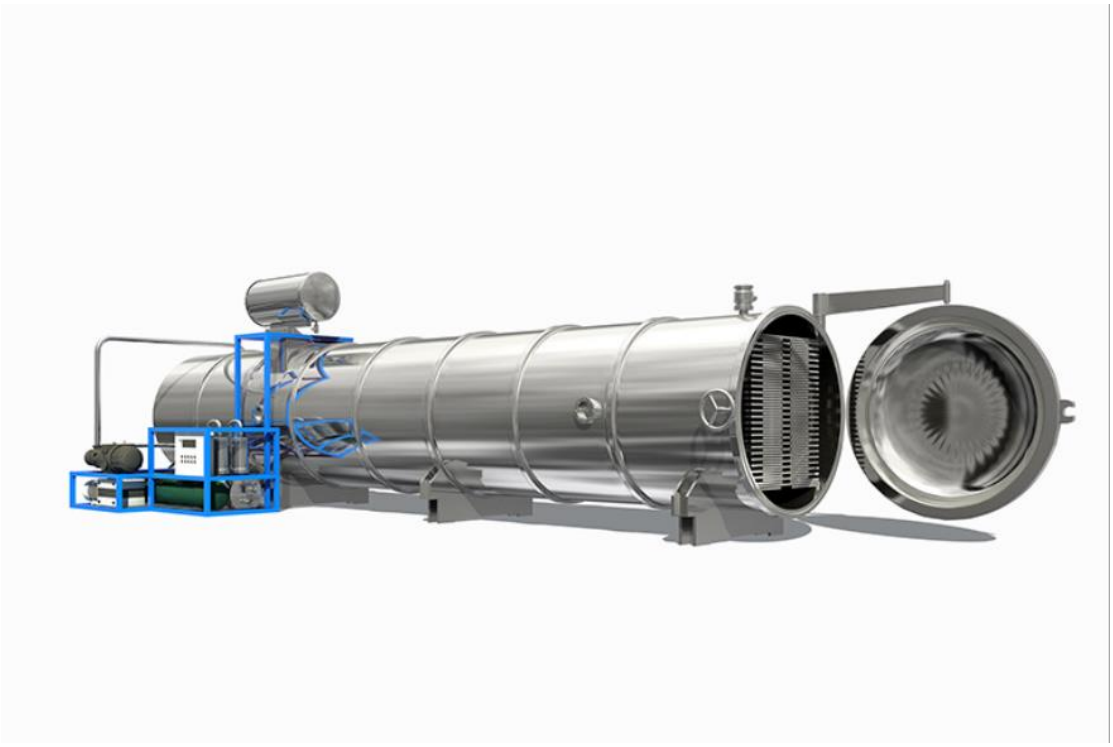
This equipment can filter and remove residual organic solvents and substances with small molecular weight to ensure that organic substances and by-products in products are removed clean.

3.3.3 Preparation of camptothecin coated nanospheres

According to the process route, we should first disperse the complex grafted in the previous step evenly in dimethyl sulphone in a stirred container, and then add deionized water in a ratio of 1:1. The addition of deionized water should be controlled within 30-40 minutes. Adding too fast will affect the self-assembly of nanospheres and have too great influence on particle size. Here we need a precision instrument to control the droplet acceleration of deionized water, which needs to be customized according to the actual situation.

3.4 Freeze drying process

Freeze-drying technology is the main production process of biological agents, which can keep the original physical and chemical properties and biological activity of products, and the loss of active ingredients is very little. After drying, the shape, volume, crystal shape and other physical and chemical indicators of good uniformity. The product is easy to be preserved for a long time because of its low water content. However, in the production or experiment of lyophilized preparation, we always have some problems in lyophilization, such as making the best sample as possible while reducing energy consumption. In this step, we will add 8% mannitol solution of equal volume as lyophilization protective agent. The sample picture of vacuum freeze dryer is as follows:



Freeze-dried samples need to be stored in the freezer, but in view of the large industrial output, ordinary refrigerators can not meet the demand, we need to build a cold storage below zero.

3.5 Drug packaging design

Considering that our finished products are freeze-dried in powder form and the nano properties of the drug, we plan to use capsules for packaging, so as to protect the original properties of the drug to the maximum extent and give better play to its performance. The preparation process of capsule is as follows: the preparation process of hard capsule is usually as follows: preparation and selection of empty capsule → mixing and filling of drugs and auxiliary materials → nesting and sealing → finishing and polishing of capsule → packaging → quality inspection → finished product.

Capsule preparation machine

3.6 Other points on industrial production

Site selection

The following factors should be considered in the selection of the location of the pharmaceutical factory :(1) choose a place far away from residential areas, so as not to cause ecological environment pollution; 2) Choose places far away from water sources to ensure the quality of water resources in the area; 3) Choose to hand in

Convenient places to ensure the convenient transportation of raw materials and finished products

3.7 Details of workshop design

In the design of the pharmaceutical workshop, attention should be paid to ensuring the ventilation between cars to avoid the risk of cross-contamination, confusion and errors in the drug production process. Set up dust removal room and

machine room in pharmaceutical workshop, reasonably install exhaust system. Strictly separate the flow of people in the plant from the logistics channel, consider the connection between the production clean area and the related auxiliary production area, ensure the flexibility of the spatial layout, as well as the reasonable arrangement of water, electricity, pipeline and other issues between the plants. Safety management is the key point of pharmaceutical factory design and construction. It is necessary to strictly grasp the safety problems and do a good job in safety inspection of all kinds of equipment in pharmaceutical factory to ensure normal operation. The equipment is purchased by professional personnel, and the equipment is fixed for repair and maintenance, at the same time, attention should be paid to the establishment of safe passage and alarm system, which is convenient for the pharmaceutical factory to evacuate the crowd in time when accidents occur. Finally, pharmaceutical companies need to establish sewage treatment systems to avoid a greater impact on the environment. The sewage produced by the pharmaceutical factory is treated and discharged only after meeting the national discharge standards. Effective treatment of solid waste, not littering, if the pharmaceutical factory does not have professional treatment capacity can be handed over to the relevant paper waste treatment company. The noise caused by the construction and production of the drug factory should also be dealt with, and sound insulation glass or mute measures should be set up. Generally speaking, the design of pharmaceutical factory should basically meet five aspects, health requirements, safety requirements, construction requirements, technological requirements and national pharmaceutical factory construction standards

3.7.1 Other points on industrial production

3.7.2Site selection

The following factors should be considered in the selection of the location of the pharmaceutical factory :(1) choose a place far away from residential areas, so as not to cause ecological environment pollution; （2）Choose places far away from water sources to ensure the quality of water resources in the area; （3）Choose to hand in.

Convenient places to ensure the convenient transportation of raw materials and finished products

3.7.3 Details of workshop design

In the design of the pharmaceutical workshop, attention should be paid to ensuring the ventilation between cars to avoid the risk of cross-contamination, confusion and errors in the drug production process. Set up dust removal room and machine room in pharmaceutical workshop, reasonably install exhaust system. Strictly separate the flow of people in the plant from the logistics channel, consider the connection between the production clean area and the related auxiliary production area, ensure the flexibility of the spatial layout, as well as the reasonable arrangement of water, electricity, pipeline and other issues between the plants. Safety management is the key point of pharmaceutical factory design and construction. It is necessary to strictly grasp the safety problems and do a good job in safety inspection of all kinds of equipment in pharmaceutical factory to ensure normal operation. The equipment is purchased by professional personnel, and the equipment is fixed for repair and maintenance, at the same time, attention should be paid to the establishment of safe passage and alarm system, which is convenient for the pharmaceutical factory to evacuate the crowd in time when accidents occur. Finally, pharmaceutical companies

need to establish sewage treatment systems to avoid a greater impact on the environment. The sewage produced by the pharmaceutical factory is treated and discharged only after meeting the national discharge standards. Effective treatment of solid waste, not littering, if the pharmaceutical factory does not have professional treatment capacity can be handed over to the relevant paper waste treatment company. The noise caused by the construction and production of the drug factory should also be dealt with, and sound insulation glass or mute measures should be set up. Generally speaking, the design of pharmaceutical factory should basically meet five aspects, health requirements, safety requirements, construction requirements, technological requirements and national pharmaceutical factory construction standards

3.7.4 Measures to achieve total quality management in pharmaceutical factories

1, the establishment of quality-centered corporate culture in the actual operation of pharmaceutical plants should always establish "quality first, safety first." This idea. First of all, the enterprise should compile a quality manual to facilitate the learning of employees. Then formulate a quality culture implementation plan, clear specific purpose and significance, mobilize all employees to actively participate in the corresponding discussion. Finally, specific measures should be taken to implement quality culture, such as opening speech contests related to quality and carrying out quality knowledge competitions.

2. Establish a quality management system Enterprises shall establish a drug quality management system, clarify the responsibilities of institutions and personnel at

all levels of the company, highlight the independence and importance of quality management institutions, refine the quality objectives layer by layer, and implement specific work to specific departments and relevant persons in charge. The quality control system shall cover all factors affecting drug quality, Including the product of the production process quality control and quality risk management, change control, deviation handling, corrective action and preventive action, supplier auditing, verification, sampling, and the product quality inspection, material and product release, product quality review analysis, stability test, adverse reactions to the complaint and a series of organized, planned activities.

As a special commodity, medicine is related to people's life and health.

3. Reducing the quality risk of drugs and ensuring their safety is the primary consideration of every pharmaceutical company, so it is necessary to implement quality risk management to identify, analyze, evaluate, identify, evaluate, control and review the risks of drugs, so as to effectively reduce risks. Develop quality risk management documents, establish quality risk management flow chart, use failure mode and impact analysis (FMEA), hazard source analysis and critical control point (HACCP), statistical credit analysis tools and other risk assessment methods to assess risk, risk control to acceptable range.

the conclusion

With the improvement of people's living standard, people's demand for drug quality is getting higher and higher, which also brings higher requirements to pharmaceutical enterprises. In the design and construction of pharmaceutical factory, we should pay attention to reasonable arrangement, improve the utilization rate of

space, do a comprehensive planning and design for the equipment and technology needed by the pharmaceutical factory, and strictly monitor the environmental temperature and humidity, cleanliness and room pressure difference in the process of drug production. In quality management to establish and improve the quality management system, through the monitoring of drug production process, drug quality inspection, audit of raw materials suppliers and other factors affecting drug quality management, reduce quality risk, for people to produce safe drugs.

Conclusions and Prospects

In this paper, we studied and prepared two kinds of nano-hydrogels, Lauric-acid-LeU-HES nano-hydrogels and Lauric-acid-serine-HES nano-spheres, and determined the successful preparation of nano-hydrogels through a series of characterization. The appearance, molecular weight, particle size, pH response and other characteristics of the prepared nano-hydrogel were studied and characterized. To study the suitability of the two nano-hydrogels as drug carriers, and to study the properties of the two nano-hydrogels through in vitro degradation experiment, drug delivery and release experiment, cell uptake and cytotoxic MTT.

For Lauric-acid-serine-Hes nanohydrogels, DCC and NHS facilitated condensation reaction methods were used. Leucine was modified with lauric acid, and

characterized by Fourier transform infrared spectroscopy and mass spectrometry, the characteristic peak at 1732 cm-1 corresponds to C=O, indicating that carboxyl group was introduced into leucine. The molecular weight of β -Cd was 1135, and that of lauric acid was 100.07. One lauric acid molecule was grafted to leucine, indicating the successful modification of leucine. The modified leucine was grafted onto hydroxyethyl starch. Infrared characterization showed that the presence of amide bond and the increase of molecular weight confirmed the successful grafting of leucine onto HES. The morphology of the copolymer was analyzed by SEM, TEM and AFM, and its particle size was about 110 nm by Zeta particle size analyzer. Serine was grafted to the copolymer using DIC and DAMP - promoted condensation reactions. The appearance of characteristic peak at 1700 cm-1 was analyzed by Fourier transform infrared spectroscopy (FTIR), and the successful grafting of lysine was judged. The morphological characteristics of the copolymer were analyzed by SEM, TEM and AFM. The results showed that the copolymer had a three-dimensional structure, spherical shape, relatively uniform size, and suitable for drug loading. Zeta analyzer was used to analyze the particle size and Zeta potential of the nano-hydrogel, and the particle size of the nano-hydrogel under different pH values was analyzed. The particle size of the prepared nano-hydrogel aqueous solution was about 150 nm, and the nano-hydrogel could remain stable in acidic environment. The carboxyl and amino groups on the surface of the prepared nano-hydrogel provide excellent properties. The size varies in different pH environments. When the pH value is 7, the particle size is the smallest, 140.1 nm, and the isoelectric point is between pH6 and 7. At this time, the nano-hydrogel has no charge, no charge repulsion inside, and the minimum particle size is suitable for human environment.

For lauric-acid-serine-HES nanospheres, the same preparation method was used for Lauric-acid-LeU-HES nanospheres.

Degradation, drug delivery and cytotoxicity of Lauric-acid-Leu-HES and Lauric-acid-serine-HES nanospheres were investigated, respectively. The degradation time of Lauric-acid-LeU-HES nanohydrogel was 12 days after the in vitro degradation experiment. It can be judged that the nanohydrogel prepared by us can be degraded in vitro and is a suitable drug carrier for human body. In the drug loading and drug release experiment, the lauric-acid-LeU-HES nanohydrogel was loaded with camptothecin. The standard curve of camptothecin aqueous solution was simulated. The encapsulation rate and drug loading rate of camptothecin were 60% and 37.44% respectively. The cumulative release of camptothecin and ibuprofen reached 40.2% and 52.0% respectively. The results showed that the two kinds of nanospheres had obvious slow-release effect on the drug. Cytotoxic MTT assay was performed to detect cell viability. Cytotoxicity experiments show that the two kinds of nano-hydrogels prepared by us have no toxic and side effects on cell growth, have good biocompatibility and can promote cell growth. The prepared Lauric-acid-Leu-HES and Lauric-acid-serine-HES nanospheres can be used as drug carriers.

Degradation, drug delivery and cytotoxicity of Lauric-acid-Leu-HES and Lauric-acid-serine-HES nanospheres were investigated, respectively. The performance of the two kinds of hydrogels was studied, and it was found that the nano-hydrogels prepared by us could be degraded within 12 days with good degradation effect. Strong drug delivery and release capacity. In cytotoxic MTT test, with the increase of the concentration of the nano-hydrogel, the cell survival rate was more than 90%, indicating

that the two kinds of microspheres prepared by us had no toxicity to cells and were beneficial to cell growth. It can be determined that the Lauric-acid-LeU-HES and Lauric-acid-serine-HES nanospheres prepared by us can be used as drug carriers. Finally, we made a preliminary design for industrial production, hoping to translate the results into large-scale production.

Looking forward to

The application of nanotechnology in drug delivery has promoted innovation and development in the field of drug delivery and pharmaceutical production. The future prospect of nanogels should mainly focus on the clinical application of nanogels. Before the application of nanogels in clinical practice, precise control of their properties and more detailed studies on the pharmacokinetics and pharmacodynamics of nanogels are needed. The detailed research on the mechanism and physicochemical properties of nanogels will provide broad prospects for developing different biomedical applications. The research and application of nanoscale drug carrier should be further developed to give play to its unique performance in drug delivery field. At the same time, there are still some problems in the research on the preparation of nano-hydrogel drug carrier with biopolysaccharides. In the modification of biopolysaccharides, the purification of the product needs to further explore appropriate and effective methods. The problem of intelligent nano-hydrogel, pH and temperature sensitivity still need to be strengthened, so that it can reach the level of real intelligent nano-hydrogel; The types and methods of drug loading of the prepared nano-hydrogels still need to be

explored and experimented.

We hope that researchers will further study the performance and application of nanoscale drug carriers, and be able to accurately and efficiently target and slow and control the release of drugs, contributing to the development of science and technology and the cause of human health.

Reference

[1] Buss B, Beck L, Miyake G. Synthesis of Star Polymers using Organocatalyzed Atom Transfer Radical Polymerization Through a Core-first Approach[J]. Polymer Chemistry, 2017,9(13):10-1039

[2] Lange S C, Unsleber J, Drücker P, et al. pH response and molecular recognition in a low molecular weight peptide hydroge.[J]. Organic & Biomolecular Chemistry, 2014, 13(2): 561-569

[3] Zhang E, Wang T, Lian C, et al. Robust and thermo-response graphene–PNIPAm hybrid hydrogels reinforced by hectorite clay[J]. Carbon, 2013, 62(5): 117-126

[4] Wei X, Lv X, Zhao Q, et al. Thermosensitive β-cyclodextrin modified

poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) micelles prolong the anti-inflammatory effect of indomethacin following local injection[J]. Acta Biomaterialia, 2013, 9(6): 6953-6963

[5] Zhang H, Li J, Cui H, et al. Forward osmosis using electric-responsive polymer hydrogels as draw agents: Influence of freezing–thawing cycles, voltage, feed solutions on process performance[J]. Chemical Engineering Journal, 2015, 259: 814-819

[6] Wu H, Yu G, Pan L, et al. Stable Li-ion battery anodes by in-situ polymerization of conducting hydrogel to conformally coat silicon nanoparticles[J]. Nature Communications, 2013, 4(3): 1943

[7] Dong W, Bente M, Yurong Z, et al. Preparation and Properties of Semi-Self-Assembled Lipopeptide Vesicles[J]. Langmuir, 2019, 20:56-59

[8] Abirami S, Marco M, Aldo R. Boccaccini. Preparation and characterization of mesoporous calcium-doped silica-coated TiO2 scaffolds and their drug releasing behavior[J]. International Journal of Applied Ceramic Technology, 2018, 15:4

[9] Tan L, Xu X, Jia S, et al. Synthesis, Characterization, and Acute Oral Toxicity Evaluation of pH-Sensitive Hydrogel Based on MPEG, Poly(ε-caprolactone), and Itaconic Acid[J]. BioMed Research International, 2013, 2013(9): 229-238

[10] Flory P J, Rehner J J. Statistical Mechanics of Cross-Linked Polymer Networks I.

Rubberlike Elasticity[J]. Journal of Chemical Physics, 2004, 11(11): 521-526

[11]Flory P J. Statistical Mechanics of Swelling of Network Structures[J]. Journal of

Chemical Physics,1950, 18(1): 108-111

[12] Peppas N A. Physiologically Responsive Hydrogels[J]. Journal of Bioactive &Compatible Polymers, 1991, 6(3): 241-246

[13] Way A E, Hsu L, Shanmuganathan K, et al. pH-Responsive Cellulose Nanocrystal Gels and Nanocomposites[J]. Acs Macro Letters, 2012, 1(8): 1001-1006

[14] Zhang Y, Yang B, Zhang X, et al. A magnetic self-healing hydrogel.[J]. Chemical Communications, 2012, 48(74): 9305-9307

[15] Liu H, Wang C, Gao Q, et al. Magnetic hydrogels with supracolloidal structures prepared by suspension polymerization stabilized by Fe2O3 nanoparticles[J]. Acta Biomaterialia, 2010, 6(1): 275

[16] Li L, Wang Y, Pan L, et al. A nanostructured conductive hydrogels-based biosensor platform for human metabolite detection.[J]. Nano Letters. 2015, 15(2): 1146.

[17] Qiu Y, Park K. Environment-sensitive hydrogels for drug delivery[J]. Advanced Drug Delivery Reviews, 2001, 53(3): 321

[18] Teßmar J, Brandl F, Göpferich A. Hydrogels for Tissue Engineering[Z]. Springer Berlin Heidelberg, 2009, 1869-1879

[19] Concheiro A, Alvarezlorenzo C. Chemically cross-linked and grafted cyclodextrin hydrogels: from nanostructures to drug-eluting medical devices[J]. Advanced Drug Delivery Reviews, 2013, 65(9): 1188-1203

[20] He H, Guan J, Lee J L. An oral delivery device based on self-folding hydrogels[J]. Journal of Controlled Release Official Journal of the Controlled Release Society, 2006, 110(2): 339-346

[21] Rathbone M J, Martinez M N. Modified release drug delivery in veterinary medicine[J]. Drug Discovery Today, 2002, 7(15): 823-829

[22] Bronich T K, Satya B, Shlyakhtenko L S, et al. Template-assisted synthesis of nanogels from Pluronic-modified poly(acrylic acid)[J]. Journal of Drug Targeting, 2006,14(6):357-366

[23] Jin W, Xu P, Zhan Y, et al. Degradable Cisplatin-Releasing Core-Shell Nanogels from Zwitterionic Poly(β-Aminoester)-Graft-PEG for Cancer Chemotherapy[J].

Drug Delivery, 2007,14(5):279-286

[24] Yan M, Ge J, Liu Z, et al. Encapsulation of single enzyme in nanogel with enhanced biocatalytic activity and stability[J]. Journal of the American Chemical Society, 2006,128(34):11008-11009

[25] Allen T M. Ligand-targeted therapeutics in anticancer therapy[J]. Nat Rev Cancer, 2002;2(10)：750-763

[26] Ming Y, Zhi X L, Diannan L, et al. Fabrication of single carbonic anhydrase nanogel against denaturation and aggregation at high temperature[J].Biomacromolecules, 2007,8(2):560-565

[27] Khmelnitsky Y L, Neverova I N, Gedrovich A V, et al. Catalysis by α-chymotrypsin entrapped into surface-modified polymeric nanogranules in organic solvent[J]. European Journal of Biochemistry, 1992,210(3):751-757

[28] Jun H, Peijun G, Dongmei X, et al. Stabilization of alpha-chymotrypsin by covalent immobilization on amine-functionalized superparamagnetic nanogel[J].

Journal of Biotechnology, 2007,128(3):597-605

[29] Bronich T K, Vinogradov S V, Kabanov A V. Interaction of Nanosized Copolymer Networks with Oppositely Charged Amphiphilic Molecules[J]. Nano Letters, 2001,1(10):535-540

[30] Vinogradov S V. Colloidal microgels in drug delivery applications.[J]. Current Pharmaceutical Design, 2006,12(36):36-89

[31] Kabanov V A, Skobeleva V B, Rogacheva V B, et al. Sorption of Proteins by Slightly Cross-Linked Polyelectrolyte Hydrogels: Kinetics and Mechanism[J].

Journal of Physical Chemistry B, 2004,108(4):1485-1490

[32] Oh K T, Bronich T K, Kabanov V A, et al. Block polyelectrolyte networks from poly(acrylic acid) and poly(ethylene oxide): sorption and release of cytochrome C[J]. Biomacromolecules, 2007,8(2):490

[33] Vinogradov S, Batrakova E, Kabanov A. Poly(ethylene glycol)–polyethyleneimine NanoGel particles: novel drug delivery systems for antisense oligonucleotides[J].Colloids & Surfaces Biointerfaces, 1999,16(1–4):291-304

[34] Karen M A, Peter S, Mirielle A, et al. Polymeric nanogels produced via inverse microemulsion polymerization as potential gene and antisense delivery agents[J].Journal of the American Chemical Society, 2002,124(51):15198-15207

[35] Vinogradov S V, Bronich T K, Kabanov A V. Self-assembly of polyamine-poly(ethylene glycol) copolymers with phosphorothioate oligonucleotides.[J]. Bioconjugate Chemistry, 1998,9(6):805-812

[36] Missirlis D, Kawamura R, Tirelli N, et al. Doxorubicin encapsulation

and diffusional release from stable, polymeric, hydrogel nanoparticles[J]. European Journal of Pharmaceutical Sciences, 2006,29(2):120-129

[37] Kabanov A V, Valery Yu A. Pluronic block copolymers in drug delivery: from micellar nanocontainers to biological response modifiers[J]. Critical Reviews in Therapeutic Drug Carrier Systems, 2002,19(1):1-72

[38] Niren M, Ming C X, Stephany S, et al. A macromolecular delivery vehicle for protein-based vaccines: acid-degradable protein-loaded microgels[J]. Proceedings of the National Academy of Sciences of the United States of America, 2003,100(9):4995-5000

[39] Vinogradov S V, Kohli E, Zeman A, et al. Chemical engineering of nanogel drug carriers: Increased bioavailability and decreased cytotoxicity[J]. Papers Presented at the meeting.american Chemical Society.division of Polymer Chemistry, 2006, 47(2):27-28

[40] Kohli E, Han H, Zeman A, et al. Formulations of biodegradable Nanogel carriers with 5-triphosphates of nucleoside analogs that display a reduced cytotoxicity and enhanced drug activity[J]. Journal of Controlled Release, 2007,121(1):19-27

[41] Oh J K, Siegwart D J, Lee H I, et al. Biodegradable nanogels prepared by atom transfer radical polymerization as potential drug delivery carriers: synthesis, biodegradation, in vitro release, and bioconjugation[J]. Journal of the American Chemical Society, 2007,129(18):5939-5945

[42] Lee H, Mok H, Lee S, et al. Target-specific intracellular delivery of siRNA using degradable hyaluronic acid nanogels[J]. Journal of Controlled Release, 2007, 119(2):245-252

[43] Yu S, Hu J, Pan X, et al. Stable and pH-sensitive nanogels prepared by self-assembly of chitosan and ovalbumin.[J]. Langmuir the Acs Journal of Surfaces & Colloids, 2006,22(6):2754

[44] Varga I, Szalai I, Mészaros R, et al. Pulsating pH-responsive nanogels.[J]. Journal of Physical Chemistry B, 2006,110(41):20297-20301

[45] Cong C, Zong C W, Chang Y Q, et al. Fabrication of a novel pH-sensitive glutaraldehyde cross-linked pectin nanogel for drug delivery[J]. Journal of Biomaterials Science Polymer Edition, 2007,18(12):1591-1599

[46] OISHI, Motoi, SUMITANI, et al. On-off regulation of 19F magnetic resonance signals based on pH-sensitive PEGylated nanogels for potential tumor-specific smart 19F MRI probes[J]. Bioconjug Chem, 2007,18(5):1379-1382

[47] Jain K K. Nanomedicine:application of nanobiotechnology in medical practice[J].Med Prine Pract. 2008;17(2)：89-101

[48] Allen T M. Ligand-targeted therapeutics in anticancer therapy[J]. Nat Rev Cancer. 2002;2(10)：750-763

[49] Liu T Y, Hu S H, Liu T Y, et al. Magnetic-Sensitive Behavior of Intelligent Ferrogels for Controlled Release of Drug[J]. Langmuir, 2006, 22, 5974–5978

[50] Giri A, Bhowmick M, Pal S, et al. Polymer Hydrogel from Carboxymethyl Guar Gum and Carbon Nanotube for Sustained Trans-Dermal Release of Diclofenac Sodium[J]. Int. J. Biol. Macromol, 2011, 49, 885–893

[51] Zhang Qiang, ZHU Chun-ft, LI Liu-jing. Synthesis and Characterization of β -Cd/acrylamide hydrogel [J]. Liaoning Chemical Industry,2009 (7) : 437-441

[52] Nagahama K, Kawano D. Self-Assembling Polymer, Micelle/Clay Nanodisk/Doxorubicin Hybrid Injectable Gels , for Safe and Efficient Focal Treatment of Cancer[J]. Biomacromolecules, 2015, 16, 880–889

[53] Sutani, K. The Synthesis and the Electric-Responsiveness of Hydrogels Entrapping Natural Polyelectrolyte[J]. Radiat. Phys. Chem, 2001,61,49–54

[54] Indermun S,Choonara Y E, Kumar P. Patient-Controlled Analgesia: Therapeutic Interventions Using Transdermal Electro-Activated and Electro-Modulated Drug Delivery[J] . Pharm, 2014, 103, 353–366

[55] Servant, A. Electroresponsive Polymer-Carbon Nanotube Hydrogel Hybrids for Pulsatile Drug Delivery in Vivo[J]. Adv. Healthcare Mater, 2013, 2, 806–811

[56] Li Y, Huang G, Zhang X, et al. Magnetic Hydrogels and Their Potential Biomedical Applications[J]. Adv. Funct. Mater, 2013, 23, 660–672

[57] Liu T Y, Hu S H, Liu T Y, et al. Magnetic-Sensitive Behavior of Intelligent Ferrogels for Controlled Release of Drug[J]. Langmuir, 2006, 22, 5974–5978

[58] Anirudhan T S, Sandeep D. Synthesis and Characterisation of Chitosan Crosslinked-β-Cyclodextrin Grafted Silylated Magnetic Nanoparticles for Controlled Release[J]. 2013, 25, 806–811

[59] Allen C, Maysinger D, Eisenberg A. Nano-engineering block copolymer aggregates for drug delivery[J]. Colloids & Surfaces B, 1999,16(1-4):3-27.

[60] Zhang Y, Guan Y, Zhou S. Permeability control of glucose-sensitive nanoshells.[J].

Biomacromolecules, 2007,8(12):3842-3847

[61] Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue.[J]. Adv Drug Deliv Rev, 2012, 64(3): 329-347

[62] Beningo K A, Wang Y L. Fc-receptor-mediated phagocytosis is regulated by mechanical properties of the target.[J]. Journal of Cell Science, 2002, 115(Pt 4): 849

[63] Ahmad Z, Pandey R, Sharma S, et al. Pharmacokinetic and pharmacodynamic behaviour of antitubercular drugs encapsulated in alginate nanoparticles at two doses.[J]. International Journal of Antimicrobial Agents, 2006, 27(5): 409-416.

[64] Yoo J W, Doshi N, Mitragotri S. Adaptive micro and nanoparticles: Temporal control over carrier properties to facilitate drug delivery [J]. Advanced Drug Delivery Reviews, 2011, 63(14-15): 1247

[65] Hamidi M, Azadi A, Rafiei P. Hydrogel nanoparticles in drug delivery[J]. Adv Drug Deliv Rev. 2008, 60(15): 1638-1649

[66] Knuth K, Amiji M, Robinson J R. Hydrogel delivery systems for vaginal and oral applications: Formulation and biological considerations[J]. Advanced Drug Delivery Reviews, 1993, 11(1–2): 137-167

[67] Nagai T, Machida Y. Buccal delivery systems using hydrogels[J]. Advanced Drug Delivery Reviews, 1993, 11(1–2): 179-191.

[68] Kashyap N, Kumar N, Kumar M N. Hydrogels for pharmaceutical and

biomedical applications.[J]. Critical Reviews in Therapeutic Drug Carrier Systems, 2005, 22(2): 107

[69] Lin C C, Metters A T. Hydrogels in controlled release formulations: network design and mathematical modeling[J]. Advanced Drug Delivery Reviews, 2006, 58(12–13): 1379-1408

[70] Peppas N A, Bures P, Leobandung W, et al. Hydrogels in pharmaceutical formulations[J]. European Journal of Pharmaceutics & Biopharmaceutics, 2000, 50(1): 27-46

06, 851(3): 1157