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Faculty of Chemical and Bio pharmaceutical Technologies
Department of Industrial Pharmacy

Master's thesis

on the topic STUDY ON THE FORMULATION TECHNOLOGY OF SILYBIN NANOCRYSTALS GEL FOR RESIST SKIN INJURY

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

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АННОТАЦІЯ

Xiang LIU. Дослідження технології виготовлення нанокристалічного гелю силібіну для захисту шкіри від пошкоджень. – Рукопис

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

Магістерська робота присвячена приготуванню нанокристалічного гідрогелю сілібіну для запобігання пошкодженню шкіри та вивченню цього процесу. В ході експерименту було приготовлено наносуспензія силібіну, нанокристалічний гідрогель силібіну та досліджено вплив нанокристалічного гідрогелю силібіну на шкіру, пошкоджену УФВ-променями. Встановлено, що середній розмір частинок наносуспензії силібіну дорівнює 41,36 нм, а індекс дисперсності складає 0,2848. Розмір частинок 10 % наносуспензії силібіну становить менше 14,21 нм; 50% - менше 27,02 нм; 90% - менше 51,46 нм. У процесі приготування нанокристалічного гідрогелю силібіну одержано гідрогелевий продукт з високою стабільністю та відчуттям освіження шкіри. В експериментах на мишах було доведено, що нанокристалічний гідрогель силібіну має очевидний захисний ефект при нанесенні на шкіру, пошкоджену при УФВ опроміненні. Розглянуто також перспективи подальшої розробки нанокристалічного гідрогелю силібіну. Наносуспензія має багато переваг, яких не мають традиційні рецептури. Подальші дослідження приготування наносуспензії може підвищити її стабільність і має хорошу біосумісність.

Ключові слова: нанокристалічний гідрогель силібіну, стабілізатори, методи приготування

SUMMARY

Xiang LIU. Study on the formulation technology of silybin nanocrystals gel for resist skin injury

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

The master's thesis is devoted to the preparation of nanocrystalline hydrogel silibin to prevent skin damage and study this process. During the experiment, nano-suspension of silibin, nanocrystalline hydrogel of silibin was prepared and the effect of nanocrystalline hydrogel of silibin on UV-damaged skin studied.

It has established that the average particle size of the silybin nanosuspension is 41.36 nm, and the dispersion index is 0.2848. The particle size of the 10% silybin nanosuspension is less than 14.21 nm, 50% is less than 27.02nm, and 90% is less than 51.46 nm. In the preparation process of silybin nanocrystalline hydrogel, a hydrogel product with strong stability and refreshing skin feel has obtained. Finally, in nude mice experiments, it is proved that silybin nanocrystalline hydrogel has obvious protective effect on UVB-induced skin damage.

Prospects for further development of nanocrystalline silibin hydrogel are also considered. Nanosuspension has many advantages that traditional formulations do not have. Further preparation of nanosuspension can expand the stability of the suspension and has good biocompatibility.

Key words: *silybin nanocrystals hydrogels, stabilizers, preparation methods*

List of abbreviations, symbols

English abbreviations	English full name	The full name in Chinese
TAA	Thioacetamide	硫代乙酰胺
MRSA	Methicillin-resistant Staphylococcus aureus	耐甲氧西林金黄色葡萄球菌
SD	Solid dispersion	固体分散体
SDS	Sodium dodecyl sulfate	十二烷基硫酸钠
PVP	Polyvinylpyrrolidone	聚乙烯吡咯烷酮
TPGS	Succinate	琥珀酸盐
PEG	Polyethylene glycol	聚乙二醇
PLC	Poly（ε-caprolactone）	聚（ε-己内酯）
MPEG	Hydrophilic block	亲水性嵌段
BCS	Biopharmaceutics Classification System	生物药剂学分类
PDI	Polydispersity	粒度分布
PVA	Polyvinyl alcohol	聚乙烯醇
PTK	Tyrosine kinase	酪氨酸激酶
DNA	Deoxyribonucleic acid	脱氧核糖核酸
RNA	Ribonucleic acid	核糖核酸
SA	Staphylococcus aureus	金黄色葡萄球菌
TPC	Total plate count	细菌总数
IDD-PTM	Insoluble Drug-Delivery Particles	微流体技术对粗品混悬液进行均质化处理
CMX	Cefmenoxime	头孢氨噻肟唑
AUC	Authentication Center	鉴权中心

RES	Reticuloendothelial System	网状内皮系统
IPN	Interpenetrating network	互穿网络
SEMI-IPN	Semi-interpenetrating network	半互穿网络材料
MPEG	Moving Picture Experts Group	运动图象专家组
MPS	Mononuclear phygocyte system	单核吞噬细胞系统
HPMC	Hydroxy propyl methyl cellulose	羟丙基甲基纤维素
MDR	Multiple Resistant Bacteria	多重耐药细菌
MBIC	Butyl imidazole	丁基咪唑
DAPI	4,6-diamidino-2-phenylindole	4,6-二脒基-2-苯基吲哚
AAP	American Academy of Pediatrics	美国儿科学会
TCM	Traditional Chinese medicine	中医
MIC	Military-Industrial Complex	军界，工业界集团
ROS	Reactive oxygen species	活性氧
MMP	Matrix metalloproteinase	基质金属蛋白酶
TLR	Toll-like receptor	Toll-样受体
UV	Ultraviolet	紫外线
UVA	Ultraviolet A	长波紫外线
UVB	Ultraviolet B	慢性中波紫外线
UVC	Ultraviolet C	短波紫外线
SPF	Sun protection factor	防晒指标

CONTENT

INTRODUCTION

More than 35% of the potential drugs have the problem of poor water solubility, which makes some potential drugs with key effects unable to be sold or produced good therapeutic effects. Company Elan cooperated with a few large pharmaceutical companies and successfully developed two new formulations and put them on the market using nano-crystallization technology. Using this technology, micron-sized drug crystals are wet-processed into nano-sized particles and adsorbed on the surface of the particles. The stabilizer can improve the stability of nanoparticles and inhibit the agglomeration of crystals.

For many years, as a functional biomass matrix material, hydrogel has been endowed with unique functions, such as biocompatibility, strong water absorption and water retention, responsiveness, adsorption, chemical modification, etc. It has a huge range of added and application value in the fields of pharmacy, chemistry, electronics, environment, etc. According to different synthetic materials, hydrogels can be divided into two types: natural polymer hydrogels and synthetic polymer hydrogels. Drug nano-crystallization, also known as nanosuspension, is a submicrocolloid dispersion system prepared by self-assembly or fragmentation technology by dispersing drug particles in a medium with a small amount of surfactant or polymer as a stabilizer. Drug nanocrystals can be administered in various ways, oral dosage forms are relatively rare, and transdermal dosage forms are mostly in the exploratory stage. However, these two methods of drug delivery are difficult to directly enter cells in the form of nanocrystals, resulting in low cell uptake rates. In contrast, intravenous injection significantly increases the rate of cell uptake. The development of nanocrystals for intravenous injection can reduce the adverse reactions of existing intravenous products and achieve targeted drug delivery. The reduction of drug particles from micrometer to nanometer will cause special changes in their physical and chemical properties, making them have different physical and chemical properties from ordinary preparations. For example, making water-insoluble drugs into nanocrystals can effectively increase their

solubility and dissolution rate, thereby significantly improving their oral bioavailability. The skin permeability of the drug increases with the increase in saturated solubility. The adhesion of the drug to cell membranes or biofilms is significantly increased. The preparation of isotonic nano-aqueous suspension can effectively reduce the dose of intravenous medication and the incidence of adverse reactions. Although some insoluble drugs can also be improved by adding surfactants (such as polyoxyethylene castor oil EL) or adding solvents.

Studies have shown that drug nanocrystals prepared by Tween-80 or Poloxam 188 can avoid the above-mentioned adverse reactions and are well tolerated by intravenous injection [1-6]. Currently, there are two basic processes for the preparation of drug nanocrystals: bottom-up (self-assembly technology) and top-down (crushing technology). The latter is more widely used in the pharmaceutical industry.

A hydrogel is a gel that uses water as a dispersion medium. The water-soluble polymer with a network cross-linked structure introduces a part of hydrophobic groups and hydrophilic residues. The hydrophilic residues and water molecules are combined in the network, the water molecules are combined in the network, and the hydrophobic residues are in the water of the cross-linked polymer. It is a polymer network system that is soft, can maintain a certain shape, and can absorb a lot of water. The formation principle is that all water-soluble or hydrophilic polymers can form hydrogels through certain chemical or physical cross-linking. These polymers can be divided into two categories: natural and synthetic according to their source. Natural hydrophilic polymers include polysaccharides (starch, cellulose, alginic acid, hyaluronic acid, chitosan, etc.) and polypeptides (collagen, polylysine, polyglutamic acid, etc.). Synthetic hydrophilic polymers include alcohols, acrylic acid and their derivatives (polyacrylic acid, polymethacrylic acid, polyacrylamide, etc.). Hydrogels can be divided into physical gels and chemical based on the different bonding of the hydrogel network gel. The physical gel is formed by the physical forces such as electrostatic interaction, hydrogen bonding and chain entanglement. This kind of gel is non-permanent and can be converted into a

solution by heating, so it is also called pseudo gel or thermo-reversible gel. Many natural polymers are in a stable gel state at room temperature, such as K2 carrageenan, agar, etc. A typical example of synthetic polymer is polyvinyl alcohol (PVA), which can be frozen and thawed to form a stable hydrogel below 60° C. Chemical gel is a three-dimensional network polymer formed by cross-linking of chemical bonds. It is permanent and is also called true gel.

According to the size and shape of hydrogels, it can be divided into giant gels and micro-gels (microspheres). Giant gels can be divided into columnar, porous sponge, fibrous, membrane, spherical, etc. according to their shape. The prepared microspheres can be divided into micro-level and nano-level. According to different synthetic materials, hydrogels are divided into synthetic polymer hydrogels and natural polymer hydrogels. Natural polymers have received more and more attention from scholars because of their good biocompatibility, environmental sensitivity, abundant resources and low prices. However, natural polymer materials have poor stability and are easily degraded.

Silybin properties: silybin is white crystalline powder. No peculiar smell, smell a bit bitter, strong hygroscopicity. Easily soluble in acetone, ethyl acetate, methanol, ethanol, slightly soluble in chloroform, almost insoluble in water. Silybin is suitable for the treatment of liver cirrhosis, acute and chronic hepatitis, liver poisoning and other diseases. Silybin has an effective anti-inflammatory effect. Current research shows that silybin has good biological activities, such as liver protection, anti-tumor, cardiovascular protection, antibacterial and so on. Studies have shown that silybin can treat some common problems of ordinary women, such as vaginal ulcers, uterine fibroids, and cervical erosion [9-13].

Ultraviolet rays (UVB) under the sun is one of the main environmental causes of skin damage, which can induce complex changes in skin cells, including DNA damage, oxidative stress, inflammation and apoptosis. The prevention and repair of UVB damage requires a variety of interventions, such as DNA repair, removal of reactive oxygen species (ROS) and inhibition of inflammation. Silybin is a traditional flavonoid hepatoprotective agent, which has a significant protective

effect on UVB-induced skin cell damage, but its mechanism is not fully understood. Previous studies in vivo have shown that silybin can inhibit UVB-induced skin inflammation in mice. UVB irradiation reduces the autophagy of epidermal cells and increases the autophagy of dermal cells, while silybin regulates autophagy in both directions to inhibit epidermal and dermal inflammation. In order to clarify the protective mechanism of silybin on UVB-damaged skin cells, it is necessary to study epidermal cells and dermal cells separately.

The purpose of this paper is to prepare a silybin nanocrystalline hydrogel that can effectively prevent the skin from the damage by ultraviolet rays. This research takes the plant silybin as the main body and the mouse epidermis as the research object. The mid-grinding method was used to prepare nanocrystalline silybin hydrogel. Milk thistle nanocrystalline hydrogel is a traditional Chinese medicine cosmetic. Compared with modern cosmetics Silybum marianum hydrogel has the characteristics of a long history, diverse functions, pure naturalness, and overall treatment. It is more in line with the modern concept of natural health and has a potential commercial market. In the field of pharmacy, this experiment mainly uses pure plant formulas of traditional Chinese medicine. Chinese medicine can further nourish the skin by nourishing qi and blood, nourishing the spleen and stomach, nourishing the lung and nourishing the kidney.

SECTION 1 PRESCRIPTION STUDY

1.1 Introduction to Silybin

Silybin, which belongs to the genus Compositae, Silybum marianum, is widely found in North Africa and southern Europe. It is a folk medicinal herb suitable for the treatment of liver diseases. It was not until the 1960s that German pharmacists successfully extracted the effective ingredients of silybin, that my country used silybum marianum as a medicinal plant to promote liver protection on a large scale. Silybum marianum is shown in Figure 1.1.

Figure 1.1 – Silybum marianum

Silybin is white crystalline powder. Slightly bitter, odorless, hygroscopic. Its melting point is 167°C, and it melts when heated to about 80°C.

Silybin is an active ingredient extracted from the fruit or seeds of the silybin plant. It belongs to flavonoids and consists of one molecule of Taxifolin (flavonoids) and one molecule of Coniferyl (lignans). Silybin is a mixture of two stereoisomers. It is insoluble in chloroform and water, easily soluble in aqueous solutions with a pH value of 7-12, and more easily soluble in organic solvents such as ethyl acetate, acetone, ethanol and methanol. Silymarin, silymarin A, silymarin B are isomers of silybin. Silybin has a history of more than 2,000 years in the treatment of liver and gallbladder diseases in ancient China [15-23]. Clinical studies have shown that liver damage caused by various

types of carbon tetrachloride, muscarinine, TAA and other liver poisons, such as hepatitis and cirrhosis. The mechanism of silybin action is to remove active oxygen free radicals in the body and protect liver cells from damage. Promote the repair and regeneration of liver cells. Silybin has protective and therapeutic effects, and it inhibits the increase in ALT caused by tetratryptamine also [1]. This year's study found that silybin also has anti-prostate cancer, bladder cancer, breast cancer and colon cancer effects, which has attracted widespread attention at home and abroad. In addition, silybin has a very strong antioxidant function, which can eliminate free radicals in the human body, anti-aging, health food, biopharmaceuticals, food, cosmetics, skin care products and other fields. The molecular formula of silybin is shown in Figure 1.2.

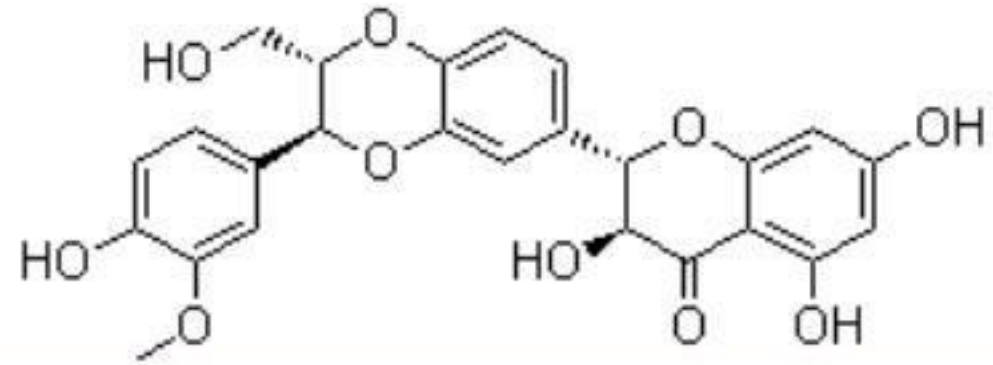

Figure 1.2 – Silybin molecular formula

1.1.1 Pharmacological activity of silybin

(1) Anti-tumor

As we all know, silybin is a broad-spectrum anti-tumor drug, which acts at different stages of the tumorigenesis process [2]. Its mechanism of action is to inhibit the activity of tumor receptors (tyrosine kinase, PTK), such as inhibiting the transformation of epidermal growth factor and insulin growth factor and their molecular signals, and inducing apoptosis of cancer cells by promoting the expression of apoptosis regulators. Regulate the cell cycle to prevent the proliferation of cancer cells. By regulating and promoting autophagy factors to

induce cancer cells to swallow themselves, it has a strong inhibitory effect on prostate cancer, breast cancer, colon cancer and lung cancer. In the treatment of type 2 diabetes, significant clinical effects have been achieved by reducing glycolytic pathways.

(2) Liver protection

Silybin has many physical and chemical properties such as scavenging free radicals, anti-lipid, anti-oxidation, maintaining cell membrane stability, and reducing lipid content in blood. Silybin also has a strong protective effect on the liver, so silybin has a good protective effect on liver damage caused by some hepatic toxins. The liver protection mechanism of silybin can be divided into:

① Protective effect on liver membrane and protect liver function. Maintain the fluidity of the cell membrane through peroxidation, prevent damage to the liver cell membrane, and enhance the ability of the liver cell membrane to resist related hepatic toxins.

② Silybin promotes the repair and regeneration of liver cells, and can enhance the expression of RNase in the nucleus, thereby promoting cell repair and regeneration.

③ Anti-liver fibrosis, silybin can directly inhibit the activation of cell-related factors and inhibit the occurrence of liver fibrosis.

In terms of function, silybin is commonly used to treat liver damage such as alcoholic liver disease, liver fibrosis, and non-alcoholic fatty liver.

(3) Cardiovascular protection

Cardiovascular diseases threaten human life and health, and are the main cause of human health deaths. Silybin can increase the efficiency of protein expression in mitochondria, reduce the damage of cardiomyocytes, improve mitochondrial function, and protect cardiomyocytes. At the same time, silybin can reduce the damage of cardiomyocytes caused by hypoxia and lack of sugar. Silymarin regulates the activity of antioxidant enzymes, blocks oxidative stress, improves hemodynamics, and blocks myocardial ischemia-reperfusion injury by regulating the expression of inflammatory factors and NADPH oxidase II, and improves

cardiac complications of sepsis.

(4) Antibacterial

There are two main mechanisms of action of natural medicine on bacteria, one is to destroy the cell wall of bacteria, the other is to destroy the integrity of cell membrane, hinder the energy metabolism of bacteria, and affect the synthesis of bacterial protein, RNA or DNA. Many studies have proved that silybin can inhibit bacterial protein expression and destroy bacterial cell walls through the following measures [3]. Silybin can inhibit the synthesis of nucleic acid and protein of Staphylococcus epidermidis, destroy the structure of the biofilm, and weaken the growth of the biofilm of Staphylococcus epidermidis and the activity of topoisomerase. In addition, silybin inhibits the efflux gene expression of methicillin-resistant Staphylococcus aureus quaternary ammonium salt resistance protein and quinolone resistance protein, which can inhibit the efflux system and restore the antibiotic sensitivity of MRSA.

In summary, although silybin has a variety of pharmacological activities, its water solubility is poor, and its equilibrium solubility in distilled water at 25°C is only 40 mg/ml. Silybin has a short half-life in plasma, low bioavailability in the body, and low utilization rate, which hinders the application and development of silybin [4]. At present, the bioavailability of soy milk thistle can be improved by the formation of salt, cyclodextrin inclusion, solid liposomes, etc., but the use of these technologies has a certain range, beyond which they are no longer applicable. These technologies will cause some adverse reactions, such as poor physical stability, low drug-polymer interactions or excessive addition of accessories can cause adverse reactions.

1.2 Introduction to nanocrystals

1.2.1 Research progress of nanocrystals

At present, more and more new active drug molecules are discovered, and about 40% of the drugs under development and 70% of the synthetic drugs under

high-throughput screening are insoluble drugs with low solubility and high permeability. According to incomplete statistics, the clinical loss caused by insoluble drugs is as high as 60 billion U.S. dollars each year. At the same time, due to poor water solubility, low oral bioavailability, and unstable absorption, patients can only achieve therapeutic effects by increasing the dose of the drug, and large doses may cause toxic side effects and cause great physical harm to the patient. The increase in solvents may greatly cause thorny safety issues such as patient allergies, irritation, drug release, and concentration reduction. The preparation methods to improve bioavailability mainly include salt method, cyclodextrin inclusion method, emulsion method, solid dispersion method and so on. Although these methods can improve the insolubility of drugs, they also have some drawbacks. For example, the cyclodextrin inclusion technology has high requirements for drug molecules, solid dispersion, and poor physical stability.

The basic structure difference between nanocrystals and other nano-drug delivery systems is shown in Figure 1.3.

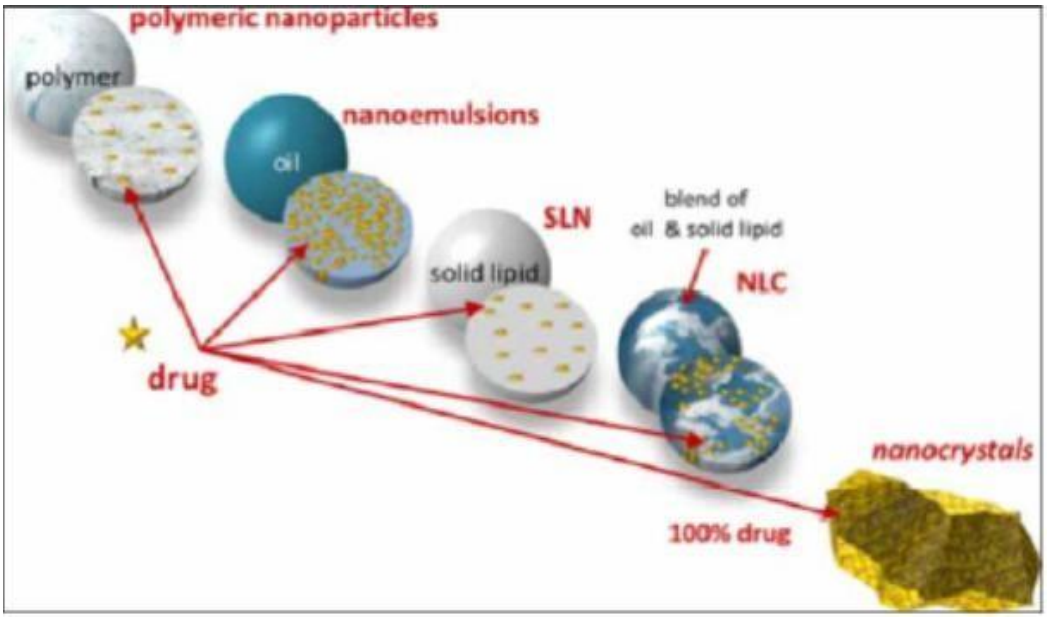

Figure 1.3 – The basic structure difference between nanocrystals and other nano drug delivery systems

Drug nanocrystals, also called nanosuspensions, use a small amount of surfactants or polymers as stabilizers to disperse the drug particles in a medium and process them through some physical or chemical methods. The drug nanosuspension is a submicron colloidal dispersion system prepared by self-assembly or fragmentation technology, which disperses the size of the drug particles in a fixed partition. The particle size range is 20-500 nm. According to the Noyes-Whitney equation, small particles with a large specific surface area can increase the solubility and dissolution rate of insoluble drugs in vivo and in vitro, thereby increasing the bioavailability of insoluble drugs in the human body. Macromolecules and surfactants should be selected as stabilizers to reduce toxicity, improve efficacy, and improve patient compliance.

Nanocrystals can be used as the final dosage form or as an intermediate dosage form, which can be further processed into tablets, capsules, patches or sprays, etc., and can be administered orally by injection, pulmonary inhalation or ocular administration. Among them, oral dosage forms are few, and transdermal dosage forms are mostly in the research stage. However, it is difficult for drugs to enter cells directly in the form of nanocrystals, and the cell uptake rate is low. In contrast, intravenous injection significantly increases the rate of cell uptake. The development of nanocrystals for intravenous injection can reduce the adverse reactions of existing intravenous products and achieve targeted drug delivery.

1.2.2 Preparation method of nanocrystalline

Currently, there are two basic processes for the preparation of drug nanocrystals: bottom-up (self-assembly technology) and top-down (crushing technology). The latter is more widely used in the pharmaceutical industry.

(1) Self-assembly technology

The basic principle of this method is to dissolve the drug in a solvent, and to precipitate the drug to form nanocrystals by adding a non-solvent. The key to this process is to control the crystal structure (amorphous or crystalline) to avoid crystal growth beyond the nanometer scale. There are many kinds of self-assembly

technologies, but there are no mature products published, because the technology has some insurmountable defects, such as residual organic solvent, poor physical stability of the preparation, and easy austenitization. Therefore, the actual first choice is to use crushing technology.

Precipitation technology: The drug is first dissolved in a suitable organic solvent, and then the organic solvent containing the drug is added to the aqueous solution containing the stabilizer. The drug is supersaturated and precipitated and crystallized to form nanocrystals. Stable nanocrystals can be obtained by controlling the rate of crystal formation and growth. For example, rapid nucleation and slow nucleation are ideal conditions for preparing nanocrystals. The advantage of this method is that it consumes less mechanical energy and is suitable for water-soluble drugs and insoluble drugs. The disadvantage is the use of organic solvents in the preparation process, and there are safety problems such as solvent residues, which are not widely used in actual production.

(2) Crushing technology

Nanocrystals are made of ground crystals, and there are two basic types: dry grinding and wet grinding. After the wet milling drug particles are dispersed in the surfactant to form a mixed suspension mill, the efficiency is higher than that of dry grinding. The container is stirred, and the crystals in the medium are separated by grinding, and finally nanocrystals are formed. Most commercially available nano-products are manufactured using this technology. Crystals can also be ground by high-pressure homogenization technology, such as IDD-PTM (Insoluble Drug Delivery-Particle) technology of suspension fluid collision, and the crystals form nanocrystals under the effect of collision and cavitation. DissoCubes technology can grind crystals into nanometers through cavitation, collision and liquid shearing, where the suspension can pass through the narrow homogenizer pores at high speed. At present, the above-mentioned technologies must be carried out in a suspended state, and the non-aqueous medium homogenization technology is still in the research stage.

The high-pressure homogenization method uses a high-pressure homogenizer to

collide and shear larger drug particles through a homogenization valve, thereby reducing the size of the drug particles and obtaining nanoparticles. The advantage of this method is that the particle size distribution obtained is uniform, and it is easy to scale up industrial production. It is suitable for the preparation of nanocrystals for sterile injection. The disadvantage is that the preparation of nanocrystals requires multiple homogenization cycles and high homogenization pressure to obtain, and the preparation cycle is long.

In the media grinding method, the drug is first added to an aqueous solution with a stabilizer, and then is placed in a grinder equipped with a grinding medium to cause the drug particles to collide and shear with the grinding medium and the wall of the grinding chamber. Then the filter is used to separate the particles, leaving the large particle size grinding media and drugs in the grinding chamber. The drug with a small particle size enters the recirculation chamber, and can not be directly taken out from the recirculation chamber until the required particle size is reached to obtain drug nanocrystals. The method has a wide range of applicable drugs, simple preparation process, easy industrial production, and small particle size difference between batches. The disadvantage is that the medium will fall off and dissolve during the drug grinding process, so that the prepared nanocrystals contain a certain amount of impurities, which are not suitable for injection administration.

(3) Combination process

Using a single preparation method is not only suitable for a narrow range of drugs, but also difficult to prepare nanocrystals with uniform particle size and good stability, and the preparation effect is not ideal. Therefore, the combination of two or more methods is not only suitable for a wide range of drugs, but also can better prepare nanocrystalline drugs. Compared with a single technology, a combination of several technologies is usually used to improve the safety and effectiveness of the product.

The combined process usually includes pretreatment and high-pressure homogenization, that is, high-pressure homogenization after crystals are precipitated to form a suspension. The advantage of this combination is flexible operation. In the

presence of solvent and no solvent, crystals precipitate at the two countercurrent interfaces, and form nanocrystals after high-pressure homogenization. Combining multiple technologies, including different patents, these technologies can reduce the number of homogenizations and produce nanoparticles with a particle size of less than 100 nanometers. In practical applications, combining H 96 technology (freeze-drying technology and high-pressure homogenization technology) can prepare amphotericin B nanocrystals with an average diameter of only 50 nm. For example, the high-pressure homogenization method combined with the precipitation method. First, the precipitation method is used to obtain larger particles, and then the high-pressure homogenization method is used to obtain products with uniform particle size distribution. The combination of these two methods not only avoids the problem of uneven particle size caused by the precipitation method, but also overcomes the disadvantage of poor physical stability. Therefore, combining different preparation methods to prepare nanocrystals has good development prospects.

1.2.3 Application advantages of nanocrystals

(1) Improve the dissolution rate of insoluble drugs and further increase the bioavailability.

Sandalwood and Astragalus are a kind of plant antitoxin, which is a natural derivative compound. It has anti-inflammatory, analgesic, and anti-tumor effects, but its characteristics such as easy oxidation and poor water solubility are severely restricted in clinical practice. Chen Zhe et al. prepared Astragalus into nanocrystals [61-64]. In vivo pharmacokinetic experiments in mice showed that the CMX and AUC (0-T) of oral nanocrystals were improved, which were 3.38 times and 1.47 times that of the crude drug, respectively, and the bioavailability was significantly improved.

(2) Increase gastrointestinal adhesions, reduce body differences in drug absorption, and reduce the impact of dietary consumption on bioavailability.

Small-sized nanocrystals can increase the adhesion of drugs, and especially are suitable for pulmonary, gastrointestinal and nasal administration, and can significantly improve the absorption of drugs. The adhesion mechanism of nanocrystals mainly includes electrostatic theory, diffusion theory, and adsorption theory. Li Qian et al. orally administered Aprepitant (20 um, 1um) and Aprepitant nanocrystals (230nm) of different particle sizes to rats, and the bioavailability was 33.1%, 46.7%, and 67.5%, respectively. Therefore, nanocrystals can increase the adhesion of drugs and improve bioavailability.

(3) Increase the stability of the formulation

10-Hydroxycamptothecin is easy to open under alkaline conditions, resulting in the loss of original pharmacological activity. The ring-opening rate of nanocrystals prepared by Zhang Yi et al. is less than 10% at pH 7.0-8.0 [9-10].The results show that nanocrystalline technology can improve the stability of preparation.

(4) Increase the safety of the preparation, reduce or even no use of solvents, thereby reducing the toxicity of the drug

Insoluble drugs usually add a large amount of solvent to increase its solubility in water, but adding a large amount of solvent can cause adverse reactions to the human body. For example, the preparation of etoposide injection requires the addition of a large amount of solvents such as Tween-80 to improve the solubility of the drug, but the injection is more irritating and can cause bone marrow suppression. Zhang Dong et al. prepared etoposide into nanocrystals, and the results showed that etoposide significantly reduce the inhibitory effect on bone marrow [19-21]. Therefore, the preparation of insoluble drugs into nanocrystals can reduce the dosage of surfactants, reduce the dosage, and reduce the probability of adverse reactions, thereby improving the safety of preparation.

(5) Targeted drug delivery can be achieved

Nanocrystals can improve the targeting ability of drugs and achieve targeted drug delivery in specific tissues and organs. After intravenous injection or inhalation of drug nanocrystals, when the drug particles are less than 1 nm, they are easily

recognized and ingested by the reticuloendothelial system (RES), and passively gather in the liver, spleen and bone marrow to achieve passive drug targeting. At the same time, after surface modification of drug nanoparticles, active targeting can be achieved. Muller et al. used polyethylene glycol to modify the surface of drug nanoparticles, which can prolong the residence time of the drug in the body. So as to achieve the purpose of long-term circulation of drugs in the body.

(6) Because the drug nanosuspension contains fewer excipients, the carrier and drug loading are higher by changing the size of the insoluble drug crystal particles, dividing the area, and changing the drug metabolism mode, different injections and administration can be achieved the purpose of long-term efficacy.

1.2.4 Biopharmaceutical characteristics of nanocrystals

There are many ways to use nanocrystalline drugs. The most of them are oral, intravenous injection, transdermal administration, inhalation, etc., which have expanded the use of nanocrystalline drugs. Different administration methods will also lead to certain differences in the behavior of nanocrystalline drugs in the body.

(1) Oral

At present, the most reliable and convenient method of administration is oral administration. In oral preparations, dissolution is a key factor affecting drug bioavailability. After mixing and preparing the nanomedicine suspension, its dissolution rate increases a lot, and the absorption capacity of the mucosa increases. At the same time, it can also increase the residence time of the drug in the gastrointestinal tract, which significantly improves the bioavailability of the drug. Targeted delivery of drugs to lymphatic vessel-mediated diseases.

(2) By intravenous injection

The advantages of intravenous administration are rapid action, low dosage and good bioavailability. However, in the production process, for insoluble drugs, it is often necessary to use a small amount of preparations and solvents that may cause serious adverse reactions, and the use of insoluble intravenous preparations is greatly

inhibited. In the production process, the nanocrystalline medicine does not need to be added to the formula, the toxicity and adverse reactions are small, and it is especially suitable for intravenous administration.

(3) Inhalation administration

Compared with the total area of human skin, the total area of alveoli is very large, about 100 square meters. After the medicine enters the lungs, it can quickly enter the large circulation, and the effect is quicker. According to data, the bioavailability of drug nanocrystalline aerosols is significantly higher than that of powder inhalers.

(4) Eye medication

Ocular administration has always been a very difficult way of administration, possibly because of the disorder of the blood-eye barrier around the eyes. Nanocrystalline drugs occupy a large area and form attraction between different molecules in the eye, which can increase the absorption range of the drug and increase the action time [12].

(5) Transdermal administration

The delivery of drugs in the outermost layer of the epidermis plays a crucial role in the bioavailability of transdermal drug delivery. This may be due to the small size of the nanocrystalline drug particles, which can penetrate the gaps in the outermost layer of the epidermis and significantly expand the transdermal ability. After the nanocrystalline drug diffuses, it can stay in a certain part of the skin for a long time, release the drug slowly, and then increase the action time.

1.3 Introduction to hydrogels

1.3.1 Research progress of hydrogel

Generally, a hydrogel is a hydrophilic hydrogel, which is a three-dimensional network polymer with a large amount of water or water as a dispersion medium in the structure. Over the years, researchers have proposed different definitions of hydrogels. The most common definition is the next: a hydrogel is a polymer

network with a cross-linked structure produced by a simple reaction of one or more monomers, that swells in water [43-44]. Another common definition is that hydrogel is a polymer material with hydrophilic groups in the structure, which has a significant water retention effect and can swell in water without dissolving [47]. Many materials, including synthetic materials and natural materials, meet this hydrogel definition.

Wichterle and Lim introduced a hydrophilic gel with biological applications in the early 1960s [27-31]. In the past 50 years, due to the natural tissue flexibility of hydrogels such as high water content, many scientific researchers have conducted a lot of research to improve the performance of hydrogels and promote use of hydrogel. The hydrophilic groups of the polymer backbone determine the water absorption capacity of the hydrogel, and the cross-linking points in the hydrogel network define its insolubility in water. Therefore, the biopolymer hydrogel, due to its high water content, water retention, water swelling characteristics, and biomolecule biocompatibility, flexibility, degradability, and biospecificity, makes the biopolymer hydrogel Glue is of great significance in biomedical fields such as biomaterials, regenerative medicine, biological tissue engineering, and gene therapy. However, poor mechanical strength and high production cost have always been important factors restricting the application and development of biopolymer hydrogels.

The anti-bacterial hydrogel has a certain degree of biocompatibility, can disperse macromolecules of polymer compounds into small molecules, and inhibit bacteria. Therefore, hydrogels have been developed and used in biopharmaceutical fields, such as drug delivery, wound or burn coating, tissue engineering scaffolds, and so on. According to the hydrogel matrix that has been explored for many years, it can be divided into the following types: the hydrogels that inhibit bacteria (alginate hydrogel, chitosan hydrogel, cellulose hydrogel, gelatin hydrogel) and other types of hydrogels (polyvinyl alcohol gels, nano-carbon hydrogels, etc).

1.3.2 Preparation method of hydrogel

According to the different induction systems, the preparation methods of cellulose hydrogel can roughly divide into three methods, namely, chemical initiation method, radiation initiation method and ionized initiation method. The preparation of cellulose hydrogel can also divide into physical cross-linking method and chemical cross-linking method [15]. In the preparation of physical cross-linked hydrogels, although it has an impact on the environment, the mechanical function of the hydrogels is low, and the chemical cross-linking method is used. However, it is a hydrogel prepared by a dynamic chemical free radical graft polymerization method. In terms of structure, the key to chemical crosslinking is stability. The surface groups are more diverse and easier to react with other groups [17] and very conducive to the development of responsive smart hydrogels [16].

1.3.3 Advantages of hydrogel

(1) No greasy feeling, very convenient to apply on the skin, and easy to wash.

(2) It can absorb tissue exudates without affecting the general function of the skin.

(3) Low fluidity is beneficial to the release of drugs, especially the release of water-soluble drugs. The disadvantage is poor lubricity, easy to dehydrate and mold, often need to mix a lot of moisturizers and preservatives.

1.3.4 Classification of hydrogels

Hydrogels can be classified in many ways. According to the different sources of hydrogels, hydrogels can be divided into natural hydrogels and synthetic hydrogels. Among them, natural polymer hydrogels have good biocompatibility and degradability, and there are abundant sources of materials [24-29]. Natural polymer proteins (collagen, gelatin, etc.) and polysaccharides (sodium alginate, chitosan, agarose, etc.) can be used as hydrogels. However, the strength of natural hydrogels is generally poor. Synthetic polymer hydrogels are the opposite.

Hydrogels can be divided into single-component hydrogels, two-component

hydrogels and multi-component hydrogels according to their composition. Among them, the one-component hydrogel is usually a cross-linked skeleton structure formed by the same monomer through its own characteristics or polymerization process. A two-component polymer hydrogel is a polymer network formed by free or alternately connecting two different monomers through polymer chains. The multi-component polymer hydrogel network is a very important hydrogel. They interpenetrate and interact with each other to form an interpenetrating network (IPN) [42] or a semi-interpenetrating network (SEMI-IPN) [36]. In a semi-interpenetrating network, one component is a cross-linked polymer and the other component is a non-cross-linked polymer.

Hydrogels can be divided into amorphous hydrogels, semi-crystalline hydrogels and crystalline hydrogels according to their structure and chemical composition.

Hydrogels can be divided into two types, physical crosslinking and chemical crosslinking, according to their crosslinking types. The hydrogel obtained by chemical cross-linking has a permanent cross-linked structure, while physical cross-linking refers to the physical interaction caused by polymer chain entanglement or hydrogen bond, ionic bond or hydrophobic bond interaction [38]. Hydrogels can be prepared with different physical forms by different polymerization processes during the preparation process. According to different, hydrogel can be divided into hydrogel matrix, hydrogel film or hydrogel microsphere.

According to the different charges of the gel network, hydrogels can be divided into non-ionic hydrogels, ionic hydrogels (including negative ions and cations), amphoteric electrolyte hydrogels containing acidic groups and basic groups, zwitterionic water Each structural unit in the gel contains carboxyl and amino groups [39].

According to the size of hydrogels, hydrogels can be classified into bulk hydrogels, porous hydrogels, microgels, nanogels, etc [40].

1.4 Application of stabilizer

Due to the small size of nanocrystals, large specific surface area, and thermodynamically unstable classification system (referring to chemical reactions, the reaction speed is faster on a macroscopic level), the physical stability of nanocrystals has become the main obstacle to its wide application. The probability of particles sticking to one another increases, and irreversible aggregation can occur.

Nanosuspension is a relatively new drug system, which provides new research ideas for solving the problem of poor water solubility of drugs, and has become a topic of great interest to researchers in the field of biopharmaceuticals. At the same time, there may be physical stability problems in the development, delivery and storage of nanocrystalline drugs. In order to solve the problem of poor physical stability of nanocrystals, it is usually necessary to add one or more polymers or surfactants to reduce the increased electrostatic repulsion and surface free energy between particles. There are two commonly used stabilizers: charge stabilizers, such as SDS. In the actual production process, the addition of polymer materials or multiple substances can significantly reduce the surface tension of the solution, which is likely to cause drug safety issues.

Chitosan is a natural linear polymer, which has the advantages of analgesia, lowering cholesterol, and anti-oxidation. As a biocompatible polymer, it is widely used in the field of biopharmaceuticals. Due to the simultaneous action of electrostatic repulsion and steric hindrance, it can inhibit the agglomeration of particles and the irreversible formation of flocs, and can be used to expand the physical stability of the drug nanocrystalline system. In addition, chitosan and its derivatives are positively charged in the environment and can interact with the negatively charged phospholipids on the tumor surface to collect drugs at the tumor site [40-43]. However, chitosan has poor solubility in an aqueous solution with pH of 7, which greatly limits its application and development in the field of biopharmaceuticals.

Polyethylene glycol has good water solubility and biocompatibility. It has been widely used in the field of biopharmaceuticals. Many studies have proved that peg-modified chitosan molecules can increase their dissolution rate in aqueous solutions

and reduce their metabolic rate in the liver, so that the drug cannot be recognized and absorbed by human cells. Therefore, polyethylene glycol modified chitosan molecules can be used to expand elasticity and water absorption capacity. PCL has good biocompatibility in vivo and is often used as a drug release medium. However, PCL's poor toughness, low dissolution rate, and slow decomposition rate hinder its application and promotion. Using polyethylene glycol to modify PLC [44], prepared copolymers, which improved the dissolution rate and decomposition rate of PLC. Polyethylene glycol-poly(ε-caprolactone) can spontaneously attach to the surface of the non-hydrophilic material drug molecules in the aqueous solution, the drug is wrapped in the core, not only can expand the water solubility of the drug, but also prevent the drug from passing through the body's liquid substance , Enzymes, etc. decompose, and drug activity is lost. MPEG refers to the process of water-absorbent materials made of aqueous solutions, preventing the drug from being dispersed in the body and being recognized and absorbed by the system [45-46], so that more drugs can be delivered to diseased tissues and organs in such a way as to achieve the best therapeutic effect.

1.5 Overview of skin

1.5.1 The physiological composition of the skin

The skin is the first line of defense against environmental damage and the largest organ of the human body [2-3]. It covers the whole body and protects various tissues and organs from physical, mechanical, chemical and pathogenic microorganisms. The skin is mainly composed of epidermis and dermis (Figure 1.4), including subcutaneous fat tissue and its attached hair follicles, sweat glands, fingers (toes) and other organs. In addition, there are abundant blood vessels, lymphatic vessels and nerves running through it. The thickness of the skin varies, the thickest skin is on the sole of the foot, up to 4mm, and the thinnest skin is on the eyelids, less than 1mm [1]. The epidermis is a multilayer flat epithelium, located in the outermost layer of the skin, mainly composed of keratinocytes (derived from the ectoderm)

[4], melanocytes [5] and cells with phagocytic ability. The dermis is located under the epidermis and is mainly composed of collagen fibers and elastic fibers, forming a dense network structure. The most important cell type is fibroblasts [6], which have the function of growing and maintaining the extracellular matrix [17-34]. Derived from the mesoderm. In addition, there are a large number of blood vessels, lymphatic vessels and nerve bundles in the dermis, which are responsible for the support, immunity and sensory functions of the skin. The part where the epidermis and the dermis are connected is called the true epidermal junction or basement membrane. The basement membrane is mainly composed of type IV collagen [9]. The cells of the basal layer of the epidermis are

connected to the basement membrane through hemidesmosomes, and the dermal tissue is mainly connected to the basement membrane through anchoring fibers and elastic microfibers [1-10].

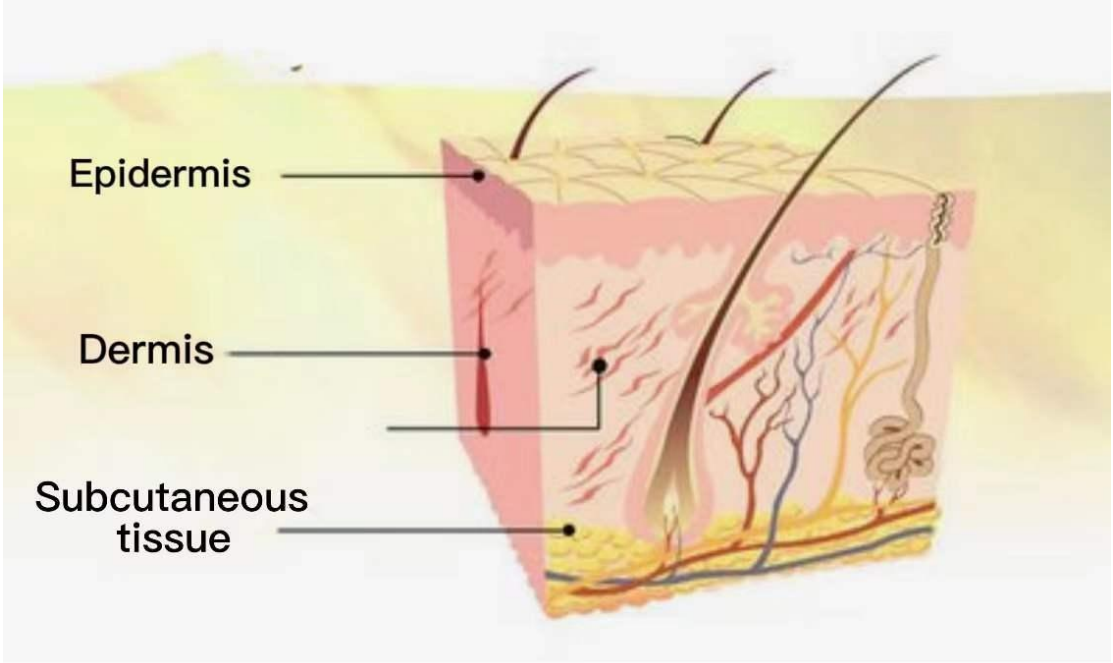

Figure 1.4 – The physiological composition of the skin

1.5.2 Epidermis

The epidermis is a constantly renewing organ composed of layers of constantly dividing keratinocytes. According to the different stages of cell development and

morphological and functional characteristics, from the base to the surface, the epidermis can be divided into basal layer, spinous layer, granular layer, transparent layer and stratum corneum. Basal cells are mainly actively proliferating keratinocytes with the lowest degree of keratinization. There are more free ribosomes and mitochondria in the cytoplasm, and the Golgi apparatus and endoplasmic reticulum are underdeveloped. There are many tendrils in the basal cells, which are perpendicular to the surface of the epidermis. There are also many actin and microtubules, which can make the basal cells

move upward after dividing [1]. There are many organelles in spinous cells, and there are abundant keratin filaments in the cytoplasm. As the degree of differentiation increases, keratinocytes continue to form a granular layer and a transparent layer (only in the palms and soles) until the stratum corneum is completely differentiated. The stratum corneum is the outermost layer of the epidermis. The cells have died and there is no nucleus or other cellular structures. The intercellular layer of the stratum corneum is filled with keratin and amorphous matrix. At the same time, the stratum corneum cells also contain filaggrin and envelope protein, which further enhances the resilience of the cells. The stratum corneum cells continue to fall off and are replaced by newly generated and differentiated cells, keeping the skin cells in a viable state. This is of great significance for the skin to resist external stimuli and can effectively prevent the invasion of exogenous damage. Healthy skin completes a renewal cycle every 28 days, but with age, the renewal cycle of the skin will become longer and longer, leaving too many dead skins that have not been treated for a long time and accumulating skin aging.

1.5.3 Dermis

The dermis is mainly composed of a variety of fibers, matrix and scattered cells. The dermal papilla close to the epidermis is the superficial dermis, also called the papillary layer, underneath it is the reticular layer, but there is no strict boundary between the two [17]. There are mainly three types of fibers in the dermis: collagen

fibers, elastic fibers and reticular fibers. The collagen fibers in the dermis are mainly type I collagen, forming criss-cross collagen bundles, which play the most important supporting role in maintaining the shape of the dermal tissue. The lower part of the reticular layer has a higher density of elastic fibers, most of which are located around the collagen fiber bundles and skin appendages, assisting the formation of the scaffold. It also gives the skin elasticity. The reticular fiber is not an independent fiber component, it is just a simple, thin collagen fiber, and its fiber bundle is spiral and has a certain degree of flexibility. These three main fiber components together form and maintain the shape of the dermal tissue, and provide a living space for the cells in the dermal tissue. The amorphous and uniform colloidal matrix existing between the fibers provides material support for various skin components and provides a place for their material metabolism.

The main functional cells of the dermis are fibroblasts. Responsible for the secretion and maintenance of collagen fibers, elastic fibers, matrix, etc. Abnormal functions will significantly affect the functional state of the skin, leading to skin aging [8-11]. In addition, the mast cells and tissue cells in the skin are responsible for the skin's defense against foreign microorganisms and immune regulation [61]. The vascular network in the dermis can provide nutrients and oxygen to various cells in the skin tissue. The lymphatic system regulates the immune function of skin tissues. The nervous system is responsible for perceiving external sensations, such as heat, touch, and pain, in order to avoid the continuation of trauma [34].

1.5.4 Immunological functions of the skin

In addition to acting as a natural barrier against environmental toxins, the skin can also function as an immune agent. Not only are the immune cells present in the skin from full-time, namely Langerhans cells in the epidermal and dermal lymphatic system, etc., there are also many "part-time" immune cells, such as keratinocytes, fibroblasts, etc., which can receive external stimulation and immunity. The expression of factors affects the chemotaxis and activation of lymphocytes, so that the immune function can be fully exerted.

Keratinocytes: Keratinocytes express MHC-II antigens and produce a variety of cytokines through the toll-like receptor (TLR) pathway. Fibroblasts: Fibroblasts are the resident alarm cells in connective tissue. Substances released during tissue injury or substances secreted by infected microorganisms, such as polysaccharides and autoinducible factors, as well as certain changes in local microenvironmental factors, may activate fibroblasts, produce chemokines, and recruit leukocytes to tissue injury or infection Location. MMP (matrix metalloproteinase), etc., which triggers an inflammatory response. Fibroblasts can determine the cell type and cytokine microenvironment of the inflammation site by expressing certain cytokines such as prostaglandins. As a whole, the epidermis of the skin exists as a defensive barrier, while the dermis mainly supports, maintains and nourishes. They form an interdependent, coordinated and unified overall functional structure, and together work to protect the health of the human body.

1.6 An overview of UVB

1.6.1 Basic features of UVB

Ultraviolet (UV, wavelength 100-400nm) is the ambient light radiation that people are inevitably exposed to in daily life, work and travel. Ultraviolet rays can be divided into short-wave ultraviolet (UVC, wavelength 100-280nm), medium-wave ultraviolet (UVB, wavelength 280-315nm) and long-wave ultraviolet (UVA, wavelength 315-400nm) according to wavelength [26]. The ultraviolet rays in the sun must pass through the atmosphere to reach the surface of the earth. In this process, the short-wavelength UVC is completely absorbed by the ozone layer, and part of the longer-wavelength UVB and most UVA can reach the ground and affect the living things on the earth. On the one hand, ultraviolet light is necessary for the human body, and the synthesis of vitamin D in the human body largely depends on UVB radiation [25]. But at the same time, ultraviolet radiation, especially UVB, is closely related to human skin diseases and even skin cancer [22-24].

1.6.2 UVB protection

UVB radiation can cause skin erythema, inflammation and even cancer, so people have always been concerned about natural radiation to prevent [31]. At present, the protection of UVB mainly focuses on sunscreen and sun protection factor. The sun protection factor specifically refers to the protection ability of sunscreen products against UVB [31]. Current sunscreen products are mainly based on the physical barrier and chemical absorption of UVB, and the cell damage caused by UVB has no repair ability. The ideal sunscreen method should be to protect and nourish the skin cells affected by radiation while blocking ultraviolet rays, so as more comprehensively and thoroughly resist the adverse effects of ultraviolet rays on the human body.

To repair damaged skin cells or pharmacologically improve the ability of cells to respond to UVB, it is necessary to clarify the mechanism by which UVB causes skin cell damage. The main harm of UVB radiation to cells is to affect the normal structure and function of protein and DNA. Serious damage to DNA by UVB can directly lead to cell death or even necrosis.

CONCLUSIONS TO SECTION 1

Silybin, a white crystalline powder. Odorless, slightly bitter, hygroscopic. Easily soluble in acetone, ethyl acetate, methanol, ethanol, slightly soluble in chloroform, almost insoluble in water. Silybin is mainly used for the treatment of liver cirrhosis, acute and chronic hepatitis, liver poisoning and other diseases. For patients with liver function and hepatitis, silybin does have a good therapeutic effect. With anti-shooting effect. Current research shows that silybin has good biological activities, such as liver protection, anti-tumor, cardiovascular protection, antibacterial and so on. Through researches, it is believed that silybin also has a certain therapeutic effect on gynecological diseases such as vaginal ulcer, uterine fibroids, and cervical erosion.

More than 35% of the potential drugs have the problem of poor water solubility, which makes some potential drugs with key effects unable to be sold or produce good therapeutic effects. Elan cooperated with a small number of large pharmaceutical companies to use nanocrystalline technology and successfully developed two new formulations and put them on the market. Using this technology, micron-sized drug crystals are wet-processed into nano-sized particles and adsorbed on the surface of the particles. The stabilizer can improve the stability of nanoparticles and inhibit the formation of agglomerates.

For many years, as a functional biomass matrix material, hydrogel has been endowed with unique functions, such as biocompatibility, strong water absorption and water retention, responsiveness, adsorption, chemical modification, etc. [49]. It has a huge range of added value and application value in the fields of pharmacy, chemistry, electronics, environment, etc. According to different synthetic materials, hydrogels can be divided into two types: natural polymer hydrogels and synthetic polymer hydrogels.

SECTION 2 RESEARCH AND ANALYSIS

2.1 The design idea of this paper

In order to solve the problem of poor water solubility of silybin and improve its water solubility and stability, this article studied the preparation and quality analysis of insoluble silybin nanocrystalline hydrogel. Referring to a large number of literature sources regarding the pharmacological activity of silybin [50-52] and how to improve its solubility and stability, the content of this thesis mainly includes the following parts.

(1) Prescription research. Check the literature to understand the appearance of silybin nanosuspensions and hydrogels

(2) Preparation of silybin nano-suspension. The silybin nano-suspension was prepared, and the particle size distribution and stability of the prepared nano-suspension were studied.

(3) Research on silybin nanocrystalline hydrogel. Before the experiment, we must first consult the information about the hydrogel, investigate the hydrogel process, and then evaluate the quality of the hydrogel, including appearance and stability.

2.2 Experimental instruments and experimental drugs

Experimental apparatus

The instrument	Model	Number
Table top ultrasonic cleaner	JP060（15L）	1
Laboratory ultrapure water unit		1
Electronic balance	400613095095	1
Heated magnetic stirrer		1
Spectrophotometer		1

Measuring cylinder (50ml), pipette, beaker, sposable sterile syringes (5ml), tweezers, microporous membrane (organic) 0.45 μm.

Laboratory reagents

Drug	Standard
Silymarin	FY16070302
PVP K-30	Lot.No.20121206
CH_3CH_2OH	AR 500ml
H_2O	Purified
$NaHCO_3$	AR
Glycerol	AR，99%
Acrylic acid Polymers	
Benzoic acid	>=99%
Ethyl 4-hydroxybenzoate	AR，99%

2.3 Preparation of silybin nanocrystals

The silybin nanoparticles were prepared by reversed-phase solvent ultrasonic precipitation method. The silybin is dissolved in a suitable organic solvent, and the stabilizer is dissolved in distilled water. During the mixing process, the organic phase and the anti-solvent phase must have a certain degree of mutual solubility to prevent extraction during the experiment. The organic solution containing the drug is injected into the aqueous solution containing the stabilizer, and the drug is precipitated in the anti-solvent phase due to oversaturation in concentration. By optimizing the preparation process and conditions, the growth process of the particles is controlled, and the ideal nanoparticle size is obtained.

2.3.1 Silybin solubility test

Take distilled water, pH2.0, pH4.0, pH6.0, pH8.0, pH10.0, pH12.0. The excess silybin was added to the phosphate buffer in a 30mL Erlenmeyer flask and rotated at 37° C until the drug no longer dissolved. Do three sets in parallel. After equilibrating for 48h, filter the supernatant with a 0.45um filter membrane and measure the

absorbance at 288nm. The external standard method was used to calculate the concentration of silybin solution. The experimental results are shown in Table 2.1 and Figure 2.1.

Table 2.1 – Solubility of silybin at different pH (n=3)

Media	Water	pH2.0	pH4.0	pH6.0	pH8.0	pH10.0	pH12.0
Solubility	38.43	42.48	45.21	44.68	99.54	194.35	289.38
($\mu g \cdot ml^{-1}$)	±2.3	±4.9	±5.3	±7.2	±9.6	±24.41	±34.39

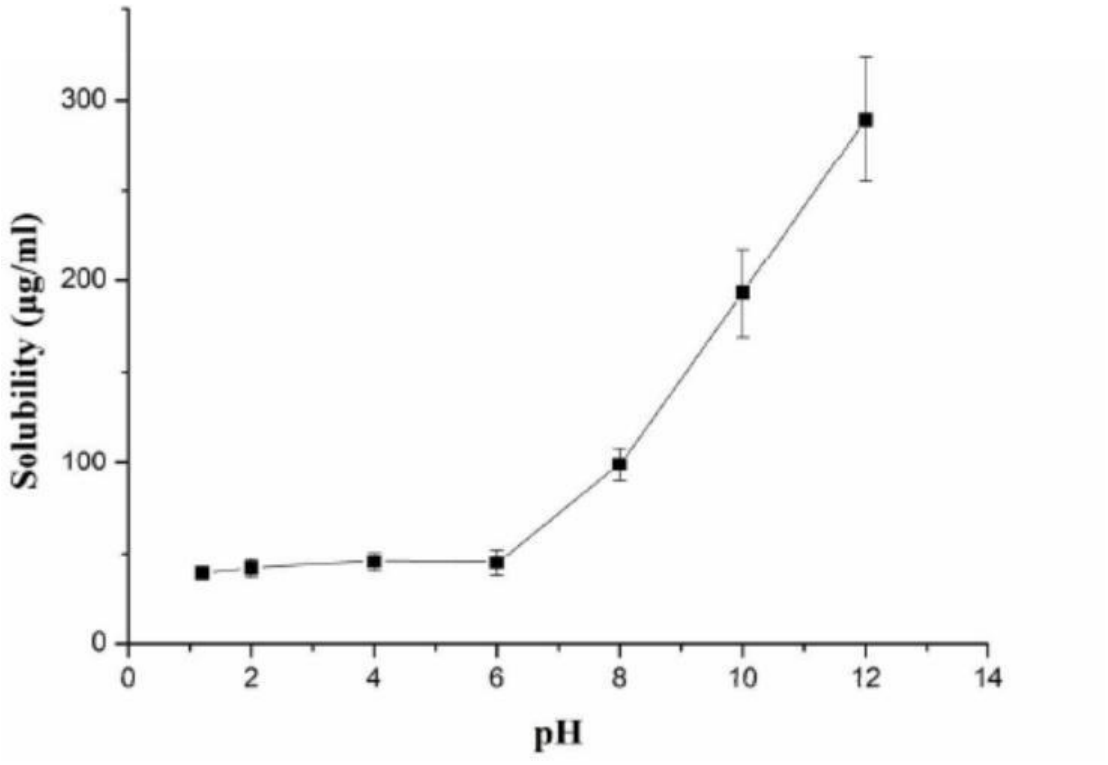

Figure 2.1 – Solubility curves of silybin at different pH (n=3)

According to the experimental results, the solubility of silybin in the buffer solution is very low at different pH values, but as the pH value increases, the

solubility of silybin increases from 38.43 μg·mL^{-1} to 289.38 μg·mL^{-1}. This is due to the influence of the molecular structure formula, which contains a large amount of hydroxyl (-OH) and which can promote the dissolution of the drug in a high pH environment, thereby increasing the solubility.

2.3.2 Screening of organic solvents

Add 5 mL of methanol, ethanol, ethyl acetate, and acetone into the silybin bottle, put it into a magnetic stirrer, and stir at 37°C for 72 hours. Take 1 mL of the sample solution, centrifuge for 15 min, 5500 rmin^{-1}, and stand still for layering to take the supernatant, and filter with a microporous membrane (0.45um). After quantitative dilution, measure the absorbance at 288nm and calculate the saturated solubility, see Table 2.2.

Table 2.2 – Saturated solubility data of silybin in different organic solutions (n=3)

Number.	Organic solvent	Saturation solubility, ± SD(μg · ml^{-1})
1	CH_3COCH_3	7937±574.39
2	CH_3OH	3740±351.73
3	CH_3CH_2OH	2632±336.46
4	$CH_3(CH_2)_3OH$	2159±274.38
5	$CH_3COOCH_2CH_3$	1658±253.32

As shown in Table 2.4, silybin has the highest solubility in acetone solution, but acetone as a solvent has higher toxicity in the pharmaceutical field, and its solubility in ethanol is second only to acetone and methanol. Considering comprehensively, this study chose ethanol as the solvent for the organic phase of silybin.

2.3.3 Influence of stabilizer and drug carrier on particle size

The silybin nanocrystals prepared by traditional stabilizers were compared and

tested. The silybin nanocrystals prepared with different stabilizers were placed for 3 days and tested by the nano-particle-size analyzer, and their particle size, multiple dispersion coefficient (PDI) and potential data were recorded (Table 2.3).

Table 2.3 – Silybin nanocrystals prepared with Different Stabilizers (n=3)

Stabilizer	0 day			3 day		
	Particle size (nm)±S.D.	PDI±S.D.	Zetapotential (mV)±S.D.	Particle size (nm)±S.D.	PDI±S.D.	Zetapotential (mV)±S.D.
Poloxamer188	239.23±1.39	0.134±0.019	-11.26±2.39	397.39±7.48	0.342±0.072	-5.39±1.44
SDS	387.54±8.72	0.274±0.021	-17.26±5.46	594.92±8.31	0.463±0.087	-9.39±1.91
HPMC	287.63±3.46	0.193±0.035	-10.85±3.72	342.16±7.37	0.284±0.052	-6.96±0.86
PVP K30	245.23±4.32	0.242±0.051	-9.48±2.71	438.59±5.72	0.361±0.063	-3.57±1.37
PEG-CS	157.28±3.43	0.109±0.011	-19.82±5.93	183.35±4.43	0.114±0.013	-17.57±4.28
PEG-CS+ PEG-PCL	103.21±2.34	0.094±0.017	-22.64±6.24	123.57±2.41	0.102±0.021	-21.84±5.84

It can be seen from Table 2.5 that the traditional stabilizer has a general stabilizing effect on silybin nanocrystals, and different degrees of precipitation occur within three days after being placed at room temperature. Among them, SDS has the worst stability to silybin nanocrystals, with a large amount of flocculation at the bottom, the largest change in particle size, and a large change in polydispersity coefficient. The HPMC group and PVP K-30 group silybin had the best stabilizing effect, with less bottom sediment. Therefore, PVP K-30 was selected as the stabilizer of silybin nanocrystals.

2.3.4 Influence of injection speed on particle size

40 mg of silybin was accurately weighted and dissolved in 10 mL of ethanol. Magnetic stirring was performed at room temperature until it was completely dissolved, and a 4 mg/mL silybin methanol solution was obtained as the solvent phase. Use a syringe with a microporous filter membrane (0.45um) to absorb a

quantitative anti-solvent phase solution and inject it into a cylinder bottle. Use a microporous filter membrane (0.45um) syringe to absorb a quantitative amount of silybin ethanol solution, and inject it into the cylinder bottle containing the anti-solvent phase at injection speeds of 60 mL/min and 1 mL/min. After magnetic stirring for 1 min and ultrasonic treatment for 15 min, silybin nanocrystals were obtained. The ratio of solvent phase to anti-solvent phase is set to 1:4, with 3 parallel groups for each group. The particle size and PDI were measured with a laser particle size analyzer. The experimental results are shown in Table 2.4.

Table 2.4 – Effect of injection rate on the size of nanometer grain

Injection speed ($ml \cdot min^{-1}$)	The average particle size (nm)	Polydispersion coefficient PDI
60	173.74 ± 9.81	0.127
1	395.85 ± 18.67	0.395

The experimental results show that when the injection rate of the organic phase to the anti-solvent phase is increased from 1 mL/min to 60 mL/min, the particle size of the silybin nanocrystals decreases from 395 nm to 173 nm, and the polydispersity coefficient decreases from 0.395 to 173 nm. 0.127. This is because a fast injection speed will lead to high supersaturation, a large number of small-sized crystal nuclei will be precipitated, so that the particle size of the nanocrystals is smaller, the PDI is smaller, and the physical stability of the sample is higher. Therefore, the injection speed is chosen to be 60 ml/min.

2.3.5 Influence of ultrasonic time on particle size

40 mg of silybin was accurately weighed, dissolved in 10 mL of ethanol, and magnetically stirred at room temperature until it was completely dissolved to obtain a 4 mL/min ethanol solution of silybin as the solvent phase. Use a syringe with a microporous filter membrane (0.45um) to absorb 4 mL of the anti-solvent phase

solution and inject it into a cylinder bottle. Use a microporous filter membrane (0.45um) syringe to draw 1 mL of silybin ethanol solution, and quickly inject it into the cylinder bottle containing the anti-solvent phase. After magnetic stirring for 1 min, silybin nanocrystals were obtained by ultrasonic treatment. The ultrasonic treatment time was set to 5, 10, 15, 20 minutes, and 3 parallel groups were set for each group. The particle size and PDI were measured with a laser particle size analyzer, and the experimental results are shown in Figure 2.2.

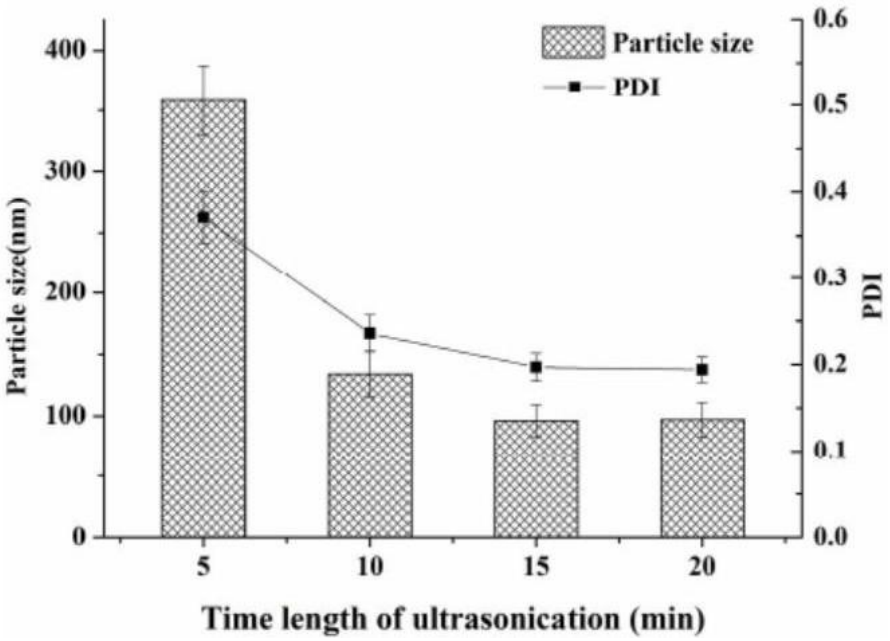

Figure 2.2 – Time length of ultrasoication

As shown in Figure 2.2, the particle size and dispersion coefficient of nanocrystals decrease as the ultrasound time increases, when the particle size and PDI reach the minimum, the ultrasound time is 20 minutes, because ultrasound treatment can inhibit the growth and aggregation of crystals and reduce the surface free energy High, improve their physical stability. When the ultrasonic time was continuously increased to 20 min, the particle size and polydispersity coefficient of the particles did not decrease significantly. This phenomenon indicates that 20min of ultrasonic treatment is sufficient to reduce the particle size of nanocrystals and maintain the stability of the nanocrystal system. Therefore, 20min is selected as the best ultrasound time for silybin nanocrystals.

2.3.6 Influence of ultrasonic temperature on particle size

Accurately weigh 40 mg of silybin, dissolve it in 10 mL of ethanol, and stir magnetically at room temperature until it is completely dissolved to obtain a solvent phase of 4 mL/min. Use a syringe with a microporous filter membrane (0.45um) to absorb 4 mL of the anti-solvent phase solution and inject it into a cylinder bottle. Use a microporous filter membrane (0.45um) syringe to draw 1ml of silybin ethanol solution, and quickly inject it into the cylinder bottle containing the antisolvent phase. After magnetic stirring for 1 min, ultrasonic treatment was performed for 20 min. Obtained silybin nanocrystals. The ultrasonic temperature was 4℃, 25℃ and 40℃, and 3 parallel groups were set up for each experiment. The particle size and PDI were measured with a laser particle size analyzer. The experimental results are shown in Figure 2.3.

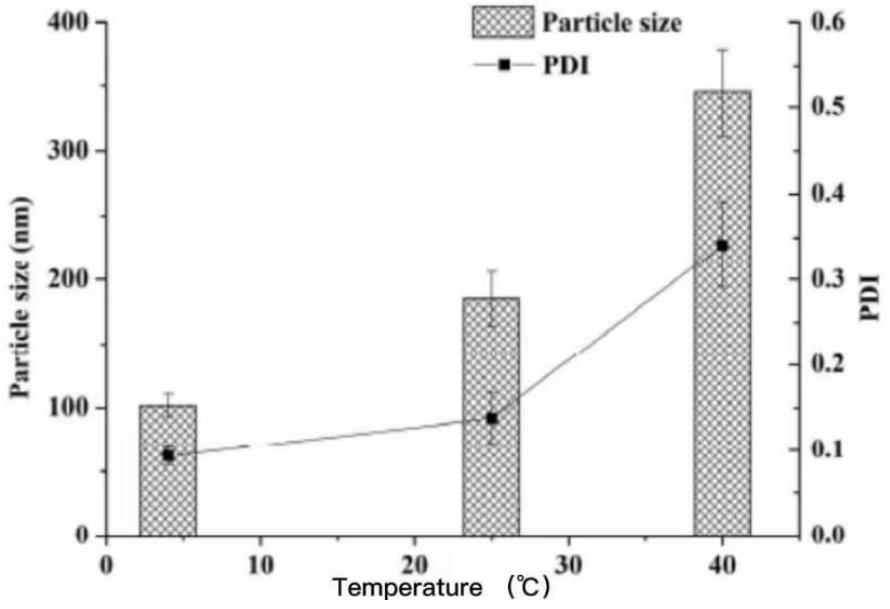

Figure 2.3 – Influence of ultrasonic temperature on particle size

Ultrasonic temperature is also an important parameter that affects the stability of nanocrystals. As shown in Figure 2.3, when the temperature increased from 4°C to 25° C and then to 40 ° C, the particle size and polydispersity coefficient of silybin

nanocrystals gradually increased. It can be seen that at a lower temperature, a narrower particle size distribution and a smaller particle size can be obtained. The decrease of ultrasonic temperature reduces the equilibrium solubility of silybin and increases the supersaturation of silybin. Too high supersaturation will increase the nucleation rate of silybin nanocrystals and reduce its solubility. Therefore, a lower ultrasonic treatment temperature can inhibit the aggregation of drug crystals, make the particle size distribution narrower, and form stable nanocrystals. Based on the above results, 4 ° C was selected as the ultrasonic temperature of the silybin nanocrystals.

2.4 Appearance study of silybin nanosuspension

References indicate that the silybin nano-suspension should be milky white liquid.

Appearance study of silybin nanocrystalline hydrogel

According to the data, the silybin nanocrystalline hydrogel should be milky white and thick.Figure 2.4 shows the Preparation of silybin nanosuspension.

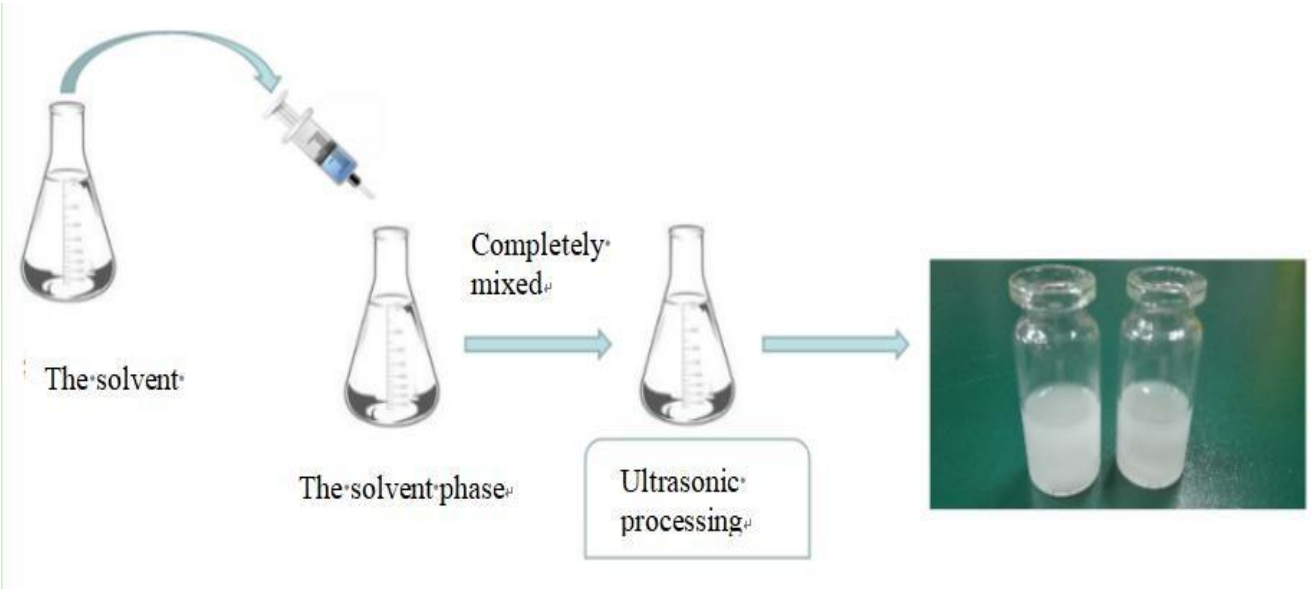

Figure 2.4 – Preparation of silybin nanosuspension

2.5 Preparation of silybin nanocrystalline suspension

（1） Preparation of the organic phase: accurately weigh out silybin with an electronic balance, weigh 0.2g for the first time, 0.1g for the second time, weigh 20ml of ethanol solution with a 50mL graduated cylinder, first dissolve 0.2g of silybin Mix well in 20ml ethanol solution, add a magnet to the beaker where the silybin and ethanol solution are mixed, seal the beaker with plastic wrap, then stir with a magnetic stirrer, stir for 2~3 minutes, then weight it for the second time The silybin taken is added to the mixed solution and stirred until it is completely dissolved. The organic phase is shown in Figure 2.5.

Figure 2.5 - Organic phase solution

（2）Preparation of the inorganic phase: accurately weigh 0.5g polyvinylpyrrolidone K-30 with an electronic balance, add polyvinylpyrrolidone K-30 to 300ml ultrapure water, and mix it with polyvinylpyrrolidone K-30 and ultrapure water Add a magnet to the mixture, seal the beaker with plastic wrap, and stir with a magnetic stirrer until it is completely dissolved. The inorganic phase

solution is shown in Figure 2.6.

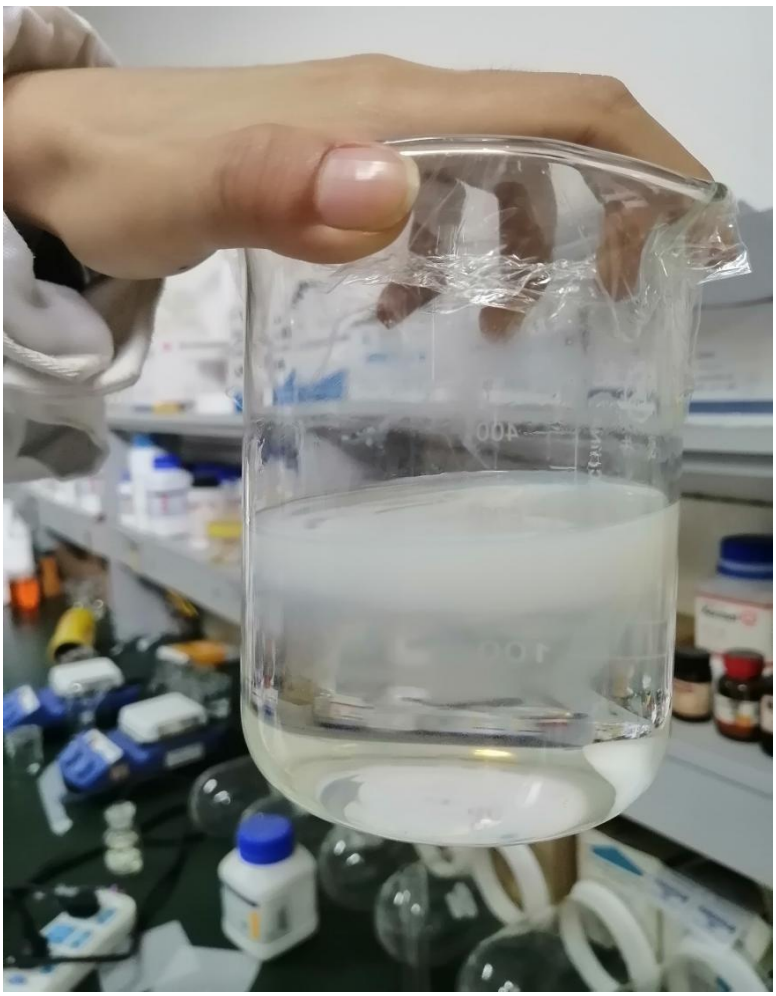

Figure 2.6 – Inorganic phase solution

（3）Use a syringe to add a 0.45μm microporous filter membrane to add the organic phase (mixture of silybin and ethanol solution) to the aqueous phase (inorganic phase) containing the stabilizer. Add a magnet to the mixed solution of phase and organic phase, seal it with plastic wrap, stir with a magnetic stirrer for 10 minutes, after stirring, put it in an ultrasonic cleaner for 10 minutes, and circulate twice. The silybin nanocrystal suspension is shown in Figure 2.7.

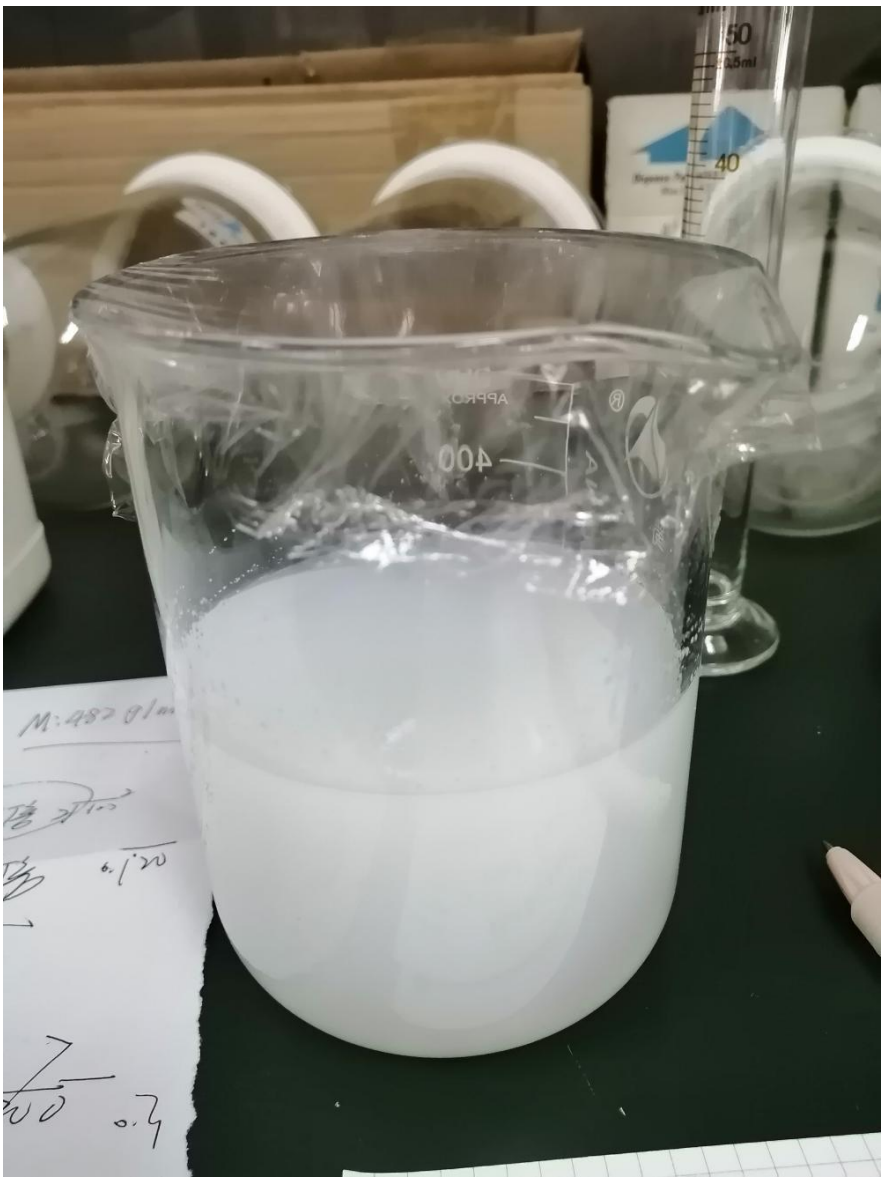

Figure 2.7 - Silybin nanocrystalline suspension

Stability is one of the important indicators for evaluating the quality of nanosuspensions [22]. Weight the newly prepared silybin nanosuspension, and measure the particle size of the sample with a photon-related nanoparticle sizer. The test results indicate With the passage of ultrasound time, silybin nanosuspension may produce a certain degree of precipitation, the average particle size decreases, the dispersion index decreases, and the stability is better. Therefore, the prepared silybin nano-suspension has suitable particle size and relatively uniform distribution, which meets the preparation requirements. The particle size distribution of the silybin nanosuspension is shown in Figure 2.8.

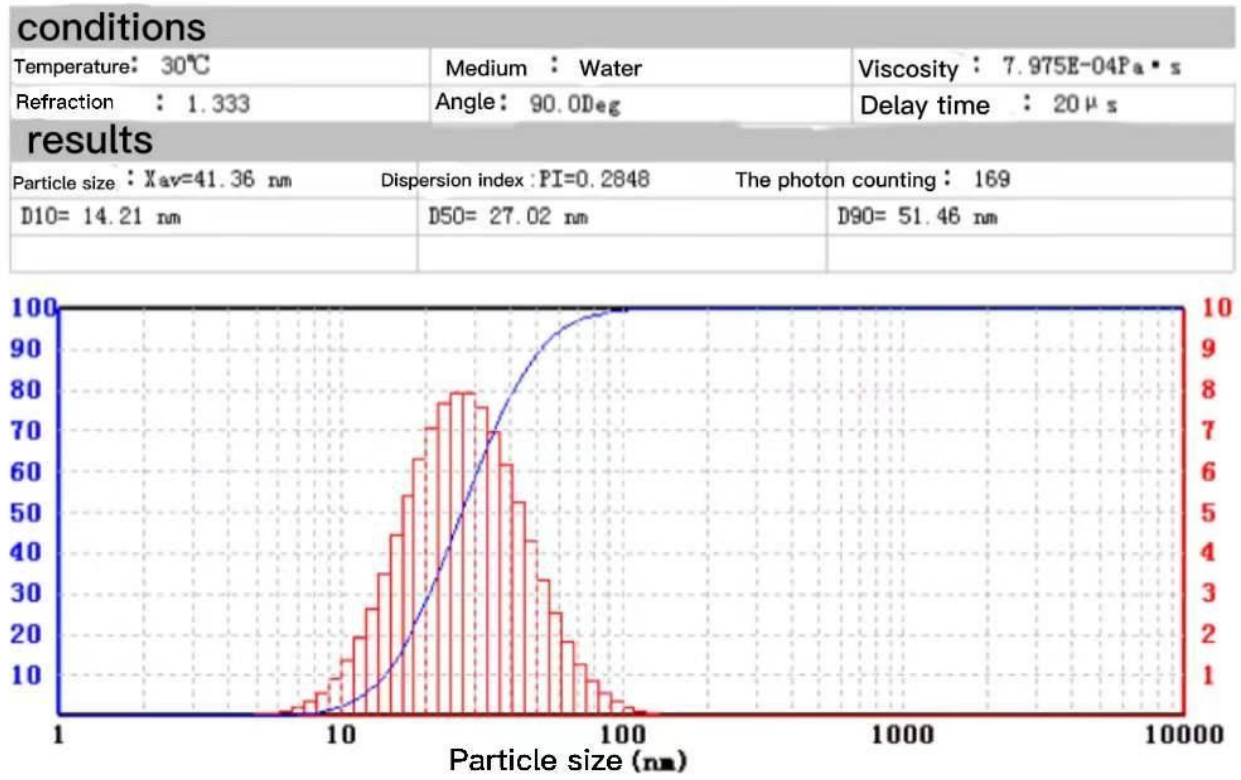

Figure 2.8 – Particle size distribution of silybin nanosuspension

Result analysis: According to the analysis of the particle size distribution diagram of the silybin nanosuspension, the particle size of 10% silybin nanosuspension is less than 14.21nm, and the particle size of 50% silybin nanosuspension is less than 27.02nm, 90% The particle size of the silybin nano-suspension is less than 51.46nm, and the average particle size of the silybin nano-suspension is 41.36nm. The dispersion index of the silybin nano-suspension is 0.2848, the particle size is suitable and the distribution is uniform, which meets the preparation requirements.

After preparing the silybin nano-suspension, the particle size distribution and stability of the nano-suspension were investigated. Note:

①Slowly filter the sample liquid with the microporous membrane, otherwise the membrane will be damaged, which will affect the results of the experiment.

② The filter membrane needs to be replaced every time it is used [23].

③ Before measuring the particle size of the suspension, it is necessary to rinse the contrast cuvette with distilled water three times and then rinse the contrast

cuvette with the test agent three times.

2.6 Study on silybin nanocrystalline hydrogel

2.6.1 Experimental instruments and experimental drugs

Experimental apparatus

The instrument	Model	Number
Table top ultrasonic cleaner	JP060（15L）	1
Laboratory ultrapure water unit		1
Electronic balance	400613095095	1
Heated magnetic stirrer		1
Spectrophotometer		1
Measuring cylinder (50ml), pipette, beaker, sposable sterile syringes (5ml), tweezers, microporous membrane (organic) 0.45 μm		

Laboratory reagents

Drug	Standard
Silymarin	FY16070302
PVP K-30	Lot.No.20121206
CH_3CH_2OH	AR 500ml
H_2O	Purified
$NaHCO_3$	AR
Glycerol	AR，99%
Acrylic acid Polymers	
Benzoic acid	>=99%
Ethyl 4-hydroxybenzoate	AR，99%

2.6.2 Preparation of silybin nanocrystal hydrogel

Measure 40 ml silybin nano-suspension with a graduated cylinder, accurately weight 0.02g (about 0.05% ~ 0.15%) ethyl paraben, add ethyl paraben into the silybin nano-suspension. Add magnets, seal with cling film, stir for 10 min, rotate at 3 mot, after it is completely dissolved, first add 2 ml (about 5%) glycerin, then add 0.6g (about 1.5%) carbomer, stir for 10min, then add 0.4g (approximately 1%) of carbomer, and then let it stand for one night. Finally, adjust the pH value with sodium bicarbonate solution. The preparation of sodium bicarbonate solution: accurately weigh 1g (about 2.5%) of sodium bicarbonate, measure 50ml of water, pour the sodium bicarbonate into the water and mix well to obtain bicarbonate Sodium solution. While adding sodium bicarbonate solution dropwise, the pH was measured at the same time. Finally, the pH of the hydrogel was measured to be about 6, and a total of 5 ml of sodium bicarbonate solution was used.

2.6.3 Appearance inspection

As shown in Figure 2.9, observe the appearance of the silybin nanocrystalline hydrogel. The sample is viscous, the appearance is crystal clear, the surface is smooth and moist but has a certain degree of toughness, it is milky white, and the appearance inspection effect is ideal.

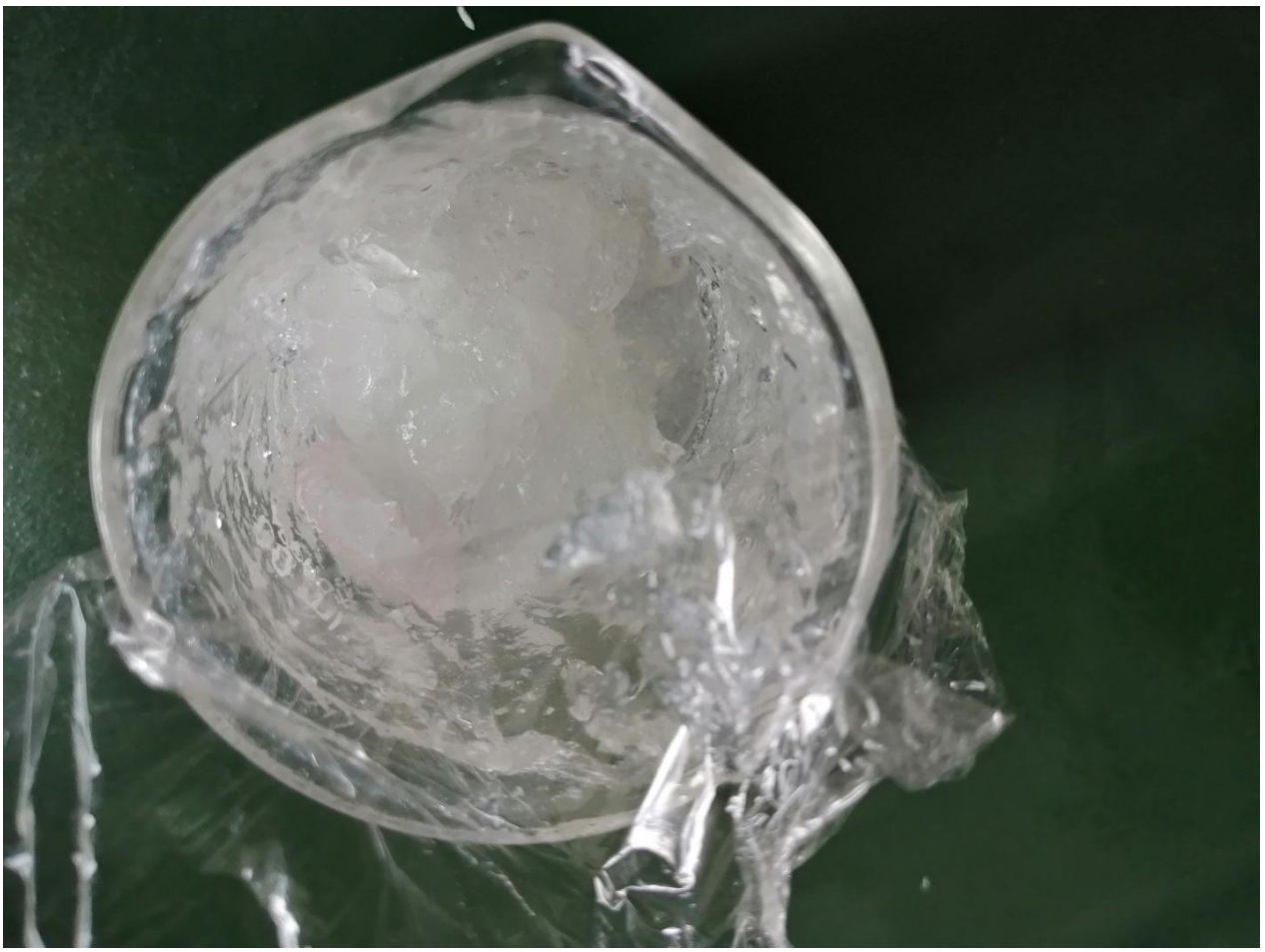

Figure 2.9 – Stability Investigation

There are two choices of preservatives for preparing silybin nano-crystalline hydrogel, one is benzoic acid and the other is ethyl paraben. Benzoic acid is very irritating. During the experiment, we made them separately Three parts of silybin nano-crystalline hydrogel were prepared, one part was added with benzoic acid as preservative, one part was added with ethyl paraben, and the other part was not added with any preservative. The silybin nano-crystalline hydrogel with benzoic acid as preservative has many flocs. the silybin nano-crystalline hydrogel with ethyl paraben as the preservative has a good gel effect. No flocs appeared. Without adding any preservatives, the effect is worse than adding benzoic acid, but not as good as adding ethyl paraben. Therefore, the choice of preservative will affect the stability, and the silybin nano-crystalline hydrogel with ethyl paraben as the preservative has the most ideal effect.

2.7 Silybin nanocrystalline hydrogel anti-skin damage

2.7.1 Experimental materials and methods

Healthy nude mice with SPF level 20, weighing 11~13g, silybin nano hydrogel, ultraviolet radiation meter and skin tester produced by CK company in Germany.

Twenty nude mice were divided into a blank group and a silybin group. Silybin hydrogel is applied to the back skin of the silybin group every day until the coverage is even and the thickness is moderate. The coated nude mice were exposed to medium-wave ultraviolet radiation. The blank group was not irradiated with UVB.

2.7.2 Moisture measurement

Moisture measurement: When measuring, press the water measuring probe vertically on the skin surface of the back of the nude mouse, and press the top of the probe back for a certain distance. A beep will be heard within 1 second, and the result will be displayed on the host. Measure 3 times at each site and take the average value. The results are shown in Table 2.5.

Table 2.5 – Effects of silybin hydrogel on epidermal moisture in nude mice

Group	Number	Moisture
The blank group	10	35.86±2.41
Silybin formation	10	68.08±3.86

2.7.3 Determination of melanin

MX18 has two measurement parameters, where M is the amount of melanin. The higher the value, the higher the melanin content in the skin. Attach the instrument probe vertically to the skin surface of the back of the nude mouse. The spring in the probe ensures that the pressure of the probe is constant during each

test. One second after the test, the instrument emits a beep and reads the data displayed on the screen. The results are shown in Table 2.6.

Table 2.6 – Effects of silybin hydrogel on melanin in nude mouse epidermis

Group	Number	Melanin
The blank group	10	195.47±8.9
Silybin formation	10	129.98±4.3

2.7.4 Conclusion

The skin moisture and melanin index of silybin hydrogel coated nude mice were significantly higher than those of the control group. It shows that silybin hydrogel can effectively prevent skin moisture loss and inhibit the formation of melanin.

In summary, the silybin hydrogel has a very good effect on protecting the skin from UVB damage.

CONCLUSIONS TO SECTION 2

Using silybin as a model drug, silybin nanocrystalline hydrogel was successfully prepared. This experimental research is mainly divided into four parts:

The first part is pre-prescription research. The silybin nano-suspension is a milky liquid, and the silybin nano-crystalline hydrogel is a milky viscous substance.

The second part is the preparation of silybin nanocrystal suspension. The silybin nanocrystal suspension has good stability.

The third part is the preparation of silybin nanocrystalline hydrogel. The silybin nanocrystalline hydrogel has a stable texture and feels good on the skin.

The fourth part is the research on anti-UVB skin damage of silybin nanocrystalline hydrogel. The experimental results show that silybin nanocrystalline hydrogel has good anti-UVB skin damage effect.

SECTION 3 PROJECT DEVELOPMENT AND SUGGESTIONS

3.1 Current research results

Silybin is an effective traditional Chinese medicine ingredient extracted from the seed coat of Silybum marianum. It has the functions of scavenging free radicals, anti-oxidation, anti-fibrosis, anti-tumor, and protecting liver function. It is especially suitable for the treatment of viral hepatitis. Poisonous and highly effective liver-protecting drugs, and have a strong inhibitory effect on the proliferation of prostate cancer, colon cancer, and breast cancer. However, due to poor water solubility, the current commercially available dosage forms are tablets and capsules with low bioavailability, which seriously affects the development of clinical efficacy and severely restricts its application and promotion. Nanocrystalline technology improves the solubility and dissolution rate of the drug by reducing the particle size of the drug and increasing the surface area of the drug particle, thereby improving its bioavailability and drug efficacy in the human body. Nanocrystalline technology can also improve the patient's tolerance to drugs, reduce the amount of excipients such as solvents and potential solvents, and avoid adverse reactions caused by excipients.

Silybin, white crystalline powder. No peculiar smell, smell a bit bitter, strong hygroscopicity. Easily soluble in acetone, ethyl acetate, methanol, ethanol, slightly soluble in chloroform, almost insoluble in water. Milk thisbin and the main suitable for the treatment of cirrhosis of the liver, acute and chronic hepatitis, liver poisoning and other diseases. Silybin is a good medicine for treating liver function and hepatitis. It has an effective fire prevention effect. Current research shows that silybin has good biological activities, such as liver protection, anti-tumor, cardiovascular protection, antibacterial and so on. Studies have shown that silybin can treat some common problems of ordinary women, such as vaginal ulcers, uterine fibroids, cervical erosion and so on.

More than 35% of potential drugs are more or less water-soluble, which makes it difficult for some key potential drug products to enter the market or play a good role as

a treatment method. Company Elan has cooperated with a few large pharmaceutical companies. By using nanocrystals, they have successfully developed two new formulations and put them on the market. Using this technology, the micron-scale crystals are wet-processed into nanoparticles, and the drug is adsorbed on the surface of the particles. The stabilizer increases the stability of the nanoparticles and prevents the crystals from re-aggregating. Rapamycin is an immunosuppressive drug and the first drug to be marketed using Elan's patented nanocrystal technology. In addition, nano-suspension is also attracting attention.

Over the years, as a functional and specious biomass matrix material, hydrogel has unique functions in the fields of medicine, chemical industry, electronics, etc., such as biocompatibility, strong water absorption and water retention, responsiveness, adsorption, chemical modification, etc. The environment has a great range of appreciation and great application value. According to different synthetic materials, hydrogels can be divided into two types: natural hydrogels and synthetic hydrogels. According to the different initiation systems, there are three methods for the preparation of cellulose hydrogels: radiation initiation, chemical initiation and ionized initiation.

The main content of this research: pre-prescription research, research and preparation of silybin nanocrystal hydrogel, research the chemical properties of silybin nanocrystal suspension, including morphology and particle size analysis.

3.2 Storage of silybin nanocrystals

The nanocrystals prepared by milk thistle are prepared in the liquid phase by solvent precipitation, which is convenient for long-distance transportation and long-term storage. The liquid phase nanocrystals are prone to austenite aging phenomenon, which leads to the aggregation and precipitation of nanoparticles. In order to further improving milk thistle for the physical stability of silybin nanocrystals, it is a necessary method to convert silybin nanocrystals into solid preparation. At present, there are many drying methods, such as vacuum freeze drying, spray drying, supercritical drying, etc., but for heat-sensitive drugs and oxidizable drugs, vacuum

freeze drying is the best choice.

The vacuum freeze-drying method is a method in which liquid materials are first frozen into a solid state, water is sublimated under low temperature and reduced pressure conditions, and a solid powder is directly formed by dehydration under low temperature conditions. This method is used for the drying of drug nanocrystals, which can avoid the decomposition and deterioration of heat-sensitive drugs and the oxidation of easily oxidizable drugs, and is beneficial to the transportation and storage of the drugs. The dried medicine will be in a loose and porous state without changing the original skeleton structure. The same volume of water for injection can quickly dissolve and form a suspended state.

The phase change of the nano-suspension during the freeze-drying process will affect the particle size and stability of the drug. In the process of dehydration, the drugs are under the pressure of drying, and the structure of the drugs can destroy. In order to avoid these problems, a freeze-drying protective agent is added to the drug nanocrystals before freeze-drying to prevent freezing stress and drying pressure from affecting the stability and structure of the drug. Common freeze-dried protective agents are sugars, such as trehalose, lactose, glucose, sucrose, and mannitol.

There are three main evaluation methods for freeze-dried products:

① Appearance: complete appearance, smooth surface, no shrinkage or collapse, no obvious change in volume before and after freeze-drying.

② Color: No spots, uniform color, it can form powder when lightly touched.

③ Re-dispersion: It is better to add the freeze-dried product to the original volume of ultrapure water, and it is better to quickly return to the suspended state with slight vibration.

Description of evaluation methods for freeze-dried products is as follows. Add 5% trehalose to prepare silybin nanocrystals. Mannitol, lactose and glucose are freeze-dried protective agents. 2ml lactose and glucose are respectively absorbed into a bottle and freeze-dried. The appearance, color and redispersibility of the freeze-dried products were used as evaluation indicators, and each indicator was evaluated by a 10-point scoring method. The final results are the sum of the three

indexes. The screening procedure of freeze-dried protective agents helps to select the best agent. The higher the score, the better the effect of the freeze-dried protective agent.

The results show that the freeze-dried product prepared with mannitol as the freeze-dried protective agent has smooth appearance, loose texture, no spots, and good redispersibility. The freeze-dried products of the other three freeze-dried protective agents are incomplete and poor in redispersibility. Therefore, mannitol was selected as the freeze-dried protective agent of silybin nanocrystals.

3.3 Production status of silybin

Antibiotics have brought huge benefits to people's health, but with the widespread use of antibiotics, bacterial resistance has become increasingly serious, posing a huge threat to human health. The emergence of drug-resistant bacteria has brought severe challenges to clinical anti-infective treatment, especially multiple resistant bacteria (Multiple Resistant Bacteria, MDR). Extensively drug-resistant bacteria [53] and pan-resistant bacteria-FDR. Multidrug-resistant infections can cause high mortality, prolong hospitalization, increase medical costs, increase the risk of adverse antibiotic reactions, and become a source of transmission. The World Health Organization has issued a report warning that drug-resistant bacteria are spreading around the world. In the United States, at least 23,000 people die from drug-resistant infections each year. In Europe, 175,000 people die each year in the field of multi-drug resistance [54]. In China, the detection rate of multi-drug resistant bacteria is very low, but some types are increasing. Faced with such a serious problem of bacterial resistance, the world is entering a "post-antibiotic era." From the 1970s to the mid-1990s, researchers used the structural modification strategies of known drugs to synthesize tens of thousands of new compounds and developed a variety of new and more distinctive antibacterial drugs.

However, in recent decades, the number of new antibacterial drugs approved for marketing is decreasing. 16 new antibacterial drugs were approved for use in the market from 1983 to 1987[55], but only 3 new antibacterial drugs were approved from 2008 to 2012, and the remaining structures were modified from existing anti-inflammatory drugs, and no drug network has a good network. The antibacterial activity and resistance to Gram-negative bacteria. Therefore, the speed of antimicrobial research and development is not balanced with the speed of bacterial resistance, with the former lagging far behind the latter. Since the 1950s [56],searching for high-efficiency and low-toxicity antibiotics from natural medicines has attracted the attention of medical workers at home and abroad, especially the research on Chinese medicine, because of its advantages, it is not easy to produce drug resistance, and its adverse reactions are low. It can also reverse bacteria and has a good application prospect in clinical antibacterial therapy. Flavonoids have anti-bacterial, anti-viral, anti-tumor, anti-oxidant, anti-cardio-cerebrovascular diseases, anti-radiation and other activities, and are currently a hot spot in medical research. Silymarin, the seed coat extract of silymarin, is a flavonoid compound consisting of 9 kinds of paclitaxel, paclitaxel derivatives, silymarin, silymarin, silymarin a, silymarin B, isosilymarin A, isosilymarin B, silymarin A, isosilymarin B, etc. The main active ingredients are silymarin, isosilymarin, silymarin and silymarin. Among them, silybin has the highest content, which is 50% ~ 70% [57-61]. At present, through extensive research on the pharmacological and physiological activities of silybin, it has been proved that it can be used in the treatment of alcoholic liver injury, fatty liver, viral hepatitis, toxic liver injury, and tumors, and can improve learning memory, movement disorders, etc.

In recent years, the antibacterial activity of silybin has received more and more attention, but there are few reports on the antibacterial activity of silybin at home and abroad. Providing a scientific basis for the research and development of new antibacterial drugs is of practical significance for slowing down the world's entry into the "post-antibiotic era" and alleviating the global bacterial resistance and shortage of antibacterial drugs.

At present, more than 40% of new chemical entities and lead compounds

through computer-aided drug design and large-scale screening are unsatisfactory properties of water-insoluble compounds in biopharmaceuticals, which not only increases the difficulty and investment in new drug development, but also brings greater development risks [62]. In 1990, the German Muller R.H. proposed the amorphous state in order to improve the insolubility of drugs. At present, dozens of drugs that rely on amorphous technology have been approved for marketing, with annual global sales exceeding US$ 6 billion.

Nanocrystals, also known as nanosuspensions, are a new type of nano-drug delivery system composed of insoluble drugs and appropriate stabilizers. They are a common strategy to improve the efficacy of biopharmaceutical classification system class II (BCSI) [60-63] drug candidates. Studies have proved that nanocrystalline technology is an important way to achieve insoluble drug delivery. Nanocrystals have the characteristics of improving the bioavailability of drugs, increasing the dosage of drugs and improving the efficacy of drugs. However, due to the physical stability of nanocrystals and the choice of dosage form, the wide application of nanocrystals in the research and development of new drugs is hindered. The application of nanocrystals in pharmaceutical preparations is affected by their thermodynamic and kinetic instability. The curing process of nanocrystals is immature and it is difficult to meet the quality requirements of physical stability of nanocrystals. The choice of stabilizers is mainly based on the "trial and error method" [64], and there is a lack of understanding of the prescription screening system rules such as the type and dosage of stabilizers [65]. In addition, because nanocrystals are different from traditional nanocarriers in drug delivery systems, their modification and controllability are weak, resulting in uncontrollable drug delivery processes after drug administration [66].

At present, the screening of antibacterial drugs from Chinese herbal medicine has gradually attracted the attention of scholars at home and abroad [67-69]. Studies have confirmed that flavonoids, volatile oils, alkaloids, organic acids, female quinones and some metabolites of Chinese medicine are the main antibacterial active ingredients. Chinese medicine or a combination of Chinese and Western

medicine can achieve better curative effects. The development and development of new antibacterial drugs is one of the keys to alleviating the global bacterial resistance and shortage of antibacterial drugs. In recent years, Chinese herbal medicine has become the focus of antibacterial drug research and development due to its wide range of sources, low price, low toxicity, and drug resistance. In this study, natural herbal extracts and isomers extracted from milk thistle were selected, and the most common clinical infections were Staphylococcus aureus and Staphylococcus epidermidis, Enterococcus faecalis, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa. As an experimental fungus, the antibacterial activity of Soy Milk Thistle was studied by the micro broth dilution method. Determine the best antibacterial curve of silybin on common clinical infection bacteria, and the combined effect of silybin and common clinical antibiotics. The purpose is to provide a scientific basis for the research and development of new antibacterial drugs [70-73]. Existing research results show that the antibacterial mechanism of drugs is mainly to inhibit the synthesis and metabolism of protein and genetic material by destroying the structure of bacteria [74-77]. In addition, it also includes the effects on respiratory metabolism, oxidation in growth metabolism, and some metabolic enzyme activities [78]. In order to study its antibacterial mechanism, with Staphylococcus epidermidis as the object, the effect of the drug on the permeability of bacterial cell membrane and the synthesis of protein and nucleic acid was systematically studied [79]. DNA topoisomerase activity and biofilm structure. The cell membrane is located on the inside of the bacterial cell wall. Under normal circumstances, it plays an important role in maintaining the stable metabolism of cells. When it is destroyed, it can weaken the ability of selective transmission, resulting in the release of macromolecules such as proteins, nucleic acids or intracellular ions (such as potassium ions, calcium ions). Therefore, changes in the concentration of extracellular protein, nucleic acid, or conductivity can be used as indicators for judging the degree of cell membrane damage. In this study, the effect of silybin on bacterial membrane was studied by measuring the extracellular protein

concentration of silybin [80-82]. The results showed that there was no significant difference in the extracellular protein concentration between the control group and the experimental group (P>0.05), indicating that silybin did not cause intracellular protein leakage after acting on bacterial cells, indicating that silybin. The antibacterial effect is achieved through other targets, rather than destroying the structure and integrity of the bacterial cell membrane. This is consistent with LeeR7's discovery that silybin and silymarin do not affect bacterial cell membranes.

3.4 Antibiotic development of silybin

The abuse of antibiotics has made the problem of bacterial resistance and drug residues in food increasingly serious, and it is urgent to find new antibacterial drugs. Natural medicines have small side effects and are not easy to produce drug resistance. They play an important role in the prevention and treatment of modern infectious diseases. The extraction of the active ingredients of natural medicines has laid a foundation for the study of antibacterial effects, and provided a foundation for exploring the antibacterial mechanism of the effective ingredients and the research and development of new antibacterial drugs. Flavonoids are secondary metabolites widely present in polyphenols and have strong antibacterial activity [83]. Silybin is a flavonoid compound. According to literature reports and preliminary research results in our laboratory, silybin has antibacterial activity against Gram-positive cocci, especially Staphylococcus epidermidis [84].

When the cell membrane is damaged, large molecules or ions (such as K+, Ca+) such as proteins and nucleic acids are released. Changes in the concentration of extracellular protein, nucleic acid, or conductivity can be used as indicators for judging the degree of cell membrane damage. The results of this experiment showed that silybin did not cause protein leakage in bacterial cells after acting on Staphylococcus epidermidis, indicating that silybin did not affect the integrity of the bacterial cell membrane and its antibacterial target was not the cell membrane. This is consistent with the results of Professor Li's research. However, it has been

reported that silymarin, which has an elution rate of 60% ethanol, has a destructive effect on the structure of bacteria.

The physiological metabolism of bacteria requires a variety of proteins to work together. When the synthesis or expression of genetic material is affected, it will cause protein abnormalities, impaired cell function, and even death. The results show that silybin can significantly inhibit the expression of soluble protein of Staphylococcus epidermidis, and the decrease in protein expression is more obvious with the increase of drug concentration. It is speculated that silybin may affect the nucleic acid synthesis of Staphylococcus epidermidis or the expression of related genes, resulting in the decrease of bacterial protein content. The effect of silybin on bacterial nucleic acid synthesis in this experiment confirmed this conclusion. However, specific gene expression suppression needs to be further studied.

4,6-diamidine-2-phenylindole (DAPI) is a blue fluorescent dye that can penetrate cell membranes. When combined with DNA or RNA, it can produce fluorescence that is more than 20 times stronger than DAPI. It is widely used in nucleic acid detection. The results showed that the number of bacteria in the DNA and RNA treatments 1MIC (50 μ g/ml) and 2MIC (100 ug/ml) was significantly lower than the control group, indicating that silybin can significantly inhibit the synthesis of bacterial DNA and RNA and enhance the inhibitory effect as the concentration of the drug increases. It may be that silybin inhibits a key enzyme in nucleic acid synthesis. However, its specific inhibitory mechanism needs to be further explored [85-86].

DNA topoisomerase catalyzes the break and excitation of DNA strands during DNA replication, transcription and recombination, thereby controlling the topological state of DNA. Topoisomerase I catalyzes instantaneous DNA single-strand disconnection and connection: Topoisomerase II can simultaneously disconnect and connect double-stranded DNA, but usually requires the participation of ATP. Flavonoids can inhibit and destroy mammalian topoisomerase I and ii, soy isoflavones and luteolin can inhibit tumor cell topoisomerase activity. The experimental results show that soy milk thistle has an inhibitory effect on

topoisomerase| and ‖ activities of grape squash. This affects the replication and transcription of DNA.

Bacterial biofilm is a growth mode corresponding to plankton formed by bacteria adhering to the surface of the carrier during the growth process. It is composed of bacteria and an extracellular polymeric matrix produced by the bacteria themselves. Bacterial biofilm not only helps bacteria adapt to different environments and is not easy to remove, but also significantly enhances the resistance of bacteria. Staphylococcus epidermidis (Staphylococcus epidermidis) is the main pathogenic hormone of Staphylococcus epidermidis, which can form a biofilm on the surface of biological materials left in the body. The formation of epidermal biofilm is a complex and orderly dynamic process, including adhesion stage (0h~12h), aggregation stage (12h~36h), maturity stage (36h~72h) and shedding stage (>72h). (atle, icaa, AAP, etc.) regulation. The results show that 1MBIC (200g/mL) and 2MBIC (400ug/mL) silybin have inhibitory effects on the adhesion, aggregation and further growth of biofilms in the mature stage of Staphylococcus epidermidis. It can inhibit (or kill) bacteria in the biofilm, destroy the structure of the biofilm, and increase its effect as the drug concentration increases [87-99]. These results provide a new way to treat Staphylococcus epidermidis biofilm-related infections. However, the destruction mechanism of silybin on the epidermal biofilm and its killing effect on the bacteria in the biofilm remains to be further studied.

CONCLUSIONS TO SECTION 3

At present, silymarin is mainly extracted from silymarin, purified and chemically modified into various medicaments. In recent years, the planting area and extractive industries abroad have gradually decreased. Some pharmaceutical companies have begun to directly import silymarin from developing countries such as China, and then resell it for profit after deep processing. My country is a big exporter of silymarin, but the total content of silymarin can only reach about 70%-80%, which is a crude product. The purification of silymarin is difficult to reach the standard. In recent years, with the development of technology, new methods such as CO_2 supercritical extraction and ultrasonic extraction have been introduced into industrial production.

In recent years, studies on flavonoids have shown that they have obvious antioxidant, anti-inflammatory, anti-ulcer, and antibacterial effects, and are widely used in medicine, food and other industries. The antibacterial research of flavonoids is mostly to test their antibacterial ability. Studies have shown that most flavonoids have varying degrees of inhibitory effects on Escherichia coli, Staphylococcus aureus and Bacillus subtilis, but their inhibitory effects on yeasts and other fungi are weak. The inhibitory effect on gram-positive bacteria is stronger than that of gram-negative bacteria. The effects of different solvents on the extraction of flavonoids from spring flowering branches and their in vitro antibacterial activities were studied. After the flavonoids extracted from leaf powder were purified by macroporous resin, they had inhibitory effects on the 6 tested bacteria, but the differences were quite large. The flavonoid glycosides extracted from mulberry seeds by supercritical extraction method have inhibitory effects on bacteria and various bacteria, and the inhibitory effect on bacteria is stronger than that on molds. The results show that flavonoids have no inhibitory effect on Bacillus subtilis and Proteus, but have inhibitory effects on Escherichia coli and Staphylococcus aureus.

Medicinal plant is a special economic plant, and its main feature is that the plant contains a variety of biologically active substances, which are used in medical

prevention and treatment. Therefore, it is the material basis of Chinese medicine, such as Mongolian medicine to treat diseases, and it is also used in the pharmaceutical industry. Important raw materials play a special role in food, brewing, health care products, cosmetics and other industries. With the widespread application of Chinese medicine worldwide, the rapid development of the domestic Chinese medicine industry and the continuous expansion of the application field of medicinal plants, research on the extraction of medicinal plants at home and abroad is also increasing year by year.

CONCLUSION

In this study, silybin was used as a model drug to successfully prepare silybin nanocrystalline hydrogels. The preparation process and quality evaluation of silybin nanocrystalline hydrogel were studied, and the UVB skin damage was studied. This article is mainly divided into three parts.

The first part is the pre-prescription research, mainly on silybin, nanocrystals, hydrogels, stabilizers, skin properties, UVB properties and so on.

The second part is the experimental part, which is mainly divided into the preparation of silybin nano-suspension, the preparation of silybin nanocrystalline hydrogel, and the research of silybin nanocrystalline hydrogel on UVB skin damage. The average particle size of the silybin nanosuspension is 41.36nm, and the dispersion index is 0.2848. The particle size of the 10% silybin nanosuspension is less than 14.21nm, 50% is less than 27.02nm, and 90% is less than 51.46nm. In the preparation process of silybin nanocrystalline hydrogel, a hydrogel product with strong stability and refreshing skin feel is obtained. Finally, in nude mice experiments, it is proved that silybin nanocrystalline hydrogel has obvious protective effect on UVB-induced skin damage.

The third part is the development prospect of silybin nanocrystalline hydrogel. Nanosuspension has many advantages that traditional formulations do not have. Further preparation of nano-suspension can expand the stability of the suspension and has good biocompatibility.

This article studies the preparation technology of silybin nanocrystals, but the in vivo pharmacokinetic study of silybin nanocrystals needs further study by the research group.

LIST OF LITREATURE SOURCES

1. Tang Hui.Study on preparation of silibin nanoparticles and capsules by supergravity reoprecipitation [D].Beijing University of Chemical Technology, 2018.
2. Shao Shuang, Liu Chunyan, Gao Yan Hang. Progress in the study of siliquibin in the treatment of drug-induced liver injury [J].Journal of Clinical Hepatobiliary Disease, 2017,33 (06): 1179-1182.
3. Guo Caiyan, Jin Chunrong, Snow White Qi, Guo Xiaoshan, Li Sijin. Clinical study of ST-T segment alteration of ECG during loading nuclide myocardial imaging [J].Journal of Integrated Traditional Chinese and Western Medicine, 2019,17 (21): 3390-3392.
4. Zhao Maoji.The antibacterial effect and antibacterial mechanism of SAB in vitro [D].Chengdu University of Traditional Chinese Medicine, 2018.
5. Mou Dongsheng, Liao Yuan, Zhou Dashun, Wan Jiangling, Yang Qingliang. Progress in nanocrystallization drugs [J]. Medical Guide, 2020,39 (09): 1257-1261.
6. Du Junfeng, Tu Lixing, Hu Kaili, Feng Jianfang.Preparation process and preparation of puerarin nanocrystalline and their characterization [J].Journal of Hunan University of Traditional Chinese Medicine, 2017,37 (04): 369-372.
7. Xiong Ruolan, Lu Weigen. Nanosuspension agent research progress [J].World Clinical Drugs, 2007 (02): 117-121.
8. Zheng Yingying, Zhao Jinrong, Li Min, Zhou Zhimin, Zhao Shaozhen.Study of tacrolimus nanocrystalline synthesis and Surface artificial tear modification [J].The International Journal of Biomedical Engineering, 2018,41 (02): 167-171.
9. Liu Meicui. Preparation and characterization of aromatic acid / chitosan nanoparticles [D].Qingdao University of Science and Technology, 2013.
10. Fu Qiang, Sun Jin, He Gui. Advances in nanocrystallization [J]. Journal of Shenyang Pharmaceutical University, 2010,27 (12): 952-960.
11. Tang Hui.Study on preparation of silibin nanoparticles and capsules by

supergravity reoprecipitation [D].Beijing University of Chemical Technology, 2018.

12. Shao Shuang, Liu Chunyan, Gao Yan Hang. Progress in the study of siliquibin in the treatment of drug-induced liver injury [J].Journal of Clinical Hepatobiliary Disease, 2017,33 (06): 1179-1182.
13. Guo Caiyan, Jin Chunrong, Snow White Qi, Guo Xiaoshan, Li Sijin. Clinical study of ST-T segment alteration of ECG during loading nuclide myocardial imaging [J].Journal of Integrated Traditional Chinese and Western Medicine, 2019,17 (21): 3390-3392.
14. Zhao Maoji.The antibacterial effect and antibacterial mechanism of SAB in vitro [D].Chengdu University of Traditional Chinese Medicine, 2018.
15. Mou Dongsheng, Liao Yuan, Zhou Dashun, Wan Jiangling, Yang Qingliang. Progress in nanocrystallization drugs [J]. Medical Guide, 2020,39 (09): 1257-1261.
16. Du Junfeng, Tu Lixing, Hu Kaili, Feng Jianfang.Preparation process and preparation of puerarin nanocrystalline and their characterization [J].Journal of Hunan University of Traditional Chinese Medicine, 2017,37 (04): 369-372.
17. Xiong Ruolan, Lu Weigen. Nanosuspension agent research progress [J].World Clinical Drugs, 2007 (02): 117-121.
18. Zheng Yingying, Zhao Jinrong, Li Min, Zhou Zhimin, Zhao Shaozhen.Study of tacrolimus nanocrystalline synthesis and Surface artificial tear modification [J].The International Journal of Biomedical Engineering, 2018,41 (02): 167-171.
19. Liu Meicui. Preparation and characterization of aromatic acid / chitosan nanoparticles [D].Qingdao University of Science and Technology, 2013.
20. Fu Qiang, Sun Jin, He Gui. Advances in nanocrystallization [J]. Journal of Shenyang Pharmaceutical University, 2010,27 (12): 952-960.
21. Yan Yu, Bai Linxi, Li Zhitao, Qing Yuling, Qin Shaorong, Zhao Jiaji General, Sun Kehong. A traditional Chinese medicine composition for the treatment of atopic dermatitis and its preparation method and application [P].Chongqing Municipality: CN110025705A, 2019-07-19.

22. Wang Jianguo, Wang Shaojuan, bangs, Wang Chunmei, Zhou Yuqi, Kuang Baoxiao.Preparation method and application of a slow-release microsphere of ivermectin [P].Shandong Province: CN112190567A, 2021-01-08.
23. Luo Kaipei, Li Xiaofang, Yang Lu, Lin Hao, Luo Jia. Preparation of silistin nanosuspension and in vitro dissolution [J]. Chinese Journal of Pharmaceutical Industry, 2016,47 (09): 1165-1170.
24. Wang Lifang, Chen Xiaonan, Li Jun, Tu Pengfei, Wang Jinling. Preparation of nanosuspension agent and in vitro olysis [J].Chinese Journal of Traditional Chinese Medicine, 2019,44 (11): 2236-2243.
25. Zhou Yuqi. Study on construction of nanosuspension with multi-effect stabilizer [D].Qilu University of Technology, 2017.
26. Lu Xiong, Xu Boy, Gan Donglin. A preparation method for imitation mussel contact antimicrobial hydrogels for tissue repair [P].Sichuan Province: CN108371728B, 2020-12-18.
27. Li Xuefeng, Shu Mengmeng, Long Shijun, Huang Zihan, Chen Shunlan, Li Jie. Preparation method of printable forming high-intensity body temperature release drug hydrogels [P].Hubei Province: CN111548454A, 2020-08-18.
28. Xu Hongshun. Nanotech in the biomedical field [J]. Zhejiang Chemical Industry, 2004 (12): 25-28 + 31.
29. Wang Yancai. Construction and evaluation of the nanocrystalline release system [D]. Shandong University, 2011.
30. Shi Haiying, Chen Jun, Fei Chaoqun, Yang Xuejuan. Preparation of slow release gel and in vitro release properties [J].Chinese patent medicine, 2013,35 (03): 491-495.
31. Zeng Longbiao, Lian Yunfei, Zhang Jiandong, Tang Wenyan, Li Juan. Research Progress in Nanocrystallization Preparation Industrialization [J].Journal of China Pharmaceutical University, 2013,44 (06): 504-510.
32. Chen Qin, Zhou Rong, Yang Fan, Lu Lina, Lu Qilin. Nanocellulose gels [J].Textile technology progress, 2020 (12): 9-13 + 18.
33. Gao Tianying, Wang Yongli, Li Xueming. Overview of the bioppothectic

properties of drug nanocrystallization [J].Chinese Journal of Pharmaceutical Industry, 2015,46 (07): 762-766 + 777.

34. Yang Minmin, Zhou Xiping, Wu Han. A method for preparing ettravirin and its intermediates [P].Jiangsu: CN102675220A, 2012-09-19.
35. Zhang Ying, Song is compatible, Chen Tong, Li Huili. Progress in non-invasive ocular nanoadministration systems [J].Chinese Journal of Pharmacy, 2013,48 (17): 1237-1241.
36. Zhao Xiaoyu, Wang Guohua, Zhang Baosheng, Li Hui, Nie Qixia, Zang Chen, Zhao Xiaomei. Preparation and characterization of lyophilized powder with thhistin nanosuspension. [J].Chinese Journal of Traditional Chinese Medicine, 2009,34 (12): 1503-1508.
37. Preparation of slow release microcapsule and pharmacokinetics and in vitro release [J].Wang Feng.The Northwest Journal of Pharmacy.2018(05)
38. Preparation of 10-characterization and pharmacokinetic evaluation of hydroxycamptothecine nanosuspensions (English) [J].Zhang Yi, Zhan Ying, Pang Ning, Liu Yujie, Cheng Shixian, Li Ji, Du Yi Tian, Qi Xianrong. Journal of Chinese Pharmaceutical Sciences.2017(08)
39. Progress in the study of siliquibin in the treatment of drug-induced liver injury [J]. Shao Shuang, Liu Chunyan, Gao Yan Hang. The Journal of Clinical Hepatobiliary Diseases.2017(06)
40. Progress in the nanosuspension delivery system [J]. Wang Lili, Zhu Meihua, Liu Zhengping, and Zhang Jianqiang. China Dispensary.2017(10)
41. The process of extraction in ultrasound assisted duplex phase system is preferred [J].Ruan Hongsheng, Jia Jinyan, Wu Zhijun, Zheng Xiaoliang. Journal of Heilongjiang Bayi Agricultural Reclamation University.2017(01)
42. Advances in studying stabilizers in nanosuspensions [J].Huang Ping, Mao Kunjun, Wang eting, Huang Daoming, Ye Yingjun.Guangzhou chemical industry.2016(20)
43. Progress in preparation of nano suspension and route of administration [J].Yuan Huiling, Yi Jiaming, Zhang Caiyun, Lu Chuan, Chen Weidong. The Chinese

Journal of New Drugs. 2014(03)

44. Progress in the preparation, characterization and application of nanosuspension agents [J].Chen Chongshu, Liang Yan, Liang Li, Chen Hong. Armed police medicine.2013(04)
45. Study on silistinin extraction and silibinbin purification [J].Ren Bingru, Zhao Youyi, Xu Baiheng, Wu Julan, Chen Jian, Liang Chengyuan, Lu Han, Li Weilin. Shi Zhen national medicine.2012(03)
46. Effect of apoptosis in human cervical cancer and gastric cancer cells [D].Zhang Yuanxin. Jilin University, 2012
47. The immunosuppressive effects of regulatory T cells in HBV-infected liver CC [D].Wang Fengmei. Tianjin Medical University. 2012
48. Construction and evaluation of the nanocrystalline Release System [D].Wang Yancai.Shandong University, 2011
49. Extraction, isolation and preliminary activity study of the active components of fly thistle seeds [D].Liu Hong. Beijing University of Chemical Technology 2009
50. Preparation and preliminary pharmacokinetic study of rosewood nanosuspension agent [D].Chen Zhe.Peking Union Medical College 2017
51. Preparation of hydroxycamptothecin semi-solid lipid nanoparticles and the in vitro pharmacological properties study [D]. Sina. First Military Medical University 2006
52. Design, optimization and in vitro - in vivo evaluation of smart nanocaged carrier delivery of multifunctional PEG-chitosan stabilized silybin nanocrystals[J] . Yangyang Liu, Yancai Wang, Juan Zhao. International Journal of Biological Macromolecule . 2018
53. Nanocrystals Technology for Transdermal Delivery of Water-Insoluble Drugs[J] Yangyang Liu,Juan Zhao,Lulu Wang,Beibei Yan,Yu Gu,Ping Chang,Yancai Wang.Current Drug Delivery . 2018 (9)
54. Nano spray drying for encapsulation of pharmaceuticals[J] . Cordin Arpagaus, Andreas Collenberg, David Rütti, Elham Assadpour, Seid Mahdi Jafari. International Journal of Pharmaceutics . 2018 (1-2)

55. Chitosan as biomaterial in drug delivery and tissue engineering[J] . Saad M. Ahsan,Mathai Thomas,Kranthi K. Reddy,Sujata Gopal Sooraparaju,Amit Asthana,Ira Bhatnagar.International Journal of Biological Macromolecule . 2018
56. Cryoprotectant choice and analyses of freeze-drying drug suspension of nanoparticles with functional stabilisers [J] . Lulu Wang, Yingying Ma, Yu Gu, Yangyang Liu, Juan Zhao, Beibei Yan, Yancai Wang. Journal of Microencapsulation. 2018 (3)
57. Drug nanocrystals – Versatile option for formulation of poorly soluble materials [J]. Leena Peltonen,Jouni Hirvonen.International Journal of Pharmaceutics . 2018 (1-2)
58. In vitro and in vivo evaluation of targeting tumor with folate-based amphiphilic multifunctional stabilizer for resveratrol nanosuspensions [J] . Lulu Wang, Yangyang Liu, Juan Zhao, Chunpeng Li, Yuqi Zhou, Juan Du, Yancai Wang. Colloids and Surfaces B: Biointerfaces . 2017
59. In vivo fate of lipid-silybin conjugate nanoparticles: Implications on enhanced oral bioavailability [J] . Yuhua Ma, Haisheng He, Fei Xia, Yingxia Li, Yan Lu, Daofeng Chen, Jianping Qi, Yi Lu, Wei Zhang, Wei Wu. Nanomedicine: Nanotechnology, Biology, and Medici . 2017 (8)
60. Nile red nanosuspensions as investigative model to study the follicular targeting of drug nanocrystals [J]. Francesco Corrias, Michele Schlich, Chiara Sinico, Rosa Pireddu, Donatella Valenti, Anna Maria Fadda, Salvatore Marceddu, Francesco Lai. International Journal of Pharmaceutics . 2017 (1-2)
61. Downstream drug product processing of itraconazole nanosuspension: Factors influencing drug particle size and dissolution from nanosuspension-layered beads [J]. Johannes Parmentier, En Hui Tan,Ariana Low, Jan Peter Mischwitzer. International Journal of Pharmaceutics. 2017 (1-2)
62. Transdermal delivery of dimethyl fumarate for Alzheimer's disease: Effect of penetration enhancers [J]. Dina Ameen, Bozena Michniak-Kohn. International Journal of Pharmaceutics . 2017 (1-2)
63. Safety of nanosuspensions in drug delivery [J]. Lulu Wang, Juan Du, Yuqi

Zhou, Yancai Wang. Nanomedicine: Nanotechnology, Biology, and Medici . 2017 (2)

64. Nanocrystals Technology for Improving Bioavailability of Poorly Soluble Drugs: A Mini-Review [J]. Zhou Yuqi, Du, Juan, Wang, Lulu, Wang,Yancai. Journal of Nanoscience and Nanotechnology . 2017 (1)
65. Facts and evidences on the lyophilization of polymeric nanoparticles for drug delivery [J] . Pedro Fonte, Salette Reis, Bruno Sarmento. Journal of Controlled Release . 2016
66. Preparation of ritonavir nanosuspensions by microfluidization using polymeric stabilizers: I. A Design of Experiment approach [J]. Alptug Karakucuk, Nevin Celebi, Zeynep Safak Teksin. European Journal of Pharmaceutical Sciences . 2016
67. Synthesis and chemical modification of poly(butylene succinate) with rutin useful to the release of silybin[J] . Letícia Pedretti Ferreira, Bruno Cunha, Ricardo Machado Kuster, José Carlos Pinto, Marcio Nele Souza, Fernando Gomes de Souza. Industrial Crops & Products . 2016
68. Influence of drug physicochemical characteristics on in vitro transdermal absorption of hydrophobic drug nanosuspensions [J]. Chengying Shen, Ruisheng Li,Bao-de Shen, Gang Shen, Li-qiang Wang, Juan Zheng, Xiao-rong Li, Hong-yan Min, Jin Han, Hailong Yuan. Drug Development and Industrial Pharmacy . 2015 (12)
69. Injected nanocrystals for targeted drug delivery [J]. Yi Lu, Ye Li, Wei Wu. Acta Pharmaceutica Sinica B . 2015
70. Application of chitosan and chitosan derivatives as biomaterials [J]. Changyong Choi, Joung-Pyo Nam, Jae-Woon Nah. Journal of Industrial and Engineering Chemistry. 2015
71. Particle size control and the interactions between drug and stabilizers in an amorphous nanosuspension system [J]. Yanping Bi, Jingjing Liu, Jianzhu Wang, Jifu Hao, Fei Li, Teng Wang, Hong Wei Sun, Fengguang Guo. Journal of Drug Delivery Science and Technology. 2015

72. Formulation and process optimization of naproxen nanosuspensions stabilized by hydroxy propyl methyl cellulose [J]. Bibaswan Mishra, Jagannath Sahoo, Prasanna Kumar Dixit. Carbohydrate Polymers. 2015
73. pH-sensitive nanoparticles of poly(l -histidine)–poly(lactide- co-glycolide)–tocopheryl polyethylene glycol succinate for anti-tumor drug delivery[J] . Zhen Li, Lipeng Qiu, Qing Chen,Tangna Hao, Mingxi Qiao,Haixia Zhao, Jie Zhang, Haiyang Hu,Xiuli Zhao, Dawei Chen,Lin Mei.Acta Biomaterialia . 2014
74. Engineered nanocrystal technology: In-vivo fate, targeting and applications in drug delivery [J] . Vivek K. Pawar, Yuvraj Singh, Jaya Gopal Meher, Siddharth Gupta, Manish K. Chourasia. Journal of Controlled Release . 2014
75. Linolenic acid-modified PEG-PCL micelles for curcumin delivery [J]. Zhimei Song, Wenxia Zhu, Na Liu, Fengying Yang, Runliang Feng. International Journal of Pharmaceutics . 2014 (1-2)
76. Nanonization of curcumin by antisolvent precipitation: Process development, characterization, freeze drying and stability performance [J]. Deepak Yadav, Neeraj Kumar. International Journal of Pharmaceutics. 2014 (1-2)
77. Study on formability of solid nanosuspensions during solidification: II novel roles of freezing stress and cryoprotectant property [J]. Peng-Fei Yue, Gang Li, Ji-Xiu Dan, Zhen-Feng Wu, Chang-Hong Wang, Wei-Feng Zhu, Ming Yang. International Journal of Pharmaceutics. 2014 (1-2)
78. Pharmaceutical cocrystals and poorly soluble drugs [J]. Ranjit Thakuria, Amit Delori, William Jones, Maya P. Lipert, Lilly Roy, Naír Rodríguez-Hornedo. International Journal of Pharmaceutics. 2013 (1)
79. Bottom-up approaches for preparing drug nanocrystals: Formulations and factors affecting particle size [J]. Biswadip Sinha, Rainer H. Müller, Jan P. Mschwitzer. International Journal of Pharmaceutics. 2013 (1)
80. Stability of nanosuspensions in drug delivery [J]. Yancai Wang, Ying Zheng, Ling Zhang, Qiwei Wang, Dianrui Zhang. Journal of Controlled Release . 2013 (3)
81. Study on formability of solid nanosuspensions during nanodispersion and

solidification: I. Novel role of stabilizer/drug property [J]. Peng-Fei Yue, Yu Li, Jing Wan, Ming Yang, Wei-Feng Zhu, Chang-Hong Wang. International Journal of Pharmaceutics . 2013 (1)

82. Dissolution Studies of Poorly Soluble Drug Nanosuspensions in Non-sink Conditions [J]. Peng Liu, Odile Wulf, Johanna Laru, Teemu Heikkil, Bert Veen, Juha Kiesvaara, Jouni Hirvonen, Leena Peltonen, Timo Laaksonen. AAPS PharmSciTech . 2013 (2)
83. Mechanism of freeze-drying drug nanosuspensions [J]. Nae-Oh Chung, Min Kyung Lee, Jonghwi Lee. International Journal of Pharmaceutics. 2012 (1-2)
84. Drug nanocrystals: In vivo performances [J]. Lei Gao, Guiyang Liu, Jianli Ma, Xiaoqing Wang, Liang Zhou, Xiang Li. Journal of Controlled Release. 2012 (3)
85. Twenty years of drug nanocrystals: Where are we, and where do we go ? [J] . R.H. Müller,C.M. Keck.European Journal of Pharmaceutics and Biopharmaceutics . 2011 (1)
86. Effect of the non-ionic surfactant Poloxamer 188 on passive permeability of poorly soluble drugs across Caco-2 cell monolayers [J]. Sarah Maud Fischer, Martin Brandl, Gert Fricker. European Journal of Pharmaceutics and Biopharmaceutics . 2011 (2)
87. State of the art of nanocrystals – Special features, production, nanotoxicology aspects and intracellular delivery [J]. Rainer H. Müller, Sven Gohla, Cornelia M. Keck. European Journal of Pharmaceutics and Biopharmaceutics . 2011 (1)
88. Surface modified nevirapine nanosuspensions for viral reservoir targeting: In vitro and in vivo evaluation [J]. Ranjita Shegokar, Kamalinder K. Singh. International Journal of Pharmaceutics. 2011 (2)
89. In vitro antitumor activity of silybin nanosuspension in PC-3 cells [J]. Dandan Zheng, Yancai Wang, Dianrui Zhang, Zhaoping Liu, Cunxian Duan, Lejiao Jia, Feihu Wang, Yue Liu, Guangpu Liu, Leilei Hao, Qiang Zhang. Cancer Letters . 2011 (2)
90. Production methods for nanodrug particles using the bottom-up approach [J]. Hak-Kim Chan,Philip Chi Lip Kwok. Advanced Drug Delivery Reviews . 2011

(6)

91. A novel method for synthesizing PEGylated chitosan nanoparticles: strategy, preparation, and in vitro analysis [J]. Satya Prakash, Shyamali Saha, Catherine Tomaro-Duchesneau, Ciaran Lane, Meenakshi Malhotra. International Journal of Nanomedicine. 2011 (defa)
92. Preparation of stable nitrendipine nanosuspensions using the precipitation – ultrasonication method for enhancement of dissolution and oral bioavailability [J]. Dengning Xia, Peng Quan, Hongze Piao, Hongyu Piao, Shaoping Sun, Yongmei Yin, Fude Cui. European Journal of Pharmaceutical Sciences . 2010 (4)
93. Development and in vitro evaluation of deacety mycoepoxydiene nanosuspension [J]. Yancai Wang, Zhaoping Liu, Dianrui Zhang, Xihui Gao, Xiaoyu Zhang, Cunxian Duan, Lejiao Jia, Feifei Feng, Yaojian Huang, Yuemao Shen, Qiang Zhang. Colloids and Surfaces B: Biointerfaces 2010 (2)
94. Physical stability of nanosuspensions: Investigation of the role of stabilizers on Ostwald ripening [J]. Sudhir Verma, Sumit Kumar, Rajeev Gokhale, Diane J. Burgess. International Journal of Pharmaceutics. 2010 (1)
95. Understanding the structure and stability of paclitaxel nanocrystals [J]. Jiexin Deng, Leaf Huang, Feng Liu. International Journal of Pharmaceutics. 2010 (2)
96. Methoxy poly(ethylene glycol)-grafted-chitosan based microcapsules: Synthesis, characterization and properties as a potential hydrophilic wall material for stabilization and controlled release of algal oil[J]. Hailong Peng, Hua Xiong, Jinhua Li, Lingxin Chen, Qiang Zhao. Journal of Food Engineering . 2010 (1)
97. Crosslinkable polymers for nanocrystal stabilization [J]. Kathrin Fuhrmann, Marc A. Gauthier, Jean-Christophe Leroux. Journal of Controlled Release . 2010 (1)
98. Preparation and characterization of silybin-loaded nanostructured lipid carriers [J]. Le-Jiao Jia, Dian-Rui Zhang, Zhen-Yu Li, Fei-Fei Feng, YanCai Wang, Wen-Ting Dai, Cun-Xian Duan, Qiang Zhang. Drug Delivery. 2010 (1)
99. Targeted cancer therapy with novel high drug-loading nanocrystals [J].

Feng Liu, JiYoung Park, YongZhang, Christine Conwell, Yang Liu, Surendar Reddy Bathula, Leaf Huang. J. Pharm. Sci. 2010 (8)

100. Determination of entrapment efficiency and drug phase distribution of submicron emulsions loaded silybin [J]. Xiaoliang Liu, Yu Zhang, Xing Tang, Hongyao Zhang. Journal of Microencapsulation . 2009 (2)
101. Multitargeted therapy of cancer by silymarin [J]. Kumaraguruparan Ramasamy,Rajesh Agarwal.Cancer Letters . 2008 (2)
102. Nanosizing - Oral formulation development and biopharmaceutical evaluation [J]. Filippos Kesisoglou,Santipharp Panmai,Yunhui Wu.Advanced Drug Delivery Reviews . 2007 (7)
103. Salt formation to improve drug solubility [J]. Abu T.M. Serajuddin. Advanced Drug Delivery Reviews . 2007 (7)
104. Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates [J]. N. Blagden, M. de Matas, P.T. Gavan, P. York. Advanced Drug Delivery Reviews . 2007 (7)
105. Role of polymeric stabilizers for drug nanocrystal dispersions [J]. Ji-Yeun Choi, Ji Youn Yoo, Hae-Soo Kwak, Byeong Uk Nam, Jonghwi Lee. Current Applied Physics . 2005 (5)
106. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs [J]. Jun-ichi Jinno, Naoki Kamada, Masateru Miyake, Keigo Yamada, Tadashi Mukai, Masaaki Odomi, Hajime Toguchi, Gary G. Liversidge, Kazutaka Higaki, Toshikiro Kimura. Journal of Controlled Release. 2005 (1)
107. Regiochemical functionalization of a nanoscale cage-like structure: Robust core-shell nanostructures crafted as vessels for selective uptake and release of small and large guests[J] . Jeffrey L. Turner, Zhiyun Chen, Karen L. Wooley. Journal of Controlled Release . 2005 (1)
108. Production and Characterization of a Budesonide Nanosuspension for Pulmonary Administration [J] . Claudia Jacobs, Rainer Helmut Müller. Pharmaceutical Research . 2002 (2)

109. Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide [J]. C. Jacobs, O. Kayser, R.H. Müller. International Journal of Pharmaceutics . 2000 (2).