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KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty of Chemical and Biopharmaceutical Technologies
Department of Industrial Pharmacy

Master's thesis

on the topic PREPARATION AND PROPERTIES OF POLYMERIC BACTERIOSTATIC COMPOSITE HYDROGEL

Completed: student of the group MPhch-20

of the speciality 226 Pharmacy, industrial pharmacy
(code and title of the speciality)

Guoqiang SHI
(first name, last name)

Supervisor Tetiana DERKACH
(first name, last name)

Reviewer Volodymyr BESSARABOV
(first name, last name)

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Institute, faculty Chemical and Biopharmaceutical Technologies
Department Industrial Pharmacy
Speciality 226 Pharmacy, industrial pharmacy
(code and title)

Approve
Head of Department of Industrial Pharmacy,
Professor, Doctor of Pharmaceutical Science
Vladyslav STRASHNYI

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ASSIGNMENTS FOR THE MASTER'S THESIS

Guoqiang Shi,
(Full Name)

1. Thesis topic Preparation and properties of polymeric bacteriostatic composite hydrogel

2. Scientific supervisor Tetiana Derkach, Doctor of Science, Professor
(first name, last name, patronymic, academic degree, academic title)

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4. Initial data for work: scientific and information sources, methodological and technological literature, scientific periodicals, international and domestic regulations and standards for the development and production of medicines

5. Content of the thesis (list of questions to be developed). To prepare an antibacterial hydrogel material using PVA and PHMG as raw materials without introducing an initiator and a crosslinking agent by the freeze-thaw method. To determine the optimal composition of the hydrogel. To determine the hydrogel's light transmission, swelling rate, dissolution rate, mechanical properties, biocompatibility, and antibacterial properties in vitro.

6. Consultants of the master's thesis sections

Section	Surname, initials and position of the consultant	Signature, date	
		the task was issued	the task accepted
Section 1	Tetiana Derkach, Professor, ScD		Guoqiang Shi 24.09.21
Section 2	Jinku Xu, Associate Professor, PhD	Jinku Xu. 24.09.21	Guoqiang Shi 24.09.21
Section 3	Tetiana Derkach, Professor, ScD		Guoqiang Shi 24.09.21

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No	The name of the stages of the master's thesis	Terms of performance of stages	Note on performance
1	Introduction	24.09-01.10.2021	Guoqiang Shi
2	Section 1 Literature review	02.10-08.10.2021	Guoqiang Shi
3	Section 2 The preparation and quality evaluation of PVA hydrogels	09.10-22.10.2021	Guoqiang Shi
4	Section 3 Consumer properties and production technology	23.10-05.11.2021	Guoqiang Shi
5	Conclusions	06.11-12.11.2021	Guoqiang Shi
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8	Checking the master's thesis for signs of plagiarism (10 days before the defence)	26.11-02.12.2021	Guoqiang Shi
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10	Submission of master's thesis for approval by the head of the department (from 7 days before the defence)	10.12-19.12.2021	Guoqiang Shi

Student Guoqiang Shi Guoqiang SHI

(signature) (surname and initials)

Scientific supervisor _____ Tetiana DERKACH

(signature) (surname and initials)

Head of the Scientific and _____ Olena HRYHOREVSKA
Methodological Centre for (signature) (first name, last name)
the Management of Specialist Training

Summary

Shi Guoqiang "Preparation and properties of polymeric bacteriostatic composite hydrogel". – Manuscript.

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

The master's thesis is devoted to developing and preparing a composite hydrogel material that can meet the long-term antibacterial properties through a simple physical cross-linking method. The specific research content is as follows: Using PVA and PHMG as raw materials, without introducing initiator and cross-linking agent, through the freezing-thawing method, an antibacterial hydrogel material was prepared. By controlling the PHMG content, freezing time, the number of freeze-thaw cycles and other conditions, the performance of the hydrogel can be adjusted. The best formulation of the hydrogel is found through characterization methods such as light transmittance, swelling rate, dissolution rate, mechanical properties, biocompatibility, and in vitro antibacterial. Prove that the hydrogel has excellent performance—especially long-term antibacterial ability and safety.

Keywords: PVA, PHMG, antibacterial dressing, hydrogel

Анотація

Ші Гоцян «Отримання та властивості полімерного бактеріостатичного композитного гідрогелю». – Рукопис.

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

Магістерська робота присвячена розробці та виготовленню композитного гідрогелевого матеріалу, який може мати довготривалі антибактеріальні властивості за допомогою простого фізичного методу поперечного зшивання. Зміст дослідження полягає в наступному. Використовуючи в якості сировини ПВА та ПГМГ, без введення ініціатора та зшиваючого агента методом заморожування-розморожування було виготовлено антибактеріальний гідрогелевий матеріал. Контролюючи вміст ПГМГ, час заморожування, кількість циклів заморожування-відтавання та інші умови, можна регулювати рівень характеристик гідрогелю. Найкращий склад гідрогелю знайдено шляхом дослідження таких характеристик, як світлопропускання, швидкість набухання, швидкість розчинення, механічні властивості, біосумісність та антибактеріальний ефект in vitro. Доведено, що гідрогель має чудові характеристики, особливо довгострокові антибактеріальні властивості та безпечність у застосуванні.

Ключові слова: ПВА, ПГМГ, антибактеріальна пов'язка, гідрогель

Аннотация

Ши Гоцян Получение и свойства полимерного бактериостатического композитного гидрогеля. – Рукопись.

Магистерская работа по специальности 226 Фармация, промышленная фармация. – Киевский национальный университет технологий и дизайна, Киев, 2021.

Магистерская работа посвящена разработке и изготовлению композитного гидрогелевого материала, который может обладать длительными антибактериальными свойствами с помощью простого физического метода поперечной сшивки. Содержание исследования состоит в следующем. Используя в качестве сырья ПВА и ПГМГ, без введения инициатора и сшивающего агента методом замораживания-размораживания был изготовлен антибактериальный гидрогелевый материал. Контролируя содержание ПГМГ, время замораживания, количество циклов замораживания-оттаивания и другие условия можно регулировать уровень характеристик гидрогеля. Лучший состав гидрогеля найден путем исследования таких характеристик, как светопропускание, скорость набухания, скорость растворения, механические свойства, биосовместимость и антибактериальный эффект in vitro. Доказано, что гидрогель обладает отличными характеристиками, особенно долгосрочными антибактериальными свойствами и безопасностью в применении.

Ключевые слова: ПВА, ПГМГ, антибактериальная повязка, гидрогель

List of abbreviations

DMA – dynamic mechanical analysis

DMAEMA – dimethylaminoethyl methacrylate

DSC – differential scanning calorimetry

E.coli – Escherichia coli

GA – glutaraldehyde

OD – optical density

PVA – polyvinyl alcohol

PEG – polyethylene glycol

PHMG – polyhexamethylene guanidine hydrochloride

PGs – polyguanidine salts

PBGs – polybiguanide salts

Triton X-100 – polyethylene glycol octyl phenyl ether

TGA – thermogravimetric analysis

UV – ultraviolet rays

XRD – X-ray diffraction

Meetings attended and articles published:

1. Ruiqi Kong, T.A. Palchevska, Yifan Liu, Guoqiang Shi, Jinku Xu. Simvastatin-loaded PLGA microspheres for drug delivery. "Achievements of Modern Medical Technology" at the 9th International Science and Practical Internet Conference. November 5, 2021. P. 24-25.

2. Guoqiang Shi, Yifan Liu, Ruiqi Kong, Jinku Xu, T.M. Derkach. Preparation and properties of polymeric bacteriostatic composite hydrogel. In: Physical-Organic Chemistry, Pharmacology and Pharmaceutical Technology of Biologically Active Substances. 2001, Issue 4 (accepted for publication).

Content

INTRODUCTION

The relevance of the topic：

Bacteria are the most critical type of resident bacteria in the human body, and bacterial infection is the most common and severe type of skin disease. If not correctly diagnosed and treated in the initial stage, it will cause infection and the spread of toxins, resulting in severe consequences and even life-threatening. At present, the antibacterial dressings in the market have some problems, such as single antibacterial performance and poor biocompatibility. Therefore, it is necessary to prepare an efficient and broad-spectrum antibacterial dressing.

Among the polymer antibacterial agents, guanidine polymer antibacterial agents are essential. Most polymers containing guanidine groups have sound antibacterial effects. Among many guanidine polymers, the research on polyhexamethylene guanidine (PHMG) is the most popular. Because PHMG has good antibacterial properties, the Ministry of Health has listed PHMG as a common disinfectant in the technical specification for disinfection. PHMG is a new generation of high-efficiency broad-spectrum bactericide with high antibacterial activity and prevents mildew. Only a small amount of PHMG is needed to achieve the ideal effect. The sterilisation rate is usually 99%; Moreover, PHMG has no side effects and irritating effects on human eyes, skin and mouth.

Hydrogel is a soft, hydrophilic three-dimensional network structure of polymer materials. The polymer network can absorb large amounts of water or other liquids, resulting in swelling. However, hydrogels still retain their original structure without dissolution, and the water retention property of the hydrogel is excellent.

Polyvinyl alcohol (PVA) is a kind of general water-soluble polymer. The molecular chain contains many hydroxyl groups; therefore, it has good water solubility and wide chemical diversity. In the 1950s, Sone Yasuo, a Japanese scholar, first observed the gelation of polyvinyl alcohol (PVA) aqueous solution. In addition to the general properties of hydrogels, PVA hydrogel also has many advantages. They are low cost, easy moulding, good biocompatibility, easy crystallisation and biodegradability. It is a synthetic material that meets the requirements for biomedical polymers' needs and is widely studied as a typical biomedical material.

Therefore, it is significant to prepare high-efficiency broad-spectrum antibacterial materials by combining the advantages and characteristics of polymeric bacteriostatic agent PHMG and PVA hydrogel.

The purpose of the study：

To prepare a high-efficiency broad-spectrum antibacterial dressing and conduct quality evaluation in the form of a drug-loaded antibacterial film.

The research objectives of the study:

The goal of this study is to prepare a high-efficiency broad-spectrum antibacterial film. According to the research goal, the following experimental tasks are formulated:

1. Preparation of antibacterial film of PHMG composite PVA hydrogel.
2. Quality evaluation of a drug-loaded antibacterial film.
3. Establishment of drug quantitative analysis method.

Research methods

1. Preparation of drug-loaded hydrogel antibacterial film. The drug-loaded PVA hydrogel film was prepared by the physical crosslinking method (freeze-thaw method). The PVA hydrogel films with different freeze-thaw times and different drug-loaded concentrations were prepared by the controlled variable method.

2. Quality evaluation of the drug-loaded antibacterial film. Determine the composite film's swelling, antibacterial properties, light transmittance, mechanics, drug release, etc.

3. Establish quantitative drug analysis methods. Preparation of PHMG solution, dilute it in multiples, establish a working curve for drug determination by spectrophotometry, and establish a process for determination of drug dissolution.

Practical value

Generally, hydrogel materials have poor mechanical properties. They cannot reduce the damage to the wound surface caused by the external environment and external mechanical forces and are inconvenient for medical operations. At the same time, medical hydrogels have disadvantages such as generally expensive costs and complicated preparation methods. The freeze-thaw method is used to prepare a hydrogel dressing with a simple preparation process, meeting specific mechanical strength, low cost, simple preparation process, and excellent antibacterial ability, which has great market prospects and practical value.

CHAPTER 1. LITERATURE REVIEW

1.1 Main types and characteristics of polymer antibacterial agents

Synthetic polymer antibacterial agents have only been over 30 years, and the research history is short. However, they have received significant attention and developed very rapidly in recent years due to their outstanding advantages. At present, many polymer antibacterial agents have been used in all aspects of human life. The different active functional groups can be mainly divided into chitosan and its derivatives, polyquaternary ammonium salts, polyhaloamine salts, polyguanidine salts, etc.

1.1.1 Chitosan polymers

Chitosan is representative of a natural polymer antibacterial agent, which has the advantages of safety, non-toxicity and high antibacterial activity. However, it has poor solubility, insoluble in water and most organic solvents, has a high viscosity, and its antibacterial activity is easily affected by pH value. It is not suitable for an environment with strong acidity and alkalinity. These problems significantly limit its application [1]. Many chitosan derivatives have been designed and synthesised to overcome the above shortcomings. Among them, the most studied water-soluble chitosan derivatives can be obtained in the following three ways.

1) Controlling the deacetylation of chitin or the acetylation reaction conditions of chitosan [1].

2) The amino or hydroxyl groups on chitosan introduce hydrophilic groups and improve water solubility. For example, water-soluble chitosan can be obtained by

carboxymethylation, acylation, hydroxyethylation [2] or sulfonation [3] of chitosan (Figure 1.1).

3) Degradation of chitosan with high relative molecular weight [1]. Some groups can improve the water solubility of chitosan and cooperate with it to resist bacteria – for example, quaternary ammonium derivatives of chitosan [4].

People have done a lot of research on the antibacterial mechanism of chitosan and its derivatives and put forward various theories.

1) Adsorb on the bacterial cell membrane through electrostatic attraction to form a porous membrane, destroy the permeability of the cell membrane, make it unable to normally absorb nutrients and pump out harmful substances [5] and destroy the integrity of the cell membrane, resulting in the extravasation of substances in the cell [6].

2) Chitosan with a relative molecular weight of less than 5000 Da can diffuse into microorganisms. Interaction with DNA and other electronegative substances will inhibit RNA synthesis and destroy normal physiological activities [7].

3) Chitosan has high nitrogen content and solid chelating ability. It can selectively combine with some trace metals and inhibit the production of bacterial toxins and bacterial reproduction [5]. The actual antibacterial process may be a combination of one or more mechanisms. Their antibacterial activity is related to many factors, such as deacetylation degree, relative molecular weight, pH value, metal ions, ionic strength, etc.

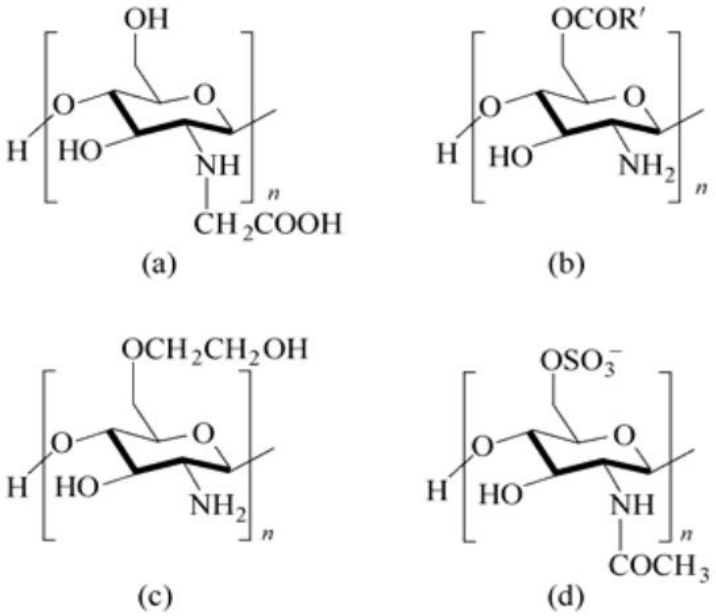

Figure 1.1 – Representative of water-soluble chitosan

1.1.2 Quaternary ammonium salt polymers

Among high molecular antibacterial agents, quaternary ammonium salt polymers are widely used [8]. They are characterised by a positive monovalent quaternary ammonium nitrogen ion (n^+) with four substituents (R1 ~ R4) and counteranions (such as Cl^-, Br^-, I^-) (Figure 1.2) [9]. The preparation of quaternary ammonium salt polymers generally adopts the route of "polymerisation before quaternisation". For example, Roy and others first synthesise the homopolymer pDMAEMA of dimethylaminoethyl methacrylate (DMAEMA) and then quaternize it with Bromoalkyl to obtain quaternary ammonium polymer [9]. However, some studies have adopted the route of "quaternisation before polymerisation". For example, Lu Guiqian et al. [10] quaternized DMAEMA with haloalkanes to obtain quaternary ammonium salt monomers and then prepared quaternary ammonium salt polymers through free radical polymerisation. The quaternisation degree of the latter is higher.

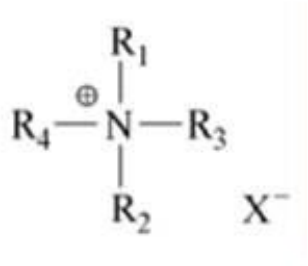

Figure 1.2 – General structure of quaternary ammonium salt compounds

Quaternary ammonium polymers have attracted much attention because of their high antibacterial activity, stable and lasting performance, residual toxicity and low irritation to human tissues [11]. Taking the polyamide amine derivative developed by Calabretta et al. [12] as an example, its half lethal dose to bacteria is about 1 / 2 of LL-37 (antibacterial peptide, a highly effective antibacterial agent). Still, its toxicity is only about 1 / 10 of LL-37. The MBC values of quaternary ammonium salt polymer pairs and synthesised by Sun et al. [13] are as low as 20 μG / ml and 50μG / ml, respectively, comparable to LL-37 and other commercially available antibacterial agents. It is generally accepted that the antibacterial properties of quaternary ammonium salt polymers depend on the positive electricity of N^+, but the specific action mode of N^+ has not been determined. Some studies believe they are adsorbed to the bacterial surface through the electrostatic attraction between N^+ and bacterial cell membrane (electronegativity). Then they penetrate and pierce the bacterial cell membrane with the help of the hydrophobic effect of substituted alkyl groups, resulting in the leakage of intracellular substances and killing bacteria [10]. It is also the most widely accepted theory at present. However, some studies believe that quaternary ammonium salt polymers rely on N^+ ion exchange with Ca^{2+} and

Mg^{2+} in the cell membrane to destroy the charge balance and structural integrity of bacteria, to exert antibacterial activity [16]. Many studies on the structure-activity relationship of quaternary ammonium salt polymers have been carried out. It is found that quaternary ammonium salt polymers show good antibacterial activity in a specific range of relative molecular weight. When the relative molecular weight is small, the density of active functional groups is low. It is insufficient to show good antibacterial activity. When the relative molecular weight is too large, the molecular size is large. Therefore, the resistance to penetrate the bacterial cell membrane is also significant, which is not conducive to the exertion of antibacterial activity [14]. At the same time, the length of the substituted alkyl chain will also significantly affect the antibacterial properties. It is generally believed that the number of carbon atoms of at least one of the four substituted alkyl chains of N^+ should reach 8 ~ 18 to enhance the compatibility with bacterial lipophilic phospholipid bilayer to destroy cell membrane better and kill bacteria [9]. In addition, the counter anion and the spacing length between the active functional group and the main chain will also affect the antibacterial properties [18-19].

1.1.3 Haloamine polymers

Among the high molecular antibacterial agents, haloamine polymers are relatively new [20-21]. The structural feature is that the repeating unit contains one or more haloamine bonds [20]. Theoretically, the hydrogen on amide n can be replaced by halogen to form a haloamine bond. Badrossamay et al. [22] grafted acrylamide on the polyethylene (PE) surface and obtained antibacterial activity after halogenation. But frankly speaking, more research is on heterocyclic acetolactate. Figure 1.3 illustrates a standard route for

preparing a heterocyclic acetolactam monomer. Jang et al. [23] synthesised ADMH monomer. They initiated the copolymerisation of ADMH with methyl methacrylate (MMA) on the surface of silica microspheres and then halogenated it. The final microspheres have good antibacterial properties against Gram-positive bacteria (G+ bacteria) and Gram-negative bacteria (G− bacteria), especially G+ bacteria.

Haloamine polymers have high antibacterial activity. They can release highly oxidising halogen cations into microorganisms, destroy cellular enzyme activity and metabolic process [24], and exert antibacterial activity through direct contact with bacteria [25]. Some studies believe that microorganisms cannot form drug resistance to haloamine polymers, and their antibacterial activity is renewable. When the antibacterial performance cannot meet the demand, simple halogenation treatment can improve the halogen stock and restore the antibacterial performance [26]. However, it should be noted that the halogenation step has a significant impact on antibacterial activity. The study also found that the better the hydrophilicity of the polymer, the lower the G, the easier it is for the amide compound to form a stable haloamine structure, and there is no apparent change in the FT-IR spectrum after soaking in water for two months [27]. At the same time, the better the hydrophilicity, the easier it is to release halogen and contact with bacteria, and the higher the antibacterial activity, but their antibacterial ageing will be relatively shortened [26].

It should be noted that haloamine polymers release halogen during the antibacterial process. Existing studies have proved that halogen can react with organics in the water to produce carcinogenic and mutagenic by-products, which affect human health and environmental safety. Therefore, it is necessary to comprehensively evaluate the safety

performance of haloamine polymers and pay attention to the selection of application fields.

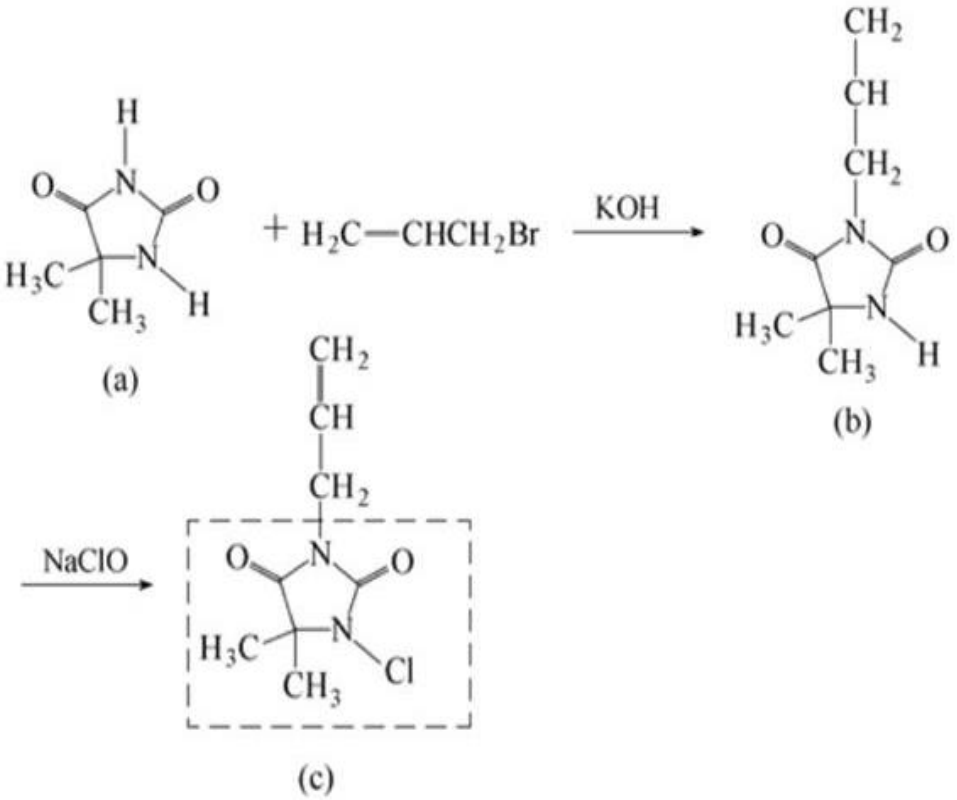

Figure 1.3 – Synthesis of ADMH monomer

1.1.4 Guanidine polymers

Guanidine polymeric antimicrobial agents include polyguanidine salts (PGs) and polybiguanide salts (PBGs) [27]. It is generally prepared by polycondensation, and the control of relative molecular weight and shape is poor. For example, Wei et al. [28] synthesised polyhexamethylene guanidine hydrochloride (PHMG) using hexamethylene diamine and guanidine hydrochloride polycondensation. The synthesised compound has three linear and four cyclic or branched structures. Guanidine polymers belong to strong cationic compounds. They can quickly adsorb to the negatively charged bacterial cell membrane, exchange ions with Ca^{2+} and Mg^{2+} in the cell membrane. So, they destroy the

charge balance of the cell membrane. They can also eliminate phospholipids biological activity and cell membrane structure. Their antibacterial properties depend on the structure of repeating units, relative molecular weight, polymer structure and so on. It was found that PHMG had good antibacterial properties when the relative molecular weight was 640 ~ 956 da. Cyclic or branched polymers have higher charge density and better antibacterial properties than linear polymers [28]. The increase of chain length of diamine will increase the distance between guanidine groups, resulting in the decrease of antibacterial energy. Guanidine carbonate reacts with a diamine to form urea, which is unsuitable for preparing guanidine antibacterial polymers, but guanidine hydrochloride is suitable.

Most polymers containing the guanidine group have a good antibacterial effect among high molecular antibacterial agents. Among many guanidine polymers, the research on polyhexamethylene guanidine (PHMG) (Figure 1.4) is the most popular. Because PHMG has good antibacterial properties, the Ministry of Health has listed PHMG as a common disinfectant in the technical specification for disinfection.PHMG is a new generation of high-efficiency broad-spectrum bactericide with high antibacterial activity and prevents mildew. Only a small amount of PHMG is needed to achieve the ideal effect, and the sterilisation rate is usually 99%. Moreover, PHMG has no side effects and irritating effects on human eyes, skin and mouth.

PHMG attracts and closely combines with the positive guanidine group through electrostatic interaction with the electronegative cell membrane of the outer layer of the bacteria. Such an interaction results in the rupture of the bacterial cell membrane, the massive outflow of internal nutrients, and the death of the bacteria due to the failure of

normal reproduction. The unique feature of this sterilisation mechanism is that the bacteria will not produce drug resistance. Nowadays, people pay more and more attention to protecting the environment. It is necessary to study and synthesise green antibacterial agents that are friendly to the environment, long sterilisation time, have a wide range of sterilisation, and are less harmful to the human body. Introducing PHMG into other materials to prepare antibacterial composites has become an important research topic [29, 30, 31].

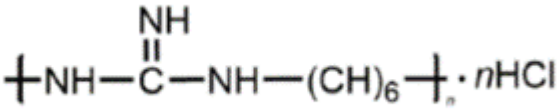

Figure 1.4 – Structure diagram of PHMG

1.2 Hydrogel

Because of their unique properties, hydrogels have been extensively studied as antibacterial materials in recent years. In summary, hydrogels have the following advantages in anti-infection treatment: they can release antimicrobial agents at the infection site and avoid repeated administration. It has good tissue adhesion and can promote tissue repair [32-35]; When used as an antibacterial coating, it can protect medical devices or implanted devices from causing infection in patients [36, 37]. The definition and preparation methods of hydrogels are introduced in detail.

1.2.1 Definition of hydrogel

Hydrogels are polymers that contain large amounts of water and have three-dimensional network crosslinking structures. Hydrogel has attracted wide attention for its unique properties. First of all, because of its high water content and similar structure to the human extracellular matrix, hydrogels are closer to living tissues than other materials, thus having good biocompatibility. Secondly, due to the softness of hydrogel, the mechanical damage to the surrounding tissues is relatively small. Moreover, the unique porous structure gives it excellent permeability, convenient for transporting drugs and nutrients. Its remarkable plasticity makes it possible to meet more needs in biomedicine. In recent years, hydrogels have been widely studied and applied as excellent biomedical materials, such as drug carrier tissue engineering, cell culture matrix, coating and wound dressing.

1.2.2 Preparation of hydrogel

The preparation methods of hydrogels are generally divided into physical crosslinking and chemical crosslinking [38], as shown in Figure 1.5.

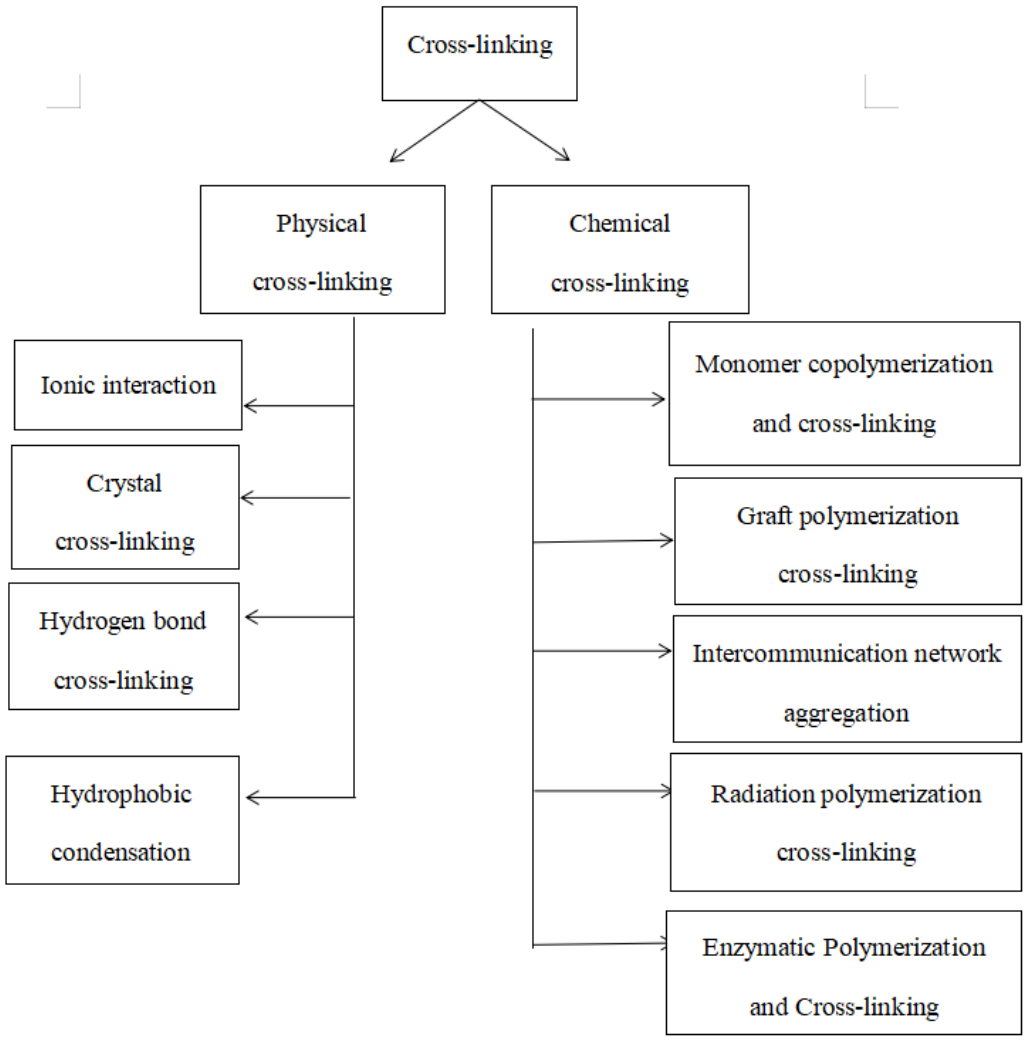

Figure 1.5 – The preparation methods of hydrogels

1.2.3 Antibacterial hydrogel

Antibacterial hydrogel, which has bacteriostatic or bactericidal functions, has essential research significance as a new antibacterial material. The preparation of antibacterial hydrogel is usually introduced into the hydrogel system using physical encapsulation or chemical crosslinking. According to the reported antibacterial hydrogel, according to its antibacterial ingredients, it can be classified into four main categories: antibiotic antibacterial hydrogel, antibacterial gel containing inorganic nanoparticles,

antibacterial peptide hydrogel and cationic polymer antibacterial hydrogel. Cationic polymers are widely studied as new antibacterial agents.

Cationic polymer antibacterial hydrogels, such as dendrimer, polyethyleneimine and polylysine, have attracted the attention of researchers because of their unique properties. Such properties are stable structure, antibacterial durability and ease of modification. Studies on hydrogels with cationic polymers and their derivatives as antibacterial agents have been reported extensively. Zhou et al. [39] reported a lysine based photo-polymerisation of poly (lysine) antibacterial hydrogel. The results show that the gel can be used to prepare antibacterial coating on the surface of medical equipment or equipment. It has broad-spectrum and high-efficiency antibacterial activity, and its sterilisation mode is contact sterilisation. Lee et al. [40] have prepared a broad-spectrum antibacterial hydrogel that can degrade mature biofilms. Firstly, two polymers were synthesised. The first was ABA triblock copolymer, in which the intermediate segment was a hydrophilic peg, and the two ends were vitamin E (VE) - functionalised polycarbonate. The second is an antibacterial cationic polymer containing VE. Hydrogels can be formed by hydrophobic interaction self-assembly after mixing the aqueous solutions of these two polymers. The gel has over 99.9% sterilisation effect on Staphylococcus aureus and Escherichia coli through contact sterilisation. The synergistic antibacterial effect of fluconazole is introduced to Candida albicans, and the gel is proved to remove bacterial biofilms.

Giano et al. [41] reported a hydrogel formed by direct crosslinking of oxidised dextran and cationic polymer branched polyethyleneimine via Schiff base bonds. The gel can be injected in situ in the infected area and showed excellent anti-infection

performance in mouse skin infection and cecal ligation models. Liu and others have prepared polycarbonate and PEG in situ antibacterial hydrogel [42] by ring-opening polymerisation, quaternary amination and two-step Michael addition reaction of cyclic carbonates. They have broad-spectrum antibacterial activity and can achieve about 99.9% bactericidal efficiency for bacteria and fungi, and the antibacterial activity is durable. In addition, the gel can also be used to prepare antibacterial coating on the surface of the catheter material and shows a good antibacterial and antifouling effect.

1.3 PVA hydrogel

1.3.1 Introduction to PVA

The structural formula of polyvinyl alcohol (PVA) is shown in Figure 1.6. PVA contains more -OH between molecules, so PVA is easy to form a hydrogen bond and then form a macromolecular network structure. It has good water absorption and an excellent gas barrier. PVA solution has suitable film-forming properties and excellent mechanical properties. The most important thing is that PVA can be thoroughly degraded and has good solvent resistance [43].

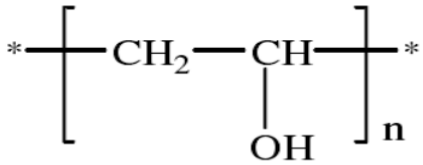

Figure 1.6 – Structure diagram of PVA

The performance of PVA is between plastic and rubber, with water solubility and no toxicity. PVA has a large amount of -OH in the molecule and hydrogen bond between -

OH, so the chemical properties of PVA are relatively stable [44-46]. In recent years, PVA has been more widely used due to the improvement of PVA synthesis technology. PVA has high dielectric constant and insulation properties, so PVA is also used in the packaging and electronic industries [47]. For PVA, its degree of polymerisation can be divided into four categories: ultra-high, high, neutralisation and oligomerisation. The increase of PVA polymerisation degree will reduce its water solubility and increase its viscosity [48].

1.3.2 The film formation of PVA

The solubility of PVA in water depends on its alcoholysis degree. When the alcoholysis degree of PVA is between 87% ~ 89%, its water solubility is the best. Therefore, in terms of water solubility, the alcoholysis degree of PVA is better in the range of 87% ~ 89% [49]. The molecule of PVA has a linear structure; -OH in the molecule will form a hydrogen bond with -OH in H_2O [50]. At the same time, PVA has good resistance to organic solvents and is suitable for film materials. The film formed by PVA has strong toughness and mechanical properties [51]. At present, there are four methods for preparing PVA films.

1. Casting method

The casting method is to pour the configured PVA mother liquor into the roller casting machine, heat dry the roller of the casting machine, and automatically peel it into film after cooling. It has the advantages of high efficiency and high precision. The process flow is shown in Figure 1.7. The technical key point is to control the drying temperature and drying time in the whole process and grasp the glue feeding amount [52-53].

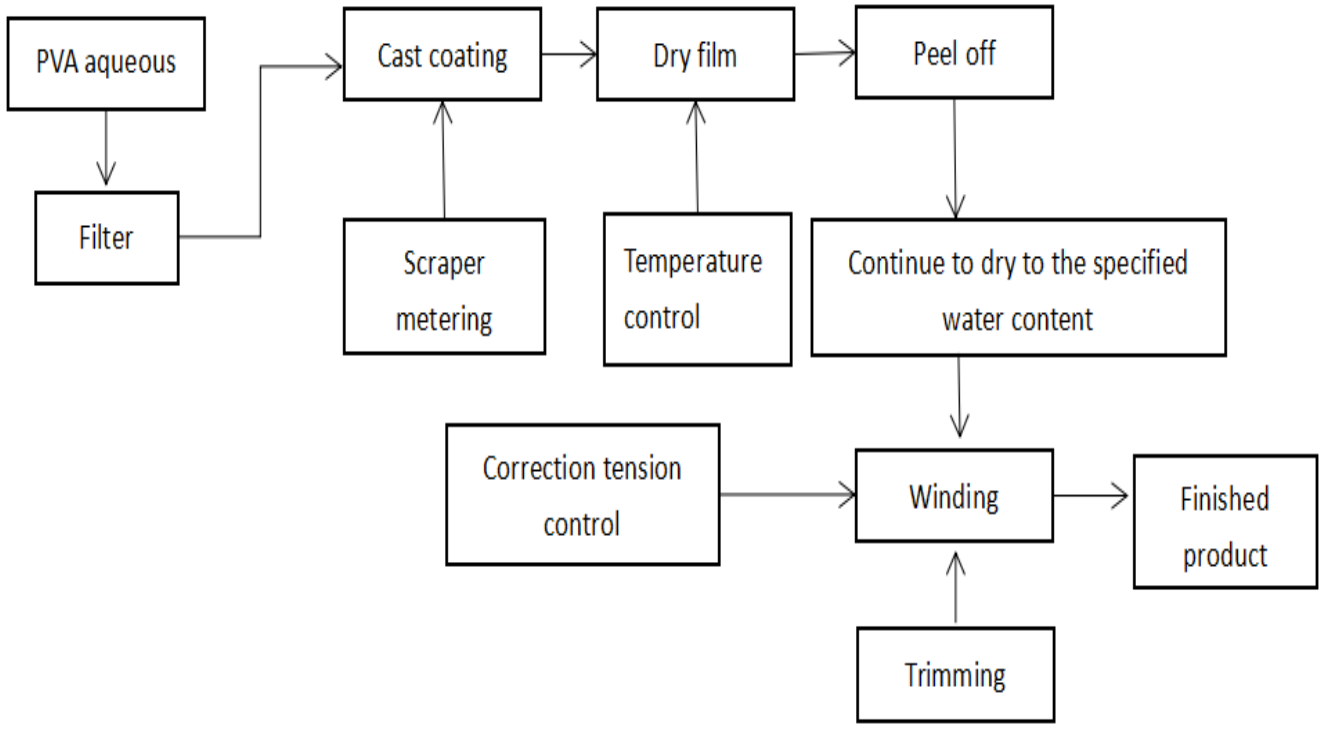

Figure 1.7 – Flow chart of casting method

2. Wet extrusion blow moulding

Figure 1.8 shows the preparation process of the PVA blown film forming process. Firstly, PVA was blended with water and additives, swelled for a while, then extruded and melted, and finally formed a film by defoaming and quantitative blow moulding [53].

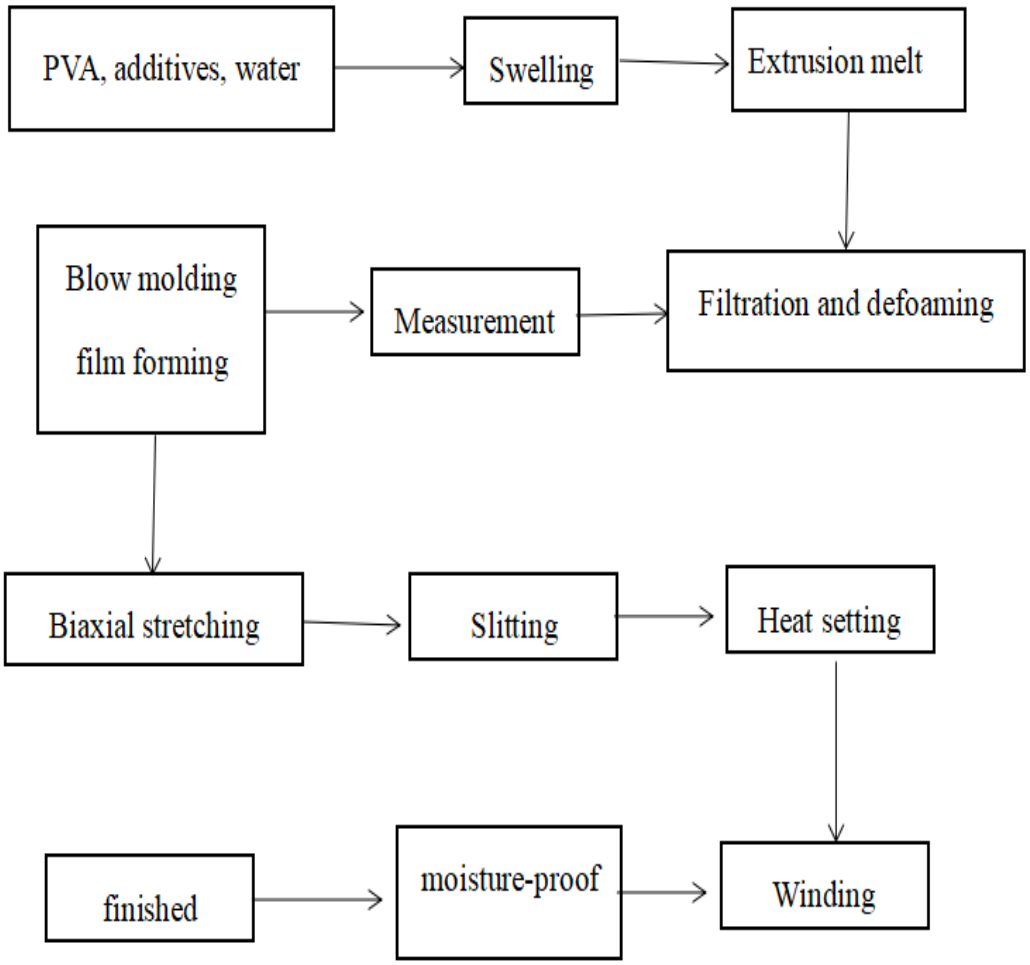

Figure 1.8 – Flow chart of wet extrusion blow moulding method

3. Dry extrusion blow moulding

Figure 1.9 shows the preparation process of PVA by dry extrusion blow moulding. The dry extrusion blow moulding method is to vacuum dry PVA particles for 24 hours, mix them with various additives, extrude and granulate them with a single screw extruder, then defoamer and blow film, and obtain the finished products after post-treatment. This method has the advantages of low cost, simple operability and can be carried out on a large scale.

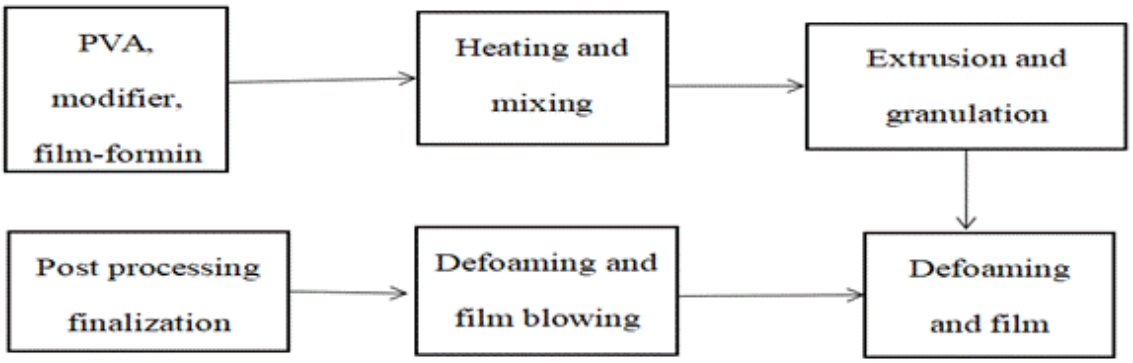

Figure 1.9 – Flow chart of dry extrusion blow moulding method

4. Plate coating method

The plate coating method is a simple film-forming method. It adopts coating PVA mother liquor on a smooth plate and uncovering the film after natural air drying. It is simple and easy, but the efficiency is low. It is suitable for small-scale experiments, and it is a feasible scheme for preparing films in general laboratories.

1.3.3 Preparation of PVA hydrogel

Polyvinyl alcohol (PVA) is a water-soluble polymer obtained by the alcoholysis of polyvinyl acetate. Polyvinyl alcohol has good water solubility, non-toxic to the human body, has no side effects, has good histocompatibility and has little environmental pollution. PVA hydrogel is widely used in ophthalmology, artificial cartilage, wound dressing, biomedicine and other fields. The preparation methods of PVA hydrogel are mainly divided into three types: physical crosslinking, chemical crosslinking and radiation crosslinking.

1. Chemical crosslinking method

Polyvinyl alcohol (PVA) hydrogel was prepared in [55] by Jun-Seo Park et al. with polyvinyl alcohol (PVA) as raw material and chemical crosslinking with glutaraldehyde (GA) under acidic conditions. Chemical crosslinking agents form the crosslinking point between PVA molecules, and then the PVA hydrogel is formed. The chemical crosslinking method is used to enhance the properties of PVA hydrogels by adjusting chemical crosslinking agents. Chitosan and polyvinyl alcohol (PVA) blends were used to prepare PVA/ chitosan hydrogels through chemical crosslinking of glutaraldehyde by Herman S. Mansur et al. [54]. Polyvinyl alcohol and polyvinyl alcohol gel were studied by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and dynamic mechanical analysis (DMA). Their swelling characteristics and tensile strength were also determined. The results showed that gels' melting point and crystallisation temperature were lower than polyvinyl alcohol hydrogels without crosslinking agents.

Du et al. [56]: Crosslinked polyvinyl alcohol with different glutaraldehyde content to prepare shape memory polymers. The results show that the hydrogel has good shape memory and cycle performance and offers the potential application prospect of SM-PVA as shape memory material. Although chemical crosslinking makes PVA hydrogel fast and easy to react to, it is difficult to remove all the crosslinking agents entirely that remain in the gel without participating in the reaction. These small molecule crosslinking agents are mostly bioactive and have biological toxicity, such as glutaraldehyde, boric acid, etc. These disadvantages greatly restrict the application of PVA hydrogel prepared by a chemical crosslinking agent in biomedicine.

2. Radiation crosslinking method

Irradiation crosslinking means that free radicals are generated by irradiating PVA molecules by high-energy rays and PVA hydrogels are formed by combining free radicals.

Fan [57] et al.: Chitosan (CS) / gelatin / polyvinyl alcohol (PVA) hydrogel for medical dressings was prepared by irradiation. The study shows that the tensile strength of CS/ gelatin /PVA hydrogel is higher than that of gelatin /PVA hydrogel, and the maximum tensile strength can reach 2.2 Mpa. Meanwhile, CS/ gelatin /PVA hydrogel also has an excellent hemostatic effect, good pH sensitivity, swelling ability and water evaporation rate.

Mallaiah [58] et al.: Polyvinyl alcohol (PVA) hydrogel containing gold nanoparticles was synthesised by X-ray irradiation Glue nanocomposites. The addition of gold nanoparticles reduced the binding sites of the PVA matrix, resulting in enhanced swelling and mechanical properties of gold nanoparticles / PVA hydrogel nanocomposites. The radiation crosslinking method has many advantages that the chemical crosslinking method does not have, such as faster reaction speed, controllable polymerisation process in space and time, no residual crosslinking agent, etc. However, many materials can not be added to the gel system due to radiation intensity, so the application scope is limited. At the same time, the radiation crosslinking method also needs special radiation equipment, which has hidden dangers of radiation safety and environmental safety. In addition, radiation crosslinking has no noticeable effect on the mechanical properties of the materials.

3. Physical crosslinking method

Physical crosslinking, also known as freeze crosslinking, is the most commonly used method for preparing PVA hydrogels. The PVA solution was frozen at low temperature (-20^{o}C ~-80^{o}C) and then removed at room temperature by thawing. Repeated cycles of the freezing and thawing processes enhance the performance of PVA hydrogel. The mechanism of PVA hydrogel prepared by freezing-thawing is as follows.

1. At low temperatures, the PVA chain's movement speed decreases, making prolonged contact between PVA chains; the hydrogen bonds between PVA molecules and intermolecular hydroxyl groups gradually form hydrogen bonds. This structure plays the role of crosslinking point in PVA hydrogel and creates a more stable structure. This firm structure was maintained after thawing at room temperature. By repeating the freezing-thawing process, more and more hydrogen bond crosslinking points are formed, and the substance becomes denser.

2. At low temperatures, the gel phase starts to form phase separation, which creates the aggregation area and non-aggregation area of polymer segments. These aggregation regions form a gel network with non aggregated areas.

In the process of freezing, microcrystalline regions were formed between PVA molecules. These microstructures were used as crosslinking points of hydrogels. With the increase of the freezing-thawing process, the microcrystalline regions in PVA hydrogels increased, and the mechanical strength of hydrogels increased significantly. There are also theories that these mechanisms may not exist independently in the gel system but form a gel together [59]. See Figure 1.10 below for illustration.

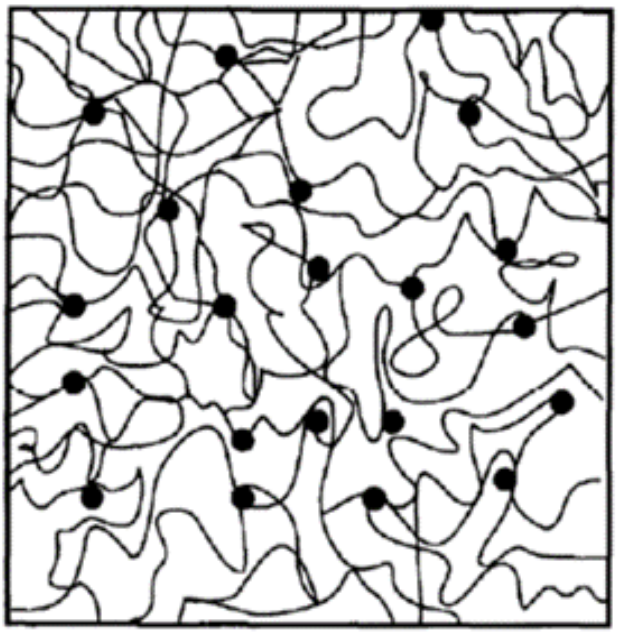

Figure 1.10 – Schematic diagram of PVA hydrogel after freezing and thawing

The preparation of physically crosslinked PVA hydrogels was prepared by freezing of PVA solution. Therefore, the factors affecting the properties of physically crosslinked PVA hydrogel mainly include freezing-thawing cycles, PVA solution concentration and solvents. Lozinsky et al. [60] studied the effect of polyols in the initial PVA water solution on the rheological properties and thermal properties of the low-temperature gel prepared by the freeze-thawing method. Polyol additives (cosolvent) are glycerol, propylene glycol, diethylene glycol, triethylene glycol and ethylene glycol oligomers (PEG-400 and peg-1000). The results showed that the shear modulus and melting temperature of PVA hydrogels were reduced by glycerol, propylene glycol and two glycols. Gels' strength and thermal stability increased with the use of three glycols and glycol oligomers as cosolvent. Y. Machida [61] et al. determined the NMR spectra of polyvinyl alcohol (PVA) hydrogel prepared by multiple freezing-thawing cycles. The results showed that after the second

freezing-thawing cycle, the PVA solution began to gel, and after the fourth cycle, a stable physical crosslinking hydrogel was formed. Therefore, the freezing-thawing cycle can effectively enhance PVA hydrogels' structure and mechanical strength.

Tatsuko et al. [62] used polyvinyl alcohol (PVA) hydrogel to carry out 1 to 5 freezing-thawing cycles. The phase transition of hydrogels was also studied by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The results show that PVA molecules aggregate during ice formation and form hydrogen bonds between molecular chains. With the increase of freezing-thawing cycles, the residual free molecular chains are contained in the binding band in turn. At the same time, PVA hydrogel exhibited a synergistic effect when the sol concentration was below 5%. Therefore, it is not easy to prepare gel with a concentration of less than 5%. The sol-gel transformation is challenging to detect if the concentration of sol decreases. The physical crosslinking method uses the freezing-thawing cycle to form a physical crosslinking point in the PVA hydrogel. It does not need to add any chemical crosslinking agent in the reaction process. It can effectively maintain the biocompatibility of the material. Meanwhile, the PVA hydrogel prepared by physical crosslinking has high water content, high mechanical strength and controllable, stable and insoluble at room temperature, and the preparation process is simple. Without special equipment and compatibility with other sustained-release drugs, the hydrogel is an ideal preparation method for PVA [63-66].

Chapter 1 conclusions

In the first chapter, I mainly review the theme from three aspects.

The first aspect is the main types and characteristics of polymer antibacterial agents. It is concluded from the overview that PHMG is a new generation of high-efficiency broad-spectrum bactericide, which has high antibacterial activity and prevents mildew. Only a small amount of PHMG is required to achieve the ideal effect, and the sterilisation rate is usually 99%. Moreover, PHMG has no side effects and irritating effects on human eyes, skin and mouth. Nowadays, people pay more and more attention to protecting the environment. It is necessary to study and synthesise green antibacterial agents that are friendly to the environment, have a long sterilisation time, and have a wide range of sterilisation and are less harmful to the human body. Introducing PHMG into other materials to prepare antibacterial composites has become an important research topic.

The second aspect is the definition, preparation and application of hydrogels in the field of antibacterial. From the summary, hydrogels have been widely studied and applied as excellent biomedical materials, such as drug carrier tissue engineering, cell culture matrix, coating and wound dressing. Especially in wound dressings, the preparation of antibacterial hydrogel materials has become a research hotspot.

The third aspect is PVA hydrogel. The properties of PVA and the method of PVA film formation, and the preparation of PVA hydrogel are introduced. It is concluded from the summary that PVA is water-soluble, non-toxic, chemically stable, and has high dielectric constant and insulation properties. Therefore, PVA hydrogel is an excellent material for preparing antibacterial dressings. Physical crosslinking, also known as freeze crosslinking, is the most commonly used method for preparing PVA hydrogels. The physical crosslinking method is easy to operate. It has good physical and chemical

properties of the prepared PVA hydrogel. The PVA hydrogel has better biocompatibility because it does not need to join a chemical crosslinking agent.

Therefore, in the above three aspects, my research goal is to prepare antibacterial dressings and study their properties by using PVA hydrogel as a carrier and adding a new generation of broad-spectrum bactericide PHMG.

CHAPTER 2. THE PREPARATION AND QUALITY EVALUATION OF PVA HYDROGELS

This experiment used the circular freezing-thawing method to prepare a three-dimensional network structure of polyvinyl alcohol hydrogel. This chapter mainly introduces the drugs and equipment used in the investigation, introduces the preparation process of hydrogels with different drug concentrations and freeze-thaw cycles, establishes quantitative drug analysis methods, and carries out a quality evaluation of the drug-loaded PVA hydrogel membrane.

2.1 Experimental raw materials and equipment

2.1.1 Experimental raw materials

The raw materials and drugs used in this chapter are shown in Table 2.1.

Table 2.1 – Drugs and reagents

Name of drug and reagent	Manufacturer
PVA	Ti xi'ai (Shanghai) Huacheng Industrial Development Co., Ltd
PHMG	Aladdin company
Beef paste	Aladdin company
Tryptone	Aladdin company
Agar	Aladdin company
Sodium chloride	Aladdin company
Escherichia coli	Aladdin company
Staphylococcus aureus	Aladdin company
Hella cell	Aladdin company
Triton X-100	Aladdin company

2.1.2 Instrumentation

The experimental instruments are shown in Table 2.2.

Table 2.2 – A list of instruments used

Name of experimental instrument	**Model**	**Manufacturer**
Ultrasonic cleaner	Kq5200 type	Kunshan Ultrasonic Instrument Co., Ltd
Intelligent magnetic stirrer	ZNCL-BS140*140	Gongyi Yuhua Instrument Co., Ltd
Electronic balance	TE124S	Saidoris scientific instrument (Beijing) Co., Ltd
Electric blast drying oven	101type	Beijing Yongguangming Medical Instrument Co., Ltd
PH meter	PHS-3E	Shanghai Leici Chuangyi Instrument Co., Ltd
Ultraviolet-visible spectrophotometer	UV-6000H	Shanghai Yuanxi Instrument Co., Ltd
Booster electric agitator	Jb50-d type	Made by Shanghai specimen model factory
Refrigerator	BCD-50WDPF	Gree Co., Ltd
Constant temperature magnetic stirring oil bath	Type D101	Saidoris scientific instrument (Beijing) Co., Ltd
Microplate Spectro-photometer	SpectraMaxRi3	Shanghai Leici Chuangyi Instrument Co., Ltd
Autoclave	YX280B	Beijing Yongguangming Medical Instrument Co., Ltd
Constant temperature incubator	HPX-9272MBE	Beijing Yongguangming Medical Instrument Co., Ltd
electron microscope	BA210	Mcdio Industrial Group Co., Ltd

2.2 Sterilization mechanism of PHMG

Polyhexamethylene guanidine (PHMG) is a new cationic polymer prepared by polycondensation of hexamethylene diamine and guanidine hydrochloride under certain conditions. The synthesized PHMG is a translucent light yellow solid, which is very soluble in water and has no pungent smell. The molecular formula is (CH: NCI) n. It is an efficient broad-spectrum bactericide and can effectively control the growth of bacteria for a long time.

According to the research of experts at home and abroad for many years, the antibacterial mechanism of PHMG is: because PHMG is positively charged, it is easy to adsorb negatively charged bacteria and viruses, which inhibits the re-division of bacteria and viruses, makes bacteria and viruses lose the ability of diffusion and reproduction, inhibits the respiratory ability of bacteria and viruses, and accelerates the rapid death of bacteria and viruses 9. The bactericidal rate of 0.02% PHMG against Escherichia coli, Staphylococcus aureus, Candida albicans and Neisseria gonorrhoeae was 100%.

2.3 Selection of PVA concentration

The effect of PVA concentration on the properties was studied by Hu Xiaoli et al. [67]. It was found that hydrogel was easy to dehydrate and shrink at low PVA concentrations. Only when PVA concentration exceeds 5wt%, stable and effective crosslinked physical hydrogel can be formed. When the concentration of PVA is 5wt% ~ 10wt%, the gel is thawed, jelly-like, and the force is liable to deformation and

fragmentation. When the mass fraction of PVA is 13%, the PVA hydrogel has good properties. Therefore, the hydrogel was prepared by PVA with a mass fraction of 13%.

2.4 The preparation principle of PVA drug-loaded hydrogel

The freezing process "freezes" the molecular motion state of the PVA aqueous solution at a particular time so that the molecular chains in contact with each other can interact and closely combine with van der Waals force and hydrogen bond. Some molecular segments can form an ordered structure in a certain micro-region to strengthen the binding. So that after the PVA solution is thawed at room temperature, these tightly bound ordered micro-regions still bind and become "entanglement nodes". When frozen again, some new closely tied ordered micro-regions are formed, called "physical crosslinking points". PHMG is easily soluble in PVA aqueous solution. When the "physical crosslinking point" is created, PHMG is fixed in an ordered micro-region. Finally, the drug-loaded PVA hydrogel was formed.

2.5 Preparation process of drug-loaded PVA hydrogel

First, weigh an appropriate amount of PVA into a wide mouth flask, measure deionized water, and thoroughly stir and dissolve it at room temperature for 30 minutes. Move the flask into an oil bath with magnetic stirring, slowly raise the temperature to 85℃, stir and dissolve at constant temperature for 5 h, then increase the temperature to 90℃ and stand for 30 min to remove the bubbles generated during stirring. Cool the completely dissolved PVA aqueous solution to room temperature. Add a certain amount of PHMG into PVA aqueous solution, stir to dissolve it and stand to obliterate bubbles.

Finally, the solution was injected into the cavity of the polypropylene mould with a thickness of 0.2mm. After freezing at - 20℃ for 13 hours, thaw at 25℃ for 20 minutes as a freeze-thaw cycle. The PVA hydrogel membrane was made several times after repeated cycles. The flow chart is shown in Figure 2.1

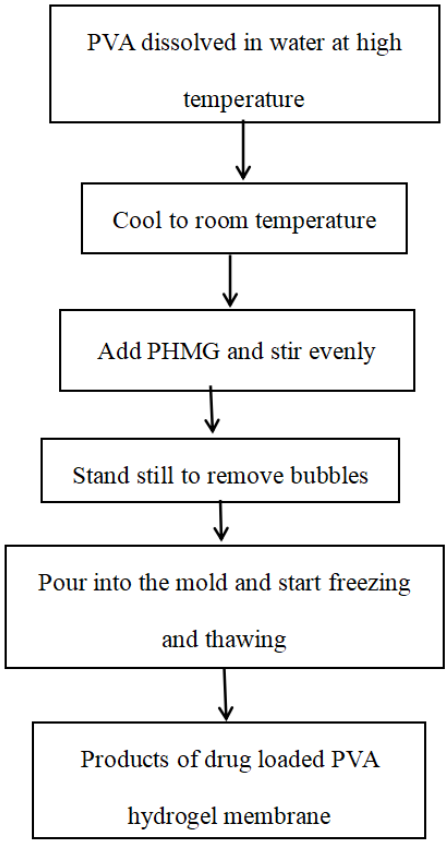

Figure 2.1 – Process flow chart for preparation of drug-loaded PVA hydrogel membrane

2.6 Experimental design of drug-loaded PVA hydrogel

Two factors are crucial to the finished product in the drug-loaded PVA hydrogel preparation process. The first is that the drug loading concentration is different, and the second is that the freezing and thawing times are different. It is found that the film cannot be formed when the freezing and thawing times are the same.

Therefore, the experiment was divided into two groups:

(1) Different drug loading concentrations: the mass fractions of PHMG loaded by A, B, C and D were 0.3%, 0.5%, 0.7% and 0 respectively. Their freezing and thawing cycles were 5 times.

(2) The freezing and thawing times are different: B1, B2 and B3 are frozen and thawed for 2, 3 and 4 times, respectively. The mass fraction of PHMG loaded on them is 0.5%, as shown in Table 2.3 below.

Table 2.3 – Experimental design of drug-loaded PVA hydrogel

Name	Mass fraction of PVA (%)	Mass fraction of PHMG loaded (%)	The number of gelatin freeze-thaw cycles
A	13	0.3	5
B	13	0.5	5
C	13	0.7	5
D	13	0	5
B1	13	0.5	2
B2	13	0.5	3
B3	13	0.5	4

2.7 Standard curve equation of PHMG concentration concerning the absorbance

(1) Determination of PHMG detection wavelength

Accurately weigh 5.00 mg PHMG drug, place it in a 100 mL volumetric flask, dissolve it with deionized water, dilute to the mark, shake the volumetric flask to make it

uniform, and use it as a stock solution (50 μg/mL). Use a pipette to take out 15 mL and transfer to a 50 mL volumetric flask, dilute the volume to the mark with deionized water, shake the volumetric flask to make it uniform, and prepare a standard solution with a concentration of 15 μg/mL. Use deionized water as a blank control solution and detect with an ultraviolet spectrophotometer. Scan the standard solution with a 190-800 nm wavelength to determine the maximum wavelength of PHMG. As shown in Figure 2.2 below, PHMG has a maximum absorbance at a wavelength of 192nm.

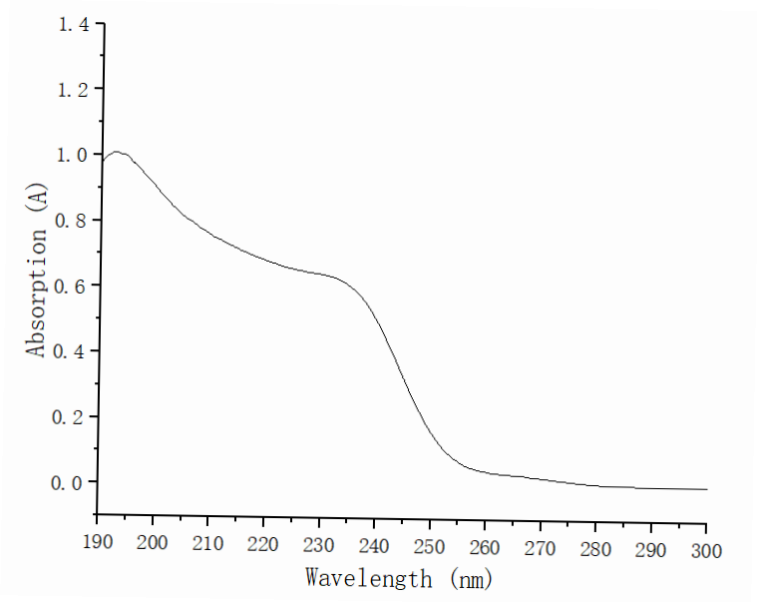

Figure 2.2 – UV scanning spectrum of PHMG

(2) Preparation of standard curve

Precisely measure 2, 4, 8, 10, and 15 mL of the standard solution, respectively, transfer to a 50 mL volumetric flask, dilute the volume to the mark with deionized water, shake the volumetric flask to make it uniform, and obtain a concentration of 2, 4, respectively, 8, 10, 15 μg/mL series of solutions. Use deionized water as a blank control solution, detect with an ultraviolet spectrophotometer, select PHMG at a wavelength of

192nm, scan the series of solutions to determine the absorbance (A). Taking the concentration (C, μg/mL) of the PHMG model drug as the abscissa (x) and the absorbance (A) as the ordinate (y), linear regression of the absorbance against the concentration was performed to obtain the standard curve equation. See Figure 2.3, Figure 2.4 and Table 2.4 below.

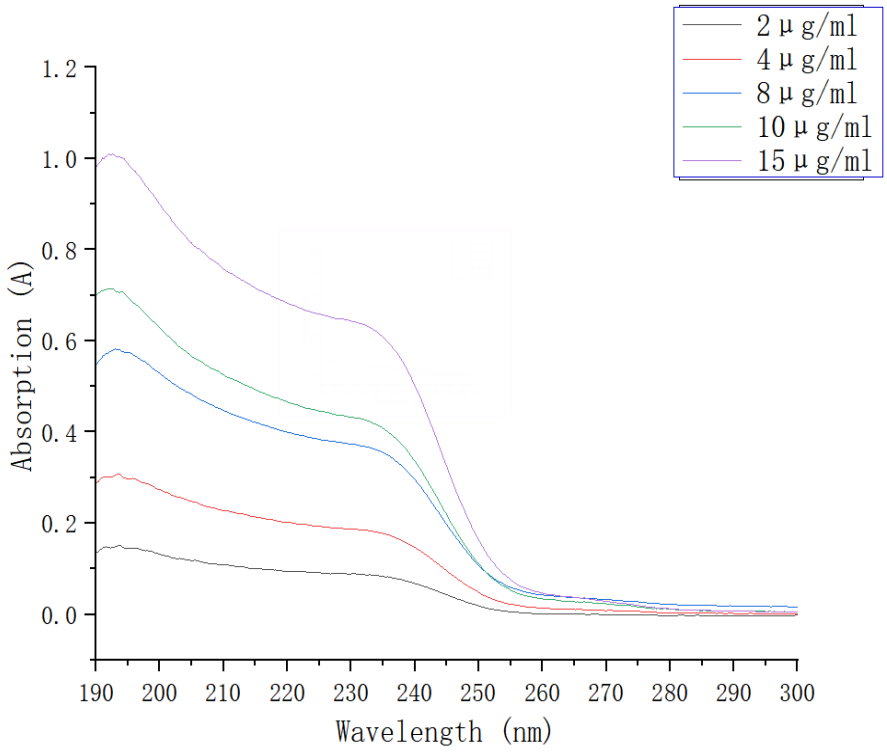

Figure 2.3 – Absorbance of PHMG at different concentrations

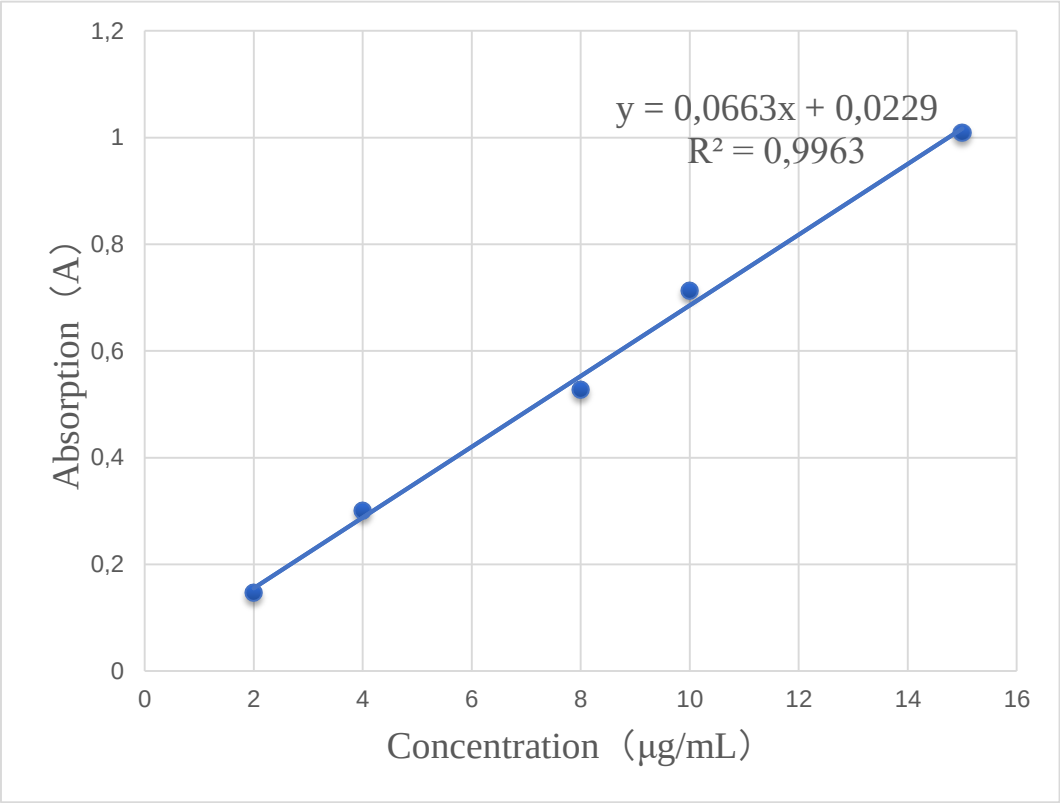

Figure 2.4 – Linear regression of absorbance at -192nm versus concentration

Table 2.4 – Absorbance of different concentrations of PHMG at 192nm

Concentration（μg/mL）	2	4	8	10	15
Absorbance (ABS)	0.1468	0.3014	0.5281	0.7136	1.0094

Linear regression is performed on the concentration C (μg/mL) by absorbance A (Absorption), and the standard curve equation is obtained: Y=0.0663X+0.0229, R^2=0.9963. The results showed that the absorbance and concentration of PHMG drugs showed a good linear relationship in the concentration range of 2-15 μg/mL. Therefore, we get the formula

$$Y = 0.0663X + 0.0229,\ 2 \leq X \leq 15, \qquad (2.1)$$

X is the concentration of PHMG in the solution, and its unit is μg/mL. Y is the absorbance at a wavelength of 192nm, and its unit is A.

2.8 Photograph of PVA hydrogel film appearance and light transmittance test

Cut the composite hydrogel film into 10 mm x 40 mm rectangles and take pictures.

The appearance of PVA with the same freezing and thawing times and different drug loading concentrations is shown in Figure 2.5.

The appearance of PVA with different freezing and thawing times and the same drug loading concentration is shown in Figure 2.7.

Scan the light transmittance of the composite hydrogel film with an ultraviolet spectrophotometer. First, take the blank film D with the same freeze-thaw times as the scanning base, and scan the three films A, B, and C with a wavelength of 200-800 to obtain the light transmittance (see Figure 2.6 below).

Then use water as the scanning substrate to scan the three films of B1, B2, and B3 at 200-800 wavelengths to obtain the light transmittance, as shown in Figure 2.8.

（1) Intuitively from Figure 2.5, the transparency of the drug-loaded PVA films with the same number of freeze-thaw cycles (5 times) and different drug-loading concentrations are very close.

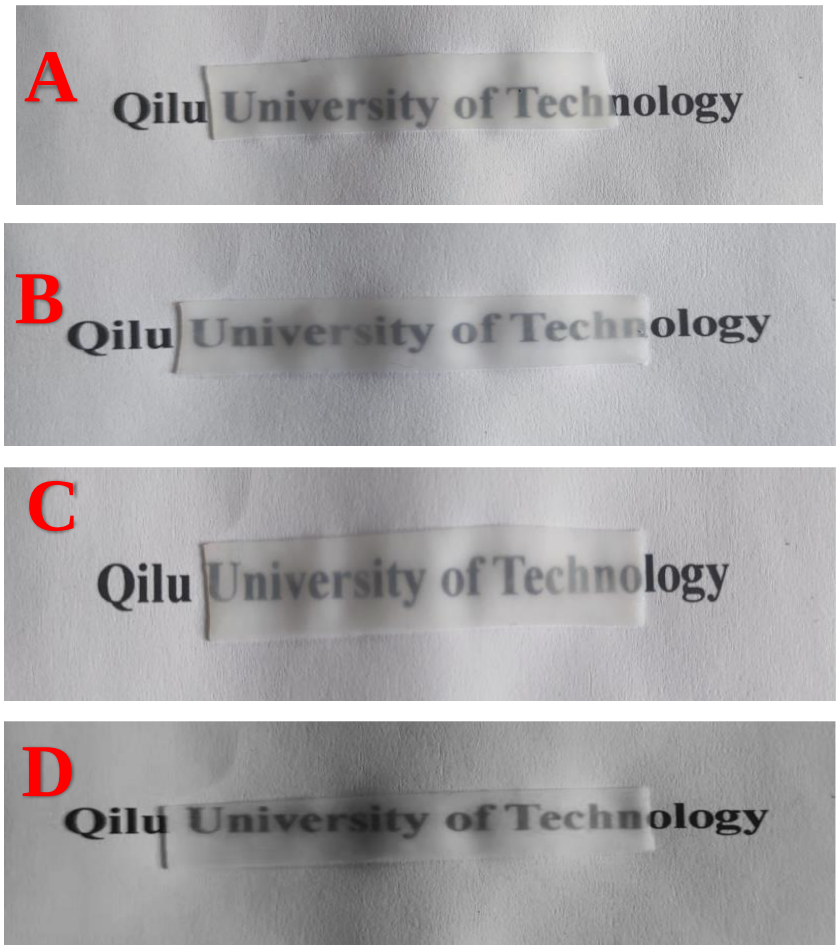

Figure 2.5 – The appearance of PVA membrane with the same freeze-thaw times and different drug loading concentrations

As seen from Figure 2.6, their light transmittance curves are almost the same.

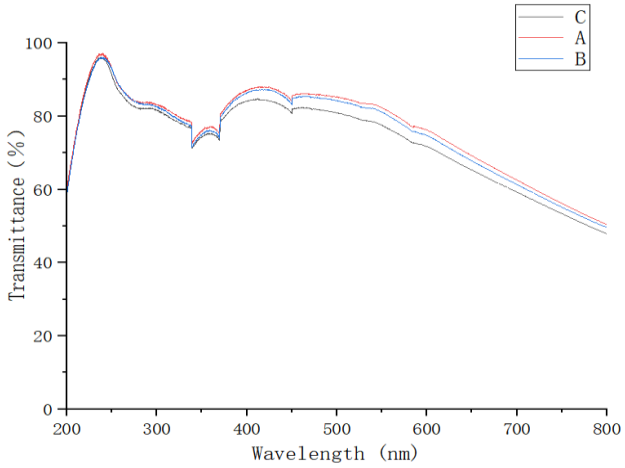

Figure 2.6 – Transmittance of PVA film with the same freeze-thaw times and different drug loading concentrations

(2) From the visual point of view in Figure 2.7, it is found that the transparency of B1, B2, and B3 are significantly different.

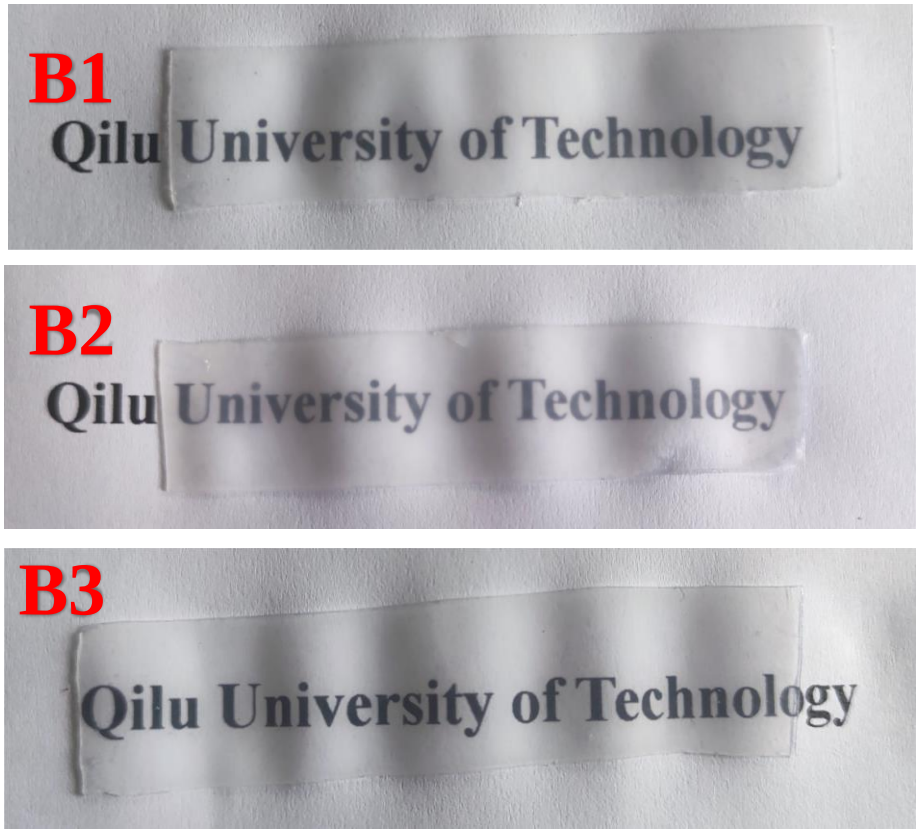

Figure 2.7 – The appearance of PVA with different freeze-thaw times and the same drug loading concentration

Combined with Figure 2.8, there is indeed a gap in their light transmittance. The light transmittance: B3 < B2 <B1.

Therefore, we found that the transparency of the film is mainly determined by the number of freeze-thaw cycles of the PVA film. The fewer the number of freeze-thaw cycles (2 times), the higher the transparency.

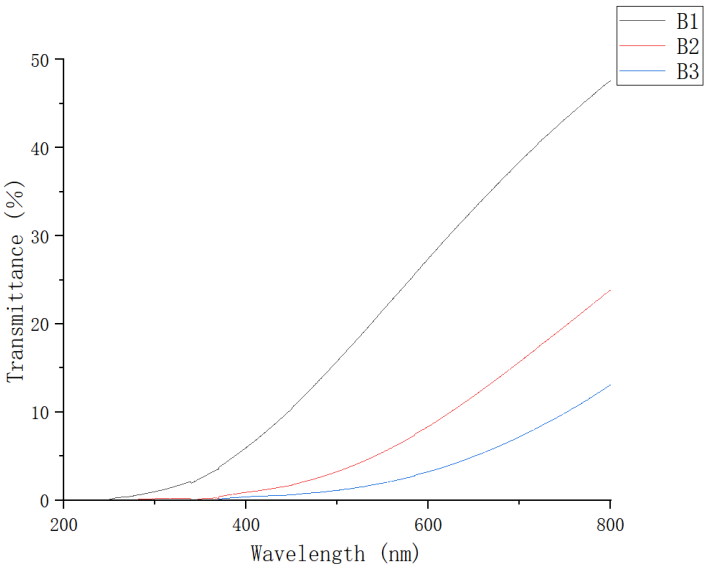

Figure 2.8 – Transmittance of PVA film with different freeze-thaw times and the same drug loading concentration

2.9 Swelling rate test of PVA hydrogel film

Soak the composite hydrogel in distilled water for 48 hours to ensure it swells to equilibrium. Wipe off the excess water on the surface of the composite hydrogel and weigh it as the mass (Ws), dry the swollen composite hydrogel membrane at 105°C for 12 hours, and weigh the mass (Wd) of the dried membrane. Calculate the swelling rate by formula (2.2). Get Figure 2.9.

$$\text{Swelling ratio (\%)} = \frac{W_s - W_d}{W_d} \times 100 \qquad (2.2)$$

The weight of Ws and Wd in the upper form is the swelling and drying state of the hydrogel, respectively.

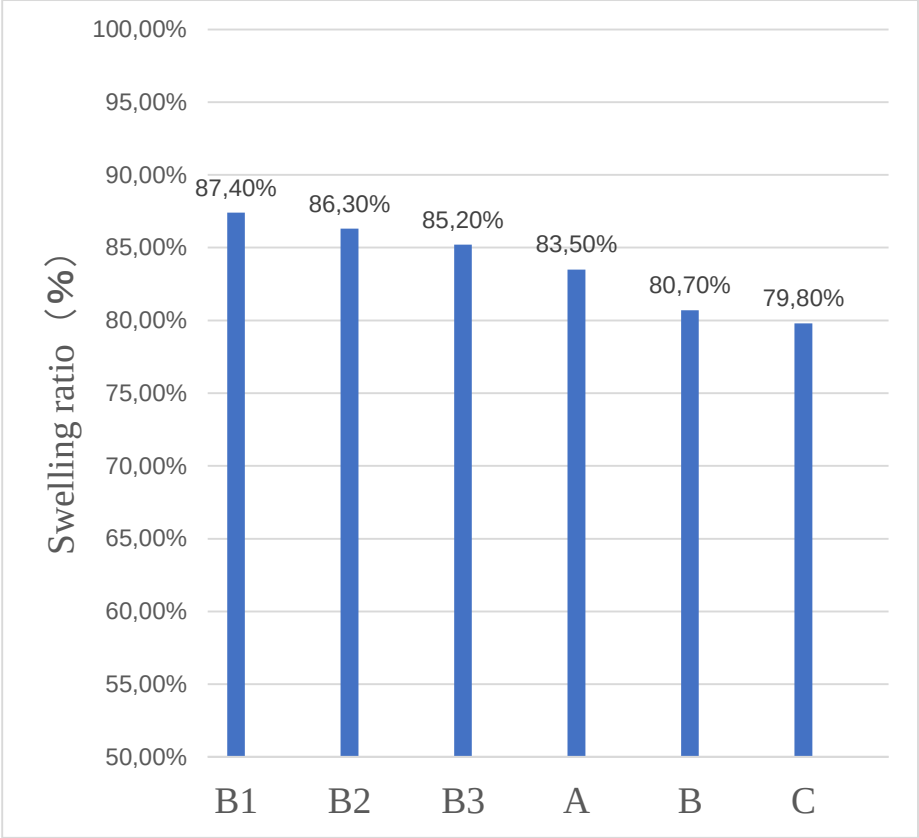

Figure 2.9 – PVA membrane swelling rate

The swelling rate of the PVA film represents the liquid absorption capacity of the film, and the liquid absorption capacity is an essential indicator of the quality of the dressing. It can be seen from Figure 2.9 that B1 has the highest swelling rate, reaching 87.40%; C, which has the lowest swelling rate, also has 79.8%. By comparing B1, B2, B2 and A, B, C, it is concluded that the membrane swelling rate decreases as the drug loading concentration increases; as the number of freeze-thaw cycles decreases（≥ 2 times), the film swelling rate increases.

2.10 Dissolution rate test of drug-loaded PVA hydrogel film

The dissolution rate of the drug-loaded hydrogel will have a more significant impact on the drug's efficacy. Generally, the prescription must be released slowly at a specific

rate to reduce the number of administrations and maintain effectiveness. Therefore, the dissolution time of the drug is affected. Research is an essential indicator for investigating the dissolution performance of drugs.

The specific steps for the dissolution rate test of the drug-loaded PVA hydrogel film: Wash the A, B, C, B1, B2, and B3 hydrogel films with water to remove PHMG not fixed in the hydrogel. Then, they were freeze-dried.

Weigh 0.4 g of the freeze-dried hydrogel films of A, B, C, B1, B2, and B3, respectively. Soak them separately in 500 mL deionized water. Take 5ml of the soaking solution at 15min, 30min, 1 h, 3 h, 6 h, 9 h, 12 h, 24 h, and test the saturated solution with an ultraviolet-visible photometer (UV-6000H).

Using the formula (2.1) obtained in section 2.5, one needs to calculate the PHMG concentration. In these calculations, X is the concentration of PHMG in the solution, and its unit is μg/mL. Y is the absorbance at a wavelength of 192 nm, and its unit is A.

Use formula (2.3) to calculate the dissolution rate of PHMG and draw the dissolution rate curve as shown in Figure 2.10.

$$\text{Dissolution rate}(\%) = \frac{V_W C_{PHMG}}{W_A} \times 100\% \tag{2.3}$$

Among them, WA represents the mass of PHMG added, Vw represents the volume of the soaking solution (mL), and C_{PHMG} represents the concentration of PHMG in the soaking solution (μg/mL).

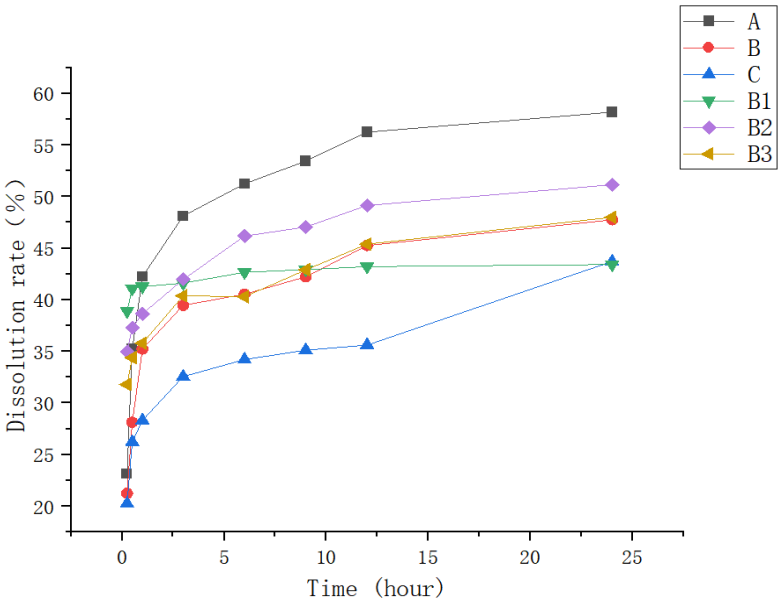

Figure 2.10 – Dissolution rate of drug-loaded PVA membrane at different times

If you want long-lasting antibacterial activity, then the PVA hydrogel film must have a slow-release effect on the drug.

First, by comparing the three curves of A, B and C, we found that:

(1) The dissolution rate of A is the largest, and the dissolution rate can reach more than 50% in 6 hours. It shows that the network structure of PVA hydrogel can effectively control the release of PHMG, effectively prolong the antibacterial time and improve long-term protection for wounds.

(2) The dissolution rate of A and B after 12 hours is almost unchanged, while the dissolution rate of C continues to increase in 12-24 hours, and the drug concentration of C is the highest. It shows that the higher the drug concentration, the longer the drug is released at the same freezing and thawing times. Although A has the highest dissolution rate, the quality of PHMG dissolved within 24 hours is still lower than that of C.

Secondly, by comparing curves B, B1, B2, and B3, we found that:

(1) The dissolution rate of B1 reached about 40% after being placed in water for 15 minutes, and the dissolution rate has hardly changed since then. It can illustrate two problems. First: After the dissolution rate reached 40%, it did not continue to increase. About 60% of PHMG is not fixed in the PVA hydrogel membrane, and it is removed at the step of washing with water. Second: B has only two freeze-thaw cycles. It shows that the physical crosslinking points formed by the two freeze-thaw cycles are not enough so that the PHMG is not firmly fixed.

(2) The curves of B and B3 almost overlap, and their freeze-thaw times are 4 and 5, respectively. It shows that when the drug loading concentration is constant, the dissolution rate remains virtually unchanged when the number of freeze-thaw cycles reaches more than 4 times.

(3) B2 has the highest dissolution rate among them, which shows that when the PVA film has the same drug loading concentration, the drug with the number of freeze-thaw cycles of 3 times has the highest dissolution rate.

To sum up: when the number of freeze-thaw cycles is three times, the higher the drug-loaded mass fraction of the PVA film, the longer the drug dissolution. Then its antibacterial effect will last longer.

2.11 Mechanical strength test of drug-loaded PVA hydrogel film

The specific steps of mechanical characterization are: cut the composite hydrogel film A, B, C, D, B1, B2, B3 into a 10 mm x 40 mm rectangle and expand to the equilibrium in distilled water. Through IX series automatic material testing system, the mechanical properties are tested at the rate of 20 mm/min at room temperature and

measured in parallel three times. During and after the test (see Figure 2.11). Elongation at the break (or breaking extension) and tensile strength are shown in Figure 2.12 and Figure 2.13.

Elongation at the break is calculated as follows:

$$E=(La-L0)/L0 \quad (2.4)$$

Where E is the elongation at the break, L0 is the original length of the sample, and the length of the La sample when it is broken.

The calculation formula for tensile strength (MPa) is:

$$T=Fb/S0 \quad (2.5)$$

Where T is the tensile strength and Fb is the maximum force (N) borne by the sample when it is pulled apart; S0 is the original cross-sectional area of the sample (mm^2).

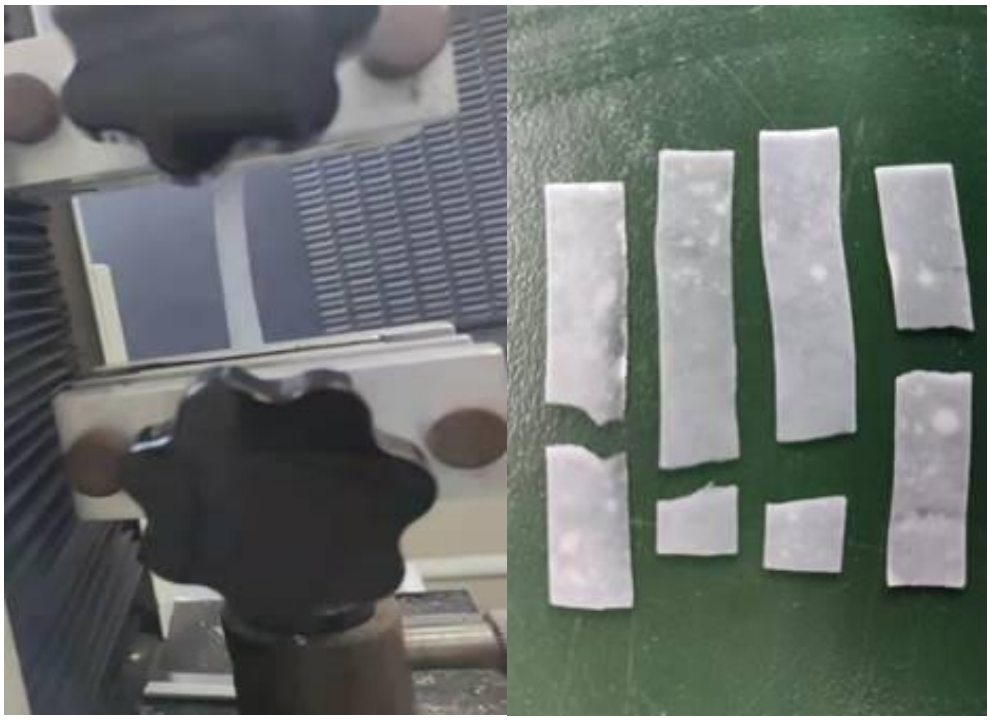

Figure 2.11 – PVA membrane mechanical test and after test

The relative elongation generally expresses the elongation at a material's break (or breaking extension). In other words, it is the ratio of the elongation of the material at the

break to its initial length expressed as a percentage. It is an index that characterizes the softness and elasticity of materials. The greater the breaking extension, the better are the softness and elasticity. Specific use of the material requires a particular level of elongation at the break.

The breaking extension is one of the critical indicators that determine the material processing conditions and the performance of its product. The material with significant breaking extension has a softer hand and can cushion the force during processing, convenient for processing.

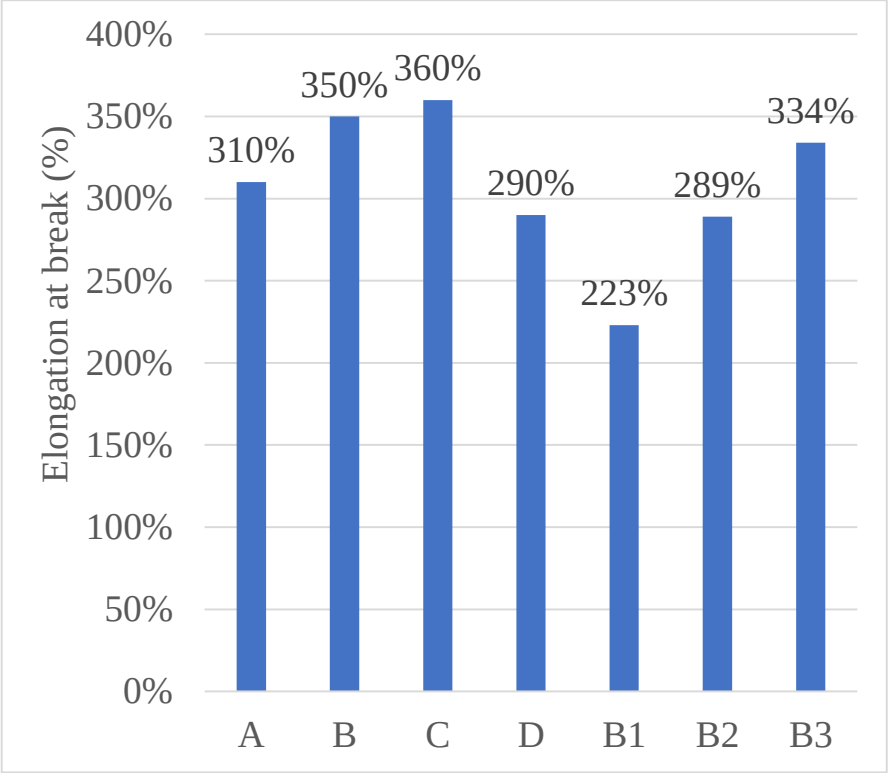

Figure 2.12 – Elongation at break of PVA film

From Figure 2.12, the elongation at break of C is the largest, and B1 is the smallest. However, their breaking extension is more significant than 200%, which shows that the softness and elasticity of these films are very good, which is very conducive to the

processing of dressings. From the comparison of B1, B2, B3, and B, as the number of freeze-thaw cycles increases, the softness and elasticity of the PVA film increase. From the comparison of A, B, and C, as the drug loading concentration increases, the softness and elasticity of the PVA film increase.

During the tensile process of the sample, the material passes through the yield stage and enters the strengthening stage. After the transverse section size is significantly reduced, the maximum force (Fb) that it bears when it breaks is divided by the sample's original cross-sectional area (So). The stress (T) is called tensile strength, and the unit is (MPa). It represents the maximum ability to resist damage under tensile force.

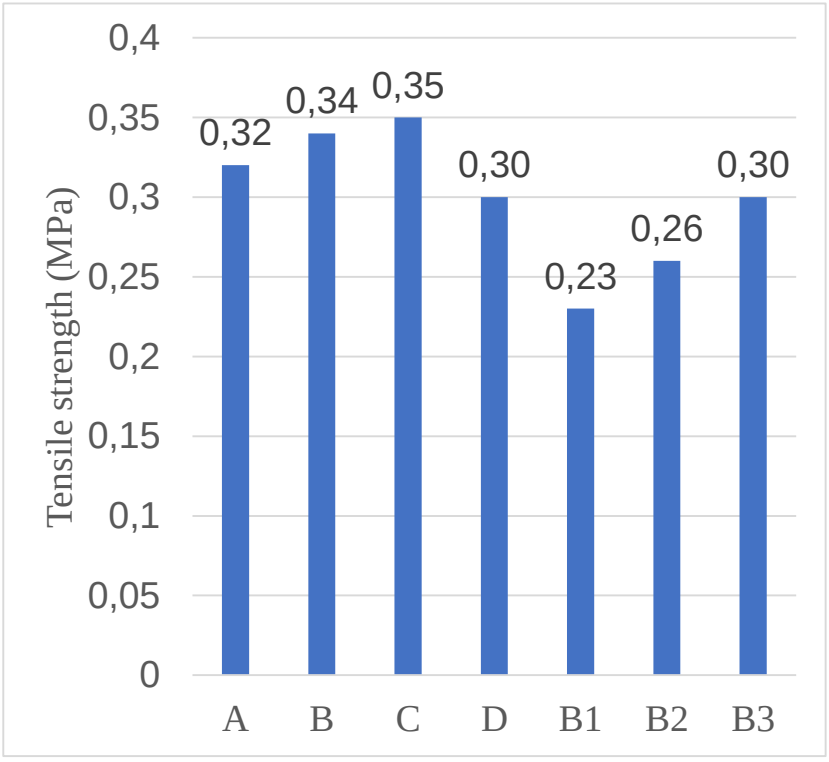

Figure 2.13 – Tensile strength of PVA film

Tensile strength (MPa) refers to the maximum stress a material can withstand before it breaks. From Figure 2.13, the tensile strength of C is the largest. From the comparison

of B1, B2, B3, and B, as the number of freeze-thaw cycles increases, the tensile strength of the PVA film increases. From the comparison of A, B, and C, as the drug loading concentration increases, the tensile strength of the PVA film rises.

To sum up: in terms of mechanical strength, the performance of A, B, C, D, B1, B2, and B3 are all very good. Among them, C has the best performance.

Chapter 2 conclusion

A series of tests were carried out to evaluate the quality of PHMG composite PVA hydrogel. The quality evaluation of drug-loaded hydrogels was carried out regarding appearance, transmittance, swelling rate, dissolution rate and mechanical properties.

We found that:

(1) The transparency of the PVA membrane is mainly determined by the freezing and thawing times of the PVA membrane. The fewer freeze-thaw times (≥2 times), the higher the transparency.

(2) With the increase of drug loading concentration, the membrane swelling rate decreased. The membrane swelling rate increased with the decrease of freeze-thaw times (⩾2 times).

(3) In terms of mechanical strength, A, B, C, D, B1, B2 and B3 have good performance. The performance of C is the best.

(4) Regarding dissolution rate, when the freezing and thawing times are three times, the higher the drug loading mass fraction of the PVA membrane, the longer the drug dissolution is. Then the longer its antibacterial effect is.

In summary, the three-dimensional network structure of PVA hydrogel is stable after three freeze-thaw cycles. Especially in terms of dissolution rate, the dissolution rate is the highest when freezing and thawing three times. A high dissolution rate means solid antibacterial ability.

When the water gel freeze-thaw was melted three times, and the mass fraction of PHMG was 0.7%, the hydrogel had an excellent swelling rate and mechanical properties.

Therefore, through the orthogonal experiment and quality evaluation of drug-loaded PVA hydrogels, we have obtained the best formula: PVA hydrogel freeze-thaw three times, and PHMG mass fraction is 0.7%.

CHAPTER 3. CONSUMER PROPERTIES AND PRODUCTION TECHNOLOGY

3.1 Antibacterial and biocompatibility properties

3.1.1 In vitro antibacterial test of PVA hydrogel membrane

(1) The inhibition zone method is used to evaluate the dissolution. The process is as follows:

1. Cut the hydrogel sample into a 1cm rectangle;

2. Sterilize the pipette tip and test tube at high temperature;

3. Pour normal strength agar into the petri dish. After cooling and solidification, pour a layer of 1/4 strength agar, cooling and solidification for later use;

4. Smear 0.1 mL10^6mL cfu/mL E. coli bacteria liquid on the agar medium, and then place the sample rectangle on the medium. Incubate at 37°C Celsius for 24 h, and observe the size of the inhibition zone.

The results of the zone of inhibition method are shown in Figure 3.1.

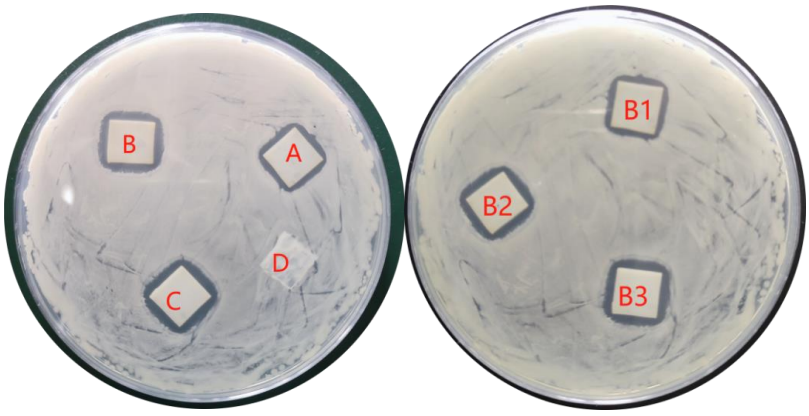

Figure 3.1 – Evaluation of the antibacterial effect of the hydrogel by the inhibition zone method

As shown in Figure 3.1, we found that the unloaded D gel has no bacteriostatic zone, and the rest of the hydrogels have formed a bacteriostatic zone. It indicates that the drug-loaded hydrogel has an antibacterial effect and is dissolvable.

(2)The test process of the shaking method is as follows:

1. Take 0.1 g freeze-dried hydrogel sample and cut into pieces;

2. Sterilize the pipette tip and test tube at high temperature;

3. The number of Escherichia coli in the solution is 10^8 cfu/mL. Take 1 mL of bacterial solution into a 9 mL PBS buffer test tube. Vortex and dilute to 10 times to 10^5cfu/mL. After adding the sample, place it in a 37°C constant temperature incubator and shake for 24 hours at a speed of 250 r/min.

4. After shaking, dilute the bacterial solution 10 times with PBS buffer solution, take 0.1 mL of each, inoculate on an agar petri dish, incubate at 37°C incubators for 24 hours, and count the colonies take the average value of 3 times.

The antibacterial rate is calculated according to formula (3.1).

$$\text{Bacteriostatic rate} = \frac{A-B}{A} * 100\% \quad (3.1)$$

A represents the number of colonies in the blank control group, and B represents the number of colonies in the antibacterial hydrogel.

As the content of PHMG gradually increases from A through B to C, the antibacterial rate increases (Figure 3.2). But even the lowest concentration of A has an antibacterial rate of 95%. Comparing B1, B2, B3, and B, it can be seen that the drug loading concentration is the same, and the PVA hydrogel structure is gradually more stable with

the increase of freezing and thawing times. Therefore, the antibacterial rate is slowly steady and can reach 99.93%.

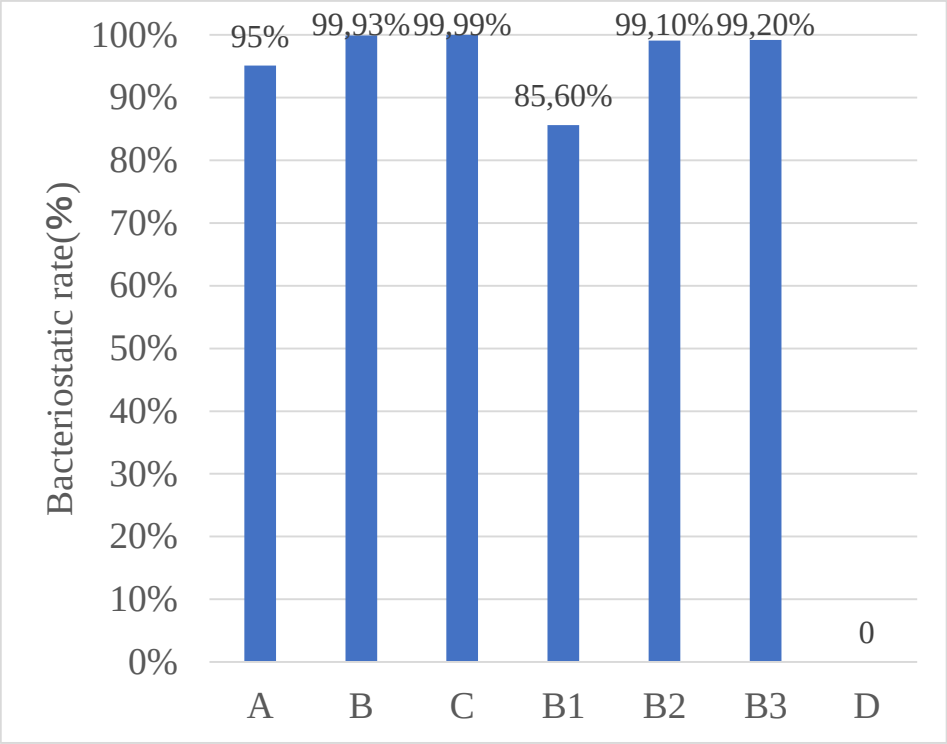

Figure 3.2 – PVA antibacterial rate of hydrogel

In summary, all PVA hydrogels containing PHMG have high-efficiency antibacterial performance. Among them, C is as high as 99.99%.

3.1.2 Biocompatibility test of PVA hydrogel membrane

(1) The specific operation of the hemolysis rate of the sample is as follows: Wash hRBC with PBS, centrifuge 3 times, and dilute with PBS to a volume fraction of 4%.

Take 2 ml of the diluted red blood cell suspension and introduce it into a 24-well plate containing 0.2 g of the sterilised hydrogel.

Set up a positive control group and a negative control group: use untreated red blood cells as a negative control (negative), red blood cells treated with 0.1% Triton X-100 as a positive control (positive), incubate in a 37°C incubator 1 h. Triton X-100 is a non-ionic surfactant, which can dissolve lipids to increase the permeability of the antibody to cell membranes, which can completely rupture red blood cells. Use it as a positive control and define its hemolysis rate as 100%.

The hRBC suspension in each well was centrifuged at 3000 r/min for 5 min, and the temperature was set to 4°C. Transfer 100 uL of the supernatant from each centrifugation to a 96-well plate. Detect its OD value at 576 nm with the microplate reader of a Microplate Spectrophotometer to calculate the hemolysis ratio. Three parallel experiments were set up for each sample. The sample's hemolysis rate (Hemolysis) can be calculated by the formula (3.2).

$$\text{Hemolysis rate} = \left(1 - \frac{OD_{sample} - OD_{negative}}{OD_{positive} - OD_{negative}}\right) \quad (3.2)$$

(2) Evaluation of hemolysis rate of samples

The hemolysis test was used to characterise the blood compatibility of the antibacterial hydrogel. The test principle of the hemolysis experiment: hemolytic agent can rupture red blood cells. A microplate reader measures the absorption value of released haemoglobin to determine the lysis and rupture of red blood cells, thereby determining the hemolysis rate of the material and evaluating its blood compatibility. The hemolysis rate of the gel and the positive control group (Triton X-100 treatment, defined as 100% hemolysis rate) for hRBC is shown in Figure 3.3.

Compared with the positive control group, all gels showed a very low hemolysis rate. The lower the hemolysis ratio, the smaller is the degree of dissolution and rupture of hRBC by the PVA hydrogel. Also, the better is the biocompatibility of the PVA hydrogel. It is generally believed that the hemolysis rate of a substance to hRBC does not exceed 20%, indicating that it has good blood compatibility. From A to B and then to C, as the content of PHMG gradually increases, the hemolysis rate gradually increases.

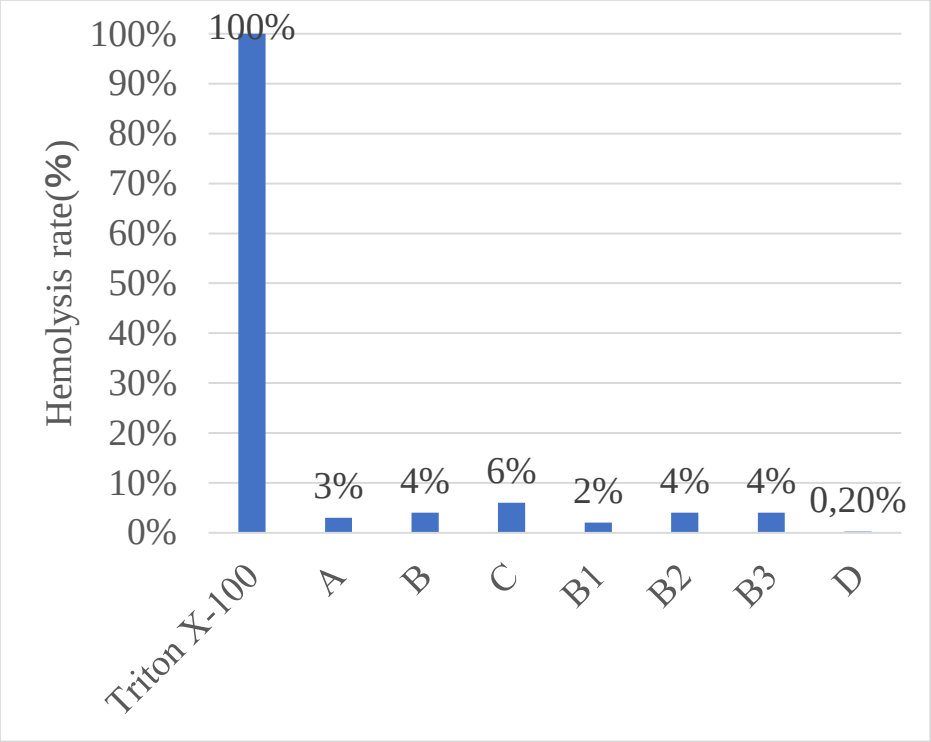

Figure 3.3 – Hemolysis rate of samples

Still, even for gel C with the highest PHMG content, the hemolysis rate does not exceed 10%. The hemolysis rate of D without PHMG is close to 0, indicating that the prepared PVA hydrogel has good biocompatibility.

(3) The specific operation steps of the cell viability test are as follows: 0.2 g sterilised hydrogel is soaked in a 2 ml extraction medium at 37°C for 24 h.

The hydrogel is then taken out, and the extract is stored for the subsequent evaluation of cell viability.

Incubate HeLa in a 96-well plate containing 10% FBS/a-MEM medium at 37°C in 5%CO_2 the humid atmosphere for 24 h. At this time, the cells are in an adherent growth state, and then the cells are extracted and cultured. The base was further incubated for 24 h.

Subsequently, aspirate the medium and replenish 100 uL, fresh medium. Add 10 uLCCK-8 reagents to each well, and place the cells in a dark environment for further incubation for 1 h.

Use the Microplate Spectrophotometer to measure the OD value of each well of the cell culture medium at 450 nm.

Untreated cells were used as negative controls, and wells without cells but containing medium were used as blank controls. Three parallel experiments were set up for each sample. Cell viability can be calculated by the formula (3.3):

$$\text{Cell viability}\ (\%) = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \qquad (3.3)$$

(4) Cell viability test evaluation:

To further confirm the good biocompatibility of the hydrogel, the cell viability of the antibacterial hydrogel to HeLa cells was characterised by testing the cell survival rate. The cell survival rate of HeLa cells to PVA gel is shown in Figure 3.4. The unloaded D gel has the highest cell survival rate (99.3%). The cell survival rate gradually decreases with PHMG content from A to B and then to C. However, C still has a higher cell survival

rate (89.50%), and the changing trend of cell survival rate is consistent with the hemolysis experiment. It shows that PHMG's PVA hydrogel maintains high antibacterial properties while having excellent biocompatibility and low toxicity.

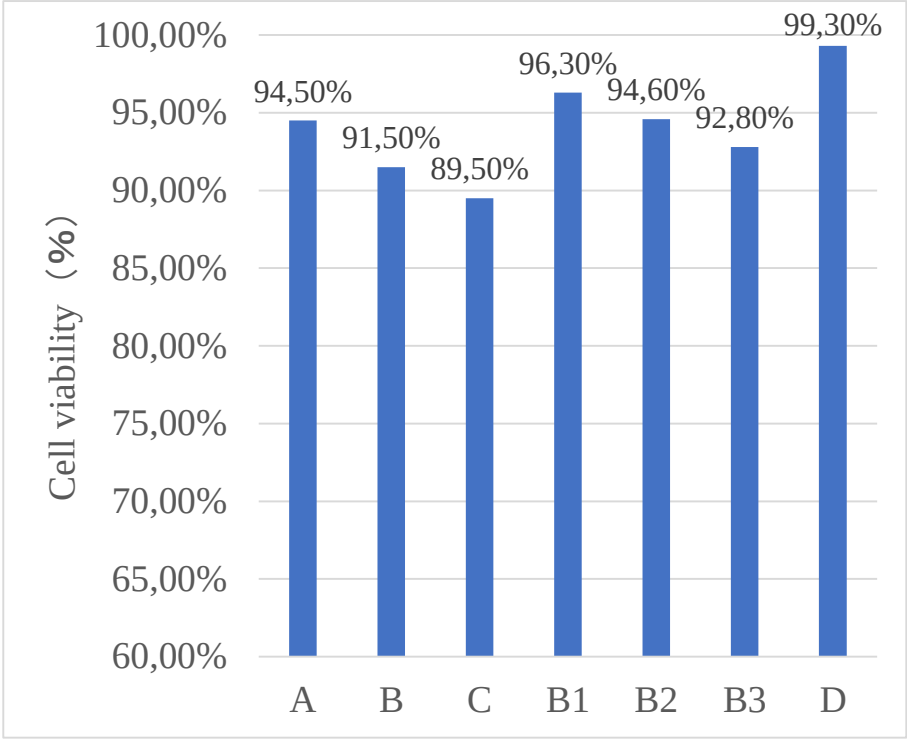

Figure 3.4 – Cell viability of samples

In summary, both the hemolysis and the cell survival rate tests show that the PHMG-containing PVA hydrogel has a good antibacterial effect and is a very safe material.

3.2 Equipment to implement production technology

3.2.1 Main equipment characteristics of industrialized production of drug-loaded PVA membrane

1. Large industrial refrigerator, model: GY-6580N (-65～-20°C), power: 7.5kW, cooling method is air cooling. It can accurately control the temperature and has a large

volume, up to 1600 L. It also has a low-temperature automatic defrost function (can be designed once per hour or manual button defrost according to actual needs). It can work at a low temperature when the door is frequently opened and closed for a long time. It can meet the needs of multiple freezing and thawing. With self-diagnosis function; leakage protector, refrigerating machine overload protection; high and low safety protection pressure switch, overload relay, thermal protection device, independent temperature limit high-temperature protection, sensor failure protection and other security functions.

2. Electric dumping furnace, model: HZP-25. It can work uninterrupted for 24 hours, and the melting is even and longer. The device is environmentally friendly and meets environmental protection requirements, eliminating the trouble of ecological protection inspection; it saves electricity compared to the thyristor intermediate frequency. It is convenient to maintain and can save electricity by 15-20%. With an electromagnetic stirring effect, it is conducive to the complete melting of PVA.

3. Lifting dispersing kettle is especially suitable for mass single-machine operation products. The dispersing shaft can be lifted and stirred evenly, and the dispersing and stirring is clean and environmentally friendly. The product can be selected in different capacities and configurations according to the characteristics and capacity of the materials. Select volume: $10m^3$, main motor power: 132-160 kW, lifting stroke: 1000 mm, speed: 0-800 rpm/min.

4. The moulds used are customized and made of high-strength stainless steel materials. These moulds can be used repeatedly, and there is almost no wear. And it won’t be deformed even after experiencing a considerable temperature difference.

3.2.2 Analysis of the status quo of the product market

(1) Overview of dressing hydrogel: Hydrogel material contains a lot of water and has good biocompatibility. It is the material closest to living tissue, so it has many biomedical applications, such as drug carriers and artificial cartilage, wound dressings and tissue adhesives, etc.

As early as the 1960s, polymer dressings were considered potential materials for wound healing. Winter et al. introduced polymer dressings into wound healing for the first time. In the mid-1970s, wound dressings made of polymer materials were incredibly developed. In 1978, several chitin-based polymer materials were used as the earliest wound dressing materials due to their excellent biological activity, demonstrating the superb development prospects of polymers in the field of wound dressings.

Most polymer dressings, such as hydrogels, foams, films, hydrocolloids, etc., have advantages. Compared with other polymer dressings, wound dressings made of hydrogel materials have the following unique benefits:

1. The hydrogel can achieve a cooling and analgesic effect through its water volatilization, and the analgesic effect is sustainable.

2. The hydrogel can only rely on its water to keep the wound moist, thereby accelerating wound healing and improving healing quality.

3. The hydrogel material does not adhere to human tissues. It is painless to change the dressing and does not cause secondary damage to the wound.

4. Hydrogel has a variety of structures and properties to meet the needs of different wound environments.

As a wound dressing hydrogel material, it is best to have both good mechanical strength and anti-bacterial and anti-inflammatory properties at the same time. Since hydrogel materials contain a large amount of free water in their structure, they generally have poor mechanical strength and paste. However, when used as a wound dressing, they need to meet mechanical properties and particular mechanical strength.

Preventing wound infection is the basic requirement for dressings in clinical practice. Dressing hydrogels are mainly divided into two categories according to the difference of matrix and antibacterial components: one is hydrogels with antibacterial properties; the other is hydrogels loaded with antibacterial agents. The methods for preparing antibacterial wound dressing hydrogel mainly include loading antibiotics, organic antibacterial agents, inorganic nano-metal particles or semiconductor materials with photocatalytic antibacterial effect, etc. They use antibacterial cationic electrolyte monomers for endogenous antibacterial and the construction of sexual hydrogel, endogenous antibacterial hydrogel while loading antibacterial agents, etc.

(2) Research status: currently reported or applied anti-harmful microbial medical dressings, on the one hand, most of them use modified chitin or nanosilver system, which is not only expensive, but also slow in anti-microbial effect, and the result is not ideal; on the other hand. Many organic small molecule antibacterial agents, such as quaternary ammonium salts, quaternary biguanide antibacterial agents, etc., have the advantages of fast sterilization and a wide antibacterial range. However, the antibacterial agent is added

to the dressing by blending or dipping and exudation. The antibacterial agent may be harmful to human health.

PHMG can inhibit and kill microorganisms with high efficiency and broad-spectrum, is safe and non-toxic to the human body, and has a low price. It is widely used in medicine, water treatment, pulp and other fields. However, because PHMG is easily soluble in water and easily lost, there are few reports on its research and application in dressings. This study successfully solved this problem by freezing and thawing and endowed the hydrogel dressing with long-lasting antibacterial properties.

(3) Advantages for consumers: With the development of the economy, people's material life has been greatly satisfied. Therefore, people's consumption needs have undergone tremendous changes in food, clothing, shelter, transportation, and the medical field. It is not enough to have sound curative effects, and consumers are also paying attention to the more negligible side effects. No chemical cross-linking agent is added in the production process of this product. And in the biocompatibility experiment, the performance is excellent. Therefore, the safety of the human body is more guaranteed. This is good news for consumers who pay more attention to product safety.

(4) Advantages of the product to the enterprise: The enterprise first pays more attention to economic benefits. This is the driving force for their production and sales. In the production process of this product, the method used is simple. Therefore, the production process is simple and can save a lot of labour. The raw materials are also cheaper than other products. Therefore, the cost of the product is lower. Moreover, the product has an excellent performance in terms of antibacterial and safety performance. It

will definitely be recognized by consumers, thus forming a virtuous circle. While bringing benefits to the enterprise, it also gains a good reputation. Therefore, the product has excellent production prospects.

In short, this product has up-and-coming application prospects in the field of anti-harmful microorganism medical dressings.

3.3 Proposed scheme to production technology

3.3.1 Industrialized production process of drug-loaded PVA hydrogel membrane

The flat film coating method is adopted in the laboratory, which is simple and easy to implement, but the efficiency is low and suitable for small-scale experiments.

Large-scale production of products requires us to choose the right equipment. And a suitable production process.

Industrialization of products. Firstly, we use an electric dumping furnace to melt PVA and prepare an aqueous PVA solution. Secondly, the PVA aqueous solution is poured into the lift-type dispersion kettle and stirred by the electric dumping furnace. After the solution is cooled to room temperature, add an appropriate amount of PHMG and stir to dissolve to remove air bubbles. Then pour the solution into the mould. Finally, put the mould into a large industrial freezer to freeze. After freezing at -20°C for 13 hours, thawing at 25°C for 20 minutes, as a freeze-thaw cycle. This cycle is repeated three times to form a PVA hydrogel film. The flow chart is as follows:

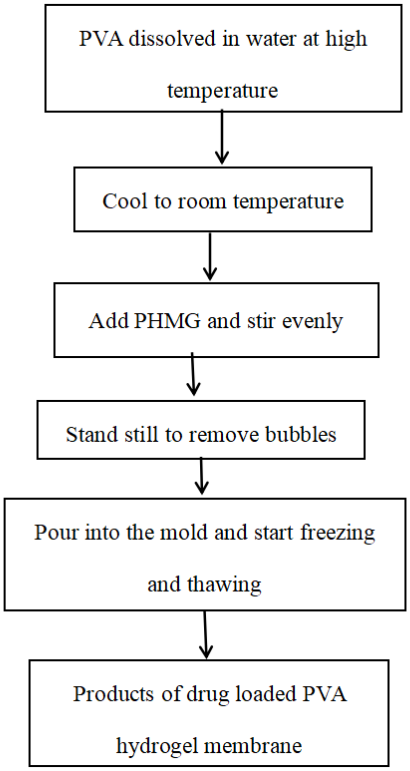

Figure 3.5 – Process flow chart of preparation of PVA hydrogel film

3.3.2 Safety of industrial production

Safety is an eternal topic of industrial enterprises. Without safety, enterprises can't talk about production, let alone economic benefits. The focus of enterprise safety work lies in the team, the direct executor of various production tasks and the primary carrier for safe production. To do a good job in team safety, we must make team members pay attention to safety. Team members must master the basic skills of safe production and whether they can be used proficiently. The following describes their responsibilities from the perspective of front-line operators and managers.

(1) As an operator, pay attention to the following points:

1. Strictly observe the operating procedures and safety systems of this post;

2. Make safety inspections and records carefully;

3. Use and wear labour protection equipment following the meeting;

4. Avoid violations. Risky and brutal behaviour;

5. Stop others' illegal operations and command violations;

6. Reflect safety issues to superiors promptly;

7. Know your safety responsibilities in emergencies;

8. Take relevant training and education seriously, and truly master safety skills;

9. Actively put forward safety and rationalization suggestions.

(2) Production management personnel must do the following:

1. Assist enterprise leaders to organize safety work during production and implementing labour protection laws and regulations.

2. Organize and assist relevant departments in formulating safety production systems and technical operating procedures and supervising their implementation and inspections.

3. Perform on-site inspections, assist and solve problems frequently. In case of a particularly urgent and unsafe situation, they have the right to order to stop first and then report to the business leaders for handling.

4. Conduct publicity and education on production safety for employees.

5. Guide the safety production, publicity, and education of various departments and teams.

6. Supervise relevant departments and teams to distribute labour protection supplies following applicable regulations.

7. Participate in the investigation and handling of casualties; analyze, make statistics, and report safety production.

(3) Production management personnel should have the following qualities and conditions:

1. They should have relatively extensive and solid safety knowledge.

2. Should have a strong sense of responsibility and a serious and meticulous work style.

3. You should be good at collecting safety information.

4. There should be a hard-working and dedicated spirit.

5. Should have a healthy physique and solid psychological quality.

(4) During the production process, the following points should be paid attention to when the equipment is overhauled:

Before equipment maintenance:

1. Preparation of labour protection supplies. Before entering the workshop, you must wear work clothes, labour protection gloves, protective glasses and other labour protection equipment. When entering the access control workshop, wear the personnel information card and swipe the card to enter the access control workshop.

2. Analyze the maintenance work to be carried out according to the five-step method, and take corresponding measures for possible hidden risks.

3. If hot work is required, review and approve the "Fire Management Regulations", issue a fire order, equip with on-site guardians and fire-fighting equipment, clean up all flammable and explosive materials, and leave a necessary safety distance.

4. For those who need to climb high, by the "Climbing Management Regulations" requirements, issue a climbing order, wear climbing safety equipment and place warning signs under the climb to remind passers-by to pay attention to falling objects.

Equipment under repair:

1. It is strictly forbidden to repair equipment and parts that directly contact the agent while in motion.

2. When using human resources to move parts during the work process, the personnel must cooperate adequately. When multiple people move and lift, one person should command them, and the actions should be consistent, steady, stable, and steadily moving forward.

3. Pay attention to the safety of surrounding personnel and yourself during work to prevent damage caused by waving tools, tools falling off, and workpiece splashing. Pay attention to cooperation when working with more than two people, and place the workpieces neatly and steadily.

4. When using electric tools, pay attention to checking the fastening of fasteners and rotating parts at any time, and use them after ensuring that they are in good condition.

5. Check whether the seat belts are hung securely at any time during climbing operations to ensure safe use.

6. It is forbidden to work on rotating or rotating equipment and its auxiliary circuits under normal circumstances. Suppose it is necessary to perform inspections, cleaning and investigations on rotating or rotating equipment. In that case, attention must be paid

to fastening the cuffs and wearing a work cap to prevent the rotating part from being caught by twisting or bumping.

After the equipment is repaired:

1. Before starting the equipment, check whether the protective device, fastening screw, electric, hydraulic and pneumatic power source switch are intact, and then conduct commissioning inspection. It can be put into use only after passing the operation. During operation, strictly abide by the safety operation procedures of the equipment.

2. After the hot work is completed, the operation site shall be cleaned in time, the fire-fighting equipment shall be put in place, and the safety officer shall check it to ensure safety before leaving.

3. After the work is completed, the site shall be cleaned in time and kept clean and tidy. Oil and sewage shall not be left on the ground to prevent people from being hurt by anti-skid.

4. After the team members complete the patrol inspection and maintenance, the maintenance personnel shall fill in the inspection and maintenance records timely and carefully. The records shall be kept for future reference.

5. The team leader shall carefully review the patrol inspection and maintenance completed by the personnel on duty and maintenance personnel
Record to ensure that the document is accurate and effective.

Chapter 3 conclusions

The hydrogel was tested by the inhibition zone and oscillation methods for antibacterial testing. The experimental results show that the prepared antibacterial hydrogels have sound antibacterial effects, and the antibacterial rate of C is as high as 99.99%.

The hydrogel was tested by cell survival rate and hemolysis rate in terms of biocompatibility. The experimental results show that the prepared antibacterial hydrogels have good biocompatibility. C, which contains the highest drug content, has a hemolysis rate of 6% and a cell survival rate of 89.5%.

After analyzing the antibacterial dressing market, it was found that our products are simple to prepare and low in cost. In the case of excellent antibacterial effect, the biocompatibility is also very good, satisfying consumers who attach great importance to safety. Moreover, it is a safe and highly effective antibacterial, so it must be very competitive in the market.

In terms of mass production of products, we introduced some equipment parameters. Also put forward some reasonable suggestions, especially in terms of safety. Whether an enterprise can produce safely is a significant issue.

GENERAL CONCLUSIONS

First of all, in the first chapter, we discussed the advantages of PHMG and PVA through a review.

Nowadays, people are paying more and more attention to protecting the environment. The research and synthesis of green antibacterial agents that are environmentally friendly, have a long sterilization time, have a wide range of sterilization, and have less harmful effects on the human body is a direction that needs to be worked hard. The introduction of PHMG into other materials to prepare antibacterial composite materials has become an important research topic.

Therefore, my research goal is to use PVA hydrogel as a carrier, add a new generation of high-efficiency broad-spectrum bactericide PHMG, prepare antibacterial dressings, and study its performance.

Secondly, in the second chapter, the quality of the PHMG composite PVA hydrogel is evaluated through a series of tests. A comprehensive quality evaluation of the drug-loaded hydrogel was carried out from the aspects of appearance, light transmittance, swelling rate, dissolution rate, mechanical properties, etc.

Through orthogonal experiment and quality evaluation of drug-loaded PVA hydrogel, we have obtained the optimal formula: PVA hydrogel is frozen and thawed three times, and the mass fraction of PHMG is 0.7%.

In the third chapter, conduct antibacterial experiments and biocompatibility experiments. It is found that the prepared antibacterial hydrogel has an excellent

antibacterial effect, and its biocompatibility is also excellent. Among them, the hemolysis rate of C with the highest drug content was 6%, and the cell survival rate was 89.5%.

Finally, combining the overview in Chapter 1 and the experiments in Chapter 2, I made some suggestions regarding the selection of equipment, production process and production safety when the product is mass-produced, especially safety issues in production.

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