

# Properties and Application of protein-based metal gel

Yuanman Zhang

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# Properties and Application of protein-based metal gel

## 基于蛋白质金属凝胶的性质与应 用

学院名称 化学与化工学院  
专业班级 应用化学（国际班）17-1  
学生姓名 张媛曼  
学 号 201704300039  
导师姓名 李桂华  
专业技术职务 副教授

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指导教师签名：李桂华

2021年6月8日

毕业设计（论文）作者签名：张媛曼

2021年6月8日

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## **Abstract**

A gel is an inelastic semisolid that is homogeneous in shape and can maintain a certain shape. There are many kinds of gels and protein gels are one of them. One of the important functional properties of proteins is gelation and the formation of protein gelation is affected by many factors, such as pH value, ion concentration, heating temperature and time. The gel properties of proteins are the decisive factor in the distinctive texture, flavor and feel of some foods. The formation of protein gels can be defined as the aggregation of protein molecules. In the process of aggregation, a highly ordered three-position network structure which can store a large amount of water is formed. The gelation behavior of protein has been studied deeply for a long time, but the mechanism of protein gelation has not been fully understood. Metal ions can affect the characteristics of protein gels and make protein gels get better performance.

**Key words:** Gel; Metal ion; Protein

## 摘 要

凝胶是一种非弹性半固体，形状均匀，并能保持一定的形状。凝胶有很多种而蛋白质凝胶是其中一种。凝胶化是蛋白质重要的功能特性之一，而许多因素如 pH 值、离子浓度、加热温度和时间等都可以影响蛋白质凝胶化的形成。蛋白质的凝胶特性是决定某些食物独特质地、风味和口感的决定性因素。蛋白质凝胶的形成可以定义为蛋白质分子的聚集。在聚集过程中，形成了高度有序的可储存大量水分的三维网络结构。长期以来，人们对蛋白质的凝胶行为进行了深入的研究，但对蛋白质的凝胶机理尚未完全了解。而金属粒子的存在，可以影响蛋白质凝胶的特点，使其获得更好的性能。

**关键词：**凝胶 金属离子 蛋白质

# Chapter 1 Introduction

## 1.1 Introduction

Gel is something that looks both liquid and solid. Tofu, meat, contact lenses, superabsorbent resins which are common things in life are gel. There are many kinds of gels, and because of their various properties, they can be used in medicine, health, food, agriculture and forestry and other fields. As a kind of gel, protein gel has good biocompatibility and environmental friendliness. This article mainly records the preparation of protein metal gel and the determination of some of its properties.

## 1.2 Gel

Gel plays an important role in the composition of organisms. Muscle, skin, cell membrane, blood vessel wall, hair, nail and so on can be regarded as gel. Protein is a natural hydrophilic polymer compound, so protein gel as a kind of natural gel is non-toxic, biodegradable and highly biocompatible. Nowadays, people pay more and more attention to environmental problems, because of these advantages of proteins gel, protein gels are considered as environmentally friendly water absorbent gels and biomaterials.

### 1.2.1 The Classification of Gel

Gel can be divided into natural gels and synthetic gels according to their source. Natural gels are made from organisms such as AGAR, konjac, muscle, protein, etc.. Synthetic gels are made by synthesizing crosslinked polymers and simultaneously absorbing solvents like contact lenses, super absorbent resin and so on. There are many kinds of natural gels, many of which are used in human foods. In general, organisms including humans are made of gels. In order to improve the biological adaptability of synthetic gels, synthetic gels are often combined with biological components to perform specific biological functions. It is called a hybrid gel. Hybrid gels can be used as filling materials that can fuse with biological tissues and artificial viscera devices, such as

artificial skin, artificial cornea and other medical materials, and have attracted great attention.

Gel is divided into gels and xerogels according to whether the medium is a gas or a liquid, while liquids are divided into water and organic solvents. Hydrogels are water-based gels and most natural and synthetic gels are hydrogels. Aerogels are gels with gas as medium, such as frozen tofu, silica gel and dried AGAR. In addition, organic gels with organic solvents as the medium also play a lot of roles, such as oil-absorbing resin form organic gels by absorbing oil, which play a great role in removing oil pollution.

Gel can be divided into chemical cross-linking and physical cross-linking according to the cross-linking methods of the cross-linking polymers that constitute the gels. The cross-linking of polymer segments by covalent bonds is called chemical crosslinking and this bond is very strong. Most synthetic gels are chemically cross-linking. Molecular cross-linking formed by hydrogen bonds, Coulomb forces, coordination bonds, and physical entanglement are called physical cross-linking. Most natural gels are cross-linked by hydrogen bonds formed between polymer segments. Like protein gel, its hydrogen bonds are broken by heating and turns a gel into a sol [1].

In addition to these classifications, there are other classification criteria, such as gels are classified into micro-gel and macro-gel according to their size. Microgels are extremely small and do not cross - link between molecules, but cross - link within molecules. Macroscopic gels are further divided into opaque non-homogenous gels and transparent homogenous gels. And gels are classified according to their cross-linking structure and size.

### 1.2.2 Preparation of the Gel

The essence of gel preparation is to prepare polymer network or form crosslinked polymer. The cross-linking structure of polymers can be roughly divided into two categories: those formed by covalent bonds and those formed by intermolecular binding. The gels formed by the former can be called chemical gels, while those formed by the latter can be called physical gels.

Through heat, initiator, light, ray, plasma, electric field and other energy can form the cross-linking structure composed of covalent bonds and divided into two ways, one is to form the cross-linking of polymerization at the same time, the other is to form a

linear polymer, and then through the polymer reaction to make them cross-linked. The first one is simple and suitable for all kinds of monomers. It can be made by chain addition polymerization of olefin monomers with crosslinking agent, polycondensation or polymerization addition of functional compounds. Common simultaneous polymerization crosslinking methods include thermal polymerization initiator initiates the polymerization [2,3], photopolymerization and irradiation polymerization [3-5], Plasma initiated polymerization and electrolytic polymerization [3]. The latter is able to maintain the advanced structure and orientation of linear polymers, so that it can make any form of gel fiber, film and so on. The method of forming polymer bonds and then crosslinking include Chemical reaction method, optical crosslinking, irradiation crosslinking, plasma crosslinking.

The cross-linking structure of physical gel is mainly formed by intermolecular hydrogen bond, coordination bond, electrostatic coupling, hydrophobic aggregation, van der Waals force binding and so on. Polysaccharide, protein and other natural polymer gels mostly belong to this type.

### 1.2.3 Properties and Application of Gel

The gel is composed of two components, the polymer network structure and the solvent. The polymer network envelopes the solution and does not let the liquid flow out, which plays the role of the container. In general, gels have both Solid and Liquid Properties. For example, in a highly swollen gel, the solvent has a high diffusivity, which is a property of the liquid. But gels have a shape that can be cut by force to change shape, which is a property of solids. Gels are generally soft and elastic, which gives them a lot of similarities with living organisms, so they are mostly Biocompatible.

Gel is a kind of polymer material with good Water Absorption (Hygroscopicity, Water-Retention). Although the construction units form an insoluble network structure through physical or chemical crosslinking, they still have good water absorption. Because gels exhibit excellent water retention, they have great potential application value as a water-retaining agent for forestry and agricultural soils [6].

The interaction between gel and the outside world has been found to be able to carry out the exchange of energy and material information, function as a sensor and chemical reaction site, and the gel has the characteristics of changing its shape in response to the external environment, that is, it has the functions of holding, separating

and Releasing substances. Based on its unique internal structure and performance, it has been widely used in the field of drug sustained-release materials. [7,8]

Gels are very useful in terms of practicality and functionality in skin care cosmetics to give these products a beautiful appearance, good feeling, stable properties and better results.

The properties and applications of gels are actually very wide, covering all aspects of people's lives, such as sanitary products, daily necessities, food packaging, medical treatment, agriculture and horticulture, civil construction, chemical industry, sports and entertainment industry, etc. Table 1 lists some of the properties of gels and their applications.

Table 1. Properties of Gels and Their Applications

Properties	Application
<b>Water absorption</b>	Water absorbent, Diapers, Sanitary napkins
<b>Hygroscopicity</b>	Desiccant
<b>Sustained release</b>	Drug carrier, Aromatic agent.
<b>Thickening</b>	Food materials, Cosmetics
<b>Light permeability</b>	Artificial vitreous, Optical lens
<b>Biocompatibility</b>	Cell cultures, Artificial skin, Contact lenses
<b>Sound absorption</b>	Sound insulation material, Insulator
<b>Elasticity, Fluidity, Softness</b>	Deformation material, Filling material
<b>Expansibility</b>	Toys, Filling materials, Sealing materials
<b>Chemical mechanical responsiveness</b>	Artificial muscles, Switching components

### 1.3 Protein

Protein is a kind of important biological macromolecules, is the material basis of all life, is an important part of the body cells. It is, therefore, a substance that is intimately associated with life and all forms of life.

#### 1.3.1 Gelation of Protein

Functional properties of proteins refer to the non-nutritional properties of proteins

used as food additives, such as gelation, viscosity, emulsification and foaming.

Gelation refers to the transformation of the system from a molecular dispersion state to a three-dimensional network of molecular cross-linking. Edible gels are made up of cross-linked polymers of sugar, protein, or a mixture of both, immersed in water. The precondition for gel formation is that the polypeptide chain of a part of the protein unfolds through denaturation, and then the partially unfolded peptide chain molecules interact with each other to form a three-dimensional network structure. Gelation is based on the separation and extension of the peptide chain, and further through the disulfide bond, salt bond or hydrogen bond to form a relatively solidified network structure, and water is combined, surrounded in the network structure [9].

### 1.3.2 Effect of Metal Ions on Protein Gels

The difference of the positive charge and diameter of metal ions can change the electrostatic repulsion force between proteins or shield the charge of proteins, so that the attraction is in a state of balance, which significantly affects the protein-protein, protein-solvent interaction and thus affects the deformation, expansion and aggregation process of proteins [10-12]. On the other hand, ionic strength has a significant effect on the solubility of protein [13], resulting in the change of protein content, thus affecting the properties of protein gel.

Metal ions have an effect on the microstructure, physical and chemical properties, aggregation behavior, molecular force and molecular conformation of protein gels.

Changes in the concentration of metal ions have an impact on the network structure. The low concentration ( $<0.1\text{mol/L}$ ) of monovalent metal ions promotes the formation of a fine beam-like structure. When the concentration is greater than  $0.1\text{mol/L}$ , the gel network structure becomes chaotic [14]. Within a certain range of ion concentration, increasing the ion concentration can significantly increase the gelation and water holding capacity of the protein. At the same time, the ionic strength has a greater impact on the water absorption and solubility of the protein [15]. The type and strength of metal ions have a greater impact on the microstructure and other characteristics of the gel, and the more complex the protein, the greater the impact of metal ions on the protein gel.

Metal ions affect the type of gel formation by affecting the aggregation rate of protein molecules, and the type and concentration of metal ions have different effects on the aggregation form and degree of protein molecules. Metal ions can increase the

solubility of the protein in the solution by reducing the hydrophobicity of the protein surface, and increase the protein concentration, and promote the hydrophobic aggregation of protein molecules.

The formation of protein gels has a very important relationship with the electrostatic repulsion between proteins. The addition of metal ions will interact with the oppositely charged protein groups to form an electric double layer of electron groups, which weakens the electrostatic repulsion between protein molecules, and promote protein-protein and protein-solvent interactions.

In the process of protein gelation, metal ions change the molecular conformation of the protein and part of the chemical force of the gel or combine with protein molecules by influencing the denaturation and unfolding of protein molecules, the degree of rearrangement and aggregation of protein linear molecules. In the end, the gel hardness, microstructure and water holding capacity are improved to a large extent, and different types and concentrations have different effects [16].

### 1.3.3 Application of Protein Gel

Water absorbent gel is a kind of insoluble hydrophilic polymer which rapidly absorbs a large amount of water by hydration. And it contains a variety of hydrophilic groups, which can swell in water to form a three-dimensional network structure. It can absorb tens of times or even thousands of times its own weight of liquid, at the same time has a strong liquid retention ability. At present, the most studied and applied at home and abroad are mainly polyacrylic acid and polyacrylonitrile type of super absorbent gel, which has a good water retention ability. The disadvantages are that they are expensive, not biodegradable, have poor salt resistance, contain unreacted toxic monomers and easy to cause pollution, so their application is limited. In the past 10 years, chemically modified protein gels have been used in the preparation of water absorbent materials, because protein is pollution-free and is a natural biodegradable substance [17].

Intelligent hydrogel is a kind of hydrogel that can respond sensitively to external stimuli (such as temperature, pH, solvent, salt concentration, light, chemical substances, etc.). Intelligent gels are mainly made of synthetic materials. Proteins are amphoteric molecules with properties that can affect the external environment. Therefore, in recent years, gels with sensitive response to the environment produced by proteins or modified

proteins began to appear. Walters Christina find that LEA-1protien can be added to water to form a hydrogel. The hydrogels can be used as an effective component of absorbent materials, skin therapeutic agents, excipients for cosmetics, additives for improving the hygroscopic and hydrating properties of foods, antifreeze agents for maintaining the integrity of biomolecules, and materials for enhancing the resistance to dry penetration, heat resistance and cold resistance of organisms [18].

Protein gel has good biocompatibility and can simulate the tissue characteristics of the organism. It can be applied to the diagnosis, treatment, repair and replacement of human tissues and organs in biomedical engineering, especially tissue engineering, or improve their functions. It can also be used for growth factors, drugs, gene carriers, etc. [19]. Collagen sponge prepared by freeze-drying technology using glutaraldehyde as crosslinking agent can be used in the study of tissue engineering of biological hybrid artificial skin [20]. It has been reported in foreign literatures that the transforming growth factor— $\beta_1$  (TGF $\beta_1$ ) in collagen sponge can release TGF $\beta_1$  which has biological activity, and this kind of sponge material is an ideal material for bone repair [21].

## Chapter 2 Experimental section

### 2.1 Experimental Drugs and Instruments

#### 2.1.1 Experimental Drugs

##### (1) Silver nitrate ( $\text{AgNO}_3$ ).

Purity: AR (Analytical Pure).

Manufacturer: Shanghai Maclean Reagent Co., Ltd.

Silver nitrate is a colorless crystal with a chemical formula of  $\text{AgNO}_3$ , which is easily soluble in water. Pure silver nitrate is stable to light, but because the purity of general products is not enough, its aqueous solution and solids are often stored in brown reagent bottles.

##### (2) Trypsin.

Purity: AR (Analytical Pure).

Manufacturer: Tianjin Komiou Reagent Co., Ltd.

Trypsin is a kind of protease. In vertebrates, it functions as a digestive enzyme. Trypsin can hydrolyze natural protein, denatured protein, fibrin and mucin into peptides or amino acids. And trypsin does not digest normal tissues, but can decompose sticky sputum, purulent sputum and other viscous secretions, and can promote the penetration of antibiotics and chemotherapeutic drugs into the lesion.

Trypsin is a white or quasi-white crystalline powder. As a protein, trypsin is easily contaminated and inactivated at room temperature.

##### (3) Bovine serum albumin (BSA).

Purity: AR (Analytical Pure).

Manufacturer: Tianjin Komiou Reagent Co., Ltd.

BSA is generally used as a stabilizer in storage solutions and reaction solutions for restriction enzymes or modified enzymes, because some enzymes are unstable or have low activity at low concentrations. After adding BSA, it may play a "protective" or "carrier" role. Many enzymes can greatly increase their activity after adding BSA. Enzymes that do not need to add BSA will not be affected by the addition of BSA.

Generally, Bovine serum albumin is a milky white powder in the form of small flakes.

**(4) RO water (Reverse osmosis water).**

Purity:  $\mu=2.9$ .

Manufacturer: Self-made.

Reverse osmosis water is a kind of laboratory water that is more and more widely used in laboratories. The production process of reverse osmosis water consumes low energy, produces water quickly, is safe, and is a purely physical process without chemical reactions. Therefore, this economical, practical, safe and efficient water production method is now being used in more and more laboratories.

Reverse osmosis water can effectively remove impurities such as dissolved salts, viruses, bacteria, colloids, bacterial endotoxins and most organic matter in the water, and overcomes many shortcomings of distilled water and deionized water.

### 2.1.2 Experimental Instruments

**(1) Ultrasonic cell crusher**

Model: JY96-IIN.

Manufacturer: Ningbo Xinzhi Biological Technology Co., Ltd.

The ultrasonic cell disruptor has the functions of crushing tissues, bacteria, viruses, spores and other cell structures, homogenizing, emulsifying, mixing, degassing, disintegrating and dispersing, leaching and extracting, and accelerating the reaction. Therefore, it is widely used in biology, medicine, Laboratory research and enterprise production of chemistry, pharmacy, food, cosmetics, environmental protection, etc.

**(2) Constant temperature water bath**

Model: EX-8L.

Manufacturer: Changzhou Boyuan Experimental Analytical Instrument Factory.

Constant temperature water bath is widely used in drying, concentration, distillation, dipping chemical reagents, dipping medicines and biological preparations. It can also be used for constant temperature heating in water baths and other temperature tests. It is an indispensable tool for biology, medicine, hygiene, biochemical laboratories, etc. The main features are: the studio water tank is made of stainless steel, which has superior corrosion resistance. The temperature control is accurate, with digital display, and automatic temperature control. It is easy to operate and safe to use.

### **(3) Magnetic stirrer**

Model: DF-101S.

Manufacturer: Zhengzhou Yuhua Instrument Co., Ltd.

Magnetic stirrer is a laboratory instrument used for liquid mixing, mainly used for stirring or simultaneously heating and stirring low-viscosity liquid or solid-liquid mixtures. The basic principle is to use the principle of repulsion of the same sex and attraction of the opposite sex of the magnetic field, and use the magnetic field to push the magnetic stirrer placed in the container to rotate in a circle, so as to achieve the purpose of stirring the liquid.

### **(4) Ultra-pure water meter**

Model: UPR-1-15T.

Manufacturer: Chengdu Zhuoyue Technology Co., Ltd.

Ultra-pure water meter is mainly used in areas where water quality requirements are quite high.

### **(5) Constant temperature drying oven**

Model: DHG-9053A.

Manufacturer: Zhengzhou Yuhua Instrument Co., Ltd.

Constant temperature drying oven is used for various experiments in scientific research institutes, professional colleges, and other laboratories, and production sites: such as drying, curing, baking, and disinfection. Note that it cannot be used for combustibles and explosives.

### **(6) EYEL mixer**

Model: NZ-1100.

Manufacturer: Shanghai Ailang Instrument Co., Ltd.

## **2.2 Experimental Method**

In this experiment, trypsin and silver nitrate complex hydrogels were synthesized by one-step hydrothermal method. This method is simple and easy to operate. The experimental steps are green and environmental friendly, no waste pollutants are produced, and the reaction conditions are mild. It is a very simple method to synthesize protein metal hydrogels.

## 2.3 Experimental Procedure

### 2.3.1 Preparation Solution

Firstly, 0.4g, 0.2g, 0.1g trypsin was weighed and prepared into an aqueous solution with a volume of 10ml, and then placed in the refrigerator for preservation, because trypsin, as a protein, is easily contaminated and deactivated at room temperature. Then 0.0169g silver nitrate solids and 10ml deionized water were weighed to prepare a 0.01mol/L solution, which is placed it in a brown bottle, and stored away from light.

### 2.3.2 Reaction

Take 0.4g/10ml, 0.2g/10ml, and 0.1g/10ml of three different trypsin solutions and place 2ml each in the reaction vessel. After preheating the trypsin to 60°C, add 1ml of 0.01mol/L silver nitrate solution. Trypsin is gradually added dropwise to the solution under vigorous stirring to ensure that the two solutions can fully react. The reaction was carried out for two hours under dark conditions during the whole process. After the reaction was completed, the samples were placed in the refrigerator and kept under cold storage.

And it is worth noting that the container used for the reaction should be transparent in order to facilitate the observation of the gel formed later. But the silver nitrate solution is unstable and easy to decompose when exposed to light, which will affect the reaction result. Therefore, the method of wrapping the reaction container with tin foil during the experiment is adopted to prevent it from being exposed to light during the reaction process, and the gel can be easily observed by removing the tin foil after the reaction.



Fig. 1. Flow Chart of the Experiment

## Chapter 3 Results and Discussion

### 3.1 Characterization Methods

#### 3.1.1 Photographing

Trypsin and silver nitrate complex hydrogel samples are placed upside down in a small bottle with a dark background, partially shaded to avoid overexposure, and the camera lens is kept at an appropriate angle to the sample for shooting, to observe whether the sample flows under the action of gravity.

#### 3.1.2 Analysis of Fourier Infrared Spectroscopy

Fourier Transform infrared spectroscopy is an analysis and identification method that combines Fourier transform mathematical processing and computer technology with infrared spectroscopy. It can verify the functional groups of unknown substances, determine the chemical structure, observe the chemical reaction process, distinguish isomers, analyze the purity of the substance, etc.

Type NICOLET Is10 (American) infrared spectrum analyzer was used to record the data. Specific operation is that grinding the sample of the powder mortar and after calcining dry potassium bromide in accordance with the proportion of 1:100 dose is mixed grinding, and be piece after the tablet press pressure. It is placed on the perpendicular to the path of the specimen holder, then the final data is derived by the computer.

#### 3.1.3 UV-visible Spectrum Analysis

UV-visible Spectrum Analysis is widely used in the qualitative and quantitative determination of organic and inorganic substances. This method has the characteristics of high sensitivity, good accuracy, excellent selectivity, easy operation, and good analysis speed.

Instrument: UV-2600. Before the measurement, the liquid background was calibrated. After the baseline was stabilized, the liquid absorption mode was selected,

and trypsin and silver nitrate complex hydrogel were placed on the test fixture for testing. The wavelength range of 200nm~800nm was the absorption mode.

#### 3.1.4 Zeta Potential

ZETA potential refers to the potential of the shear surface, also called electromotive potential, which is an important indicator of the stability of colloidal dispersions.

The instrument used is Zetasizer Nano ZS90(Malvern Inc.). A 1-2ml sample of CNCS dispersion is placed in a Darwin potentiometer for measurement and repeated about three times.

### 3.2 Results and Discussion

As shown in the figure, we took physical pictures of the three samples respectively. Through observation, we found that when the concentration of trypsin was increased, or when the content of trypsin was increased, it was conducive to the formation of protein metal ion hydrogel. At the same time, we consider that the presence of metal ions may also increase the formation of gel. As can be seen from the figure, the protein metal ion hydrogel can be formed when the concentration of trypsin reaches 0.2g. With the increase of trypsin concentration, the shape of the protein metal ion hydrogel is better maintained and the fluidity is worse, indicating that the strength of the gel is also better.

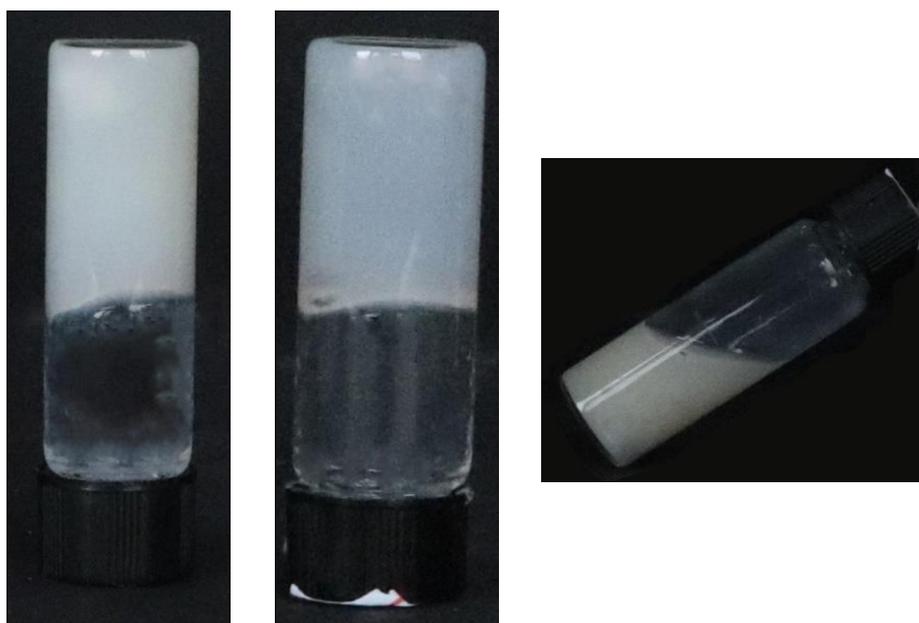


Fig. 2. Photographing of Protein Metal Ion Hydrogel

In order to characterize the gel properties of protein metal ion hydrogels, we carried out rheological tests on the gel. From the figure, we can see the viscoelastic properties of protein metal ion hydrogels. From the frequency scan (Figure 3), we observe that the hydrogels with trypsin concentration of 0.4g/10ml and 0.2g/10ml at frequencies above 1 rad/s, the material behaves more like a solid rather than sticky liquid. It is worth noting that at higher frequencies, both the loss modulus and the storage modulus remain in a similar range, thus supporting the hypothesis of the viscoelastic gum-like and viscoelastic material of the protein metal ion hydrogel. These measurements also prompted us to use 1rad/s in downstream rheology experiments because this value is in the range of viscoelastic solids, not in the range of liquids. Strain sweep measurement allows us to determine the linear viscoelastic region (LVE) in which  $G'$ (storage modulus) and  $G''$  (loss modulus) are almost constant, corresponding to the undisturbed material. A lot of information can be obtained from this area. If  $G' > G''$ , the material is a viscoelastic solid.

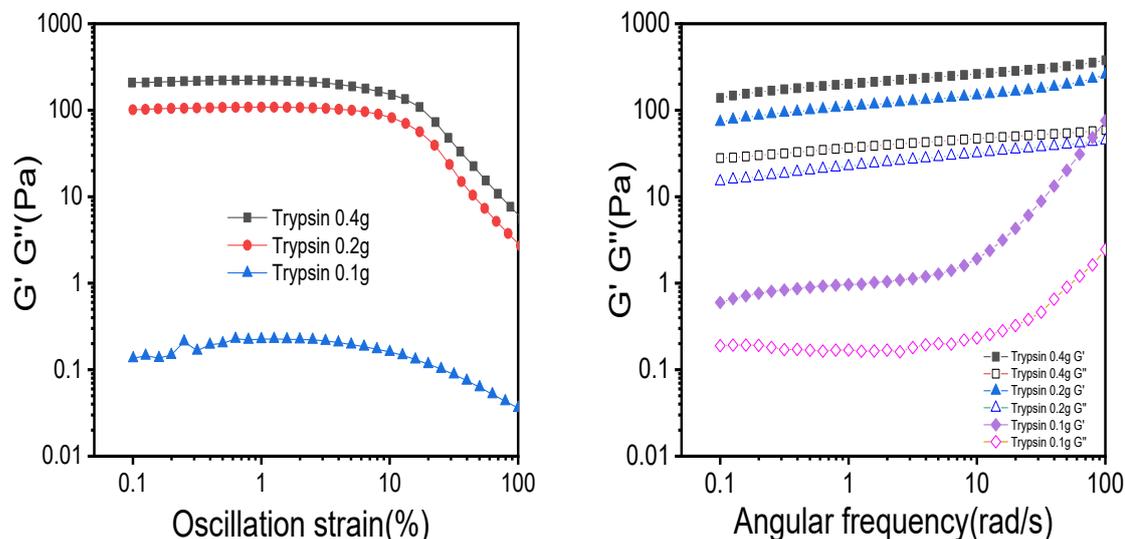


Fig. 3. Rheological Test Diagram of Gel

In addition, in order to characterize the presence of silver ions inside the protein metal ion hydrogel, we performed UV-Vis tests on the hydrogel samples and partially diluted samples. We took the trypsin solution as the baseline, from the UV absorption Peak we see the absorption peak of silver ions.

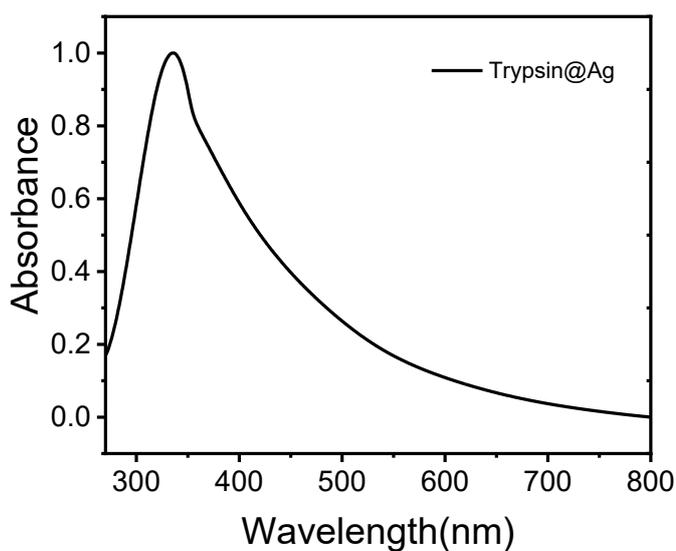


Fig. 4. Ultraviolet Spectrogram of Trypsin with Ag<sup>+</sup>

In order to explore the application of protein metal ion hydrogels, we conducted experiments on its antibacterial properties. Compared with penicillin, which has the strongest antibacterial properties, protein metal ion hydrogels still showed good antibacterial properties. The bacteria used Gram Negative, Gram-positive Escherichia coli and Staphylococcus aureus. This also provides a wider range for its practical application.

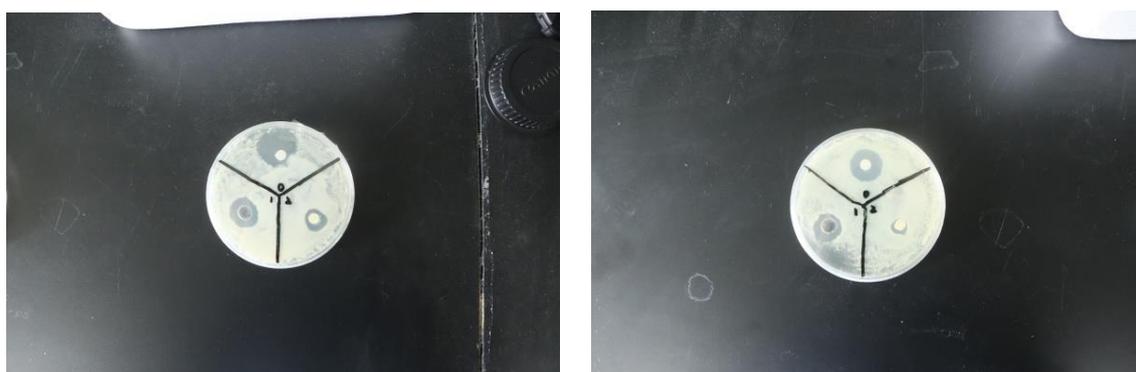


Fig. 5. Testing of the Antibacterial Properties of Gel

## Chapter 4 Conclusion

Gelation is one of the functional properties of proteins, and protein gelation is affected by many factors. The influence of metal ions is one of the factors affecting protein gelation.

Trypsin was used to make protein-metal hydrogel in the experiment. When the trypsin content (trypsin concentration) increases, protein hydrogels are more likely to be formed. Although the gel formation can be increased under the influence of metal ions, different concentrations of trypsin all use the same concentration of metal ion solution.

From the frequency sweep test, it can be seen that the storage modulus ( $G'$ ) of each group is greater than the loss modulus ( $G''$ ), that is, the sample will not flow within a short period of time when the force is applied, and it is in a colloidal (solid) state.

Finally, the antibacterial properties of the protein-metal hydrogel were tested. Compared with penicillin, which has the strongest antibacterial properties, protein metal ion hydrogel still exhibits good antibacterial properties. As mentioned earlier, protein gel is a kind of natural gel in gels, and its biggest feature is non-toxic, pollution-free, environmentally friendly, biodegradable and biocompatibility. Its biocompatibility determines that protein gels can be applied to biomaterials, such as tissue engineering substrates, wound dressings, alloy membranes, etc. For this application, the protein metal gel is also required to have good antibacterial properties.

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