Research on the Preparation of Poloxamer-based Strong Intensity Injectable In-Situ Gel

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Abstract: Injectable in-situ gel can be converted into solidified state by liquid, which can form a specific form according to the characteristics of injection site, and can also achieve the target drug target release. However, the common temperature sensitive injectable gel has a small mechanical strength. This experiment takes Poloxamer P407 as the basic material, combined with sodium alginate and Ca2 + into half interpenetrating network (IPN) in order to increase its strength and toughness, in situ gel preparation temperature response type, in order to apply in clinical, such as the symptomatic treatment of senile knee arthritis. Through to the in situ gel forming time, mechanical strength, SEM, XRD, FT - IR and characterizing the properties such as biocompatibility, results show that with mooring Poloxamer P407 as base material, the joint temperature sensitive in situ of the preparation of sodium alginate gel has good mechanical strength and elasticity, gel phase transition time is less than 2 min. SEM and other properties show that its microstructure has porous structure and can be used as an effective carrier for clinical therapeutic drugs.

1. Introduction

Gel as a new drug carrier, at present can be used to slow release, controlled release and pulsed release new drug delivery system, with its excellent medicine features and widely applied to the skin, eye, nose, mouth, vagina, rectum and so on many kinds of ways to medicine, it can be divided into temperature sensitive type, pH sensitive type and ion sensitive type. The main material of in situ gel preparation with polyoxyethylene - oxygen poly propylene block copolymer (poloxamer), polyethylene glycol (peg) block copolymer poly (lactic acid) (PLA), acrylic polymer, chitosan, alginate, etc. As injectable hydrogels, the formation of the hydrogel elastomers have also had physical properties similar to that of the articular cartilage, can be used as a substitute to repair articular cartilage defect of articular cartilage, and restore the articular surface integrity, maintain joint function; Alternatively, it can be used as a body implant for filling or targeting drugs for specific parts of the body, such as lacrimal ducts and dry eye diseases. The injectable thermo-sensitive hydrogel mainly include: poloxamer thermo-sensitive hydrogel, polyvinyl alcohol gel, hyaluronic acid gel, fibrin gel, chitosan gel, magnetic water gel, etc. Now take the mooring poloxamer P407 and P188 share, more suitable for eye ophthalmic gel preparation, micro gel network structure for drug release form certain resistance load, so as to achieve the purpose of slow release. But to berth poloxamer for material preparation in situ gel mixing temperature sensitivity exists low mechanical strength and wear resistance of faults, at present only in theoretical research, limited the scope of its clinical application. Using Sodium Alginate Sodium Alginate, SA) and calcium ion crosslinking, and can also be the preparation of injectable hydrogels, but commonly used calcium Alginate gel, the calcium ion source for $CaCl_2$, belongs to the strong electrolyte, so after contact with SA immediately after mixed gel formation, characterized by lack of injectable. This experiment using the mooring poloxamer P407 and P188 as base material, joint and difficult to soluble Ca alginate sodium salt forming half interpenetrating network (IPN) to increase its strength and toughness, and at the same time with a small amount of carbomer 941 as viscosity regulator, through the study of gelation mixed gel time, gel strength, micro morphology, FT - IR and biocompatibility, inspection as an injectable

win min the possibility of in situ gel.

2. Laboratory Instruments and Materials

SHZ-82 constant temperature oscillator (Changzhou Guohua Instrument Co., Ltd.); IKA-EW120 electric mixer (Aika Equipment Co., Ltd., Aika Guangzhou); DF-101S collector constant temperature water bath (Zhengzhou Kefeng Equipment Co., Ltd.), A scanning electron microscope (SEM, model: S-4800, Hitachi, Japan), Fourier transform infrared spectroscopy (Thermo Nicolet, NEXUS), x-ray diffractometer (XRD, D / Max-3c, Japan), Cryogenic high-speed refrigerated centrifuge (Model: 3H20RI, Hunan Hexi Equipment Co., Ltd.), 722N UV-Vis spectrophotometer (Shanghai Jingke Instruments), electronic universal tensile machine (Model: XMN-GB1040, Changchun Xinke Experimental Instrument Co., Ltd.).

Poloxamer P188 (Pluronic® P188) and P407 (Pluronic® P407) (BASF Corporation, Germany); Carbopol 941 (Hangzhou Cabot Corporation); Sodium alginate (Pharmaceutical Group Chemical Reagent Co., Ltd., model number: 1788), calcium carbonate (Tianjin Chemical Reagent Factory). The remaining reagents are of analytical grade.

3. Experimental Method

3.1 Preparation of Thermo-Sensitive in Situ Gel

Weigh a certain amount of P407, low temperature conditions, according to the proportion of ultra-pure water, stir, stand overnight at 4 ° C, were different concentrations of clear and transparent solution. Prepare 2% of P188 solution according to the above method and prepare 2% of sodium alginate (SA), 2% of Cappa 941 solution and saturated solution of CaCO3, mix well and reserve. First, P407 and poorly soluble CaCO3 solution is thoroughly mixed to form A solution; P188, SA and Kappa 941 are thoroughly mixed to form B solution; and then the two mixed solutions are mixed in proportion to obtain the target solution. The target solution Injection in the body specific parts. At body temperature, a gelled state can form Figure 1.

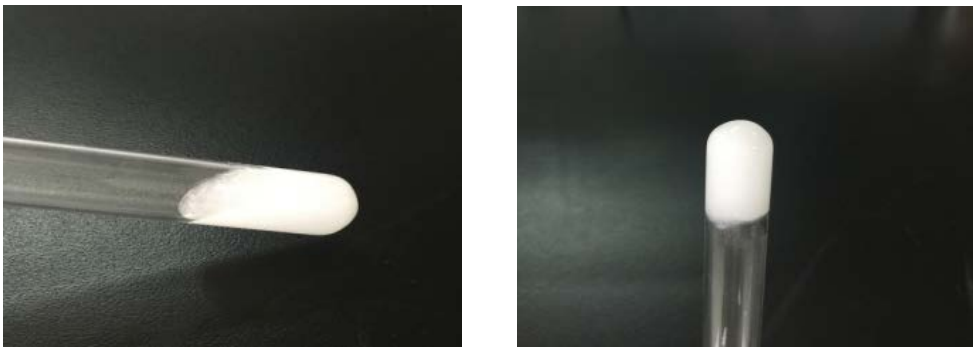

Figure 1. Gel under different temperature conditions (left for the gel state at room temperature, the right is the gel state after transformation under the conditions of 37 ℃)

3.2 Determination of Gel Intensity

The mixed solution into the cylindrical mold, placed in a constant temperature water bath. After a constant temperature of 37 ° C for 30 min, the gel was removed and the weight gradually added until the gel cylinder collapsed, recording its maximum load (Figure 2). The mixed gel at 37 ℃ constant temperature at different times, with the law on the strength of the test to examine the gel strength changes in different time. The gel strength is defined as the minimum pressure required for the gel to rupture. Each sample is done three times and averaged. In the meantime, the strength of the mixed gel obtained by mixing P407, P188 and Kaibo 941 at the same concentration and ratio was compared with that of the sample.

The mixed gel was loaded into a bar-shaped mold, heated at 37 ° C for 30 min, gelatinized in situ,

and tested for tensile strength in an electronic universal tensile machine. Tensile test speed: 10mm / min, room temperature conditions, each sample three times, take the average.

3.3 Gel Characterization

After the gel samples were freeze-dried, the samples were analyzed for their phase structures using a Rigaku Rotalflex X-ray diffractometer. The working conditions were: Cu target Kα radiation, scanning range 5 ~ 40 °, scanning speed 6 (°) / min; the mixed gel in a 37 °water bath gel 24h, immediately placed in-80 ℃ ultra-low temperature refrigerator 24h, After freeze-drying, freeze-drying 60h, the surface and cross-section were sprayed gold, observed under a scanning electron microscope microscopic morphology; sample after drying, the sample and the raw material XRD patterns were detected by X-ray diffractometer. XRD test conditions: Cu-Ka target, the measurement range 2θ at 10 ~ 60, the speed of 10 ° / min. Determination of raw materials and samples of crystallinity and changes.

3.4 Hemolysis Experimental Study

In accordance with "GB/T16886.1-1997 medical device biological evaluation of the gel sample hemolysis experiments take anticoagulant rabbit blood 5mL, rabbit blood diluted to 15mL with normal saline, spare the gel product material into the test tube , The test group was added 10mL normal saline tube, and the other two test tubes for the control, the negative control added 10mL saline, positive control added 10mL distilled water after the completion of the incubation by low temperature centrifugation (1500r / min, 10min, 4 ℃) Serum, measured at 520nm solution absorbance value of each material to take five samples were repeated test, the results take the average, the following formula to calculate the percentage of hemolysis:

$$Hemolysis\ ratio = \frac{OD_{Sample} - OD_{Negitive}}{OD_{Positive} - OD_{Negitive}} \quad (1)$$

4. Experimental Results and Analysis

Concentration of SA was 0.5%. The concentration of SA had little effect on the phase transition time of the gel system. However, if the concentration of SA was too small, the interlaminar strength would not increase obviously. However, the concentration of SA Too high will also increase the viscosity of the system and increase the resistance at injection, so the concentration of SA is chosen to be 2%.

4.1 Determination of Gel Strength

4.1.1 Compression Mechanical Strength of Gel

The gels prepared with P407 and P188 and their modulating agents, though they could form in situ gels at body temperature, had weak mechanical strength and poor elasticity. However, the gels with P407, P188 and SA-Ca formed interpenetrating network gels, after the force showed significant compressive strength, and remove the external force, the gel can restore the original, showing good flexibility, this feature is conducive to the sample used in clinical. The simple preparation of SA-Ca gel, its hardness is too large; flexibility and strength are not suitable for the body.

As can be seen from Figure 2a, in the early gel formation, the P407 concentration of 25% of the samples with higher mechanical strength, but with the extension of time, the brittleness increases, the overall strength decreased, but when the P407 concentration of 25%, P407 The formation of cross-linking speed too fast, and Ca2 + and alginic acid formed slower, so there is no effective interpenetrating cross-linked system, non-homogeneous cross-linked gel makes the pressure can withstand less; with 20% And P407 at 15%, the mechanical strength of P407 samples increased with time, and the P407 samples with 15% concentration had better mechanical strength. The increase of mechanical strength shows that in addition to the microscopic cross-linking effect of P407 formed in

the sample, the calcium alginate cross-linking is effectively formed and the interpenetrating cross-linking system is formed to a certain extent. The micropore results in the system can make the coagulation Glue with a certain degree of compression capacity.

4.1.2 Tensile Strength Testing of Gels

For injectable gels, the tensile strength can reflect the size of its elastic strength, the higher the elasticity of the gel, in clinical applications, the better the body and adaptability. The tensile strengths of the three concentrations of gel are shown in Figure 2b. As can be seen from Figure 3b, the in-situ gel formed by P407, P188 and Kappa 941, combined with the mixed gel of SA-Ca interpenetrating network, showed a better stretching effect. The higher tensile strength of 15% samples was probably due to the slow release of Ca2 + and the formation of gel velocities with SA at the time of gel formation when the cross-linking of the P407 block chains was formed. Good interpenetrating network structure, an increase of the intermolecular force within the chain or chain, showing better stretching effect.

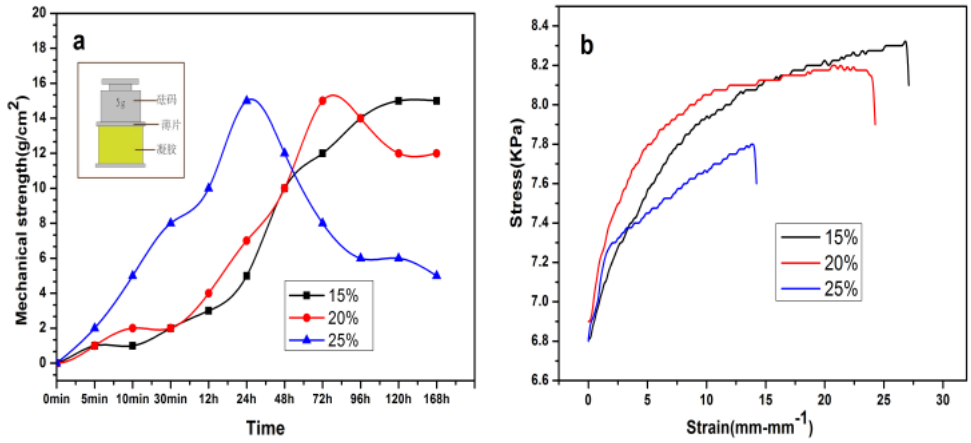

Figure 2. Determination of mechanical strength and tensile strength of gel samples

4.2 XRD Analysis

XRD analysis of P407, P188 and samples using X-ray diffractometer results are shown in Fig.3. It can be seen from Fig. 3 that P188 has obvious crystal diffraction peaks at 14.70, 19.25, 23.47, 26.36 and 36.81 °. P407 has obvious crystal diffraction peaks at 19.01 and 23.30 °, while SA has an absorption peak at 13.6 °. The samples have crystal diffraction peaks at 19.23, 23.34, 29.47, 36.52, 39.51, 43.25 and 47.58 °. The diffraction peaks at 19.23 and 23.34 are in the same position as P407 and P188, but their intensities are weakened. The absorption of the crystal diffraction peak of the corresponding group is weakened due to the enhanced intermolecular interaction between the chains. However, the absorption peak of SA disappears at 13.6 °, which indicates that the possible formation of intermolecular SA crystals may occur when SA forms cross-links with Ca^{2+} or forms interpenetrating networks with poloxamers.

4.3 FT-IR Results Analysis

As can be seen from Figure 3, the characteristic bands of P407 mainly include: 3363cm-1, an absorption peak of -OH, and an absorption peak of -CH3 at 2879cm-1. The characteristic peaks of SA at 3302, 1602, 1413 and 1061 cm-1 are the stretching vibration peaks of -OH, C-H, -COO- and C-O-C, respectively. In the sample, the characteristic peak of -OH of SA was obviously weakened, indicating that the amount of water contained in the sample decreased after the formation of cross-linking, which was consistent with the phenomenon of a small amount of water during the experiment. Due to the crosslinking of Ca^{2+}, the characteristic peak position moving from 1602 cm-1 to 1554 cm-1, cross-linking weakens the stretching vibration.

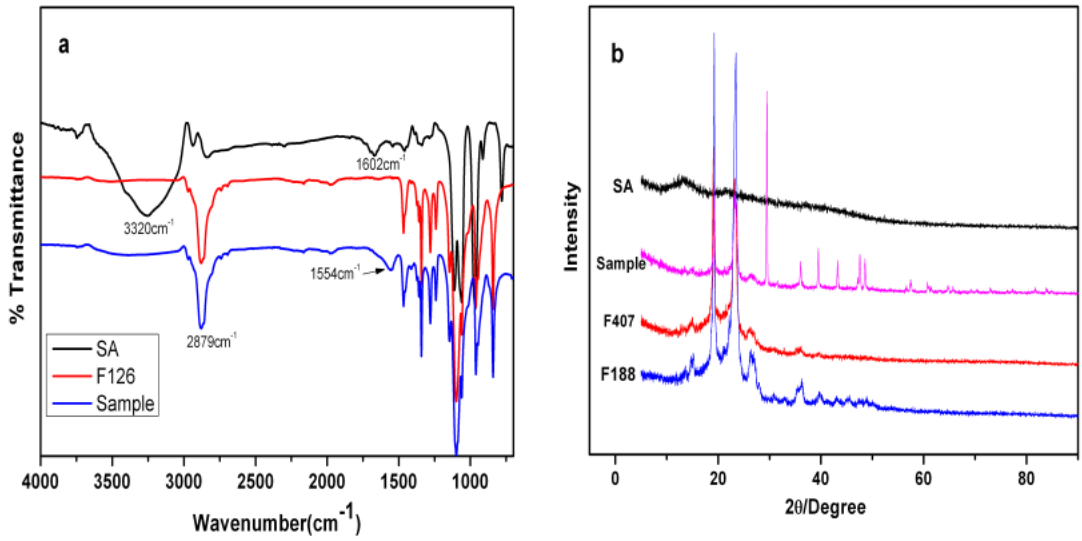

Figure 3. FT-IR and XRD patterns of raw materials and samples

4.4 Scanning Electron Microscopy (SEM) Analysis

Through the sample SEM test, the results are shown in Figure 4. It can be seen from Figure 4 that the samples are well mixed and there is no obvious delamination between the materials, indicating that the compatibility between the raw materials is better. Figure a is the surface map of the sample, the surface is smooth, with different size of the aperture, the cross section Figure b has a porous network similar to briquette, physical crosslinking may be physical so that the physical strengthening of the gel, the degree of crosslinking Increase, forming a more intensive network structure. It can be seen from the internal structure that the gel forms a three-dimensional porous network structure with interconnected pores, which can serve as a channel for the diffusion of drugs and small molecules and is favorable for the controlled release of the drug and is an excellent potential carrier material.

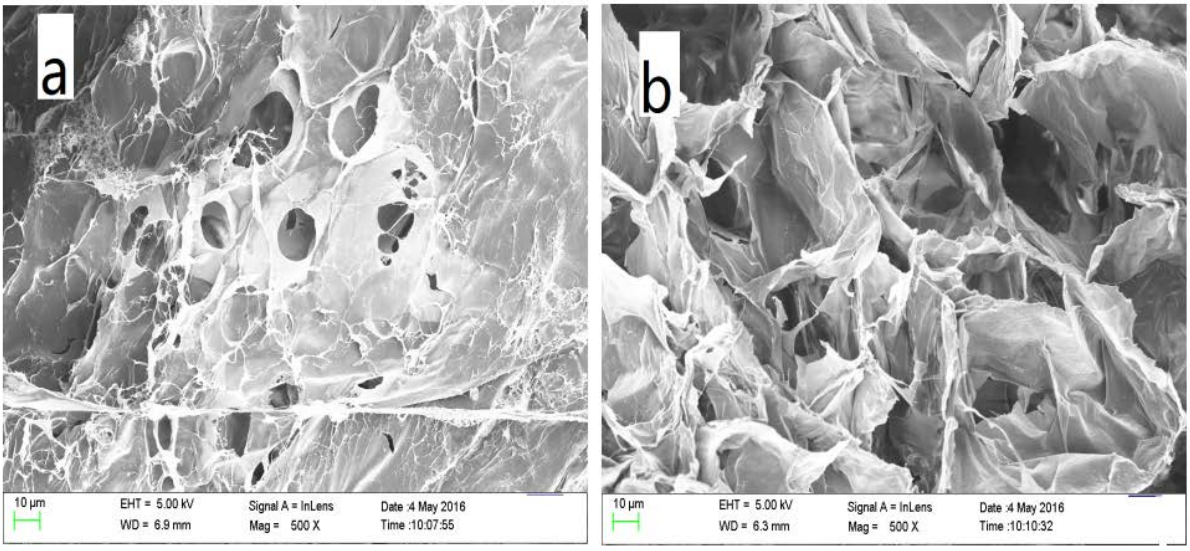

Figure 4. SEM image of the sample (Figure a is the sample surface; Figure b is the sample cross-section)

4.5 Hemolysis Test Results

Under the conditions of 37 ℃ incubated at different times of the sample extract for hemolysis test, the results calculated by Equ 1, the sample hemolysis rate in Table 1, we can see that different time samples hemolysis rate of less than 5% of national standards, you can The sample is considered to be

free of hemolysis. Generally believed that when the hemolytic value of medical materials is less than 5%, the material can be considered safe for the human body. This experiment poloxamer as the material, prepared in situ gel hemolysis rate of 2.35%, can be considered that the material has no damaging effect on the red blood cells, the above results can be judged that the material will not hemolysis, for injectable The material in the human body is safe.

5. Conclusions

In this experiment, we use $CaCO_3$ as a weak electrolyte to release Ca^{2+} slowly and sustainably, which makes the gel possess injectable characteristics and form an interpenetrating network structure with P407. By FT-IR and XRD, we can conclude that the formation of interpenetrating network did not affect the cross-linking reaction. SEM analysis showed that the mixed gel formed a uniform three-dimensional porous interpenetrating network structure, which the uniform three-dimensional network structure can be used as a good carrier of medicine. By testing the mechanical strength, poloxamers are used as base material, and the gels with sodium alginate and Ca^{2+} are added at the same time, the mechanical strength and elasticity are obviously increased, especially the products with 15% P407 as the main material, Under the condition of body temperature, P407 dehydrated to form gel, the Ca^{2+} released slowly and formed Alg-Ca cross-linking with SA to form a stable interpenetrating network structure, and the rate of Ca^{2+} release was the same as that of P407 Therefore, its mechanical strength will increase slowly with time. This spatial result is very favorable for clinical applications and drug carriers and may be used as a physical therapy for clinical diseases such as senile knee osteoarthritis. Later, further study of its clinical application as a drug carrier features.

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