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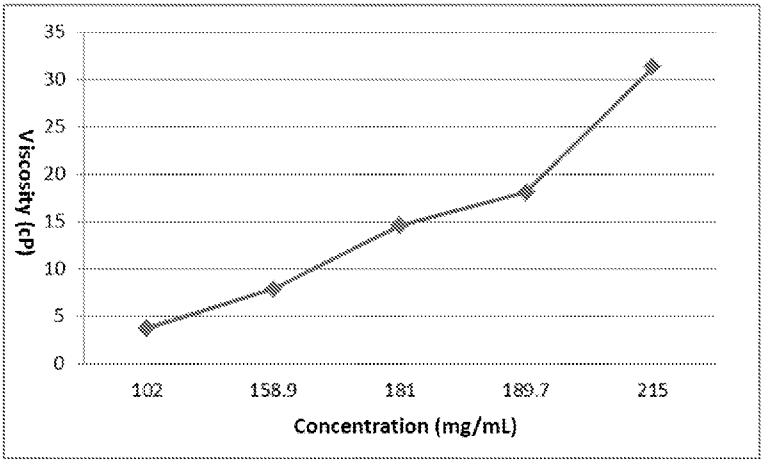

Fig. 1

(57) **Abstract:** Provided are liquid formulations comprising high concentrations humanized antibodies for treating an IL-6-related disease. In some embodiments, the liquid formulation comprises 120-300 mg/mL of the antibody, 5-30 mM of a histidine salt buffer or 5-30 mM sodium acetate buffer, 0.1-1.0 mg/mL surfactant, 70-200 mM stabilizer and water for injection without antioxidant. The antibody formulation provides good antibody stability, acceptable viscosity for subcutaneous injection, prevents aggregation and degradation of the monoclonal antibody, and acidic isomer increase. The preparation is applicable in stabilizing the structure and function of the humanized antibody.

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LIQUID FORMULATIONS COMPRISING HIGH CONCENTRATIONS HUMANIZED ANTIBODIES FOR TREATING IL-6 RELATED DISEASES

Technical Field

The invention relates to liquid formulations of high concentrations of humanized anti-interleukin 6 receptor (IL-6R) antibodies for treating interleukin 6 (IL-6) related diseases.

Background Art

Humanized anti-interleukin 6 receptor antibodies are useful for the treatment of rheumatoid arthritis (RA) [Josef S Smolen, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update, Ann Rheum Dis, 2014; 73(3): 492–509] whose mechanisms are binding soluble and membrane-bound IL-6 receptors (sIL-6R and mIL-6R) and inhibiting sIL-6R and mIL-6R mediated signaling.

Pharmaceutical antibodies are typically administered by injection. Subcutaneous injections allow faster administration as compared with intravenous injections, and self-injection by patients. However, for antibodies that require a high dosage (e.g., over 100 mg per dose), the antibody concentration in a subcutaneous formulation needs to be high due to the limited volume that can be administered by each subcutaneous injection, which presents formulation challenges. For example, due to high concentration, antibodies tend to aggregate which leads to reduced stability and shorter self-life. High concentration also increases the viscosity of the formulation which can make manufacturing and injection through a needle difficult. To increase shelf-life, formulations can be prepared as powders by the lyophilization. However, lyophilized powder requires reconstitution in water, making administration more complicated. Therefore, there is a need for high concentration antibody liquid formulations having long term stability and suitable for subcutaneous injection that do not require reconstitution.

Summary

It is an objective of the present invention to provide a stable liquid formulation comprising a high concentration of monoclonal antibodies that is suitable for subcutaneous injection.

The objective of the present invention is achieved by the following technical means:

In one aspect, the invention provides an antibody formulation comprising a high concentration of a humanized anti-interleukin 6 receptor antibody, a buffer system, a stabilizer and a surfactant. The formulation does not comprise an antioxidant, such as methionine. Specifically, the antibody formulation of present invention comprises, consists essentially of, or consists of the following ingredients:

(1) 120-300 mg/mL humanized anti-IL-6 receptor antibody;

(2) a buffer system comprising 5-30 mM histidine and a salt thereof or 5-30 mM acetic acid and a salt thereof;

(3) 0.1-1.0 mg/mL of a surfactant;

(4) 70-200 mM of a stabilizer;

(5) water for injection.

The pH of the antibody formulation is 5.0-7.0.

In some embodiments, the antibody comprises a heavy chain shown in SEQ ID NO. 1 and a light chain shown in SEQ ID NO. 2. In some embodiments, the antibody comprises two heavy chains shown in SEQ ID NO. 1 and two light chains shown in SEQ ID NO. 2, which is denoted as BAT1806. In some embodiments, the antibody is tocilizumab or a biosimilar thereof, such as BAT1806. In some embodiments, humanized anti-interleukin 6 receptor antibody is expressed in CHO cells by genetic engineering methods and purified by a series of standard chromatographic steps. After the antibody is prepared, a pharmaceutical formulation is prepared.

In some embodiments, the concentration of the humanized anti-IL-6 receptor antibody in the formulation is 130-280 mg/mL. In some embodiments, the concentration of the humanized anti-IL-6 receptor antibody in the formulation is 140-250 mg/mL. In some embodiments, the concentration of the humanized anti-IL-6 receptor antibody in the formulation is 160-200 mg/mL. In some embodiments, the concentration of the humanized anti-IL-6 receptor antibody in the formulation is 180 mg/mL.

In some embodiments, the stabilizer is selected from arginine or a salt thereof (e.g., arginine hydrochloride) or a combination of arginine or a salt thereof and sucrose, or trehalose (which may be added as a hydrate). In some embodiments, the stabilizer is a combination of 70-200 mM of arginine and/or a salt thereof (e.g.,14.746-42.132 mg/mL of arginine hydrochloride) and 29-87 mM (10-30 mg/mL) sucrose; or the stabilizer is a combination of 70-200 mM of arginine and/or a salt thereof (e.g., 14.746-42.132 mg/mL of arginine hydrochloride) and 26-79 mM trehalose (e.g., 10-30 mg/mL trehalose dihydrate); or the stabilizer is 70-200 mM of arginine and/or a salt thereof (e.g, 14.746-42.132 mg/mL arginine hydrochloride). In some embodiments, the stabilizer is a combination of 80-100 mM of arginine and/or a salt thereof (e.g., 16.853-21.066 mg/mL of arginine hydrochloride) and 44-73 mM (15-25 mg/mL) sucrose; or the stabilizer is a combination of 80-100 mM of arginine and/or a salt thereof (e.g., 16.853-21.066 mg/mL of arginine

hydrochloride) and 40-66 mM trehalose (e.g., 15-25 mg/mL trehalose dihydrate); or the stabilizer is 110-130 mM of arginine and/or a salt thereof (e.g, 23.173-27.386 mg/mL arginine hydrochloride). In some embodiments, the stabilizer is a combination of about 90 mM of arginine and/or a salt thereof (e.g., about 18.959 mg/mL of arginine hydrochloride) and about 58.4 mM (about 20 mg/mL) sucrose; or the stabilizer is a combination of about 90 mM of arginine and/or a salt thereof (e.g., about 18.959 mg/mL of arginine hydrochloride) and about 52.9 mM trehalose (e.g., about 20 mg/mL trehalose dihydrate); or the stabilizer is about 120 mM of arginine and/or a salt thereof (e.g, about 25.279 mg/mL arginine hydrochloride).

In some embodiments, the surfactant is polysorbate-80. In some embodiments, the surfactant is 0.1-0.7 mg/mL polysorbate-80. In some embodiments, the surfactant is 0.45-0.55 mg/mL polysorbate-80. In some embodiments, the surfactant is about 0.5 mg/mL polysorbate-80.

In some embodiments, the buffer system comprises 5-20 mM histidine and a salt thereof or 5-20 mM acetic acid and a salt thereof. In some embodiments, the buffer system comprises 8-15 mM histidine and a salt thereof or 8-15 mM acetic acid and a salt thereof. In some embodiments, the buffer system comprises 10 mM histidine and a salt thereof. In some embodiments, the buffer system comprises 10 mM acetic acid and a salt thereof.

In some embodiments, the pH of the antibody formulation is 5.5-6.5; or, the pH of the antibody formulation is between 5.6 and 6.4; or, the pH of the antibody formulation is between 5.8 and 6.2; or, the pH of the antibody formulation is between 5.8 and 6.0; or, the pH of the antibody formulation is between 6.0 and 6.2; or, the pH of the antibody formulation is about 5.8.

In some embodiments, the antibody formulation has a viscosity of from 0 to 20 cP. In some embodiments, the antibody formulation has a viscosity of from 5 cP to 15 cP. In some embodiments, the antibody formulation has a viscosity of from 8 cP to 12 cP. In some embodiments, the antibody formulation has a viscosity of about 10 cP.

In some embodiments, the antibody formulation comprises, consists essentially of, or consists of:

(1) 160-200 mg/mL humanized anti-IL-6 receptor antibody;

(2) 8-15 mM histidine salt buffer;

(3) 0.45 mg/mL to 0.65 mg/mL polysorbate-80;

(4) 80-100 mM arginine hydrochloride;

(5) 15-25 mg/mL sucrose;

(6) water for injection;

the pH is 5.6-6.4.

In some embodiments, the formulation comprises, consists essentially of, or consists of:

(1) 160-200 mg/mL humanized anti-IL-6 receptor antibody;

(2) 8-15 mM sodium acetate buffer;

(3) 0.45-0.65 mg/mL polysorbate-80;

(4) 80-100 mM arginine hydrochloride;

(5) 40-66 mM trehalose (e.g., 15-25 mg/mL trehalose dihydrate);

(6) water for injection;

the pH is 5.6-6.4.

In some embodiments, the formulation comprises, consists essentially of, or consists of:

(1) 160-200 mg/mL humanized anti-IL-6 receptor antibody;

(2) 8-15 mM sodium acetate buffer;

(3) 0.45-0.65 mg/mL polysorbate-80;

(4) 110-130 mM arginine hydrochloride;

(5) water for injection;

the pH is 5.6-6.4.

In some embodiments, the formulation comprises, consists essentially of, or consists of:

(1) 180 mg/mL humanized anti-IL-6 receptor antibody;

(2) 10 mM histidine salt buffer;

(3) 0.5 mg/mL polysorbate-80;

(4) 90 mM arginine hydrochloride;

(5) 20 mg/mL sucrose;

(6) water for injection;

the pH is about 5.6-6.4.

In some embodiments, the formulation comprises, consists essentially of, or consists of:

(1) 180 mg/mL humanized anti-IL-6 receptor antibody;

(2) 10 mM sodium acetate buffer;

(3) 0.5 mg/mL polysorbate-80;

(4) 90 mM arginine hydrochloride;

(5) 52.9 mM trehalose (e.g., 20 mg/mL trehalose dihydrate);

(6) water for injection;

the pH is about 5.6-6.4.

In some embodiments, the formulation comprises, consists essentially of, or consists of:

(1) 180 mg/mL humanized anti-IL-6 receptor antibody;

(2) 10 mM sodium acetate buffer;

(3) 0.5 mg/mL polysorbate-80;

(4) 120 mM arginine hydrochloride;

(5) water for injection;

the pH is about 5.6-6.4.

In some embodiments, the pH of the antibody formulation is about 5.8. In some embodiments, the pH of the antibody formulation is about 6.

In some embodiments, the viscosity of the antibody formulation is 5 cP to 15 cP. In some embodiments, the viscosity of the antibody formulation is 8 cP to 12 cP. In some embodiments, the viscosity of the antibody formulation is about 10 cP.

In some embodiments, a base is added to the antibody formulation for adjusting the pH. In an exemplary embodiment, the base is NaOH.

The antibody formulation is an aqueous formulation for injection. The formulation is suitable for subcutaneous injection.

In another aspect, the invention also provides a method to prepare the antibody formulation, comprising the steps of:

(1) dissolving the buffer, stabilizer and surfactant in water for injection to form a high concentration stabilizer solution having the desired concentrations of the buffer and surfactant and a high concentration of the stabilizer;

(2) adding the surfactant into a buffered antibody solution comprising a high concentration of the antibody and the desired concentration of the buffer to form an antibody surfactant solution;

(3) mixing the high concentration stabilizer solution prepared in the step (1) with the antibody surfactant solution prepared in the step (2) to provide the formulation.

In some embodiments, the pH of the solution prepared in the step (1) is adjusting with an aqueous sodium hydroxide solution.

In some embodiments, the method further comprises filtering the solution prepared in step (1) through a filter membrane into an aseptic container before adding to the antibody solution. In some embodiments, the pore size of the filter membrane being 0.22 μm for filtering bacteria and fungi. In some embodiments, in step (2), the surfactant is added to the buffered antibody solution as a surfactant solution.

The formulations disclosed herein are prepared without lyophilization.

The antibody formulation is a pharmaceutical formulation for treating IL-6 related diseases. In some embodiments, provided is a method for treating IL-6 related diseases comprising administering an effective amount of a formulation described herein to a patient in need thereof. In some embodiments, the IL-6 related diseases include: adult rheumatoid arthritis, systemic juvenile idiopathic arthritis, polyarticular juvenile idiopathic arthritis, giant cell arteritis, giant lymph node hyperplasia, cytokine storm caused by immunotherapy, cytokine release syndrome, adult Still's disease, recurrent polychondritis, type II diabetes, ankylosing spondylitis, thyroid-associated ocular diseases, cardiovascular diseases caused by rheumatoid arthritis, polymyalgia rheumatica, acute graft-versus-host disease, non-ST-segment elevation myocardial infarction, systemic lupus erythematosus, schizophrenia, uveitis, ovarian cancer, anti-neutrophil cytoplasmic antibody-associated vasculitis, neuromyelitis optica, chronic glomerulonephritis, colorectal cancer and the like. In some embodiments, the IL-6 related disease is selected from rheumatoid arthritis, cytokine release syndrome, systemic juvenile idiopathic arthritis, polyarticular juvenile idiopathic arthritis, giant cell arteritis, and giant lymph node hyperplasia. In some embodiments, the effective amount of the formulation is an amount that comprises 120-300 mg per dose of the antibody once every 1 to 3 weeks.

The antibody formulations provided by the invention comprise a high concentration of antibody with an acceptable viscosity, low turbidity and high stability suitable for administering high doses of the antibody by subcutaneous injections. The antibody formulations have been developed to remarkably inhibit the formation of acidic peaks, dimers, aggregates, degradants and insoluble microparticles during freezing/thawing cycles, long-term storage and temperature variation processes. In particular, the humanized anti-interleukin 6 receptor antibodies remain stable in the above formulations after at least 3 freeze-thaw cycles, stable at room temperature for at least 3 months. Therefore, the antibody formulations of the present invention can be used for stably storing the humanized anti-interleukin 6 receptor antibody for treating IL-6-related diseases by subcutaneous injections of the antibody in high doses required for treatment.

Brief Description of the Drawings

Figure 1. The relationship between antibody concentration and solution viscosity.

Detailed Description of the Invention

The present invention provides antibody formulations by selecting buffer solutions and stabilizers, to enhance the stability of the high concentration humanized anti-interleukin 6 receptor antibody formulation, and prevent monoclonal antibody aggregation, degradation and acidic isomer increase, while having low

turbidity and viscosity acceptable for subcutaneous injection.

The term "about" when used before a numerical value indicates that the value may vary within a reasonable range, such as within ±10%, ±5% or ±1% of the stated value, and include the stated value.

As used herein, the term "comprising" is intended to mean that the compositions or methods, etc., include the recited elements, but do not exclude others. "Consisting essentially of" when used to define compositions or methods, shall mean excluding other elements of any essential significance to the combination for the intended use, but not excluding elements that do not materially affect the characteristic(s) of the compositions or methods. "Consisting of" shall mean excluding elements not specifically recited. Embodiments defined by each of these transition terms are within the scope of this invention.

Terms as "stability" and "stable" used herein refer to the situation that in a liquid formulation comprising an antibody, the antibody does not, or only rarely, aggregate, degrade, or fragment under given production, formulation, transportation, and/or storage conditions. "Stable" formulations maintain biological activity under given production, formulation, transportation and/or storage conditions. The stability of the antibody, can be assessed by measuring the degree of aggregation, degradation or fragmentation and the like of the formulation by techniques such as SEC-HPLC, IEC-HPLC, CE-SDS, etc. In some embodiments, the main antibody peak in a stable formulation does not decrease more than 5 % after storing at room temperature for at least 3 months.

It should be noted that in the present invention, where a buffer or buffer system is included in a formulation, it is also meant that the buffer is included in the formulation and the buffer system is formed in the formulation by the buffer. A buffer is typically a combination of a weak acid or a weak base and its salt. For example, a histidine buffer (also called a histidine salt buffer) is typically formed by histidine and one or more of its salts, such as histidine hydrochloride, an acetate buffer is typically formed by acetic acid and one or more of its salts, such as sodium acetate. Unless otherwise specified, regardless of whether the buffer is in the form of acid and/or salt, the molar concentration of the buffer includes both the free acid/base and the salts.

In one embodiment of the invention, the concentration of the monoclonal antibody in the antibody formulation is about 120-300 mg/mL. In some embodiments, the concentration of the monoclonal antibody in the antibody formulation is about 130-280 mg/mL. In some embodiments, the concentration of the monoclonal antibody in the antibody formulation is about 140-250 mg/mL. In some embodiments, the concentration of the monoclonal antibody in the antibody formulation is about 160-200 mg/mL. In some embodiments, the concentration of the monoclonal antibody in the antibody formulation is about 180 mg/mL. In some embodiments, the buffer system is selected from histidine (His) buffer, citrate (CB) buffer, sodium

acetate (NaAC) buffer, and succinic acid (Sua) buffer. In some embodiments, the buffer system is histidine buffer. In some embodiments, buffer system is sodium acetate buffer.

In some embodiments, the stabilizer comprises arginine hydrochloride, proline, lysine hydrochloride, glycine, histidine, or sodium glutamate. The addition of the stabilizers further decreases the viscosity of the formulation. In some embodiments, the stabilizer is arginine hydrochloride.

In some embodiments, the surfactant is polysorbate-80.

Preferred formulations were selected by selecting buffers and viscosity reducing agents. In some embodiments, the formulation consists essentially of: about 180 mg/mL humanized anti-IL-6 receptor antibody, about 10 mM histidine salt buffer, about 0.5 mg/mL polysorbate-80, about 90 mM arginine hydrochloride, about 20 mg/mL sucrose, water for injection, with pH 5.8-6.0, and viscosity 8-12 cP. In some embodiments, the formulation consists essentially of: about 180 mg/mL humanized anti-IL-6 receptor antibody, about 10 mM sodium acetate buffer solution, about 0.5 mg/mL polysorbate-80, about 90 mM arginine hydrochloride, about 20 mg/mL trehalose dihydrate, water for injection, with pH 5.8-6.0, and viscosity 8-12 cP. In some embodiments, the formulation consists essentially of: about 180 mg/mL humanized anti-IL-6 receptor antibody, about 10 mM sodium acetate buffer solution, about 0.5 mg/mL polysorbate-80, about 120 mM arginine hydrochloride, water for injection, with pH 5.8-6.0, and viscosity 8-12 cP.

In some embodiments, the formulation is Formulation 1. In some embodiments, the formulation is Formulation 2. In some embodiments, the formulation is Formulation 3.

According to the evaluation of the stability of the above formulations, the antibody formulations of the present invention can remain stable for at least 3 months at room temperature, and remain stable after at least 3 freeze-thaw cycles.

The liquid formulations containing the monoclonal antibodies provided by the invention provide formulation combinations capable of stably storing the active ingredients.

Table 1 shows the ingredients of Formulations 1, 2, 3 and 4:

Table 1.Formulation Ingredient List

Ingredient	Formulation 1	Formulation 2	Formulation 3	Formulation 4
BAT1806	180 mg/mL	180 mg/mL	180 mg/mL	180 mg/mL
Sodium acetate buffer	10 mM	10 mM	-	-
Histidine hydrochloride buffer	-	-	10 mM	20 mM

Arginine hydrochloride salt	90 mM	120 mM	90 mM	100 mM
Methionine	-	-	-	30 mM
Trehalose dihydrate	20 mg/mL	-	-	-
Sucrose	-	-	20 mg/mL	-
Polysorbate-80	0.5 mg/mL	0.5 mg/mL	0.5 mg/mL	0.5 mg/mL
pH	5.82	5.81	6.10	6.06

The present invention provides formulation comprising a high concentration antibody for protecting monoclonal antibody, which may comprise about 120-300 mg/mL antibody, about 5-20 mM histidine salt buffer, about 0.25-1 mg/mL polysorbate-80, and a stabilizer selected from the group of about 70-200 mM arginine hydrochloride with/without combination of about 10-50 mg/mL sucrose, or about 10-50 mg/mL trehalose dihydrate, with a pH of about 5.0-7.0. In some embodiments, the formulation comprises about 180 mg/mL antibody, about 10 mM histidine salt buffer, about 0.5 mg/mL polysorbate-80, about 90 mM arginine hydrochloride, about 20 mg/mL sucrose, with pH 5.6-6.4. In some embodiments, the formulation comprises about 180 mg/mL antibody, about 10 mM sodium acetate buffer, about 0.5 mg/mL polysorbate-80, about 90 mM arginine hydrochloride, about 20 mg/mL trehalose dihydrate, with pH 5.6-6.4. In some embodiments, the formulation comprises about 180 mg/mL antibody, about 10 mM sodium acetate buffer, about 0.5 mg/mL polysorbate-80, about 120 mM arginine hydrochloride, with pH 5.6-6.4.

The technical solution of the present invention is further illustrated by the following specific examples, which are not intended to limit the scope of the present invention. Other non-essential modifications and adaptations of the invention according to the inventive concept are within the scope of the invention.

It should be noted that in the present invention, the “mass to volume ratio” is the ratio of the mass of the ingredients to the volume of the formulation.

Antibody Sample

The BAT1806 antibody is a humanized anti-interleukin 6 receptor antibody prepared through antibody preparation technology. A CHO cell line capable of stably expressing BAT1806 was constructed, and the supernatant was collected and purified through PROTEIN A.

Example 1. Formulation Preparation

The antibody formulations can be prepared by a method that comprises the steps of:

As an exemplary example, Formulation 3 was prepared as follows:

(a) The following amounts of ingredients were measured: 0.31 g histidine, 1.68 g histidine hydrochloride, 189.6 g arginine hydrochloride, 200 g sucrose, 0.5 g polysorbate-80.

The above measured ingredients were dissolved in water for injection to obtain 1 L solution having 10 mM histidine buffer, 900 mM arginine hydrochloride, 200 mg/mL sucrose, and 0.5 mg/mL polysorbate-80, the sequence of adding the ingredients of the formulation does not affect the quality of the formulation, and can be flexibly selected. The solution prepared in (a) was then filtered through a hydrophilic polyvinylidene fluoride filter membrane of 0.22 μm pore size into an aseptic container.

(b) A buffer solution having 10 mM histidine buffer and a surfactant solution having 100 mg/mL polysorbate-80 were prepared by dissolving a desired amount of histidine, histidine hydrochloride or polysorbate-80 in water for injection.

(c) An antibody concentrate containing a total of 1800 g antibody was thawed in a water bath (room temperature) followed by buffer exchange with the buffer solution prepared in (b) and concentration by ultrafiltration through 30 KD polyether sulfone ultrafiltration membrane (Millipore, C9MA73422) to obtain 9L high concentration antibody solution (200 mg/mL) with histidine buffer (10 mM).

(d) To the antibody solution obtained in (c) was added the surfactant solution (100 mg/mL polysorbate-80) prepared in (b) such that the surfactant concentration was 0.5 mg/mL.

(e) To 9 L of the antibody solution obtained in (d) was added 1 L of the solution obtained in (a) with stirring to obtain the liquid Formulation 3, which was packaged for use in vials or pre-filled syringes.

The skilled artisan will appreciate that the weight, weight to volume ratio, volume to volume ratio referred to herein may be converted to moles and/or molar concentrations using well-known molecular weights of the ingredients. The skilled artisan will also appreciate that the weight of the ingredients may be proportionally adjusted when different formulation volumes are required. For example, 16 L, 14 L, 12 L, 10 L, 5 L of the formulation comprises 1.6, 1.4, 1.2, 1.0, 0.5 times of the cited weights, respectively.

The methods to prepare the other formulations are similar to the method to prepare Formulation 3, and the amounts of the ingredients are adjusted accordingly.

Example 2. Analytic Methods

The following methods were used to analyze the formulations disclosed herein.

SEC-HPLC analysis method:

A sample of a formulation being tested was diluted with water to an antibody concentration of 5 mg/mL and

tested by SEC-HPLC. The chromatographic column was TSK-GEL G3000SWXL 7.8×300 mM, 5 μm (TOSOH). The mobile phase was 200 mM K3PO4 with 250 mM KCl (pH 7.0). The UV detection wavelength was set at 280nm, the column temperature was 30 °C, the loading quantity of sample was 40 μL (200 μg protein), and the flow was kept for 35 minutes at a rate of 0.5mL/min. The chromatography was recorded and after integration, the percentage of monomer and aggregate in the sample solution was calculated by area normalization method.

IEC-HPLC analysis method:

A sample of a formulation being tested was diluted with water to an antibody concentration of 5mg/mL and tested by IEC-HPLC. Chromatographic conditions: column, TSK-GEL CM-STAT® 4.6×100 mM, 7 μm (TOSOH); mobile phase, mobile phase A (20 mM ACES, pH 8.0) and mobile phase B (20 mM ACES+200 mM NaCl, pH 8.0); the loading quantity of sample was 50 μg; the UV detection wavelength was 280 nm; gradient elution was performed according to the elution gradient given below for 45 minutes. The chromatography was recorded. And after integration, the percentage of the main peak, the acidic region and the basic region was respectively calculated using a peak area normalization method. (The chromatography was manually integrated, a base line was drawn at a place where the base line was relatively flat, the integration starting time and the integration ending time were about 8 minutes before and after the retention time of the main peak, vertical lines were drawn at two peak valleys around the main peak, the acidic region was before the main peak that was followed by the basic region (the main peak was followed by basic peak 1, basic peak 2 and basic peak 3 in sequence), and a vertical line was drawn at each peak valley of the basic region.)

Elution Gradients

Time (min)	Mobile Phase A	Mobile Phase B	Flow Rate (mL/min)
0	90%	10%	0.6
5	90%	10%	0.6
35	70%	30%	0.6
36	0%	100%	1.0
39	0%	100%	1.0
40	90%	10%	0.6
44	90%	10%	0.6

Viscosity Determination:

Return the sample to 25°C normal temperature, and then measure it with a viscometer (Brookfield, DV2T).

About 1.2-1.5mL of the sample is slowly injected into the cone (to avoid bubbles), spread evenly, and then tested on the viscometer at a temperature of 25±2℃. The torque is between 40%-70%, and the rotation is about 7-9 times for data recording.

Turbidity Determination:

Return the sample to room temperature and use an ultraviolet spectrophotometer (Eppendorf, BioPhotometer plus) for detection. Adjust the UV spectrophotometer to 340nm, calibrate it with water, and perform sample detection only when the calibration result is 0. Rinse the disposable cuvette with 300μl sample during the test, and then slowly pour the 300μl sample into the disposable cuvette to avoid air bubbles during the process, and put the cuvette into the UV spectrophotometer for data recording.

Example 3. Buffer Selection

Studies on antibody stability were performed using a variety of buffer systems.

Based on the nature of the antibody, the inventors had empirically determined a number of buffer systems:

histidine salt buffer (His)

citrate buffer (CB)

sodium acetate buffer (NaAc)

succinic acid buffer (Sua)

Table 2 shows the types of buffer and the pH for formulations containing 180 mg/mL BAT1806, 90 mM Arg-HCl, 20 mg/mL sucrose, 0.5mg/mL polysorbate-80, and 10 mM buffer.

Table 2. The Type of Buffer and the pH Value

No.	1	2	3	4
Type of buffer	His	NaAc	CB	Sua
pH	5.93	5.80	5.83	5.84

The monomer purity (SEC-HPLC, abbreviated SEC) and charge isomer (IEC-HPLC, abbreviated IEC) of the formulations at a high temperature of 40 °C for 4 weeks (W) or at the condition of light (4500 ± 500 Lx) for 10 days (d) was investigated. The results are shown in Table 3 and Table 4.

Table 3. Formulation Buffer Selection at High Temperature (40 °C)

Sample	SEC-HPLC（%）			IEC-HPLC（%）		
	Aggregation	Main peak	Fragment	Acidic peak	Main peak	Basic peak
1-His-0d	1.31	98.62	0.07	24.28	71.74	3.99
2-NaAc-0d	1.74	98.18	0.08	24.25	71.75	3.99
3-CB-0d	2.32	97.61	0.07	24.41	71.77	3.82
4-Sua-0d	1.87	98.06	0.07	24.20	71.95	3.85
1-His-1W	1.69	98.06	0.25	-		-
2-NaAc-1W	1.66	98.05	0.29	-	-	-
3-CB-1W	2.46	97.27	0.27	-	-	-
4-Sua-1W	2.14	97.59	0.26	-	-	-
1-His-2W	1.48	96.77	1.75	31.27	63.45	5.29
2-NaAc-2W	1.57	96.67	1.77	33.51	61.32	5.16
3-CB-2W	1.34	97.13	1.53	34.15	60.92	4.93
4-Sua-2W	1.45	96.83	1.72	34.03	61.00	4.97
1-His-3W	1.58	96.64	1.78	-		-
2-NaAc-3W	1.71	96.34	1.95	-	-	-
3-CB-3W	2.52	95.81	1.67	-	-	-
4-Sua-3W	2.21	95.93	1.86	-	-	-
1-His-4W	1.90	95.71	2.39	38.64	55.63	5.73
2-NaAc-4W	1.86	95.70	2.44	42.59	51.86	5.55
3-CB-4W	2.80	95.10	2.10	45.59	49.44	4.97
4-Sua-4W	2.49	95.17	2.34	43.93	50.76	5.31

Table 4. Formulation Buffer Selection at the condition of light (4500 ± 500 Lx)

Sample	SEC-HPLC（%）			IEC-HPLC（%）		
	Aggregation	Main peak	Fragment	Acidic peak	Main peak	Basic peak
1-His-0d	1.31	98.62	0.07	24.28	71.74	3.99
2-NaAc-0d	1.74	98.18	0.08	24.25	71.75	3.99
3-CB-0d	2.32	97.61	0.07	24.41	71.77	3.82
4-Sua-0d	1.87	98.06	0.07	24.20	71.95	3.85
1-His-5d	8.65	91.18	0.17	27.79	67.05	5.16
2-NaAc-5d	6.82	93.02	0.16	24.56	70.82	4.62
3-CB-5d	6.74	93.14	0.12	25.20	69.83	4.97
4-Sua-5d	8.29	91.61	0.10	25.14	69.70	5.15
1-His-10d	11.91	86.58	1.51	29.37	58.20	12.43
2-NaAc-10d	9.75	88.59	1.65	24.59	63.81	11.60
3-CB-10d	8.83	89.84	1.34	25.84	61.87	12.29
4-Sua-10d	9.64	89.03	1.32	24.53	62.55	12.92

Example 4. pH Selection

Studies on antibody stability were performed using a variety of pH value.

In this study, the formulations of high concentration antibody contained 180 mg/mL antibody (BAT1806), 90 mM Arg-HCl, 20 mg/mL sucrose, 0.5 mg/mL polysorbate-80, and 10 mM histidine salt buffer, and pH was adjusted to 5.4, 5.6, 5.8, 6.0, 6.2, 6.4 or 6.6 by HCl or NaOH.

The monomer purity (SEC-HPLC, abbreviated SEC) and charge isomer (IEC-HPLC, abbreviated IEC) of the formulations at a high temperature 40 °C for 4 weeks or at a light test (4500 ± 500 Lx) for 10 days was investigated. The results are shown in Table 5 and Table 6.

Table 5. Formulation Buffer Selection at High Temperature (40 °C)

Sample	SEC-HPLC (%)			IEC-HPLC (%)			CE-SDS(NR) (%)			
	Aggreg-ation	Main peak	Frag-ment	Acidic peak	Main peak	Basic peak	Frag-ment	HHL	Main peak	Aggreg-ation
1-pH5.4-0d	1.13	98.79	0.08	24.41	71.75	3.84	2.25	1.58	97.75	0
2-pH5.6-0d	1.48	98.47	0.05	24.44	71.65	3.90	2.21	1.56	97.79	0
3-pH5.8-0d	1.62	98.34	0.04	24.83	71.15	4.02	2.22	1.59	97.78	0
4-pH6.0-0d	1.74	98.22	0.05	25.12	71.03	3.84	2.33	1.69	97.67	0
5-pH6.2-0d	1.74	98.19	0.07	24.95	71.16	3.90	2.47	1.64	97.53	0
6-pH6.4-0d	1.62	98.30	0.08	25.28	70.87	3.84	2.38	1.66	97.62	0
7-pH6.6-0d	1.42	98.54	0.04	25.23	70.91	3.86	2.25	1.57	97.75	0
1-pH5.4-1W	1.78	98.02	0.20	-	-	-	-	-	-	-
2-pH5.6-1W	1.59	98.11	0.30	-	-	-	-	-	-	-
3-pH5.8-1W	1.80	98.04	0.16	-	-	-	-	-	-	-
4-pH6.0-1W	1.51	98.22	0.23	-	-	-	-	-	-	-
5-pH6.2-1W	1.89	97.83	0.28	-	-	-	-	-	-	-
6-pH6.4-1W	1.86	97.96	0.18	-	-	-	-	-	-	-
7-pH6.6-1W	1.92	97.89	0.18	-	-	-	-	-	-	-
1-pH5.4-2W	1.42	96.55	2.02	31.83	61.77	6.40	4.04	1.79	95.96	0
2-pH5.6-2W	1.45	96.78	1.77	31.59	62.64	5.77	4.14	1.91	95.86	0
3-pH5.8-2W	1.50	96.74	1.75	31.53	63.14	5.34	3.66	1.96	96.34	0
4-pH6.0-2W	1.57	96.45	1.99	31.61	63.34	5.05	3.56	1.87	96.44	0
5-pH6.2-2W	1.64	96.45	1.91	32.48	62.85	4.67	3.50	1.94	96.50	0
6-pH6.4-2W	1.71	96.27	2.02	33.38	62.24	4.38	3.64	2.08	96.36	0
7-pH6.6-2W	1.79	96.27	1.93	34.78	61.21	4.00	3.54	1.86	96.46	0
1-pH5.4-3W	1.52	95.99	2.49	-	-	-				
2-pH5.6-3W	1.61	96.04	2.35	-	-	-	-	-	-	-
3-pH5.8-3W	1.59	96.14	2.26	-	-	-	-	-	-	-
4-pH6.0-3W	1.67	95.98	2.34	-	-	-	-	-	-	-
5-pH6.2-3W	1.83	95.87	2.31	-	-	-	-	-	-	-
6-pH6.4-3W	1.91	95.36	2.73	-	-	-	-	-	-	-
7-pH6.6-3W	2.04	95.20	2.76	-	-	-	-	-	-	-

1-pH5.4-4W	1.64	95.86	2.50	38.28	53.50	8.22	4.60	1.90	95.21	0.19
2-pH5.6-4W	1.70	96.07	2.23	37.96	54.89	7.14	4.52	1.94	95.28	0.20
3-pH5.8-4W	1.75	96.10	2.15	37.82	55.89	6.29	5.85	2.14	94.49	0.25
4-pH6.0-4W	1.81	96.05	2.14	38.27	56.05	5.68	5.80	2.14	94.56	0.26
5-pH6.2-4W	1.98	95.86	2.16	39.68	55.32	4.99	6.06	2.23	94.37	0.27
6-pH6.4-4W	2.05	95.63	2.32	41.00	54.30	4.70	6.12	2.12	94.17	0.39
7-pH6.6-4W	2.20	95.44	2.35	42.99	52.82	4.19	6.41	2.03	93.80	0.59

Table 6. Formulation Buffer Selection at the condition of light (4500 ± 500 Lx)

Sample	SEC-HPLC（%）			IEC-HPLC（%）		
	Aggregation	Main peak	Fragment	Acidic peak	Main peak	Basic peak
1-pH5.4-0d	1.13	98.79	0.08	24.41	71.75	3.84
2-pH5.6-0d	1.48	98.47	0.05	24.44	71.65	3.90
3-pH5.8-0d	1.62	98.34	0.04	24.83	71.15	4.02
4-pH6.0-0d	1.74	98.22	0.05	25.12	71.03	3.84
5-pH6.2-0d	1.74	98.19	0.07	24.95	71.16	3.90
6-pH6.4-0d	1.62	98.30	0.08	25.28	70.87	3.84
7-pH6.6-0d	1.42	98.54	0.04	25.23	70.91	3.86
1-pH5.4-5d	4.67	95.23	0.09	25.27	70.14	4.59
2-pH5.6-5d	5.98	93.92	0.11	26.19	69.00	4.81
3-pH5.8-5d	7.74	92.09	0.16	27.71	67.68	4.61
4-pH6.0-5d	8.34	91.48	0.19	28.70	66.94	4.37
5-pH6.2-5d	9.54	90.28	0.18	29.81	66.03	4.17
6-pH6.4-5d	8.49	91.31	0.20	30.29	65.69	4.02
7-pH6.6-5d	9.86	89.93	0.21	31.73	64.61	3.67
1-pH5.4-10d	7.09	92.71	0.20	27.28	66.57	6.15
2-pH5.6-10d	8.87	90.91	0.22	28.51	65.37	6.12
3-pH5.8-10d	10.49	89.27	0.23	30.39	63.90	5.71
4-pH6.0-10d	10.77	88.95	0.27	31.06	63.42	5.52
5-pH6.2-10d	14.70	85.03	0.28	35.32	58.99	5.69
6-pH6.4-10d	12.63	87.08	0.29	35.15	60.19	4.65
7-pH6.6-10d	15.59	84.07	0.35	38.10	57.45	4.46

The results of both SEC and IEC at high temperature or light condition show that the pH in the range of 5.4-6.4 could play a good buffer protection role as time went by. And the optimum pH range is 5.6-6.4. When the samples exposed to light, the lower pH of the buffer is, the better the stability of the antibody is. Considering the actual situation that the product can be stored in dark, the pH of the sample in the range of 5.4-6.4 is chosen to protect the antibody. The next experiments are carried out in the pH value 5.8.

Example 5. Viscosity Reducing Agents Selection

The viscosity of solutions containing a series of concentration antibody (102 mg/mL, 158.9 mg/mL, 181 mg/mL, 189.7 mg/mL, and 215 mg/mL) and 10 mM sodium acetate buffer at pH 5.8 were determined. The relationship between antibody concentration and solution viscosity is shown in Fig. 1. As can be seen from the results, the viscosity increases with the increase of antibody concentration. At the concentration of about 180 mg/mL, the viscosity is about 15 cP.

In this study, the formulation contained high concentration antibody (BAT1806), viscosity reducing agent(s), 0.5 mg/mL polysorbate-80, and 20 mM histidine salt buffer, pH 5.87. The antibody concentration, the viscosity reducing agent(s) and the viscosity are shown in Table 7.

Table 7. Viscosity results of solutions with different formulation

Sample	**Viscosity Reducing Agent(s)**	Antibody Concentration (mg/mL)	Viscosity (cP)
1	100 mM Arginine hydrochloride +30 mM methionine	202	16.58
2	100 mM Arginine hydrochloride	204.6	17.81
3	100 mM proline	201.7	19.41
4	100 mM lysine hydrochloride	200	20.40
5	100 mM glycine	203	19.54
6	100 mM histidine	188	21.07
7	100 mM sodium glutamate.	187	19.05
8	-	205.8	27.15

According to the data shown in Table 7, at 25 °C, arginine hydrochloride and the combination of arginine hydrochloride and methionine reduced the viscosity of the solution most, followed by proline, lysine hydrochloride and glycine. In the same time, the viscosity reducing agent, arginine hydrochloride, played an important role of protecting the antibody stability. It can be described as stabilizer either.

Example 6. Formulation Characteristics

Pharmaceutical Formulations 1, 2, 3, and 4 were formulated according to the method of Example 1. Actemra® (Formulation 5) was manufactured by Genentech (B1095B01).

The protein T_m (half denaturation temperature) was determined by multifunctional protein stability analysis system (UNcle, Unchained Labs) through the detection method of full spectrum of fluorescence, static light scattering and dynamic light scattering. The turbidity of the formulation was determined according to the method of Example 2 under the optical density (OD) 340 nm. And the viscosity of formulation was determined. The results are shown in Table 8.

Table 8. Formulation Characteristics

Formulation	T_m (°C)	Turbidity (OD340nm)	Viscosity (cP)
1	69.4	0.426	10.38
2	68.9	0.429	10.16
3	69.6	0.444	10.01
4	68.7	0.458	10.14
5	68.8	-	-

The T_m results show that Formulation 3 had a highest T_m value, indicating that the protein would be more stable in Formulation 3. Compared with Formulation 4, even though Formulation 3 comprised an amount of sucrose and did not comprise methionine, the viscosity of Formulation 3 was the lowest and lower than that of Formulation 4. Formulations 1, 2 and 3 had lower turbidity compared with Formulation 4. The size, turbidity and viscosity of all formulations are acceptable.

Example 7. Freeze-thaw Studies

BAT1806 antibody Formulation 1, Formulation 2, Formulation 3, Formulation 4 with an antibody concentration of about 180 mg/mL were prepared as described in Example 1. For Formulations 1, 2, 3, 4, three freeze-thaw cycles were repeated under -60 °C to 25 °C. The transparency, turbidity, SEC, IEC were observed.

Transparency was observed under the light. All the formulations are clear and transparent with a slight opalescence after 0, 1 or 3 freeze-thaw cycles.

The turbidity was measured as described in Example 4. The results are shown in Table 9. The turbidity of Formulation 4 had an increasing tend with the increase of freeze-thaw cycles, while the turbidities of other formulations did not increase.

Table 9. Turbidity after Freeze-thawing

Formulation	Freeze-thaw Cycles		
	0	1	3
1	0.426	0.425	0.425
2	0.429	0.426	0.427
3	0.444	0.440	0.442
4	0.458	0.461	0.463

SEC and IEC were measured as described in Example 2. And the results are shown in Table 10.

Table 10. SEC and IEC after Freeze-thawing

Formulation	Cycles	SEC-HPLC（%）			IEC-HPLC（%）		
		Aggregation	Main peak	Fragment	Acidic peak	Main peak	Basic peak
1	0	1.22	98.66	0.11	25.71	69.71	4.58
2	0	0.95	98.97	0.08	25.87	69.72	4.41
3	0	1.21	98.69	0.10	25.59	70.04	4.37
4	0	1.11	98.79	0.10	26.05	69.72	4.23
1	1	1.17	98.73	0.10	25.84	69.59	4.57
2	1	1.20	98.73	0.07	25.76	69.54	4.70
3	1	1.11	98.81	0.08	26.14	69.55	4.32
4	1	0.95	98.97	0.08	25.90	69.79	4.31
1	3	1.19	98.70	0.10	25.95	69.47	4.58
2	3	1.20	98.70	0.10	25.91	69.43	4.66
3	3	1.11	98.79	0.10	26.21	69.52	4.27
4	3	0.95	98.93	0.12	26.19	69.42	4.39

The results showed that although the antibody formulations thawed from -60 °C and were subjected to three freeze-thaw cycles, the SEC and IEC were not obviously changed, indicating that the samples were stable in the repeated freeze-thaw test, no precipitate was generated, the protein did not adhere to the freeze-thaw containers.

Example 8. Stability Studies under the Acceleration Conditions

Pharmaceutical Formulations 1, 2, 3, and 4 were formulated according to the method of Example 1. Actemra® (Formulation 5) was manufactured by Genentech (B1095B01). The five formulations were placed in a biochemical incubator for 3 months under the condition of (25±2) °C and sampled at the end of the 0th (0M), 1st (1M), 2nd (2M), 3rd (3M) and 6th month (6M), respectively. The samples were examined with regard

to monomer purity (SEC-HPLC) and charge isomer.

The monomer purity (SEC-HPLC) and charge isomer (IEC-HPLC) were determined as described in Example 2. The results are shown in Table 11. It can be seen from Table 11 that under the acceleration conditions, the monomer purity decreased with time over 6 months, indicating that fragments were produced under the acceleration conditions, but the SEC main peak of the antibody decreased by no more than 3.22%; with respect to IEC-HPLC, under the condition of 25 °C, the content of IEC main peaks of antibody decreased, and the degradation trend was basically the same, and the IEC main peak decreased by no more than 11.38%; the CE-SDS-NR main peak decreased by no more than 3.16%. While Formulation 3 comprises lower arginine concentration with no methionine, it displayed similar stability by SEC-HPLC and IEC-HPLC, and higher stability measured by CE-SDS compared with Formulation 5.

Table 11. Stability under the Acceleration Conditions

Formulation-Time	SEC-HPLC（%）			IEC-HPLC（%）			CE-SDS(NR)（%）			
	Aggreg-ation	Main peak	Frag-ment	Acidic peak	Main peak	Basic peak	Frag-ment	HHL	Main peak	Aggreg-ation
1 - 0M	1.05	98.95	0	23.29	69.67	7.03	1.94	1.25	98.06	0
2 - 0M	0.99	99.01	0	23.27	69.42	7.31	1.96	1.22	98.04	0
3 - 0M	0.89	99.11	0	23.75	69.66	6.59	1.99	1.24	98.01	0
4 - 0M	0.92	99.08	0	23.28	69.51	7.22	1.98	1.25	98.02	0
5 - 0M	0.79	99.21	0	18.76	67.45	13.79	2.2	1.37	97.8	0
1 - 1M	1.08	98.8	0.12	25.63	69.45	4.92	2.65	1.55	97.35	0
2 - 1M	1.43	98.37	0.2	25.4	69.68	4.93	2.94	1.78	97.06	0
3 - 1M	1.02	98.88	0.11	25.32	69.93	4.76	2.94	1.83	97.06	0
4 - 1M	0.89	99.01	0.1	25.19	69.79	5.02	3.03	1.9	96.97	0
5 - 1M	0.64	99.21	0.15	19.71	67.56	12.73	2.52	1.52	97.48	0
1 - 2M	1.37	98.46	0.17	27.27	67.98	4.75	2.77	1.66	97.23	0
2 - 2M	1.39	98.44	0.18	27.16	68.1	4.74	2.94	1.69	97.06	0
3 - 2M	1.19	98.65	0.16	26.89	68.73	4.38	2.73	1.63	97.27	0
4 - 2M	1.01	98.84	0.15	26.81	68.61	4.58	2.89	1.71	97.11	0
5 - 2M	0.8	98.99	0.21	21.7	67.05	11.26	2.62	1.44	97.38	0
1 - 3M	1.38	97.2	1.42	29.94	65.34	4.72	3.01	1.54	96.99	0
2 - 3M	1.36	97.11	1.53	29.65	65.65	4.71	2.89	1.55	97.11	0
3 - 3M	1.13	97.41	1.47	29	66.71	4.29	2.81	1.49	97.19	0
4 - 3M	0.99	97.51	1.5	29.06	66.44	4.49	2.57	1.35	97.43	0
5 - 3M	0.78	97.57	1.65	23.89	65.38	10.73	2.55	1.29	97.45	0
1 - 6M	1.77	95.85	2.38	38.13	58.29	3.58	3.7	1.63	95.91	0.39
2 - 6M	1.74	95.79	2.47	38.05	58.50	3.45	3.76	1.68	95.83	0.41
3 - 6M	1.33	96.67	2	36.13	60.49	3.39	3.39	1.53	96.34	0.27
4 - 6M	1.16	96.84	2.01	36.11	60.5	3.38	3.62	1.78	96.2	0.18
5 - 6M	0.96	97	2.04	31.20	60.14	8.66	5.18	3.03	94.64	0.18

Example 9. Stability Studies under High Temperature and Light Conditions

Formulations 1, 2, 3 and 4 were subjected to high temperature and light studies to investigate the stability of the formulations under high temperature (40 °C for 4 weeks) and light conditions (4500 ± 500 Lx). The test method is described in the other examples.

The results under high temperature conditions are shown in Table 12. It can be seen from SEC that the monomer purity decreased at 40 °C for 4 weeks, indicating that aggregations and fragments were produced under the high temperature. The main peak of Formulation 3 and 4 decreased the least. Similarly, the main peak of Formulation 3 and 4 detected by IEC or CE-SDS decreased the least, too.

Table 12. Test Results under High Temperature Conditions

Formulation-Time	SEC-HPLC (%)			IEC-HPLC (%)			CE-SDS(NR) (%)			
	Aggregation	Main peak	Fragment	Acidic peak	Main peak	Basic peak	Fragment	HHL	Main peak	Aggregation
1 - 0W	1.05	98.95	0	23.29	69.67	7.03	1.94	1.25	98.06	0
2 - 0W	0.99	99.01	0	23.27	69.42	7.31	1.96	1.22	98.04	0
3 - 0W	0.89	99.11	0	23.75	69.66	6.59	1.99	1.24	98.01	0
4 - 0W	0.92	99.08	0	23.28	69.51	7.22	1.98	1.25	98.02	0
1 - 1W	1.23	98.45	0.32	30.4	63.65	5.96	2.79	1.52	97.21	0
2 - 1W	1.23	98.42	0.35	30.3	63.34	6.35	2.84	1.52	97.16	0
3 - 1W	1.11	98.6	0.29	29.02	64.77	6.2	2.64	1.43	97.36	0
4 - 1W	0.95	98.84	0.21	28.99	65.27	5.74	2.63	1.43	97.37	0
1 - 2W	1.58	96.34	2.07	35.35	58.71	5.93	4.14	1.91	95.86	0
2 - 2W	1.31	96.9	1.79	35.29	58.41	6.3	3.66	1.96	96.34	0
3 - 2W	1.13	97.34	1.53	33.32	60.58	6.11	3.56	1.87	96.44	0
4 - 2W	1.01	97.29	1.71	33.57	60.34	6.1	3.5	1.94	96.5	0
1 - 3W	1.79	96.01	2.2	38.33	55.21	6.46	5.44	1.98	94.56	0
2 - 3W	1.79	95.9	2.31	37.94	55.46	6.6	5.69	2.08	94.31	0
3 - 3W	1.36	96.67	1.97	34.36	59.49	6.15	4.75	1.85	95.25	0
4 - 3W	1.19	96.88	1.93	34.67	59.07	6.26	4.67	1.91	95.33	0
1 - 4W	2.03	95.19	2.78	43	50.52	6.49	6.23	2.23	93.77	0
2 - 4W	1.98	95.15	2.87	42.28	51.12	6.6	6.38	2.33	93.62	0
3 - 4W	1.43	96.29	2.28	38.26	55.77	5.97	5.52	1.95	94.48	0
4 - 4W	1.26	96.49	2.25	38.17	55.57	6.25	5.65	2.16	94.35	0

The results under the conditions of light are shown in Table 13. It can be seen from SEC that the monomer purity decreased under condition of light during 8 days, small amounts of aggregations and fragments were produced. According to the results of IEC, the acidic peak has increased while basic peak has decreased. The

main peak among the formulations was basically unchanged.

Table 13. Test Results under Light Conditions

Formulation-Time	SEC-HPLC（%）			IEC-HPLC（%）		
	Aggregation	Main peak	Fragment	Acidic peak	Main peak	Basic peak
1 - 0d	1.05	98.95	0.00	23.29	69.67	7.03
2 - 0d	0.99	99.01	0.00	23.27	69.42	7.31
3 - 0d	0.89	99.11	0.00	23.75	69.66	6.59
4 - 0d	0.92	99.08	0.00	23.28	69.51	7.22
1 - 4d	2.45	97.49	0.06	24.88	70.48	4.64
2 - 4d	3.08	96.84	0.08	24.31	71.22	4.47
3 - 4d	2.67	97.25	0.07	26.82	68.72	4.46
4 - 4d	1.61	98.32	0.07	26.58	68.67	4.75
1 - 8d	3.25	96.67	0.08	25.65	69.58	4.77
2 - 8d	3.36	96.54	0.10	25.64	69.49	4.86
3 - 8d	3.07	96.83	0.10	27.20	68.04	4.76
4 - 8d	2.41	97.48	0.11	27.21	67.90	4.89

The experiments showed that the antibody Formulations 1-4 all have certain stability under the conditions of high temperature and light.

Example 10. Stability Studies under Oscillation Condition

Experimental conditions: Formulation 1, 2, 3 and 4 were flatwise placed and oscillated at 200rpm at room temperature for 48h. The solution transparency, turbidity, SEC, IEC, were measured at regular intervals. The test methods are described with reference to other examples.

Transparency was observed under the light. All the formulations are clear and transparent with a slight opalescence after oscillation for 48 hours (h). No visible foreign matter appeared.

The turbidity was determined as described in Example 4. The results are shown in Table 14.

Table 14. Turbidity after Oscillation for 48 hours

Formulation	Turbidity (OD340nm)			
	0h	8h	24h	48h
1	0.426	0.422	0.422	0.424
2	0.429	0.424	0.425	0.427
3	0.444	0.443	0.445	0.440

4	0.458	0.461	0.461	0.463

The SEC and IEC were determined as described in Example 2. The results are shown in Table 15.

Table 15. SEC and IEC after Oscillation for 48 hours

Formulation-Time	SEC-HPLC（%）			IEC-HPLC（%）		
	Aggregation	Main peak	Fragment	Acidic peak	Main peak	Basic peak
1 - 0h	1.22	98.66	0.11	25.71	69.71	4.58
2 - 0h	0.95	98.97	0.08	25.87	69.72	4.41
3 - 0h	1.21	98.69	0.10	25.59	70.04	4.37
4 - 0h	1.11	98.79	0.10	26.05	69.72	4.23
1 - 48h	1.29	98.60	0.11	25.58	69.99	4.43
2 - 48h	1.29	98.60	0.11	25.49	70.16	4.35
3 - 48h	1.42	98.50	0.08	26.22	69.57	4.2
4 - 48h	1.04	98.89	0.08	26.18	69.46	4.36

From the data in the table above, it can be seen that, after 48 hours of oscillation, compared with the data at 0 hour, the SEC-HPLC purity, IEC-HPLC main peak content and other test items showed no obvious change either, and the activity was within an acceptable range. Similar to the results of the freeze-thaw studies, the turbidity of Formulations 1, 2, and 3 remained low while the turbidity of Formulation 4 increased by oscillation, indicating that Formulations 1, 2, and 3 had better stability in the oscillation test than Formulation 4.

Example 11. Amino Acid Sequence of the Humanized Anti-interleukin 6 Receptor Antibody BAT1806

The humanized anti-interleukin 6 receptor antibody BAT1806 for the treatment of IL-6 related diseases was expressed in CHO cells by known genetic engineering technology and obtained by purifying through a series of standard chromatographic steps. BAT1806 is an IgG antibody comprising two heavy chains and two light chains, with a molecular weight of 145 kDa; each heavy chain contains 449 amino acids as shown in Table 16, with a molecular weight of 53 kDa; each light chain contains 214 amino acids shown in Table 17, with a molecular weight of 24 kDa.

Table 16. Heavy Chain Amino Acid Sequence of BAT1806

SEQ ID NO.1	5`>QVQLQESGPGLVRPSQTLSLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYS GITTYNPSLKSRVTMLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYWGQ GSLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD

	GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 3`

Table 17. Light Chain Amino Acid Sequence of BAT1806

SEQ ID NO.2	5`>DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNWYQQKPGKAPKLLIYYTSRLH SGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQGNTLPYTFGQGTKVEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC <3`

Example 12. Expression and Purification of BAT1806

With reference to the method given by *Wood et al., J Immunol. 145:3011 (1990) et al.*, the humanized anti-interleukin 6 receptor antibody BAT1806 was expressed in CHO cells. The expression vector containing the antibody gene was constructed by a conventional molecular biology method (molecular cloning) using a derived cell line of CHO-K1 cells (ATCC CCL61) as a host cell for expression. The construction of a high yield stable cell line is briefly described as follows: host cells were grown in suspension in CD-CHO medium (Gibco, CA), the host cells in logarithmic growth phase were centrifuged, resuspended in fresh CD-CHO medium, the cells were counted and the cell density was adjusted to 1.43×10^7 cells/mL, 600ul of the above cell suspension was added to an electroporation cuvette, and then 40ug linearized plasmid was added, and pipetting was used to mix the cells with the plasmid uniformly. Electroshock conversion was performed using a Bio-rad electrometer with instrument parameters set as follows: capacitance: 960uFD, and voltage: 300 V. Typically the electric shock was performed for 15-20 milliseconds. The electrically shocked cells were immediately resuspended in 37 °C pre-heated CD-CHO medium, inoculated in a 96-well plate at 100ul per well, and 2-3 days later an equal amount of selection medium (CD-CHO media + 50 μM MSX) was added. The 96-well plate cell culture supernatant was analyzed to determine the level of antibody expression. Clones with higher expression levels were transferred from a 96-well plate to a 24-well plate, and after the cells were grown to a certain amount, the cells were transferred to a 6-well plate so that 2×10^5 cells were contained in 3 mL culture medium per well, and the antibody yield and yield of the cells were measured. 20-30 clones were transferred to shake flasks for further evaluation. The final 5-8 clones with the highest expression were subcloned and further tested for expression. The culture fluid was harvested, the cells were separated from the culture medium by low-speed centrifugation, and the supernatant of the centrifugation was further clarified by high-speed centrifugation. Affinity purification with protein A and ion exchange purification were performed.

Claims

1. An antibody formulation, characterized by comprising:

(1) an antibody: 120-300 mg/mL humanized anti-IL-6 receptor antibody;

(2) a buffer system comprising 5-30 mM histidine salt, or 5-30 mM sodium acetate;

(3) a surfactant: 0.1-1.0 mg/mL;

(4) a stabilizer: 70-200 mM;

(5) water for injection;

a pH of the antibody formulation being 5.0-7.0.

2. The antibody formulation according to claim 1, wherein the concentration of the humanized anti-IL-6 receptor antibody is 160-200 mg/mL.

3. The antibody formulation according to claim 1, wherein the concentration of the humanized anti-IL-6 receptor antibody is 180 mg/mL.

4. The antibody formulation according to any one of claims 1 to 3, wherein the pH of the antibody formulation is 5.5-6.5.

5. The antibody formulation according to any one of claims 1 to 3, wherein the pH of the antibody formulation is 5.6-6.4.

6. The antibody formulation according to any one of claims 1 to 5, wherein the antibody comprises two heavy chains and two light chains, wherein the heavy chain comprises an amino acid sequence shown in SEQ ID NO. 1, and the light chain comprises an amino acid sequence shown in SEQ ID NO. 2.

7. The antibody formulation according to any one of claims 1 to 6 having a viscosity of from 0 to 20 cP.

8. The antibody formulation according to any one of claims 1 to 7, having a viscosity of from 5 to 15 cP.

9. The antibody formulation according to any one of claims 1 to 8, characterized in that, the stabilizer is selected from arginine hydrochloride, or a combination of arginine hydrochloride and sucrose, or a combination of arginine hydrochloride and trehalose dihydrate.

10. The antibody formulation according to any one of claims 1 to 9, wherein the stabilizer is selected from a combination of 70-200 mM arginine hydrochloride and 10-30 mg/mL sucrose; a combination of 70-200 mM arginine hydrochloride and 10-30 mg/mL trehalose dihydrate; and 70-200 mM arginine hydrochloride.

11. The antibody formulation according to any one of claims 1 to 10, wherein the surfactant is

polysorbate-80.

12. The antibody formulation according to any one of claims 1 to 10, wherein the surfactant is 0.1-0.7 mg/mL polysorbate-80.

13. An antibody formulation comprising:

(1) 160-200 mg/mL humanized anti-IL-6 receptor antibody;

(2) 8-15 mM histidine salt buffer solution;

(3) 0.45-0.65 mg/mL polysorbate-80;

(4) 80-100 mM arginine hydrochloride;

(5) 15-25 mg/mL sucrose;

(6) water for injection;

with a pH of 5.6-6.4;

or comprising:

(1) 160-200 mg/mL humanized anti-IL-6 receptor antibody;

(2) 8-15 mM sodium acetate buffer solution;

(3) 0.45-0.65 mg/mL polysorbate-80;

(4) 80-100 mM arginine hydrochloride;

(5) 15-25 mg/mL trehalose dihydrate;

(6) water for injection;

with a pH of 5.6-6.4;

or comprising:

(1) 160-200 mg/mL humanized anti-IL-6 receptor antibody;

(2) 8-15 mM sodium acetate buffer solution;

(3) 0.45-0.65 mg/mL polysorbate-80;

(4) 110-130 mM arginine hydrochloride;

(5) water for injection;

with a pH of 5.6-6.4.

14. An antibody formulation comprising:

(1) 180 mg/mL humanized anti-IL-6 receptor antibody;

(2) 10 mM histidine salt buffer;

(3) 0.5 mg/mL polysorbate-80;

(4) 90 mM arginine hydrochloride;

(5) 20 mg/mL sucrose;

(6) water for injection;

with a pH of 5.6-6.4;

or comprising:

(1) 180 mg/mL humanized anti-IL-6 receptor antibody;

(2) 10 mM sodium acetate buffer;

(3) 0.5 mg/mL polysorbate-80;

(4) 90 mM arginine hydrochloride;

(5) 20 mg/mL trehalose dihydrate;

(6) water for injection;

with a pH of 5.6-6.4;

or comprising:

(1) 180 mg/mL humanized anti-IL-6 receptor antibody;

(2) 10 mM sodium acetate buffer;

(3) 0.5 mg/mL polysorbate-80;

(4) 120 mM arginine hydrochloride;

(5) water for injection;

with a pH of 5.6-6.4.

15. The antibody formulation according to any one of claims 1 to 14, characterized in that, the antibody formulation is a subcutaneous injection formulation.

16. The antibody formulation according to any one of claims 1 to 15, characterized in that, the formulation remains stable for at least 3 months at room temperature.

17. The antibody formulation according to any one of claims 1 to 16, wherein the formulation remains stable after at least 3 freeze-thaw cycles.

18. The antibody formulation according to any one of claims 1 to 17, wherein the antibody formulation is a pharmaceutical formulation for treating IL-6 related diseases.

19. The antibody formulation according to claim 18, wherein the IL-6 related diseases is selected from rheumatoid arthritis, systemic juvenile idiopathic arthritis, polyarticular juvenile idiopathic arthritis, giant cell arteritis, giant lymph node hyperplasia, cytokine storms caused by immunotherapy, Cytokine release syndrome adult Still's disease, recurrent polychondritis, type II diabetes, ankylosing spondylitis, thyroid-associated ophthalmopathy, cardiovascular disease caused by rheumatoid arthritis, polymyalgia rheumatica, acute graft-versus-host disease, non-ST-segment elevation myocardial infarction, systemic lupus erythematosus, schizophrenia, uveitis, ovarian cancer, anti-neutrophil cytoplasmic antibody-associated vasculitis, neuromyelitis optica, chronic glomerulonephritis, and colorectal cancer.

20. The antibody formulation according to claim 18, wherein the IL-6 related diseases is selected from

rheumatoid arthritis, systemic juvenile idiopathic arthritis, polyarticular juvenile idiopathic arthritis, giant cell arteritis, cytokine release syndrome,and giant lymph node hyperplasia.

21. A method to prepare the antibody formulation according to any one of claims 1 to 17, comprising the steps of:

(1) dissolving the buffer, stabilizer and surfactant in water for injection to form a solution;

(2) adding surfactant into an antibody solution; and

(3) mixing the solution prepared in the step (1) with the antibody surfactant solution prepared in the step (2) to provide the formulation.

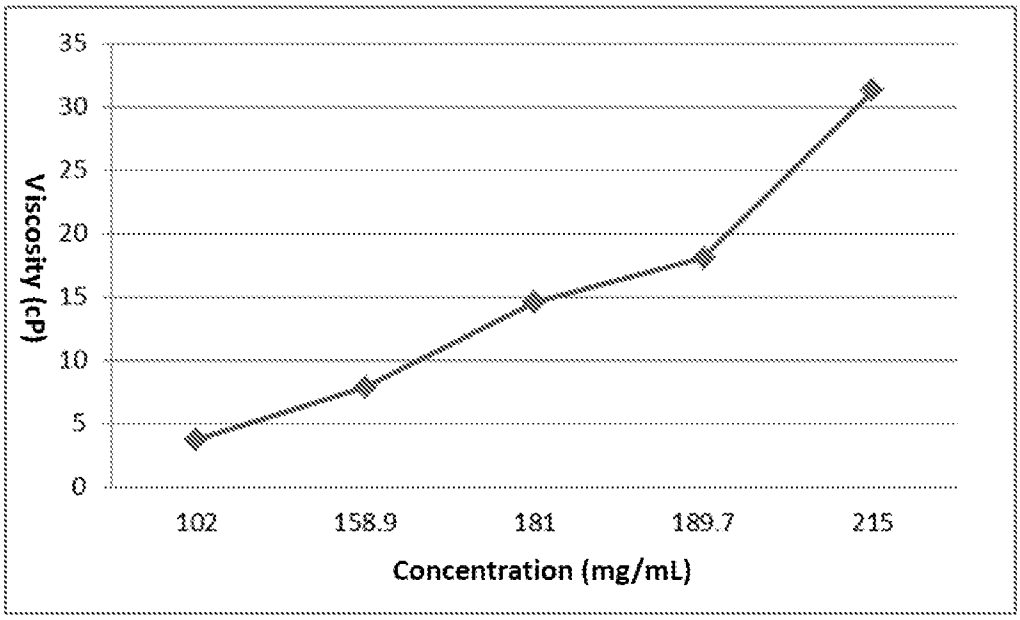
35
30
25
20
15
10
5
0
Viscosity (cP)
102
158.9
181
189.7
215
Concentration (mg/mL)

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/113241

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i; A61K 9/08(2006.01)i; A61K 47/18(2006.01)i; A61P 19/02(2006.01)i; A61P 29/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K;A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI, VEN, SIPOABS, CNABS, CNTXT, USTXT, EPTXT, WOTXT,CNKI, PubMed, ISI web of Knowledge, GenBank, EBI, STN:IL-6, antibody, formulation, buffer, surfactant,stabilizer, histidine salt, sodium acetate, sucrose, arginine hydrochloride, trehalose dihydrate, stable, 200mg,180mg,150mg,SEQ ID NO:1-2.bio thera solutions

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018060210 A1 (ARES TRADING S.A.) 05 April 2018 (2018-04-05) claims 1-11, description pages 7-13	1-5,15-21
Y	WO 2018060210 A1 (ARES TRADING S.A.) 05 April 2018 (2018-04-05) claims 1-11, description pages 7-13	6-21
Y	CN 109350740 A (BIO-THERA SOLUTIONS, LTD.) 19 February 2019 (2019-02-19) claims 1-10	6-21
Y	CN 111110842 A (BIO-THERA SOLUTIONS, LTD.) 08 May 2020 (2020-05-08) claims 1-10	1-21
Y	WO 2017147293 A1 (ELEVEN BIOTHERAPEUTICS, INC.) 31 August 2017 (2017-08-31) claims 1-65	1-21
A	WO 2017201731 A1 (BEIJING VDJBIO CO., LTD.) 30 November 2017 (2017-11-30) the whole document	1-21
A	WO 2017202387 A1 (BEIJING VDJBIO CO., LTD.) 30 November 2017 (2017-11-30) the whole document	1-21

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2021	**18 November 2021**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **LI,Shuting** Telephone No. **86-(10)-53961957**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/113241

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018218215 A1 (THE JOHNS HOPKINS UNIVERSITY) 29 November 2018 (2018-11-29) the whole document	1-21
A	SUN, Y.et al. "The effects of interleukin-6 neutralizing antibodies on symptoms of depressed mood and anhedonia in patients with rheumatoid arthritis and multicentric Castleman's disease." *BRAIN, BEHAVIOR, AND IMMUNITY.*, Vol. 66, 01 July 2017 (2017-07-01), pages 156-164	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/113241

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2021/113241

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2018060210	A1	05 April 2018	RU	2019112680	A	29 October 2020
				CN	109862880	A	07 June 2019
				US	2019218300	A1	18 July 2019
				RU	2019112680	A3	15 December 2020
				US	10961314	B2	30 March 2021
				ZA	201901590	B	28 October 2020
				BR	112019005328	A2	18 June 2019
				AU	2017333367	A1	04 April 2019
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				KR	20190062440	A	05 June 2019
CN	109350740	A	19 February 2019	IL	274813	D0	30 July 2020
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				KR	20200093628	A	05 August 2020
				AU	2018374232	A1	23 July 2020
				CA	3083971	A1	06 June 2019
				BR	112020010761	A2	24 November 2020
				WO	2019105450	A1	06 June 2019
				CN	111110842	B	11 December 2020
				CN	111110842	A	08 May 2020
CN	111110842	A	08 May 2020	IL	274813	D0	30 July 2020
				EP	3718531	A1	07 October 2020
				JP	2021504482	A	15 February 2021
				KR	20200093628	A	05 August 2020
				AU	2018374232	A1	23 July 2020
				CN	109350740	A	19 February 2019
				CA	3083971	A1	06 June 2019
				BR	112020010761	A2	24 November 2020
				WO	2019105450	A1	06 June 2019
				CN	111110842	B	11 December 2020
WO	2017147293	A1	31 August 2017	CA	3012350	A1	31 August 2017
				JP	2019510078	A	11 April 2019
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				KR	20180116359	A	24 October 2018
				EP	3419599	A4	11 September 2019
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				BR	112018017031	A2	22 January 2019
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				EP	3419599	A1	02 January 2019
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				WO	2017202387	A1	30 November 2017
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				KR	20190015380	A	13 February 2019

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2021/113241

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
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		EP 3464346 A4	22 January 2020
		CN 109563155 A	02 April 2019
WO 2017202387 A1	30 November 2017	EP 3464346 A1	10 April 2019
		WO 2017201731 A1	30 November 2017
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		JP 2019523653 A	29 August 2019
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		EP 3464346 A4	22 January 2020
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		IL 270901 D0	30 January 2020
		CA 3064869 A1	29 November 2018
		EP 3630148 A4	16 June 2021
		EP 3630148 A1	08 April 2020
		JP 2020521759 A	27 July 2020
		US 2020140547 A1	07 May 2020