INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/715, 35/12, 35/60

(11) International Publication Number: WO 96/32117

(43) International Publication Date: 17 October 1996 (17.10.96)

(21) International Application Number: PCT/RO96/00003

(22) International Filing Date: 14 March 1996 (14.03.96)

(30) Priority Data:
95-00699 11 April 1995 (11.04.95) RO


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Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: BIOACTIVE CONCENTRATE, ITS PRODUCING METHOD AND CERTAIN DRUG COMPOSITIONS CONTAINING ALSO CHONDROTIN SULPHATE

(57) Abstract

This bioactive concentrate is an active substance - aqueous solution or lyophilized powder - consisting of anti-hyaluronidase and antiinflammatory mucopolysaccharide polymers and an addition of restitutive and anti-hyaluronidase chondroitin sulphate, which has a pH = 4-6 and anti-hyaluronidase activity. The bioactive concentrate producing method consists in a two-phased treatment of animal-originated connective cartilaginous tissues such as bovine and sheep trachea, umbilical cord, young animal tendons, bowels, testicles or sea organisms, with phenol solution and the solution resulting is concentrated by vacuum evaporation and then defatted; after filtering, the supernatant obtained is treated for deproteinization, the ion excess is removed by passing through ion exchanger column, the alcohol solution is concentrated to remove alcohol until reaching a volume of 70% as against the aqueous solution subject to proteinization. Drug compositions contain the bioactive concentrate - solution or in lyophilized state - associated with synergic substances such as: heparin, sodium or lysin acetylsalicylate, ascorbic acid, vitamin E, "T"-like structured water, benzyl alcohol, propylene glycol, plant extracts (Achillea, Calendula, Matricaria, Plantago, Hypericum) and usual excipients, being conditioned in the form of intramuscular or intraarticular injections, ointment, gel and suppositories. Finally, the histamines are removed and chondroitin sulphate is added up to the concentration required.
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TECHNICAL FIELD

This invention relates to a bioactive concentrate, its producing method and drug compositions for treatment of degenerative diseases, rheumatism, arthropaties and other related ones.

BACKGROUND ART

A complex of anti-hyaluronidase substances has been derived from natural produces, such as small fish, containing amino acids, whole glucides, glycogen, chondroitinsulphate, phenol, hypotensive substances, with an anti-hyaluronidase activity of minimum 50 I.U./ml.

The method whereby the complex is produced consists in a two-phased treatment of sea fish with 0.5 % phenol solution, over a period of 24 h, the extracts brought together are vacuum-concentrated at maximum 40 °C, then they are treated with an equal ethanol 96° volume, the precipitate is removed and the supernatant achieved is celite-treated, filtered, vacuum-concentrated at maximum 40° C, then extraction solvent - treated and after solvent removing, it is phenol-treated up to a value of 4.5-5.5 %.
Drug compositions containing an anti-hyaluronidase bioactive extract, phenol and sodium metabisulphite, have been developed too.

These drugs rely on a complex of substances, natural active principles, associated in proportions close to the normal, functional ones, produced by extraction from animal tissues or biosynthesised in cell and microorganism cultures, the role of this complex being to replace, to substitute the function or even to allow recovery of the naturally existing complex in connective tissues, that had been unbalanced for various reasons and caused the disease.

The extraction and conditioning processes have been established so that losses and degradation of active substances should be as low as possible and when the ratio of raw material constituents is different from that one required to treat disease in human being, it may be set off by a proper compounding of extracts or by treatment schedule.

Now, the disease-inducing mechanisms are known to rely on a disequilibrium in the biosynthesis and breakdown of normal constituents on which the proper movement of joints depends.

Cells in the tissues of the living body are embedded and evolve within ground substance. This ground substance - a general compound of living structures-pervades every interspace and isolates every stationary cell from its neighbours. Variations in the composition of the extracellular environment exert a profound influence on cell behaviour and in turn the cells possess a powerful means of modifying their immediate environment.

The intercellular substance is a complex gel, containing water, electrolytes, metabolites, dissolved gases, enzymes, trace elements, fats, proteins, carbohydrates. This substance is rendered highly viscous by an abundance of certain long-chain acid mucopolysaccharide polymers, particularly glycosaminoglycans and the related proteoglycans, reinforced at the microscopic level by a three-dimensional network of collagen fibrils
The role of synovial fluid, found in all joints, is not only to lubricate the moving structures but also to dissipate the energy. This function is performed by the synovial fluid composition where the glycosaminoglycans (GAC) and proteoglycans (PG) such as hyaluronic acid and chondroitinsulphate inducing a high viscosity with their spiral structure, behave viscous-like when the moving frequencies are low and elastic-like when the moving frequencies are high (Ogston, A.G. and Stanier, J.E. (1953) - J. Physiol, 199, 124).


At different ages, the synovial fluid behaves similarly as it concerns viscosity, but the energy taking over is different at high moving frequencies, the synovial fluid in young people taking over 77% of the energy while in the old it takes over only 52% of the energy by elastic storage.

The synovial fluid in affected joints (osteoarthrosis) is viscous, but not elastic. The stiffness of this synovial fluid is 7 times lower than that one in the healthy old people. The hyaluronic acid concentration in these fluids is low, resulting in low viscosity indices and the protein concentration is high. The negative influence of concentration change may lower in some cases, the viscosity index up to 30 times.

Also, in joints, tendons, vitreous body of the eye - the fluid area (synovial fluid, vitreous fluid) is adjacent to the solid matrix (articular cartilage, tendon, fascia, vitreous body).

The viscous-elastic macromolecular compound - the hyaluronic acid - in the fluid matrix, penetrates the solid matrix surface. Rheologically, the major
difference between the two matrices is the presence of collagen fibrils in the solid matrix and their absence in the fluid one.

The surface layer of 1-2 μ consists of hyaluronic acid and proteins.

This layer can be removed or destroyed by the action of hyaluronidase, found in excess in the intercellular matrix.

The sublayer of 10-15 μ in thickness contains typical collagen fibrils, and the interfibrillar space is filled with approximately equal amounts of hyaluronic acid and chondroitinsulphate.

On the basis of the data known from the specialized field (Cameron, E and Pauling, L. - The Encyclopaedia of Ignorance, p. 377-385, Pergamon Press, Oxford, 1982 and Balasz, E.A. and Sweeney, D.B. - 1968 - New and Controversial Aspects of Retinal Detachement, p. 371, Ed. McPerson, Harpen and Row, New York), this invention aimed at producing a complex of ingredients in which the mucopolysaccharide polymers and proteoglycans take part under the form of hyaluronic acid and chondroitinsulphate, as well as their fragments, heparin and ascorbic acid, all of them being substances certified by recent investigations, contributing to restore the normal functions in the connective tissues, in the case of degenerative diseases. These diseases, altering the ground substance where the cells are embedded, deteriorates the relationship existing between it and these cells. The condition for a "normal" balance is for each part - the ground substance and the cell - to keep its own functions, since any change, as for instance the reduction in ground substance viscosity by hyaluronic acid depolymerizing through the action of hyaluronidase secreted in excess by cells, is followed by disease occurrence.

Another alternative to restore the balance is the association of an antienzyme called anti-hyaluronidase (hyaluronidase is after all, an enzyme group with the same functions as the connective structures), that stops or limits the hyaluronic acid depolymerization, alongside chondroitinsulphate, heparin and ascorbic acid.
Some attempts have been already known, to prepare drugs based on anti-hyaluronidase which is extracted from its containing sources, such as: bovine trachea, bovine and sheep testicles, umbilical cord, small sea fish etc.

Although important at that time (Patent RO/82273-1982 and Technological Reports drawn up by the Institute of Chemical and Pharmaceutical Research -Bucharest, No. 84740/1977 and No. 5160/1979), these attempts represented an elementary alternative solving only partially the question of both producing method and the composition of the drugs achieved.

DISCLOSURE OF INVENTION

According to this invention, the bioactive concentrate is an active substance - aqueous solution 10 % consisting of 7-9 % anti-hyaluronidase and antiinflammatory mucopolysaccharide polymers and an addition of up to 1-3 % restitutive and anti-hyaluronidase chondroitinsulphate, which has a pH = 4-6 and an anti-hyaluronidase activity of 450-750 I.U./ml, is yellowish to light brown; in a lyophilized state it contains at least 93 % active substance consisting of 65-84 % mucopolysaccharide polymers and 9-28 % chondroitinsulphate, is yellowish to light brown and contains usual conservants. The producing method for bioactive concentrate consists in the two-phased treatment of animal - originated cartilaginous connective tissues such as bovine and sheep trachea, umbilical cord, young animal tendons, bowels, testicles or sea organisms, with acid aqueous solution containing 0.5% phenol, pH = 2-4, in the first phase in a ratio of 1:3.5-4 and in the second phase in a ratio of 1:1.5, in both phases the extraction takes a period of 24 h, stirring for 30 minutes every 3 h, at a temperature between 0-4°C, and then the extract obtained in the first phase is combined with that one resulted from the second phase and adjusted to a pH = 4.5-5 % with Ca(OH)₂ solution, is allowed to
settle at 0-4°C for 8 hours, then filtered and the solution achieved is concentrated by vacuum evaporation at a maximum temperature of 45 °C until a concentration ratio of 7:1 is obtained, thereafter the solution is passed through the ion-weak anionites exchanger column and afterwards it is extraction solvent-defatted in a ratio of 1:4 in 6-10 cycles until the remanent lipids should not exceed 1-5 %. The last defatting cycle is performed with n-hexan in the same ratio, then the aqueous solution resulted is vacuum concentrated until reaching a volume of 70 % of the original solution; after filtering, the supernatant achieved is treated with 96°C ethanol for deproteinization until reaching an ethanol concentration of 50 %, then left in a cool place at 0-5 °C for 12 h, afterwards filtered and the ethanol solution concentrated to remove the alcohol until reaching a volume of 70 % as against the aqueous solution subject to deproteinization, the protein precipitate is gradient treated with ammonium sulphate and the low molecular weight protein fraction is combined with the aqueous solution from where the alcohol and the histaminic impurities were removed by successively treating of final solution with trisodium phosphate until reaching a pH = 8.5, then filtered and the solution achieved is passed through the ion-weak cationite exchanger column - pH value reverts to 4-6 and then chondroitinsulphate is added to a concentration of 1-3 %. The raw material can be in fresh, frozen or preserved state. Passage of the solution through weak cationites is performed at a flow not exceeding, per hour, 1/5 of the original solution volume.

Drug compositions contain the bioactive concentrate - solution or in lyophilized state, associated with synergic substances such as: heparin, sodium or lysin acetylsalicylate, ascorbic acid, vitamin E, "I"-like structured water, benzyl alcohol, propylene glycocoll, plant extracts (Achillea, Calendula, Matricaria, Plantago, Hypericum) and usual excipients, being conditioned in the form of intramuscular injections, intraarticular ones, ointment, gel and suppositories.
For intramuscular administration, the composition includes in 1 ml of aqueous solution, 0.1 ml bioactive concentrate solution 10 % or 11 mg lyophilized bioactive concentrate, maximum 5 mg phenol and 2 mg sodium metabisulphite, has a pH = 4-6 and an anti-hyaluronidase activity of minimum 45 I.U./ml. For intraarticular administration, the composition includes, in 1 ml of aqueous solution, 15 mg/ml dry active substance consisting of bioactive concentrate solution 10 % or 16.5 mg lyophilized bioactive concentrate, 10 mg sodium or lysin acetylsalicylate, 2 mg ascorbic acid, 0.2 mg heparin, 20 mg benzyl alcohol, 40 mg propylene glycocoll, has a pH = 4-6, an anti-
hyaluronidase activity of minimum 65 I.U./ml and the administering dose is 2 ml; other types of conservants can be used too, such as 2 mg sodium metabisulphite, 5 mg phenol, provided that the properties of active substances or the final product quality should not be altered. For conditioning in the form of ointment, gel or suppositories, the composition includes, in 100 g, 3-10 g dry substance consisting of the bioactive concentrate solution 10 % or 4-12 g lyophilized bioactive concentrate, 0.3-0.5 g sodium or lysin acetylsalicylate, 0.2-0.4 g vitamin E, 0.4-0.8 g ascorbic acid preferably 0.5 g, 0.2 g heparin, 2-5 g plant whole extract, emulsified in a cream or gel base containing 40-75 g "I"-like structured water, preferably 65g, usual conservants and flavours.

Plants used can be: Achillea millefolium, Calendula Officinalis, Matricaria Chamomilla, Plantago sp, Hypericum Perforatum. "I"-like structurated water is a water having an enhanced energetic potential, a high transmembrane penetrating capacity and a profound antiinflammatory and antiinfectious effect (Patent RO No. 109835 B.1/1995, authors: Manzatu Ioan and Ionita-Manzatu Vasile).

The solution proposed in this invention solves some of the drawbacks found in the cited works, since this is a key process, achieving a highly efficient extraction of anti-hyaluronidase alongside chondroitinsulphate, in a ratio similar to the existing one in the ground substance, therefore in joints too.
If the raw material wherefrom the extraction is made lacks the needed proportion of chondroitinsulphate, the final product is added this complex obtained from other raw material by a process of intensive utilization.

In this way, the following drugs are achieved: injectable solution as such or consisting of lyophilized powder for intramuscular administration, injectable solution as such or consisting of lyophilized powder for intraarticular administration, an ointment and gel for external, local use and suppositories for anal administration.

These drugs are used according to a treatment schedule that allows some synergetic effects to be obtained, which result in enhancing the action of each constituent, individually.

By its action, the bioactive concentrate prevents disorganization of the normally constituted macromolecular structure and stimulates the tissue and periarticular restructuring processes in inflammatory diseases at interstitial and periarticular level, as well as at the articular cartilage level, positively influences the calcium ion dynamics in antiinflammatory, regenerative, biostimulative, dermo- and tissue-restitutive effects and stimulates the metabolism of convalescent patients.

All the five drug compositions appeal to the disease cause by restoring the cellular and intercellular mechanisms at the same time with stopping the hyaluronidase excess and starting the tissue recovery process. They are easy to administrate and have no side effects.

The producing method can be used on an industrial scale, with low energy consumption and recovery of substances used.

The extraction process proposed, besides the fact that it is highly efficient, in the first phase, at a pH = 2-4, eliminates many drawbacks found, since selective purifying phases are included, such as histamine removal, a required operation since some batches of previous products had inadequate results for histamine test, leading to their throwing away for their hypotensive
effects. Also, ion exchanger deionization was included to reduce the high ion load following repeated concentration operations.

In the end, the most important process included in the extraction flow is the achievement of a parallel extraction flow for refuse recovery, thus a higher content of amino acids and chondroitinsulphate being provided, which has not occurred in the previously cited works.

Ultimately, the addition of heparin and ascorbic acid makes it possible to also achieve an injectable solution, intended to intraarticular use as well as the appropriate ointment, gel and suppositories.

BEST MODE OF CARRYING OUT THE INVENTION

Four examples of the invention accomplishment are given below:

Example 1

To achieve bioactive concentrate, the raw material subject to the extraction process consists of both the animal - originated cartilaginous, connective tissues - bovine and sheep trachea, umbilical cord, young animal tendons - and bowels, testicles or small sea fish. The raw material is the one to start with, that is small sea fish (anchovy), in an amount of 20 kg, in a fresh, frozen or preserved and desalted state, which after defreezing or desalting is chopped in sizes of 2-6 mm and the extraction is made at a temperature of 3-5°C, with 75 l aqueous solution containing 0.5 % phenol and having a pH = 2-4.

The pH value is adjusted using N (normal) HCl solution, in the phenol solution, before adding chopped raw material.

The first extraction is made in suitable glass or enamelled cast iron tanks, intermittently stirring at 90-120 rot./min. for 30 minutes, to stir every 3
hours, for 24 hours. The solution obtained is separated by centrifugation using as filtering material a fabric of more than 100 stitches/cm² and in the second extraction, with 30 l phenol solution 0.5% with a pH = 2-4 adjusted like above and the operation takes another 24 hours, stirring intermittently too.

The extracts I and II are combined and filtered using craped paper (industrial filter) and the sea paste is a refuse.

The solution extracted and filtered in this way is adjusted to a pH = 5 using Ca (OH)₂ solution, being then allowed to settle for 8 hours, at a temperature of 0-4°C.

Filtering is repeated with industrial filter in order to remove the precipitate and implicitly the chlorine ion surplus.

The filtrate, in a volume of about 95 l, is concentrated by vacuum evaporation at an internal temperature of maximum 45°C. The concentration ratio is 7:1, thus 13.5 l of a brownish - yellow suspension concentrate being achieved.

After concentrating, the pH value is controlled, which should be ranged between 4-6 and some of ions are removed by passing through a ion-weak anionite exhanger column.

The solution is passed through the ion exchanger column at a rate of about 2 l/h so that when out of the column it should have a pH = 4-6.

This solution resulted is extraction solvent-defatted, in repeated cycles, using 3 l solvent for each passage, the duration of stirring and resting process being 2 hours. This process is repeated 6-10 times and the efficiency is controlled so that finally, the remanent lipids should not exceed 1-5 %.

The lipid extract in extraction solvent is separated by settling, in suitable containers and then it is distilled for solvent recovery in a ratio of 70-75 %. Defatting can also be made with dichlorethane, ethylene trichloride or n-hexan. It is preferably for the last defatting cycle to be made with n-hexan using about 3 kg of this solvent.
The defatted aqueous solution is vacuum concentrated, to about 9 l to remove the solvent traces.

The supernatant is filtered by industrial paper filter, to remove the precipitate formed during concentration and then it is treated with ethyl alcohol, for deproteinization.

In the filtered supernatant solution 96°C ethyl alcohol is added, finely jetting under stirring until an alcohol concentration of 50 % is reached. Then it is left in a cool place at 0-5°C for 12 hours. The precipitate formed is filter-centrifugated through a fabric of minimum 100 stitches/cm², at 3,000 rot./min.

The residue found on the centrifuge filter is washed using about 3 l alcohol solution 50 %. The alcohol solutions obtained in this way are combined and vacuum concentrated to recover the alcohol. About 7 l aqueous solution containing antihyaluronidase active ingredients is finally achieved.

The proteic precipitate, achieved in the defatting phase is fractioned with ammonium sulphate and the low molecule proteic fractions resulted from the first phase of deproteinization are combined with the concentrated solution resulting from the first phase of deproteinization. This solution is purified to be free of histaminic impurities, is treated with 350 g trisodium phosphate, 200 g calcium chloride and about 300 ml sodium hydroxide solution 20 %. The trisodium phosphate and calcium chloride are added in the form of aqueous solution 30 %. The sodium hydroxide solution is added to adjust the pH value to 8.5 and it is left for 24 hours at 0-5°C, then it is filter-centrifugated through a fabric of 100 stitches/cm² and the solution is retained. The about 9 l of the solution obtained in this way are passed through an ion-weak cationite exchanger, which are regenerated using 0.5 % sodium hydroxide solution. The ion exchanger column is treated with 0.5 % phenolate water before solution passage. The operation is repeated until reaching the admissible degree of histaminic test.
The resulted solution is filtered. Chondroitinsulphate is added up, if necessary to reach a concentration of 1-3 %. About 9 l concentrated solution of about 10 % active substance, with a pH = 4-6 is achieved.

The concentrated solution is analysed, the dry substance and hyaluronidase activity are determined.

Five drugs can be produced from this concentrated solution or lyophilized powder by dilution with distilled, apyrogenic water, namely: injectable solution for intramuscular administration, injectable solution for intraarticular administration, ointment or gel for local application or suppositories.

**Example 2**

The drug composition for intramuscular administration is achieved by diluting the concentrated solution or lyophilized powder with apyrogenic water, containing maximum 5 mg/ml phenol and maximum 2 mg/ml sodium metabisulphite. The dilution ratio is established so that the injectable solution should contain, in 1 ml aqueous solution, 0.1 ml bioactive concentrate solution 10 % or 11 mg lyophilized bioactive concentrate and have an antihyaluronidase activity of minimum 45 I.U./ml.

The solution is first filtered through filters with a porosity of 0.45 μ for clarification and then through filters of 0.22 μ for sterilization.

They are aseptically packed into brown vials of 1 ml. These standard vials of 1 ml contain: 10 mg/ml active substance, consisting of 7-9 mg antihyaluronidase and anti-inflammatory mucopolysaccharide polymers and prevalently reconstitutive chondroitinsulphate added up to 1-3 mg, usual conservants and it has a pH = 4-6.

If the lyophilized bioactive concentrate is used, in vials of 10 ml containing 110 mg lyophilized bioactive concentrate and a proper conservant:
up to 25 mg, this vial is equivalent to 10 standard vials, after dissolving in distilled water.

Example 3

The drug composition for intraarticular administration is obtained by diluting the concentrated solution or lyophilized powder with apyrogenic water, which contains 20 mg/ml benzyl alcohol and 40 mg/ml propylene glycol (Rote Liste - 1993, Publishing House Cantor Aulendorf/Wurttemberg, Germany). As active, synergic substances 10 mg/ml sodium or lysine acetylsalicylate, 2 mg/ml ascorbic acid and 0.2 mg/ml heparin are added.

The dilution ratio of the concentrated solution or lyophilized powder is established so that the injectable solution should have a concentration of 1.5% active substance and an anti-hyaluronidase activity of minimum 65 I.U./ml.

The solution intended to be packed into vials is first filtered through filters with a porosity of 0.45 μ for clarification, and then through filters of 0.22 μ for sterilization and thereafter it is aseptically packed into brown vials of 2 ml.

These vials contain 15 mg/ml active substance consisting of 10-13 mg/ml anti-hyaluronidase and antiinflammatory mucopolysaccharide polymers, reconstitutive chondroitinsulphate added up to 2-5 mg/ml, 2 mg/ml ascorbic acid and 0.2 mg/ml heparin and a pH = 4-6.

Another conditioning may be performed using 0.5 % aqueous phenol solution and 0.2 % sodium metabisulphite.
Example 4

The drug composition for external, local administration - ointment, gel or suppositories - is achieved in a base of ointments, gel or suppositories, at 100g: 3-10 g dry active substance consisting of 10 % bioactive concentrate solution or 4-12g lyophilized bioactive concentrate, containing 7-9 g anti-hyaluronidase and anti-inflammatory mucopolysaccharide polymers, chondroitinsulfate added up to 1-3 g, 0.4 g vitamin E, 0.5 g ascorbic acid, 0.5g sodium or lysin acetylsalicylate, 0.2 g heparin, 2-5 g plant whole extracts (Achillea millefolium, Calendula officinalis, Matricaria chamomilla, Plantago sp., Hypericum perforatum). It is worth mentioning that the base of ointment, gel or suppositories is prepared using 60-75 % "I"-like structured water. The ointment base also contains usual conservants and flavours. The "I"-like structured water is the object of the patent no. RO 109835 B, and it is a water of an enhanced energetic potential, a high transmembrane penetrating capacity and a profound anti-inflammatory and antiinfectious action. This water is achieved by passing the filtered tap water through activating electromagnetic fields.

INDUSTRIAL APPLICABILITY

These five drugs are used as part of a treatment schedule including the administering schedule for the five or, in some cases, only two drugs recommended by the physician from case to case.
CLAIMS

1. Bioactive concentrate characterized in that it is an aqueous solution with 10% active substance, consisting of 7-9 % anti-hyaluronidase and anti-inflammatory mucopolysaccharide polymers and an addition of up to 1-3 % restitutive and anti-hyaluronidase chondroitinsulphate, has a pH value ranged between 4-6, an antihyaluronidase activity ranged between 450-750 I.U./ml and it is yellowish - to light brown and in a lyophilized state it contains minimum 93 % active substance consisting of 65-84 % mucopolysaccharide polymers and 9-28 % chondroitinsulphate, it is yellowish - to light brown and contains conventional conservants.

2. Producing method for bioactive concentrate by a two-phased treatment, over a period of 24 hours, of an organic raw-material with 0.5 % phenol solution, the vacuum concentration of the combined extracts 96°C ethanol deproteinization, defatting by means of an organic solvent such as the extraction solvent, characterized in that the animal-originated cartilaginous, connective tissues- bovine and sheep trachea, umbilical cord, young animal tendons - as well as bowels, testicles or sea organisms are subject to a two-phased treatment with acid aqueous solution that contains 0.5% phenol and has a pH = 2-4 in the first phase in a ratio of 1: 3.5-4, and in the second one, in a ratio of 1: 1.5, in both phases extraction takes 24 hours, stirring for 30 min. every 3 hours at a temperature of 0-4 °C and afterwards the extract resulted in the first phase is combined with that obtained in the second phase, they are adjusted to a pH = 4.5-5 with Ca(OH)₂ solution, being then allowed to settle for 8 hours, at a temperature of 0-4 °C, thereafter they are filtered and the resulted solution is concentrated by vacuum evaporation at an internal temperature of maximum 45 °C, until reaching a concentration ratio of 7:1; the solution is passed through a ion-weak anionite exchanger column and then it is
defatted using extraction solvent in a ratio of 1:4; in 6-10 cycles, until the remanent lipids should not exceed 1-5 %, the last defatting cycle is achieved with n-hexan in the same ratio, then the aqueous solution obtained is vacuum-concentrated, until reaching a volume of 70 % of the original solution; after filtering, the supernatant obtained is treated with 96°C ethyl alcohol for deproteinization, until an alcohol concentration of 50 % is reached, then it is left at 0-5 °C for 12 hours, and afterwards filtered and the alcohol solution is concentrated for the alcohol to be removed, until reaching a volume of 70 % as against the solution subject to deproteinization, the proteic precipitate is gradient-treated with ammonium sulphate and the low molecular weight proteic fraction is combined with the aqueous solution wherefrom the alcohol was removed; the histaminic impurities are removed by successive treatment of final solution with trisodium phosphate solutions, calcium chloride and sodium hydroxide until reaching a pH = 8.5, then filtered and the solution resulted is passed through the ion-weak cationite exchanger column, the pH value being adjusted back to 4-6 and then chondroitinsulphate is added up to a concentration of 1-3 %.

3. Process according to Claim 2, characterized in that the raw material used can be in fresh, frozen or preserved state and it is chopped before using.

4. Process according to Claim 2, characterized in that ,the solution is passed through the ion-weak cationite exchanger column, at a flow that should not exceed 1/5 an hour of the original solution volume, so that the pH value returns to a range between 4-6.

5. Drug compositions, characterized in that they contain the bioactive concentrate described in Claim 1, associated with synergic substances, such as heparin, ascorbic acid, sodium or lysin acetylsaliclylate, vitamin E, benzyl alcohol, propylene glycocoll, "I"-like structured water and plant extracts and with usual substances for their conditioning in the form of intramuscular injections, intraarticular ones, ointment, gel and suppositories.
6. Drug composition containing an anti-hyaluronidase bioactive complex, phenol and sodium metabisulphite characterized in that for intramuscular administration, 1 ml aqueous solution contains 0.1 ml bioactive concentrate solution 10% or 11 mg lyophilized bioactive concentrate, described in Claim 1, maximum 5 mg phenol and 2 mg sodium metabisulphite, has a pH = 4-6 and an anti-hyaluronidase activity of minimum 45 I.U./ml.

7. Drug composition characterized in that, for intraarticular administration, contains in 1 ml aqueous solution, 15 mg/ml dry active substance consisting of bioactive concentrate solution 10% or 16.5 mg lyophilized bioactive concentrate, described in the Claim 1, 10 mg sodium or lysin acetylsalicylate, 2 mg ascorbic acid, 0.2 mg heparin, 20 mg benzyl alcohol, 40 mg propylene glycol, has a pH = 4-6, an anti-hyaluronidase activity of minimum 65 I.U./ml and can be also associated with other types of conservants, such as maximum 2 mg sodium metabisulphite and maximum 5 mg phenol.

8. Drug composition characterized in that, for its conditioning as ointment, gel or suppositories, contains in 100 g, 3-10 g dry active substance consisting of bioactive concentrate solution 10% or 4-12 g lyophilized bioactive concentrate, described in the Claim 1, 0.3-0.5 g sodium or lysin acetylsalicylate, 0.4-0.8 g ascorbic acid preferably 0.5 g, 0.2-0.4 g vitamin E, 0.2 g heparin, 2-5 g plant whole extract, emulsified in a base of cream or gel containing 40-75 % "I"-like structured water, preferably 65 %, usual conservants and flavours.

9. Composition according to the Claim 8, characterized in that the plants used can be: Achillea millefolium, Calendula officinalis, Matricaria chamomilla, Plantago sp., Hypericum perforatum.

10. Composition according to the Claim 8, characterized in that the "I"-like structured water is a water having an increased energy potential and a high transmembrane penetrating capacity and also a profoundly antiinflammatory and antiinfectious action.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/715 A61K35/12 A61K35/60

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)

IPC 6 A61K

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 practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DATABASE WPI Section Ch, Week 8408 Dertwent Publications Ltd., London, GB; Class B04, AN 84-046876 XPD02011275 &amp; RO,A,82 273 (BIOFARM INTR MEDICAMENTE), 30 August 1983 see abstract</td>
<td>1-10</td>
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</tbody>
</table>

Further documents are listed in the continuation of box C.

| Patent family members are listed in annex. |

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document 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 21 August 1996

Date of mailing of the international search report 29.08.96

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Form PCT/ISA/310 (second sheet) (July 1992)