

The subject of the present invention are stable, non-immunogenic, physiologically readily compatible, dissolved or lyophilised galenical preparations of human proteins, as well as processes for their preparation, especially of erythropoietin.

Human proteins are proteins characteristic of the body only occurring in small amounts, such as e.g. tissue plasminogen activator (t-PA), streptokinase, urokinase, interferon, various colony stimulating factors (CSF) or erythropoietin (EPO). The present invention is to be explained in more detail using the example of EPO which is preferably used in the formulation.

Erythropoietin (EPO) is a glycoprotein which stimulates the formation of haemoglobin or erythrocytes in the bone marrow. This lipoprotein is mainly formed in the kidneys, is found in very small amounts in the serum and, under physiological conditions, is partly excreted in the urine.

The absence of EPO in the case of kidney insufficiency causes a renal anaemia. By the administration of EPO in physiological amounts, i.e. a few micrograms, in one or more dosings, in such cases the formation of erythrocytes can again be stimulated. Since the body already reacts sensitively to small dosage changes, the dosing must be exactly reproducible. EPO is usually injected either i.m. or i.v. as aqueous solution or is administered as a spray via the nasal mucous membrane.

However, it is known that EPO, namely, not only the product first obtained from human urine (Mijake et al., J. Biol. Chem., Vol. 25, 5558-5564, 1977) but also the product produced in the meantime gene technologically (WO 85-02610), are not stable in aqueous solution and, even in the case of a storage at -80°C , comparatively large activity losses occur. These two

known products differ somewhat in the glycosilation pattern and in the activity, a direct comparison with the EPO contained in the serum hitherto not being known.

5 These activity losses are to be attributed, on the one hand, to a destruction of the EPO by catalytic effects of the surface of the ampoules serving for the storage due to traces of heavy metals, atmospheric oxygen etc. but also, on the other hand, due to a
10 deposition of the EPO molecules on the vessel wall, whereby a partial denaturing can also take place. Since, as stated above, only a few micrograms are present in each dosage unit, the losses due to adsorption can be considerable, even after a short storage time.

15 Therefore, in EP-A 178,576 there has been described the inhibition of this deposition on the vessel wall by addition of polymeric compounds, such as human or bovine serum albumin, lecithin, dextran, cellulose, polyethylene glycol etc. and thus the achievement of a
20 finding again of EPO of 75 to 98% after about 2 h storage at 20°C, compared with only 16% without such addition. However, there was thereby only measured the finding again of a radio-active labelling (¹⁴C) so that these experiments say nothing about the stabilisation
25 of the EPO against decomposition.

 However, according to our findings, a long-term stabilisation with such agents is not to be achieved, i.e. the EPO effectiveness in the mouse test decreases strongly, in addition, these agents can bring about
30 immunogenic reactions in the case of the injection.

 Furthermore, from EP-A 0,178,665 are known "stabilisers", especially for lyophilised EPO preparations. Besides the polymeric substances PEG 4000, gelatine and dextran 40, there are mentioned various
35 sugars and sugar alcohols, amino acids, inorganic salts

and thiol compounds. Combinations of these materials with human serum albumin, gelatine and dextran are also mentioned. In this literature reference, too, there is only determined the finding again of the radio-
5 activities after 2 months storage of the lyophilised products. This is given as 87 - 99%, in comparison with 60% without addition. Since the lyophilised material was used directly after the production as a standard, it is not stated how high the activity losses
10 are in the case of the production of the preparations. These preparations also display a high effectiveness loss in the mouse test.

Therefore, the task exists of finding a readily compatible EPO preparation which is storage-stable, i.e.
15 ensures the in vivo effectiveness, does not lead to adsorptions on the ampoule and syringe walls and can easily be brought into an injectable form.

Surprisingly, this task is solved by a combination of various components characterised in more detail in
20 the claims. As our experiments have shown, the individual materials do not possess these properties or only to a small extent.

Decisive for the stabilisation is the addition of urea and of various amino acids. Urea is used in
25 5 - 50 g/l, preferably 10 - 15 g/l. As amino acids, there are mentioned, for example, glycine, L-alanine, L-arginine, L-isoleucine, L-leucine, L-2-phenylalanine, L-glutamic acid or L-threonine. Mixtures of various amino acids appear to have an especially advantageous
30 effect. The amino acids in question are used in amounts of 0.5 - 50 g/l, preferably 1 - 20 g/l, whereby the total amount of the amino acids is preferably to amount to 5 - 25 g/l.

Furthermore, a physiologically compatible buffer
35 is necessary which, in the small concentrations (about

20 - 100 mMol/l) necessary for injection solutions, adjusts the pH range of 6.5 - 7.4, especially 7.0 - 7.2, necessary for EPO. Apart from phosphate buffers, glycinate, carbonate, citrate and the like are usable, whereby, apart from sodium, potassium or ammonium ions can serve as counterion. A buffering is additionally brought about by the amino acids present.

The adhesion of the EPO on to the ampoule walls and syringes is substantially reduced by addition of small amounts of a detergent. Since the preparation is preponderantly to be injected, these materials must be physiologically compatible, especially i.v. Concentrations of 0.05 - 5 g/l, especially 0.1 - 0.5 g, have proved to be useful. Non-ionic wetting agents, such as the various polymacrogol types, especially polyethylene sorbitan laurate (Tween[®] 20 or 80) or sorbitan trioleate (Span[®] 35 or 80) or glycerol oleic acid polyglycol ether (Labrafil[®]) have proved to be useful for this purpose but other compatible materials can be used in the same way.

In order to reduce the influence of heavy metal ions, which are entrained almost unavoidably in the case of the working up, e.g. from the metal apparatus used, on the EPO, it has, furthermore, proved to be useful also to add to the solution 0.01 - 5 g/l of a soluble calcium salt, preferably about 0.02 - 0.2 g/l calcium chloride. Other physiologically compatible complex formers, for example citrate, EDTA, NTA, pantothenate, can also be used.

As solvent, there is used pure water for injection purposes to which, for the production of isotonia, there are also added 0.5 - 10 g/l of sodium chloride or corresponding materials, such as mannitol, sorbitol etc.

For the production of the preparations according to the invention, all adjuvant materials are dissolved

in the necessary amount of water, the EPO preparation, which should display an activity of about 100,000 to 200,000 units/mg protein, is admixed, sterile-filtered into appropriate ampoules, frozen in and gently
5 lyophilised at a low temperature. The preparations obtained can be stored under nitrogen for over 2 years at 0°C and over 1 year at room temperature. In the case of the reconstitution with water, they dissolve in a few seconds without turbidity and can thus be
10 injected either directly intravenously or intramuscularly or, after dilution with an isotonic solution, (e.g. common salt solution), infused.

The freezing in procedure has an especial importance. The adjuvant materials are so chosen in nature
15 and amount that the eutectic point of the solution to be frozen in lies between -50 and -30°C. With the help of a computer-controlled optimisation programme, there were determined the following optimum conditions for the 3 phases of the lyophilisation:

20 freezing-in time: 12 - 14 hours at -40°C
main drying: brine temperature +10°C, pressure 10^{-1} mbar,
time 48 - 60 hours
post-drying: brine temperature +20°C, pressure 10^{-3} mbar,
time 4 - 6 hours.

25 It is hereby important to recognise, with the help of a Δp -measurement device, as well as of a conductivity measurement, when the main drying is concluded in order that the product to be lyophilised is not warmed too quickly and a thawing of the frozen-
30 in solution and the activity loss involved therewith is avoided.

Thus, the adjuvant materials used are so chosen that a uniformly structured ice body to be lyophilised results and that, during the lyophilisation, a porous
35 structure (cake) is obtained from which is possible an

optimum sublimation of the ice, especially towards the end of the main drying. The post-drying takes place, as mentioned above, at only +20°C for 4 to 6 hrs. This gentle treatment is important since otherwise it results in a loss of activity in the case of the material to be lyophilised.

As a rule, the so lyophilised products have a water content of about 2 - 5% according to Karl Fischer. This residual water content depends upon the nature and amount of the adjuvant materials which are used in the formulation in question.

The aqueous solutions of the stabilised EPO can also be filled directly into ampoules and, without lyophilisation, can be made available in a form ready for use. However, the storage stability is thereby shortened, in comparison with the lyophilisate, to about 1 year at 0°C and a few months at room temperature.

The following Examples are to explain the above-described in more detail:

20

Example 1

Erythropoietin 2000 Units injection dry substance
(batch for 35,000 bottles)

In a sterile 100 l V2A double-mantle kettle provided with stirring means are dissolved the adjuvant materials:

25	urea	700.0 g
	sodium chloride	70.0 g
	Tween® 20	7.0 g
	sodium dihydrogen phosphate x 1H ₂ O	38.4 g
30	disodium hydrogen phosphate x 2H ₂ O	350.0 g
	calcium chloride x 2H ₂ O	8.4 g
	glycine	105.0 g
	L-leucine	140.0 g
	L-isoleucine	140.0 g
35	L-threonine	35.0 g

L-glutamic acid	35.0 g
L-phenylalanine	70.0 g
water for injection purposes	ad 70.0 l.

To 30 l of this adjuvant material solution are
5 added 214.3 ml of an erythropoietin crude material
batch with an EPO titre of 140,000 Units/1 ml and made
up to the end volume of 35 l and stirred up. The
filtration system used is rinsed with the remaining
adjuvant material solution. The batch solution is
10 sterile filtered over a membrane filter of 0.2 μ m pore
width. The sterile-filtered solution is filled into
1 ml injection bottles under aseptic conditions and
freeze-dried in a lyophilisation plant under the
following criteria:

15 freezing-in time: 12 - 14 hours at -40°C
main drying: brine temperature $+10^{\circ}\text{C}$, pressure
 10^{-1} mbar, time 48 - 60 hours
post-drying: brine temperature $+20^{\circ}\text{C}$, pressure
 10^{-3} mbar, time 4 - 6 hours.

20 One thus obtains a voluminous, open-pored
injection dry substance which is storage-stable for at
least 2 years in a refrigerator and for 1 year at room
temperature and dissolves within a few hours in 2 ml
of water for injection purposes without turbidity and
25 free from particles.

Example 2

Erythropoietin lyophilisate 200 Units (batch for
35,000 bottles)

erythropoietin	46.7 ml = 7 mio Units
30 sodium chloride	100.0 g
Tween [®] 20	10.0 g
sodium dihydrogen phosphate x $1\text{H}_2\text{O}$	155.0 g
disodium hydrogen phosphate x $2\text{H}_2\text{O}$	500.0 g
35 calcium chloride x $2\text{H}_2\text{O}$	10.0 g

	urea	1000.0 g
	L-leucine	150.0 g
	L-threonine	120.0 g
	L-phenylalanine	165.0 g
5	water for injection purposes	ad 70.0 l.

The adjuvant materials are dissolved in 70 l of water for injection purposes and thereafter divided into 2 portions of 35 l. The first 35 l are mixed with the necessary amount of EPO active material. The second 35 l are used for the rinsing of the filtration system. The batch solution is sterile filtered over a membrane filter of 0.2 μ m pore width. The sterile-filtered solution is filled into 1 ml injection bottles under aseptic conditions and lyophilised under the same criteria as given in Example 1. One thus obtains a white, porous lyophilisate readily soluble in 2 ml of water which can be stored for 2 years in a refrigerator or 1 year at room temperature without great activity loss.

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Example 3

Erythropoietin lyophilisate 1000 Units (batch for 35,000 bottles)

	erythropoietin	233.3 ml = 35 mio Units
25	sodium chloride	100.0 g
	Tween [®] 20	12.0 g
	sodium dihydrogen phosphate x H ₂ O	140.0 g
	disodium hydrogen phosphate x 2H ₂ O	450.0 g
	calcium chloride x 2H ₂ O	10.0 g
30	urea	700.0 g
	glycine	1050.0 g
	L-leucine	92.0 g
	L-glutamic acid	103.0 g
	L-phenylalanine	115.5 g
35	water for injection purposes	ad 70.0 l.

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Erythropoietin lyophilisate 500 Units (batch for 35,000 bottles)

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membrane filter of 0.2 μ m pore width. The sterile-filtered solution is filled into 1 ml injection bottles under aseptic conditions and lyophilised under the same criteria as given in Example 1. One thus obtains a
5 white, porous lyophilisate readily soluble in 2 ml of water which can be stored for 2 years in a refrigerator or 1 year at room temperature without great activity loss.

Example 5

10 Erythropoietin lyophilisate 750 Units (batch for 35,000 bottles)

	erythropoietin	175.0 ml = 26.25 mio Units
	sodium chloride	100.0 g
15	Tween [®] 20	12.0 g
	sodium dihydrogen phosphate x H ₂ O	140.0 g
	disodium hydrogen phosphate x 2H ₂ O	450.0 g
	calcium chloride x 2H ₂ O	10.0 g
	glycine	1250.0 g
20	L-isoleucine	98.0 g
	L-glutamic acid	130.0 g
	L-phenylalanine	145.0 g
	water for injection purposes	ad 70.0 l

The adjuvant materials are dissolved in 70 l of
25 water for injection purposes and thereafter divided into 2 portions of 35 l. The first 35 l are mixed with the necessary amount of EPO active material. The second 35 l are used for the rinsing of the filtration system. The batch solution is sterile filtered over a
30 membrane filter of 0.2 μ m pore width. The sterile-filtered solution is filled into 1 ml injection bottles under aseptic conditions and lyophilised under the same criteria as given in Example 1. One thus obtains a
white, porous lyophilisate readily soluble in 2 ml water
35 which can be stored for 2 years in a refrigerator or for

1 year at room temperature without great activity loss.

Example 6

In order to test the effectiveness of various stabilisers, a standard formulation with 1000 U EPO/ml, which contained urea as main stabiliser, was mixed with polyvinylpyrrolidone/protein or with different amino acids and the products lyophilised. The results obtained are summarised in Table 1.

After 6 weeks storage of the lyophilisate at 35°C or 0°C, the stability of EPO was determined in the mouse spleen test according to the procedure of G. Krystal in Exp. Hematol., 11, 649-660 (1983) as follows:

B₆C₃F₁ mice, female, weight of about 20 g (Zentralinstitut für Versuchstierkunde, Hannover) are injected i.p. on two successive days with 60 mg/kg phenylhydrazine hydrochloride. After 3 further days, the spleen is removed, the spleen cells are suspended in sterile complete medium (Dulbecco Modified Eagle's Medium + 584.0 mg/l L-glutamine + 0.1 mMol/l 2-mercaptoethanol + 20% foetal calf serum) and diluted to 4 x 10⁶ nucleus-containing cells/ml. The suspension, to which had previously been added the test substance or the EPO standard in, in each case, suitable concentrations, dissolved in BSA buffer, is distributed in microtitre plates (0.2 ml/cup). After incubation (22 hours, 37°C, air + 15% CO₂), 20 µl ³H-methylthymidine solution with

Ci per cup are added thereto and again incubated for 2 hours at 37°C. Thereafter, the content is transferred with the help of a cell harvester and washed with distilled water. The incorporation of ³H-thymidine is determined with a β-scintillation counter and evaluated against the standard preparation.

As working standard, there was used the "P009-EPO Standard" of Genetics Institute, Cambridge, Massachusetts, USA, which contained 112 U EPO/ml (and

503 ng protein/ml) (equilibrated against the EPO reference standard of WHO "International Reference Preparation of Erythropoietin, Human, Urinary for Bioassay (2nd I.R.P., established 1971)". The
5 standard concentration lay in the range of 10 - 100 mU/ml.

The lyophilisates to be tested for their EPO activity were first dissolved per ampoule in 2 ml of water for injection purposes, the further dilutions
10 took place - just as in the case of the working standard - with BSA buffer (8.75 g NaCl, 1.95 g $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, 1.00 g BSA (bovine serum albumin of the firm Calbiochem), water for injection purposes ad 1 l)
15 ^3H -Methylthymidine (specific activity: 2 Ci/mmol) was obtained from New England Nuclear.

Example 7

In the same way as in Example 6, a somewhat modified parent formulation was mixed with urea and various amino acids or mixtures. For comparison, two
20 mannitol-containing formulations, which correspond to EP 178,665, were also tested. The results are summarised in Table 2.

Table 1

composition mg/bottle	l 819892 G	a 819893 H	b 819894 I	ab 819895 K
erythropoietin	1000 U	1000 U	1000 U	1000 U
urea	10.0	10.0	10.0	10.0
sodium chloride	9.0	4.0	9.0	1.0
Tween [®] 20	0.1	0.1	0.1	0.1
Na dihydrogen phosphate x H ₂ O	0.55	0.55	0.55	0.55
di-Na hydrogen phosphate x 2H ₂ O	5.0	5.0	5.0	5.0
CaCl ₂ x 2H ₂ O	0.08	0.08	0.08	0.08
Kollidon 12 PF	5.0	-	5.0	-
Gelafundin	1.0	1.0	-	-
glycine	-	15.0	-	15.0
L-leucine	-	-	2.0	2.0
L-isoleucine	-	-	2.0	2.0
L-threonine	-	-	0.5	0.5
L-glutamic acid	-	-	0.5	0.5
L-phenylalanine	-	-	1.0	1.0
water	ad 1.0 ml	ad 1.0 ml	ad 1.0 ml	ad 1.0 ml
stability 25°C	493	869	934	1128
stability 0°C	985	1114	1144	1205

Table 2

Expt. No.	895	896	897	898	899	900	901	902	903
<u>Composition in mg/ml</u>									
EPO, batch: P007	250 U	250 U	250 U	250 U	250 U	250 U	250 U	250 U	250 U
sodium chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tween [®] 20	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Na dihydrogen phosphate x H ₂ O	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
di-Na-hydrogen phosphate x 2H ₂ O	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
calcium chloride x 2 H ₂ O	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
mannitol	-	-	-	-	-	-	-	-	-
urea	-	10	-	10	-	10	-	10	-
glycine	15.0	-	15	-	15	-	15	-	15
L-leucine (1/100 m molar)	-	-	1.32	1.32	-	-	1.32	1.32	-
L-isoleucine "	1.32	1.32	-	-	1.32	1.32	-	-	1.32
L-threonine "	-	-	-	-	1.2	1.2	1.2	1.2	-
L-glutamic acid "	1.47	1.47	1.47	1.47	-	-	-	-	1.47
L-phenylalanine "	-	-	-	-	-	-	-	-	1.65
L-arginine	1.74	1.74	1.74	1.74	1.74	1.74	1.74	1.74	-
NaH ₂ PO ₄ x H ₂ O	1.0	1.0	-	1.0	2	2	2	2	-
Na ₂ HPO ₄ x 2 H ₂ O	-	-	-	-	-	-	-	-	2
water p.i. ad	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
stability 25°C	164	185	150	234	170	229	226	226	229
stability 0°C	182	203	203	242	208	234	232	230	230

Table 2 (continuation)

Expt. No.	904	905	906	907	908	909	910	911	912
<u>composition in mg/ml</u>									
EPO, batch: P007	250 U	250 U	250 U	250 U	250 U	250 U	250 U	250 U	250 U
sodium chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tween [®] 20	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Na dihydrogen phosphate x H ₂ O	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
di-Na hydrogen phosphate x 2H ₂ O	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
calcium chloride x 2 H ₂ O	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
mannitol	-	-	-	-	-	-	-	-	-
urea	10	-	10	-	10	-	10	10	-
glycine	-	15	-	15	-	15	-	15	15
L-leucine (1/100 m molar)	-	1.32	1.32	-	-	1.32	1.32	2	-
L-isoleucine "	1.32	-	-	1.32	1.32	-	-	2	-
L-threonine "	-	-	-	1.2	1.2	1.2	1.2	0.5	-
L-glutamic acid "	1.47	1.47	1.47	-	-	-	-	0.5	-
L-phenylalanine "	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.0	-
L-arginine "	-	-	-	-	-	-	-	-	-
NaH ₂ PO ₄ x H ₂ O	-	-	-	-	1	-	1	-	1
Na ₂ HPO ₄ x 2 H ₂ O	5	7	5	-	-	-	-	-	-
water p.i. ad	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
stability 25°C	205	221	258	205	216	171	-	256	175
stability 0°C	167	220	291	188	210	197	240	234	218

Table 2 (continuation)

Expt. No.	982	576	575
<u>Composition in mg/ml</u>			
EPO, batch: P007	250 U	250 U	250 U
sodium chloride	1.0	4	4
Tween [®] 20	0.1	0.1	0.5
Na dihydrogen phosphate x H ₂ O	0.55	0.55	0.55
Di-Na hydrogen phosphate x 2 H ₂ O	5.0	5.0	5.0
calcium chloride x 2 H ₂ O	0.08	0.08	0.08
mannitol	-	20	30
urea	10	-	-
glycine	-	10	-
L-leucine (1/100 m molar)	-	-	-
L-isoleucine "	-	-	-
L-threonine "	-	-	-
L-glutamic acid "	-	-	-
L-phenylalanine "	-	-	-
L-arginine "	-	-	-
NaH ₂ PO ₄ x H ₂ O	1	-	-
Na ₂ HPO ₄ x 2 H ₂ O	-	-	-
water p.i. ad	1.0ml	1.0ml	1.0ml
stability 25°C	120	134	97
stability 0°C	195	172	178

Patent Claims

1. Compatible, storage-stable human protein preparation, containing a human protein, physiologically compatible buffer as well as possibly complex formers,
5 isotonia-adjusting agents, calcium chloride and other materials usual for injection purposes, characterised in that, in the injection form, it contains 5 - 50 g/l urea, 0.5 - 50 g/l amino acid, 0.05 - 5 g/l detergent, conventional adjuvant materials are added to this
10 solution and subsequently the human protein is admixed.
2. Human protein preparation according to claim 1, characterised in that the detergent is a non-ionogenic wetting agent.
3. Human protein preparation according to claim 1,
15 characterised in that the amino acid is a mixture which contains at least two of the amino acids glycine, L-alanine, L-isoleucine, L-arginine, L-leucine, L-2-phenylalanine, L-glutamic acid or L-threonine.
4. Human protein preparation according to claim 2
20 or 3, characterised in that polyethylene sorbitan laurate is used as wetting agent.
5. Human protein preparation according to one of claims 1 - 4, characterised in that the preparation is present in lyophilised form.
- 25 6. Human protein preparation according to one of claims 1 - 5, characterised in that the preparation is present in liquid form ready for injection.
7. Human protein preparation according to one of claims 1 - 6, characterised in that, per injection
30 dosage unit of 1 - 5 ml, it contains 100 to 1 million U of human protein.
8. Human protein preparation according to one of claims 1 - 7, characterised in that the human protein is erythropoietin.



9. Human protein preparation according to claim 8, characterised in that, per injection dosage unit of 1 - 5 ml, it contains 100 - 20,000 U of erythropoietin.
10. Process for the production of compatible storage-stable human protein preparations containing a human protein, physiologically compatible buffers as well as possibly complex formers, isotonia-adjusting agents, calcium chloride and other materials usual for injection purposes, characterised in that one first prepares a solution of 5 - 50 g/l urea, 0.5 - 50 g/l amino acid and 0.05 - 5 g/l detergent, adds usual adjuvants to this solution and subsequently admixes the human protein.
11. Process according to claim 10, characterised in that the detergent is a non-ionogenic wetting agent.
12. Process for the production of a preparation according to claim 10, characterised in that the human protein is erythropoietin.
13. Process for the production of an erythropoietin preparation according to claim 10, 11 or 12, characterised in that first all adjuvants are dissolved in water, the erythropoietin is added thereto, the mixture is sterile-filtered into ampoules and gently lyophilised at below -20°C.
14. Process for the production of an erythropoietin preparation according to claims 10, 11 or 12, characterised in that first all adjuvants are dissolved in water, the erythropoietin is added thereto and the mixture is filled into ampoules.

