
Liquid pharmaceutical formulation

TECHNICAL FIELD OF THE INVENTION

[001] The invention relates to a liquid pharmaceutical formulation, being essentially free of water, comprising a) at least one active pharmaceutical ingredient, selected from the group consisting of Bendamustine, Melphalan, Melflufen, Chlorambucil and Uramustine; b) one or more crystallization inhibitor(s); c) a solvent according to formula (I) and d) at least one pharmaceutically acceptable alkaline earth metal.

BACKGROUND ART

[002] Certain pharmaceutical ingredients are degradable e.g. due to hydrolysis. An exemplary group of degradable pharmaceutical ingredients are alkylating agents which are used for the treatment of various cancers. One substance of this group, for example is Bendamustine. Bendamustine comprises an alkylating $-N((CH_2)_2Cl)_2$ group, which undergoes rapid hydrolysis, by substitution of the chloride groups, to the corresponding mono- and di-hydroxy compounds. The hydrolysis of Bendamustine in water takes place in hours, therefore a solution of Bendamustine is not suitable for long term storage. Bendamustine is commercially available as powder in lyophilized form as TreandaTM. While the lyophilized form exhibits good chemical stability, with implications of chemical stability. Thus, efforts have been made to stabilize Bendamustine in liquid pharmaceutical formulations, which may be stored for longer time.

[003] In this respect, WO2011/094565 discloses a formulation for long term storage including bendamustine or a pharmaceutically acceptable salt thereof; a pharmaceutically acceptable fluid including polyethylene glycol PEG, propylene glycol (PG) and a stabilizing amount of an antioxidant such as thioglycerol. Another formulation includes bendamustine or a pharmaceutically acceptable salt thereof; a pharmaceutically acceptable fluid including polyethylene glycol PEG), propylene glycol (PG), ethanol, benzyl alcohol and glycofurol and a chloride salt such as sodium chloride, choline chloride, and hydrochloride salts of amino acids.

[004] WO 2013/112762 A1 deals with aqueous Bendamustine formulations with improved stability. These comprise a mixture of a non-aqueous solvent system and an aqueous chloride-containing water phase. The non-aqueous solvent may comprise propylene glycol or polyethylene glycol and optionally antioxidants and preservatives such as thioglycerol.

[005] It has been disclosed in WO 2019/185859 that the inventive pharmaceutical formulation therein is suitable to stabilize easy degradable active pharmaceutical ingredients in comparison to samples which did not comprise at least one pharmaceutically acceptable alkaline earth

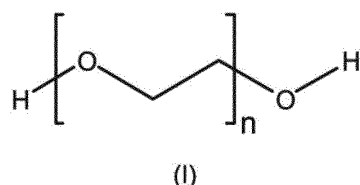
metal salt. An embodiment wherein the alkaline earth metal salt is CaCl_2 proved to be particularly effective for stabilizing easy degradable active pharmaceutical ingredients. Further, the inventive pharmaceutical formulations disclosed therein reduce or prevent color change indicating degradation during storage, in particular in comparison with other pharmaceutical formulations for the same purpose of stabilizing degradable active pharmaceutical ingredients, known in the art. Unlike organic preservatives and antioxidants the addition of alkaline earth metal salts from calcium or magnesia in the amounts described therein are regarded as physiologically safe and do not require any verification. For example, in contrast to the formulation as disclosed in WO2011/094565, the pharmaceutical formulation of the present invention does not require the presence of toxic thioglycerol as antioxidant.

[006] Polyethylene glycoles, in particular PEG400, have been found as particular useful solvents for the pharmaceutical formulation of WO 2019/185859, with earth alkali metal salts as stabilisators, in this regard, due to high solubility, a low amount of hydroxyl groups which could cause alcoholysis of Bendamustine, and extreme low toxicity. However, while commercially available Bendamustine was initially very well soluble in polyethylene glycoles in the applied amounts, after a certain time of storage of the formulation, precipitation/crystallization of an unknown compound has been observed which is undesirable for a pharmaceutical formulation. Thus, there is a need for further stabilizing the formulations of WO 2019/185859 with polyethylene glycols as solvent in order to prevent the observed precipitation/crystallization during storage.

SUMMARY OF THE INVENTION

[007] The invention relates to a liquid pharmaceutical formulation, being essentially free of water, comprising

- a) at least one active pharmaceutical ingredient, selected from the group consisting of Bendamustine, Melphalan, Melflufen, Chlorambucil and Uramustine;
- b) at least one crystallization inhibitor selected from the group consisting of (2-Hydroxypropyl)- β -cyclodextrin and DMSO;
- c) a polyethylene glycol solvent according to formula (I)



wherein n is 350 to 500

; and

d) at least one pharmaceutically acceptable alkaline earth metal.

[0008] Further, the invention is directed to the pharmaceutical formulation for use in medicine as well as to the pharmaceutical formulation for use in the treatment of cancer.

[0009] Commercially available Bendamustinehydrochlorid essentially always is in the form of its monohydrate. This monohydrate is well soluble in polyethylenglycole in the applied amounts. It has now been found, that the commercially available bendamustine, is losing this crystal water in the solution over time. The resulting Bendamustine without crystal water obviously has a lower solubility in polyethylenglycoles than the Bendamustine with crystal water and precipitates/crystallizes out as a result. It has been found that this issue can be overcome by adding at least one crystallization inhibitor selected from the group consisting of (2-Hydroxypropyl)- β -cyclodextrin and DMSO.

DESCRIPTION OF FIGURES

[0010] Figure 1 shows Bendamustine crystals formed in a PEG400 solution.

[0011] Figure 2: Diffractogram of crystallized crystals such as shown in Figure 1. Comparison with a diffractogram of Bendamustine hydrochlorid monohydrate shows that these are not identical and indeed the crystallized crystals are not Bendamustine hydrochlorid monohydrate.

[0012] Figure 3: Package analysis of Bendamustine HCl Form I and calculated diffractogram (3A).

[0013] Figure 4: Package analysis of Bendamustine HCl Monohydrate Form II and calculated diffractogram (4A).

[0014] Figure 5: Bendamustine crystals in a HPBCD-free formulation after 6 months at 40 °C (WP14 formulation #1).

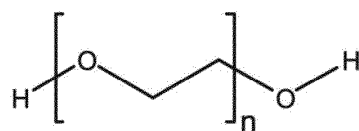
[0015] DETAILED DESCRIPTION OF THE INVENTION

[0016] The solution of the present invention is described in the following, exemplified in the appended examples, illustrated in the Figures and reflected in the claims.

□

[0017] The present invention provides a liquid pharmaceutical formulation, being essentially free of water, comprising

- a) at least one active pharmaceutical ingredient, selected from the group consisting of Bendamustine, Melphalan, Melflufen, Chlorambucil and Uramustine;
- b) at least crystallization inhibitor selected from the group consisting of (2-Hydroxypropyl)- β -cyclodextrin and DMSO;
- c) a polyethylene glycol solvent according to formula (I)



(I)

wherein n is 350 to 500

and

- d) at least one pharmaceutically acceptable alkaline earth metal.

[0018] In the context of the present invention, “essentially free of water” means a water content below 5 % (w/w), preferably below 2 % (w/w), more preferably below 1 % (w/w), most preferably below 0.5 % (w/w).

[0019] The at least one active pharmaceutical ingredient, selected from the group consisting of Bendamustine, Melphalan, Melflufen, Chlorambucil and Uramustine. Preferably, the at least one active pharmaceutical ingredient is Bendamustine.

[0020] The term “active pharmaceutical ingredient” also includes the presence of the active pharmaceutical ingredient as listed above in the form of a pharmaceutically acceptable salt, thereof.

[0021] A pharmaceutically acceptable salt” is intended to mean a salt that retains the biological effectiveness of the free acids and bases of the specified compound and that is not biologically or otherwise undesirable. A compound of the invention may possess a sufficiently acidic, a sufficiently basic, or both functional groups, and accordingly react with any of a number of inorganic or organic bases, and inorganic and organic acids, to form a pharmaceutically acceptable salt. Exemplary pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a mineral or organic acid or an inorganic base, such as salts including sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogenphosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrate, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-

dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, sulfonates, xylenesulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, γ -hydroxybutyrates, glycolates, tartrates, methane-sulfonates, propanesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates.

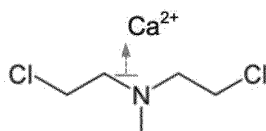
[0022] Preferably, the active pharmaceutical ingredient is present in the form of hydrochloride and/or hydrate, more preferably in the form of a hydrochloride and/or monohydrate.

[0023] In one embodiment the active pharmaceutical ingredient is Bendamustine hydrochloride monohydrate.

[0024] In one embodiment the preparation of the formulation comprises solving Bendamustine hydrochloride monohydrate in the solvent c). This does not exclude the possibility that Bendamustine hydrochloride monohydrate is converted after a certain time into other modifications of Bendamustine, such as anhydrous Bendamustine or anhydrous Bendamustine hydrochloride.

[0025] Hydrolysis in the context of the invention means cleavage of one or more bonds by addition of at least one water or alcohol (HO-R) molecule or replacement of a functional group by a hydroxyl group originating from the water molecule. The hydrolysis may include formation of small molecules formed of protons originating from the water or alcohol molecule attached and the group which has been released is for example HCl or HBr.

[0026] Degradation of nitrogen mustards (ternary 2,2'-dichloroalkylamines) is supposed to take place via the formation of a reactive three-member cyclic intermediate towards mono and dihydroxyl products (e.g. hydrolysis or alcoholysis). To start this reaction a free election pair at the nitrogen atom has to be accessible. The presence of positively charged calcium (or other alkaline earth metal) ions reduces the accessibility of the partial negatively charged election pair at the nitrogen atom by charge interactions between the calcium ion and the free electron pair. By masking the said electron pair at the nitrogen atom with calcium ions the cyclization reaction is slowed down or completely blocked resulting in a demonstrable stabilizing effect on nitrogen-mustard compounds:



[0027] The stabilizing effect is depending on the capability of the alkaline earth metal ion to undergo charge interactions to the election pair at the nitrogen atom. Thus, the degree of dissociation and solvation of the alkaline earth metal salt within the organic solvent and the cation itself (calcium, magnesium or others) as well as the counterion (chloride, bromide, acetate, etc.) have an influence on the extent of stabilization: e.g. CaBr_2 shows less effective stabilization compared to CaCl_2 , caused by the higher atomic radius of bromide compared to chloride. The stabilizing effect of chloride itself without an alkaline earth metal cation (e.g. choline chloride: (2-hydroxyethyl)-trimethylammonium) is shown to be negligible.

[0028] Preferably, the concentration of the at least one degradable active pharmaceutical ingredient in the formulation is 1 to 200 mg/g, more preferably 5 to 150 mg/g, even more preferably 10 to 100 mg/g, most preferably 20 to 40 mg/g based on the overall weight of the formulation.

[0029] Preferably, the at least one degradable active pharmaceutical ingredient in the formulation is present in a weight of 1 to 3.5 % (w/w), preferably 1.5 to 3 % (w/w), more preferably 2.16 to 2.59 % (w/w), most preferably 2 to 2.5 % (w/w) based on the overall amount of the liquid pharmaceutical formulation.

[0030] The at least one crystallization inhibitor is selected from the group consisting of (2-Hydroxypropyl)- β -cyclodextrin and DMSO (dimethyl sulfoxide), preferably (2-Hydroxypropyl)- β -cyclodextrin is present in the liquid pharmaceutical formulation. (2-Hydroxypropyl)- β -cyclodextrin and DMSO (dimethyl sulfoxide) may be applied in combination.

[0031] In one embodiment, the at least one crystallization inhibitor is present in the liquid pharmaceutical formulation in an amount of or at least in an amount of 0.5 to 5 % (w/w), preferably 0.7 to 4.3 % (w/w), more preferably 1 to 4 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.

[0032] In one embodiment, the at least one crystallization inhibitor is present in the liquid pharmaceutical formulation in an amount of or at least in an amount of 0.8 to 3 % (w/w), preferably 0.85 to 2 % (w/w), more preferably 1 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.

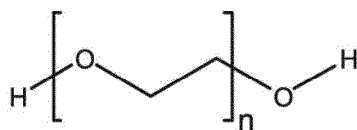
[0033] In one embodiment, the at least one crystallization inhibitor is present in the liquid pharmaceutical formulation in an amount of or at least in an amount of 1.5 to 3.5 % (w/w), preferably 1.8 to 2.5 % (w/w), more preferably 2 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.

[0034] In one embodiment, the at least one crystallization inhibitor is present in the liquid pharmaceutical formulation in an amount of or at least in an amount of 2.5 to 4.5 % (w/w), preferably 2.8 to 3.5 % (w/w), more preferably 3 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.

[0035] In one embodiment, the at least one crystallization inhibitor is present in the liquid pharmaceutical formulation in an amount of or at least in an amount of 3.5 to 5 % (w/w), preferably 3.8 to 4.5 % (w/w), more preferably 4 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.

[0036] In one embodiment, the at least one crystallization inhibitor is present in the liquid pharmaceutical formulation in an amount of or at least in an amount of 5 to 15 % (w/w), preferably 7 to 12 % (w/w), more preferably 10 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.

[0037] The solvent is a polyethylene glycol solvent according to formula (I).



(I)

wherein n is 350 to 500, preferably 390 to 430, more preferably 400. The polyethylene glycol solvent should preferably liquid at ambient temperature. Wherein ambient temperature is 18 to 28 °C.

[0038] Preferably the solvent is PEG400.

[0039] Preferably the solvent is a pharmaceutically acceptable.

[0040] Preferably, the solvent according to formula (I) represents at least 90 % (w/w), more preferably at least 95 % (w/w), most preferably at least 98 % (w/w), most preferably at least 100 % (w/w) of the solvents based on the overall weight of all solvents present in the liquid pharmaceutical formulation.

[0041] The at least one pharmaceutically acceptable alkaline earth metal salt may be any pharmaceutically acceptable alkaline earth metal known to the person skilled in the art. In the context of the present invention, "alkaline earth metal salt" means any ionic compound,

including coordination complexes and chelate complexes of an alkaline earth metal.

[0042] Preferably the at least one alkaline earth metal salt is a calcium or a magnesium salt. More preferably a calcium salt. Preferably the anion of the at least one alkaline earth metal salt is gluconate, chloride, bromide, acetate, orotate or lactate more preferably chloride, acetate or lactate, most preferably chloride.

[0043] Most preferably the at least one pharmaceutically acceptable alkaline earth metal salt is calcium chloride or magnesium chloride, particularly preferred calcium chloride.

[0044] The at least one pharmaceutically acceptable alkaline earth metal salt is preferably present in a concentration of 0.1 to 3 mol/L, more preferably 0.1 to 1.5 mol/L, even more preferred 0.3 to 1.5 mol/L, particular preferred 0.7 to 1.1 mol/L, more particular preferred 0.1 to 1.0 mol/L, most particular preferred 0.8 to 1.0 mol/L, especially preferred 0.1 to 0.5 mol/L based on the overall volume of the liquid formulation.

[0045] In a further embodiment, the at least one pharmaceutically acceptable alkaline earth metal salt is preferably present in a weight of 1 to 4 % (w/w), preferably 2 to 3 % (w/w), more preferably 2.5 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.

[0046] The at least one pharmaceutically acceptable alkaline earth metal salt in the said concentrations is preferably completely dissolved in the pharmaceutically acceptable organic solvent resulting in a clear pharmaceutical solution.

[0047] The liquid pharmaceutical formulation may comprise further additional ingredients known to the person skilled in the art. These may comprise for example buffers, detergents, antioxidants and others.

[0048] The liquid pharmaceutical formulation may further comprise an antioxidant. Preferably the antioxidant is selected from the group consisting of Thioglycerol, Butylhydroxytoluene, Thioglycolic Acid, Ascorbic Acid, Ascorbyl Palmitate, Sodium Sulfites, Butylated Hydroxyanisole, Propyl Gallate, Tocopherols more preferably the antioxidant is Butylhydroxytoluene.

[0049] Preferably, the amount of antioxidant is 0.01 to 0.1 % (w/w), more preferably 0.03 to 0.07 % (w/w), most preferably 0.05 % (w/w) based on the overall weight of the liquid pharmaceutical formulation.

[0050] In one embodiment, the liquid pharmaceutical formulation does not comprise dextran.

[0051] In one embodiment, of the liquid pharmaceutical formulation

a) the at least one active pharmaceutical ingredient is present in a weight of 1 to 3.5 % (w/w), preferably 1.5 to 3 % (w/w), most preferably 2 to 2.5 % (w/w) based on the overall amount of the liquid pharmaceutical formulation; and

b) the crystallization inhibitor is present in a weight of

i) 0.8 to 3 % (w/w), preferably 0.85 to 2 % (w/w), more preferably 1 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or

ii) 1.5 to 3.5 % (w/w), preferably 1.8 to 2.5 % (w/w), more preferably 2 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or

iii) 2.5 to 4.5 % (w/w), preferably 2.8 to 3.5 % (w/w), more preferably 3 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or

iv) 3.5 to 5 % (w/w), preferably 3.8 to 4.5 % (w/w), more preferably 4 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; and

d) the pharmaceutically acceptable alkaline earth metal salt selected from the group consisting of a calcium and a magnesium salt, preferably a calcium salt is present in a weight of 1 to 4 % (w/w), preferably 2 to 3 % (w/w), more preferably 2.5 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; and

e) optionally an antioxidant is present in an amount of 0.01 to 0.1 % (w/w), preferably 0.03 to 0.07 % (w/w), more preferably 0.05 % (w/w) based on the overall weight of the liquid pharmaceutical formulation; and

c) the amount of solvent present in the overall liquid pharmaceutical formulation corresponds to: 100 % (w/w) subtracted by the sum of the amounts of a), b), d) and e) or the sum of the amounts of a), b), d), e) and any further ingredient present in the overall liquid pharmaceutical formulation as defined above.

[0052] The liquid pharmaceutical formulation may be applied without further dilution or with prior dilution. The pharmaceutical formulation may be diluted with water for injection purposes, saline or buffer solution before administration. Preferably, the pharmaceutical formulation is diluted with water for injection purposes, saline or buffer solution, not longer than 8 hours, preferably not longer than 4 hours and most preferably not longer than 2 hours before administration.

[0053] The liquid pharmaceutical formulation may be administered in any suitable manner known to the person skilled in the art. Preferably, the liquid pharmaceutical formulation is administered parenterally, more preferably as injection, most preferably as intravenous infusion or intravenous bolus injection.

[0054] Moreover the present invention is directed to the use of the liquid pharmaceutical formulation as defined above, for use in medicine.

[0055] The inventive liquid pharmaceutical formulation may be combined with a physiologically acceptable aqueous solution prior to application to the patient. For example the liquid pharmaceutical formulation may be combined with suitable amount of isotonic sodium chloride solution. Preferably the concentration of the isotonic sodium chloride solution comprises 9 mg /L sodium chloride and preferably exhibits a pH value of 4.5 to 7.0.

[0056] The present invention further comprises a formulation comprising a) the inventive liquid pharmaceutical formulation, as described above and b) a physiologically acceptable aqueous solution, as well as its use in medicine.

[0057] Furthermore, the present invention is directed to the use of the liquid pharmaceutical formulation as defined above, optionally in the presence of water, for use in the treatment of cancer.

[0058] The term "stabilization" in the context of the invention means prevention or reduction of degradation of the easily degradable active pharmaceutical ingredient. Preferably it means maintaining a purity as determined by suitable analytical methods of at least 85%, more preferably of at least 90%, most preferably of at least 95% at the end of the defined shelf life of pharmaceutical preparation, in particular compared with a not stabilized sample. For example, the purity may be determined by high-performance-liquid-chromatography (HPLC), in particular reversed phase high-performance liquid-chromatography (RP-HPLC). Furthermore stabilization means that any visible color change of the preparation caused by degradation is prevented or reduced during product shelf life, in particular in comparison with a not stabilized sample.

EXAMPLES OF THE INVENTION

Example 1: 1.0 Stability of inventive formulations

[0059] The stability of 15 formulations for Bendamustine hydrochloride was tested in an accelerated stability study over four and eight weeks. The stabilizing effect of main solvents (PG, PEG300 and PEG400), solvent additives (DMSO, TBA, NMP, Acetone, Solketal, Glycofurol, Glycerole, polysorbate 20 / Tween 20, (2-Hydroxypropyl)- β -cyclodextrin (HPBCD)), stabilizer (CaCl_2) and antioxidant BHT was to be evaluated.

The following formulations were to be tested (composition per vial):

Table 1: Formulation variants selected for stability testing

Variant	Components [% (w/w)]						
	Bendamustine ·HCl ·H ₂ O	P300	PG	PEG400	CaCl ₂	BHT	Additive
1	2.50	97.45	-	-	-	0.05	-
2	2.50	-	95.00	-	2.50	-	-
3	2.50	-	97.45	-	-	0.05	-
4	2.50	-	94.95	-	2.50	0.05	-
5	2.50	-	-	90.20	2.50	0.05	DMSO 4.75
6	2.50	-	-	90.20	2.50	0.05	TBA 4.75
7	2.50	-	-	90.20	2.50	0.05	NMP 4.75
8	2.50	-	-	90.20	2.50	0.05	Acetone 4.75
9	2.50	-	-	90.20	2.50	0.05	Solketale 4.75
10	2.50	-	-	90.20	2.50	0.05	Glycofurole 4.75
11	2.50	-	-	90.20	2.50	0.05	Glycerole 4.75
12	2.50	-	-	94.95	2.50	0.05	-
13	2.50	-	-	92.45	2.50	0.05	Acetone 2.50
14	2.50	-	-	92.45	2.50	0.05	Solketale 2.50
15	2.50	-	-	92.45	2.50	0.05	Glycofurole 2.50
16	2.50	-	-	89.95	2.50	0.05	HPBCD 5.00
17	2.50	-	-	94.90	2.50	0.05	Tween 20 0.05

1.1 Materials and methods

[0060] Substances and Materials

Table 2: Substances and materials

Substances	Lot-no.:	Manuf. / Suppl.	Order-no.:
Purified water ($\leq 0.2 \mu\text{mS/cm}$, < 10 ppb TOC)	-	PJP	-
Bendamustine hydrochloride monohydrate	N1700362		
Polyethylene glycol 400, ROTIPURAN® Ph. Eur.	120294684	Carl Roth GmbH	0144
Butylhydroxytoluene, $\geq 99.8 \%$, for synthesis	058267347	Carl Roth GmbH	3559
Calcium chloride anhydrous, Ph. Eur.	K48950378	Merck Chemicals	102378
Ethanol pure	508278646	Carl Roth GmbH	5054.4
Glycerole	230296310	Carl Roth GmbH	6967.1
Polyethylene glycol 300, Emprove exp., Ph. Eur.	K47544702	Merck Chemicals	817002
Dimethyl sulfoxide, ROTIPURAN $\geq 98 \%$, p.a.	188270141	Carl Roth GmbH	4720.3
Acetonitrile, Chromosolv gradient grade	STBG5627V	Honeywell	34851
Trifluoroacetic acid, $\geq 99.9 \%$, for peptide synthesis	188270869	Carl Roth GmbH	P088.1
N-Methyl-2-pyrrolidone $\geq 99.8 \%$	472193375	Carl Roth GmbH	4306
Acetone, ROTIPURAN® $\geq 99.8 \%$	111168806	Carl Roth GmbH	9372
1,2-Propanediol, EMPROVE® exp Ph Eur, BP, USP	K51362178920	Merck Chemicals	107478

Tetraglycol	BCCC2236	Sigma-Aldrich	T3396
DL-1,2-Isopropylideneglycerol, 98%	STBD0082V	Sigma-Aldrich	122696
Polysorbate 20, Ph. Eur., BP, USP-NF	0001588857	AppliChem	142312.1611
(2-Hydroxypropyl)- β -cyclodextrin	BCBL4188V	Sigma-Aldrich	778966

Materials	Lot-no.:	Manuf. / Suppl.	Order-no.:
Kinetex C18, 100 x 4.6 mm, 2.6 μ m	S/N : H18-281423 B/N : 5569-0243	Phenomenex	00D-4462-E0
Glass vial 6R	2150359/1	Thüringer Pharmaglas	05000613120
Lyo stopper, Westar® RS, 1346 4023/50 GREY B2-TR	1172035601	West	7002-5042

1.2 Materials and Apparatus

[0061] HPLC Equipment

Manufacturer: Agilent Technologies (Santa Clara, California USA)

Type: 1290 Series

Chromatography analysis software: Thermo Scientific, Chromeleon 7.2.9

[0062] Additional Laboratory Equipment:

Rotary evaporator: Büchi, Rotavapor® R-100

Multistep pipette and pipette tips: Brand, Handystep

Purified water supply: Ultra Clean UV UF TM

Cytostatic safety cabinet: Berner, C-MaxPro 190

Analytical balance: Eppendorf, DE120K10A

Magnetic stirrer: 2mag, MIXdrive6

Volumetric Pipettes: Gillson/Eppendorf

1.3 Materials and Methods

[0063] Primary packing material preparation

Materials and Apparatus:**Table 3:** Primary packing material

Materials	Lot.	Manuf. / Suppl.	Order No.
6R class 1 glass vial, Fiolax clear	2170615	Thüringer Pharmaglas	41101085.1003
20 mm rubber stopper, silicone free, 1343 4023/50/Grey B2-40 Westar®RS FluroTec	1162055767	West Pharmaceutical Services	7001-8022
Alu crimp caps, tear-off 20 mm, gold	--	Adelphi	COTW20

[0064] Additional Laboratory Equipment:

Laboratory dish washer: Miele, G7783

Autoclave: Systec, DX65

Drying oven (depyrogenisation): Memmert, UFP500

All equipment that was used undergoes regular calibration and qualification according to the valid qualification and maintenance plan.

[0065] Procedure:

Vials: Vials were washed in a laboratory dishwasher with purified water and depyrogenised at 300 °C for 2 hours.

Stoppers: Stoppers were dried at 105 °C for 8 hours.

1.4 Compounding**[0066]** Procedure:

Firstly, a stock solution of CaCl₂/BHT in the corresponding solvent (PG, PEG300, PEG400) was prepared. Then, additives were added to the solvent variants to complete solvent preparation according to Table 1. The solvents were filtered through a 0.22 µm filter unit to eliminate unsolved particles. Bendamustine monohydrochloride monohydrate was weighed in a 6R vial and filled to target weight with the corresponding solvent. After complete dissolution, the variants' volumes were splitted à 4 g into 6R vials with nitrogen as an inert headspace gas.

[0067] All variants #01 to #10 and #11 to #15 were compounded in parallel. During compounding contact to atmospheric oxygen was avoided as best as possible. Compounding vessels were kept tightly closed and were selected as small as feasible to reduce headspace.

The dedicated batch size was 2 vials each variant with a fill of 4.0 g per vial.

1.5 Reversed phase chromatography (RP-HPLC)Materials and Apparatus:**[0068] Table 4:** Materials used RP-HPLC analysis

Substances	Lot-no.:	Manuf. / Suppl.	Order-no.:
Purified water ($\leq 0.2 \mu\text{mS/cm}$, < 10 ppb TOC)	-	PJP	-
Bendamustine hydrochloride monohydrate			
Dimethyl sulfoxide, ROTIPURAN $\geq 98 \%$, p.a.	188270141	Carl Roth GmbH	4720.3
Acetonitrile, Chromosolv gradient grade	STBG5627V	Honeywell	34851
Trifluoroacetic acid, $\geq 99.9 \%$, for peptide synthesis	188270869	Carl Roth GmbH	P088.1

Materials	Lot-no.:	Manuf. / Suppl.	Order-no.:
Kinetex C18, 100 x 4.6 mm, 2.6 μm	S/N : H18-281423 B/N : 5569-0243	Phenomenex	00D-4462-E0

[0069] HPLC Equipment

Manufacturer: Agilent Technologies (Santa Clara, California USA)

Type: 1290 Series

Chromatography analysis software: Thermo Scientific, Chromeleon 7.2.9

[0070] Additional Laboratory Equipment:

Rotary evaporator: Büchi, Rotavapor® R-100

Multistep pipette and pipette tips: Brand, Handystep

Purified water supply: Ultra Clean UV UF TM

Cytostatic safety cabinet: Berner, C-MaxPro 190

Analytical balance: Eppendorf, DE120K10A

Magnetic stirrer: 2mag, MIXdrive6

Volumetric Pipettes: Gillson/Eppendorf

[0071] Procedure:

The purity of Bendamustine hydrochloride was determined according to the RP-HPLC method provided by the client. DMSO was used for the dilution of the samples. The autosampler temperature was set to 25 °C to avoid crystallization of DMSO (melting point at 18 °C).

Column type: Kinetex C18, 100 x 4.6 mm, 2.6 µm (Phenomenex)

Mobile phase: A: Water/ACN/TFA = 950/50/1 v/v/v
B: Water/ACN/TFA = 50/950/1 v/v/v

Column temperature: 25 °C

Autosampler temperature: 25 °C (5 °C original value)

Flow rate: 1.2 mL/min

Detection wavelength: 234 nm

Slit: 4 nm

Reference wavelength: 550 nm

Reference slit: 100 nm

Detection range: 0-30 min

[0072] Table 5 shows a chart of the gradient.

Table 5: HPLC Gradient

Time [min]	Eluent A	Eluent B
0	95%	5%
5	95%	5%
9	80%	20%
25	55%	45%
28	20%	80%
30	20%	80%
30.01	95%	5%
35	95%	5%

1.5.1 Buffer preparation

Solution	Composition	Compounding procedure
Mobile phase A	5 % (v/v) acetonitrile and 0.1 % (v/v) trifluoroacetic acid in water	950 mL of water were mixed with 50 mL of acetonitrile and 1 mL of trifluoroacetic acid
Mobile phase B	95 % (v/v) acetonitrile and	50 mL of water were mixed

	0.1 % of trifluoroacetic acid in water	with 950 mL of acetonitrile and 1 mL of trifluoroacetic acid
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1.5.2 Sample preparation

[0073] 1 g of each vial was transferred into a 50 mL sample tube and diluted with 19 mL DMSO. The solution then was 1:10 diluted and transferred into an HPLC sample vial.

1.6. Results and Discussion

[0074] The stability of Bendamustine-HCl in the formulations listed in Table 1 was evaluated. The solutions were compounded at room temperature and filled in ISO 6R clear glass vials with FluroTec coated serum stoppers. Samples were stored at 40 °C for four weeks (T4w), eight weeks (T8w) and 12 weeks (T12w), respectively. Visual appearance (color and state of crystallization) and purity by RP-HPLC were determined. Table 6 summarizes the results.

Table 6: Summary of analytical results

variant	Visual appearance		Purity by RP-HPLC [%]			
	Color**	Crystallization*	T4w	T8w	T12w	T6m
#01	Yellow / brownish	None	>98.0	<96.0	<70.0	<10
#02	Brownish	None	<10.0	<10.0	skipped	
#03	Colorless	None	<50.0	<10.0		
#04	Colorless	None	<75.0	<15.0		
#05	Colorless	None	>98.0	>96.0	>96.0	>92.0
#06	Colorless	High	>98.0	>96.0	>96.0	>92.0
#07	Colorless	None	>98.0	>96.0	>96.0	<10
#08	Colorless	High	>98.0	>96.0	>96.0	>92.0
#09	Colorless	Medium	>98.0	>96.0	>96.0	>92.0
#10	Colorless	High	>98.0	>96.0	>96.0	>92.0
#11	Colorless	None	>98.0	>96.0	skipped	
#12	Colorless	High	>98.0	>96.0	>96.0	skipped
#13	Colorless	High	>98.0	>96.0	skipped	
#14	Colorless	Medium	>98.0	>96.0		
#15	Colorless	High	>98.0	>96.0		
#16	Colorless	None	>98.0	>96.0	~96 %	>92.0
#17	Colorless	High	>98.0	skipped		

* At T12w

[0075] The most promising formulation at the end of the stability study, T6m, are formulations #5 and #16 (colorless, crystal-free solution, ≤5 % purity decrease after six months at 40 °C).

Example 2: Determination identity of crystals crystallized/precipitated

[0076] The objective of the investigation is to determine the solid-state form (phase analysis) of precipitated crystals of the cytotoxic active ingredient Bendamustine-HCl (see figure 1).

[0077] The initial composition of the solution in the vial is as follows: Benda-H₂O-HCl 2.50% w/w CaCl₂ 2.50% w/w BHT 0.05% w/w Acetone 2.50% w/w PEG400 92.45% w/w For the PXRD measurement, the crystals are filled into a so-called Lindemann capillary (Ø 1mm) in the presence of their mother solution and a 4-hour measurement is carried out. The PXRD allows the determination of the solid-state form as well as the crystallinity (amorphous / crystalline) of the crystals. Diffractograms of Bendamustine-HCl (four crystalline forms) from patent literature US8445524B2 and calculated from single crystal data can be used as references. Suitable references for mannitol, calculated from single crystal data, are also available.

[0078] The crystals were filled into a so-called Lindemann capillary (Ø 1mm) in the presence of their mother solution and an 8-hour measurement was carried out.

[0079] The obtained diffractogram of the crystals (pjpBEN003) shows no agreement in comparison with the diffractogram of the Bendamustine hydrochloride monohydrate. Therefore it can be concluded that the precipitated solid is not the monohydrate (Figure 2)

[0080] An extended search revealed that the crystallized solid (pjpBEN003) is Form I of the Bendamustine hydrochloride (see Figure 3). According to the heat of fusion rule, Form I is the thermodynamically most stable form of Bendamustine hydrochloride (US8445524B2).

Example 3: Test of crystallization inhibition

[0081] Four different water-free Bendamustine formulations were compounded and put on a stability study at 25 °C and 40 °C.

The following formulations were tested:

Table 7: Formulation variants selected for stability testing

	Components [mg/vial]				
Variant	Bendamustine ·HCl ·H ₂ O	CaCl ₂	BHT	HPBCD	PEG400
#01	115	125	2	50	to 5 g
#02	115	125	2	100	to 5 g
#03	115	125	2	150	to 5 g
#04	115	125	2	200	to 5 g
	Components [% (w/w)]				
Variant	Bendamustine ·HCl ·H ₂ O	CaCl ₂	BHT	HPBCD	PEG400

#01	2.3	2.5	0.05	1.0	94.15
#02	2.3	2.5	0.05	2.0	93.15
#03	2.3	2.5	0.05	3.0	92.15
#04	2.3	2.5	0.05	4.0	91.15

The formulations differ in their Hydroxypropyl Betadex (HPBCD) concentration content (1 – 4 % HPBCD).

[0082] In a previous work package (WP14) the crystallization starting time point of a HPBCD-free formulation was evaluated (formulation: Bendamustine-HCl-H₂O 2.3 %, CaCl₂ 2.5 %, BHT 0.05 %, PEG400 95.15 %). After approximately four weeks storage at 40°C crystals were observed macroscopically on the vial bottom. A representative photograph at a later sampling point (six months of storage) is shown in Figure 5.

[0083] As reported in example 2, crystals were isolated and analyzed by XRPD. It has been proven that the crystals consist of pure Bendamustine hydrochloride free of crystal water. Thus, it can be concluded that the water molecule in Bendamustine*HCl*H₂O is split off during storage and that crystallization occurs due to lower solubility of Bendamustine*HCl (waterfree) in PEG400 compared to Bendamustine*HCl*H₂O.

[0084] Aim of this study is to evaluate whether addition of HPBCD can prevent this type of Bendamustine crystallization and to determine a critical / minimal Hydroxypropyl Betadex (HPBCD) concentration to prevent crystallization.

3.1 Materials and methods

Substances	Lot-no.:	Manuf. / Suppl.	Order-no.:
Purified water ($\leq 0.2 \mu\text{mS/cm}$, $< 10 \text{ ppb TOC}$)	-	PJP	-
Bendamustine hydrochloride monohydrate	N1700362		
Polyethylene glycol 400, ROTIPURAN® Ph. Eur.	120294684	Carl Roth GmbH	0144
Butylhydroxytoluene, $\geq 99.8 \%$, for synthesis	058267347	Carl Roth GmbH	3559
Calcium chloride anhydrous, Ph. Eur.	K48950378	Merck Chemicals	102378
(2-Hydroxypropyl)- β -cyclodextrin	BCBL4188V	Sigma-Aldrich	778966

Materials	Lot-no.:	Manuf. / Suppl.	Order-no.:
Glass vial 6R	2150359/1	Thüringer Pharmaglas	050006131
Lyo stopper, Westar® RS, 1346 4023/50 GREY B2-TR	1172035601	West	7002-5042
Sartopure GF+ MidiCap	648002503	Sartorius	5555305P7

Camera Equipment

Manufacturer: Canon (Tokio, Japan)
 Body: EOS 600D
 Lens: DGMacro 105mm 1:2.8

Additional Laboratory Equipment:

Rotary evaporator: Büchi, Rotavapor® R-100
 Multistep pipette and pipette tips: Brand, Handystep
 Purified water supply: Ultra Clean UV UF TM
 Cytostatic safety cabinet: Berner, C-MaxPro 190
 Analytical balance: Eppendorf, DE120K10A
 Magnetic stirrer: 2mag, MIXdrive6
 Volumetric Pipettes: Gillson/Eppendorf
 Laboratory dish washer: Miele, G7783
 Autoclave: Systec, DX65
 Drying oven (depyrogenisation): Memmert, UFP500
 Primary packing material preparation

Vials: Vials were washed in a laboratory dishwasher with purified water and depyrogenised at 300 °C for 2 hours.

Stoppers: Stoppers were autoclaved (121 °C, 2 bar, 20 min) and dried at 80 °C for 8 hours.

3.2 Compounding

3.2.1 Procedure:

[0085] Firstly, a stock solution of CaCl_2/BHT in PEG400 was prepared. Then, additives (HPBCD and additional PEG400) were added to the solvent variants to complete solvent preparation according to Table 1. The solvents were filtered through a 0.65 μm fiberglass filter unit (Sartopure GF+) to eliminate unsolved particles. Bendamustine monohydrochloride monohydrate was weighed in a 6R vial and filled to target weight with the corresponding solvent. The bulk solutions were stirred at 350 rpm for approximately 1 h. After complete dissolution, the variants' volumes were splitted à 5 g into 6R vials with nitrogen as an inert headspace gas.

[0086] All variants #01 to #04 were compounded in parallel. During compounding contact to atmospheric oxygen was avoided best possible. Compounding vessels were kept tightly closed and were selected as small as feasible to reduce headspace.

The dedicated batch size was 2 vials each variant with a fill of 5.0 g per vial.

3.3 (Accelerated) stability study

[0087] One vial per variant was stored at 25 °C, the other vial at 40 °C.

3.4 Determination of Bendamustine crystallization

[0088] At T0 and in equidistant time periods of two weeks after the last time point of analysis, all vials were examined macroscopically. The vials were put under an external light source and examined by eye at different perspectives.

3.5 Results and Discussion

[0089] The stability of Bendamustine-HCl in the formulations listed in Table 1, with special regard in crystallization, was evaluated. The solutions were compounded at room temperature and filled into ISO 6R clear glass vials with FluroTec coated serum stoppers.

Samples were stored at 25 °C and 40 °C.

3.6 Crystallization

Table 8 lists the results of visual examination for Bendamustine crystals.

Table 8: Macroscopic examination for Bendamustine crystallization

At 25 °C		Crystallization				
Variant	T0	T2w	T4w	T6w	T8w	T10w
#01	no crystals	no crystals	no crystals	no crystals	no crystals	crystals
#02	no crystals	no crystals	no crystals	no crystals	no crystals	no crystals
#03	no crystals	no crystals	no crystals	no crystals	no crystals	no crystals
#04	no crystals	no crystals	no crystals	no crystals	no crystals	no crystals
At 40 °C		Crystallization				
Variant	T0	T2w	T4w	T6w	T8w	T10w
#01	no crystals	no crystals	no crystals	no crystals	no crystals	no crystals
#02	no crystals	no crystals	no crystals	no crystals	no crystals	no crystals
#03	no crystals	no crystals	no crystals	no crystals	no crystals	no crystals
#04	no crystals	no crystals	no crystals	no crystals	no crystals	no crystals

[0090] Most likely, the crystal water of the monohydrate form gets split off at both evaluated temperatures. However, the solubility of Bendamustine anhydrate is higher at 40 °C compared to 25 °C (as usual for solid components). Thus, crystallization is observed earlier at 25 °C.

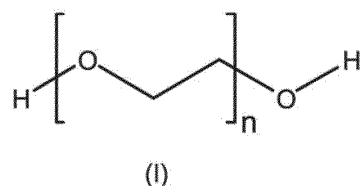
3.7 Summary

[0091] Four water-free PEG400/CaCl₂/BHT Bendamustine formulations were prepared differing in their HPBCD content (1, 2, 3 and 4 %). The vials were stored at 25 °C and 40 °C to observe the time point at which crystallization starts to occur.

[0092] Current state (T10w): Only a very small amount of crystals could be detected in the 1 %(w/w) HPBCD formulation after storage at 25 °C. A content of 2 %(w/w) HPBCD or higher in the formulation safely prevents crystallization of Bendamustine.

Claims

1. A liquid pharmaceutical formulation, being essentially free of water, comprising
 - a) at least one active pharmaceutical ingredient, selected from the group consisting of Bendamustine, Melphalan, Melflufen, Chlorambucil and Uramustine;
 - b) one or more crystallization inhibitor(s) selected from the group consisting of (2-Hydroxypropyl)- β -cyclodextrin and DMSO;
 - c) a solvent according to formula (I)



wherein n is 350 to 500

- d) at least one pharmaceutically acceptable alkaline earth metal.
2. The liquid pharmaceutical formulation according to claim 1, wherein all components of the formulation are completely dissolved resulting in a clear solution.
3. The liquid pharmaceutical formulation according to claim 1 or 2, wherein the pharmaceutical formulation comprises at least one pharmaceutically acceptable alkaline earth metal salt selected from the group consisting of a calcium and a magnesium salt, preferably a calcium salt.
4. The liquid pharmaceutical formulation according to claim 3, wherein the anion of the at least one alkaline earth metal salt is gluconate, chloride, bromide, acetate or lactate, preferably chloride.
5. The liquid pharmaceutical formulation according to any one of claims 3 or 4, wherein the at least one pharmaceutically acceptable alkaline earth metal salt is
 - i) present in a concentration of 0.1 to 3 mol/L, preferably 0.1 to 1.5 mol/L, more preferably 0.3 to 1.5 mol/L, particular preferred 0.7 to 1.1 mol/L, more particular preferred 0.1 to 1.0 mol/L, most particular preferred 0.8 to 1.0 mol/L, especially preferred 0.1 to 0.5 mol/L based on the overall volume of the liquid formulation or

- ii) present in a weight of 1 to 4 % (w/w), preferably 2 to 3 % (w/w), more preferably 2.5 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.
6. The liquid pharmaceutical formulation according to any one of claims 1 to 5, wherein the at least one active pharmaceutical ingredient is present
- i) in a concentration of 1 to 200 mg/g, preferably 5 to 150 mg/g, more preferably 10 to 100 mg/g, most preferably 20 to 40 mg/g based on the overall weight of the formulation or
 - ii) in a weight of 1 to 3.5 % (w/w), preferably 1.5 to 3 % (w/w), more preferably 2.16 to 2.59 % (w/w), most preferably 2 to 2.5 % (w/w) based on the overall amount of the liquid pharmaceutical formulation.
7. The liquid pharmaceutical formulation according to any one of claims 1 to 6, wherein
- a) the at least one active pharmaceutical ingredient is Bendamustine and/or
 - b) n is 380 to 450, preferably 390 to 430, more preferably 400.
8. The liquid pharmaceutical formulation according to any one of claims 1 to 7, wherein
- i) the crystallization inhibitor is (2-Hydroxypropyl)- β -cyclodextrin and/or
 - ii) the at least one crystallization inhibitor is present in said liquid pharmaceutical formulation in an amount of or at least in an amount of
 - i) 0.5 to 5 % (w/w), preferably 0.7 to 4.3 % (w/w), more preferably 1 to 4 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or
 - ii) 0.8 to 3 % (w/w), preferably 0.85 to 2 % (w/w), more preferably 1 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or
 - iii) 1.5 to 3.5 % (w/w), preferably 1.8 to 2.5 % (w/w), more preferably 2 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or
 - iv) 2.5 to 4.5 % (w/w), preferably 2.8 to 3.5 % (w/w), more preferably 3 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or
 - v) 3.5 to 5 % (w/w), preferably 3.8 to 4.5 % (w/w), more preferably 4 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or

- vi) 5 to 15 % (w/w), preferably 7 to 12 % (w/w), more preferably 10 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation.
9. The liquid pharmaceutical formulation according to any one of claims 1 to 8, further comprising an antioxidant, preferably the antioxidant is selected from the group consisting of Thioglycerol, Butylhydroxytoluene, Thioglycolic Acid, Ascorbic Acid, Ascorbyl Palmitate, Sodium Sulfites, Butylated Hydroxyanisole, Propyl Gallate, Tocopherols more preferably the antioxidant is Butylhydroxytoluene.
10. The liquid pharmaceutical formulation according to claim 9, wherein the amount of antioxidant is 0.01 to 0.1 % (w/w), preferably 0.03 to 0.07 % (w/w), more preferably 0.05 % (w/w) based on the overall weight of the liquid pharmaceutical formulation.
11. The liquid pharmaceutical formulation, according to any one of the claims 1 to 10, wherein the liquid pharmaceutical formulation does not comprise dextran.
12. The liquid pharmaceutical formulation, according to any one of the claims 1 to 11, wherein
- ii) the solvent according to formula (I) represents at least 90 % (w/w), more preferably at least 95 % (w/w), most preferably at least 98 % (w/w), most preferably at least 100 % (w/w) of the solvents based on the overall weight of all solvents present in the liquid pharmaceutical formulation; or
- iii) a) the at least one active pharmaceutical ingredient is present in a weight of 1 to 3.5 % (w/w), preferably 1.5 to 3 % (w/w), most preferably 2 to 2.5 % (w/w) based on the overall amount of the liquid pharmaceutical formulation; and
- b) the crystallization inhibitor is present in a weight of
- i) 0.8 to 3 % (w/w), preferably 0.85 to 2 % (w/w), more preferably 1 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or
- ii) 1.5 to 3.5 % (w/w), preferably 1.8 to 2.5 % (w/w), more preferably 2 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or
- iii) 2.5 to 4.5 % (w/w), preferably 2.8 to 3.5 % (w/w), more preferably 3 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or
- iv) 3.5 to 5 % (w/w), preferably 3.8 to 4.5 % (w/w), more preferably 4 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; or
- v) of 5 to 15 % (w/w), preferably 7 to 12 % (w/w), more preferably 10 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; and

d) the pharmaceutically acceptable alkaline earth metal salt selected from the group consisting of a calcium and a magnesium salt, preferably a calcium salt is present in a weight of 1 to 4 % (w/w), preferably 2 to 3 % (w/w), more preferably 2.5 % (w/w) based on the amount of the overall weight of the liquid pharmaceutical formulation; and

e) optionally an antioxidant is present in an amount of 0.01 to 0.1 % (w/w), preferably 0.03 to 0.07 % (w/w), more preferably 0.05 % (w/w) based on the overall weight of the liquid pharmaceutical formulation; and

c) the amount of solvent present in the overall liquid pharmaceutical formulation corresponds to 100 % (w/w) subtracted by the sum of the amounts of a), b), d) and e) or the sum of the amounts of a), b), d), e) and any further ingredient present in the overall liquid pharmaceutical formulation as defined above.

13. The liquid pharmaceutical formulation, according to any one of the claims 1 to 12, for use in medicine.

14. The liquid pharmaceutical formulation, according to any one of the claims 1 to 12, for use in the treatment of cancer.

15. The liquid pharmaceutical formulation, according to any one of claims 1 to 4, wherein the preparation of the formulation comprises solving Bendamustine hydrochloride monohydrate in the solvent c).