(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 November 2011 (10.11.2011)

PCT

(10) International Publication Number
WO 2011/138802 A1

(51) International Patent Classification:
A61K 38/26 (2006.01) *A61K 9/08* (2006.01)
C07K 14/605 (2006.01)

(21) International Application Number:
PCT/IN2011/000321

(22) International Filing Date:
6 May 2011 (06.05.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1449/MUM/2010 7 May 2010 (07.05.2010) IN

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2011/138802 A1

(54) Title: INJECTION SOLUTION

(57) Abstract: The present invention provides a stable ready-to-use solution comprising therapeutically effective amounts of glucagon together with pegylated distearoyl-phosphatidylethanolamine.

INJECTION SOLUTION

FIELD OF INVENTION

The present invention relates to stable ready-to-use solution comprising glucagon. The solution is suitable for parenteral administration, particularly suitable for self administration.

BACKGROUND OF THE INVENTION

Glucagon is a polypeptide hormone identical to human glucagon that increases blood glucose and relaxes smooth mus of the gastrointestinal tract. Glucagon is a single-chain polypeptide that contains 29 amino acid residues. It is insoluble in water but is soluble at a pH of less than 3 or more than 9.5. Due to limitations in both chemical and physical stability of glucagon, it is supplied in the form of a lyophilized powder which must be used immediately following reconstitution with its supplied aqueous diluents. It is available for use intravenously, intramuscularly, or subcutaneously in a kit that contains a vial of sterile glucagon and a syringe of sterile diluent. The vial contains 1 mg (1 unit) of glucagon and 49 mg of lactose or 107mg of lactose monohydrate (Novo nordisk). The diluent syringe contains 12 mg/mL of glycerin, Water For Injection, and hydrochloric acid or only water For Injection. Glucagon is administered as an emergency medication in case of hypoglycemic shocks. Therefore, administration of a ready-to-use aqueous solution that does not require any step of reconstitution may be a better preferred approach, particularly, in emergency cases and when the patient needs to self administer the drug. However, the biggest constraint of glucagon aqueous solution is that it is physically and chemically unstable. This is because of propensity of glucagon to aggregate resulting in the formation of fibrils and gel; a process that can be stimulated by acidic pH, increased ionic strength, peptide concentration, agitation and/or elevated temperatures. The aggregation is particularly unacceptable because aging glucagon solutions prepared at concentrations greater than 2.5 mg/ml for 24 hours have been reported to result in production of cytotoxic amyloidogenic fibrils.(by Kathy L.De Jong, Bev Incledon, Chrostopher M.Yip, Michael R. Defellippis, *Biophys J BioFAST*, June 9, 2006). It may be inferred from literature reports, that the nature of glucagon, presents a challenge for a formulator to develop glucagon solution, particularly, a ready to use solution.

The prior art references indicate that there are attempts made to tackle the problem generally associated with water insoluble drugs while making a solution. For example, United States Patent Number US 6,217,886 (herein after referred to as '886) disclosed a method of preparing a biologically active micelle product comprising one or more biologically active amphipathic compounds in association with a micelle, said method comprising steps of

a) mixing one or more lipids wherein at least one lipid component is covalently bonded to a water soluble polymer;
b) forming sterically stabilized micelles from lipids;
c) incubating micelles from step(b) with one or more biologically active amphipathic compound (s) under conditions in which said compound(s) becomes associated with said micelles in a more biologically active conformation.

The United States Patent Number US '886 and several other references, for example, Journal of pharmaceutical Sciences, vol. 93, No. 10, October 2004, pp. 2476-2487, teach that DSPE-PEG 2000 is a novel lipid based carrier for water insoluble drugs.

Also the effects of micelles of nonionic, zwitterionic, anionic and cationic surfactants and lipids on the conformation of glucagon and insulin have been investigated by circular dichroism and intrinsic protein fluorescence by Pasta et al [*Pasta et al, Biochmica et Biophysica Acta, 953 (1988) 314-320*]. Some amphipathic compounds protected glucagon against proteolysis by trypsin and chymotrypsin very markedly, whereas others did not protect at all or only slightly protected the hormone. It is reported in the literature that concentration dependent equilibrium of glucagon in mildly alkaline solution involves the formation of associated forms of higher helicity (*W.B. Gratzer and G.H. Beaven, The Journal of Biological Chemistry, vol. 244, No. 24, pp. 6675-6679, 1969*). It is also reported that monomeric glucagon in aqueous solution does not have a stable globular structure, the conformation changing continuously with change of temperature or concentration of denaturants added. (*Bhinyo Pnajipan and Walter B. Gratzer, European J. Biochemistry, 45, 547-553, (1974)*.

In attempts to develop a stable, ready-to-use solution of glucagon suitable for self parenteral administration, preferably, in emergency situations where there is no sufficient time for reconstitution, the inventors found that cyclodextrins, sugar alcohols, alcohol, proteins like human serum albumin were incapable of solubilizing the glucagon. The inventors, surprisingly, found out that aqueous solution of glucagon having distearoyl-phosphotidylethanolamine covalently bonded to polyethylene glycol was chemical and physical stability to the solution, particularly in terms of protein conformation, particularly, at a pH between 5 and 7.5.

SUMMARY OF THE INVENTION

The present invention provides a stable, ready-to-use solution which comprises therapeutically effective amounts of glucagon and pharmaceutically acceptable vehicle.

The invention can be summarized as follows:

A. A stable, ready-to-use solution which comprises therapeutically effective amounts of glucagon and pegylated distearoyl-phosphotidylethanolamine adjusted to a pH of about 5-7.5 and pharmaceutically acceptable vehicle.

B. A stable, ready-to-use solution as described in A wherein glucagon concentration ranges from 0.5 mg/ml to 5 mg/ml.

C. A stable, ready-to-use solution micellar solution as described in A wherein the pegylated distearoyl-phosphotidylethanolamine has molecular weight of about 300 to 5000 and is present in amount ranging from 1 mg/ml to 40 mg/ml.

D. A stable, ready-to-use solution as described in A wherein the vehicle comprises a solvent selected from aqueous or polar aprotic solvents such as dimethyl sulphoxide.

E. A stable, ready-to-use solution as described in D wherein the aprotic solvent is present in 5 to 15% volume by volume of the solution.

F. A stable, ready-to-use solution as described in A wherein the pH of the solution is adjusted to about 6.5.

G. A stable, ready-to-use solution as described in A wherein the solution further comprises a stabilizer is selected from the group consisting of fatty acids or zwitterionic choline derivatives or mixtures thereof.

H. A stable, ready-to-use solution as described in G wherein choline derivative is dimyristoyl phosphotidylcholine and is present in concentration of about 1 mg/ml to 2 mg/ml.

I. A stable, ready-to-use solution as described in G wherein the fatty acid is caprylic acid and is present in amount of about 0.1 mg/ml to 3 mg/ml.

J. A stable, ready-to-use solution as described in A wherein pharmaceutically acceptable vehicle comprises one or more acids, buffers, preservatives, tonicity adjusting agents and mixtures thereof.

K. A stable, ready-to-use solution as described in J wherein the buffers are selected from the group consisting of histidine succinate, phosphate, citrate salts and mixtures thereof.

DETAILED DESCRIPTION OF THE INVENTION

The term 'stable' as used herein means that the solution remains physically and chemically stable. Particularly, the solution remains clear on long term storage at either 2-8°C or preferably, at room temperature for at least three months, preferably about 6 months to 12 months. The physical stability of the solution of the present invention is determined by recording the percentage transmission at 650 nm & absorbance at 420 nm. Further the solution retains potency which is determined by bioassay methods official in pharmacopoeia as well as the chemical assay such as HPLC. The solution is said to be stable when the total impurities do not exceed more than 10 %, preferably more than 10 % when the solution is stored for a period of about three months at room temperature and/ or at 2-8°C for a period of about three month or more. Additionally, the solution is said to be stable when the related substances of glucagon, such as glucagon sulphoxide, D-trp (25)- glucagon or D- phen (22)- glucagon do not exceed 3 % at the end of storage period, such as room temperature for three months or more or when stored at 2-8°C for six months or more.

The term 'ready-to-use' as used herein means that the solution is in the form that can be administered without additional step of reconstitution. For example, product that is available in the market is in the form of a lyophilized form. Glucagon, is a therapeutic substance that is administered as an emergency medication in case of hypoglycemic shocks. Thus, it is always

desirable to have a ready to use solution that is stored in a pre-filled syringe. Depending upon on the emergency situation, the patient can self administer the glucagon solution without any delay in administration. According to one preferred embodiment, the ready-to–use stable, micellar solution of the present invention is suitable for a single dose parenteral self administration wherein the composition is dispensed in a prefilled syringe. The amount of glucagon per single administration may range from 1 mg to about 5 mg per ml of the solution, preferably, 1 mg per ml. According to another embodiment, the solution is filled in prefilled syringe with an autoinjector. Generally, an autoinjector is spring powered and designed to administer the entire contents of the prefilled syringe in one single dose. It may not have any fluid path and may not have any contact with the drug or biologic contained within the syringe. The components of autoinjector may be made up of plastic and steel. The design and performance features of the autoinjector device may include a safety mechanism to prevent inadvertent activation, automatic sheathing of the used needle, cutout window on the front assembly, locking tabs to prevent disassembly of the auto-injector device once the two sub assemblies have been connected, and self disabling to prevent reuse.

The term 'micellar' as used herein means that the solution is in the form of micelles which appears to be a clear solution by visual observation. Preferably, the micelles here are stabilized by phospholipids. The micellar solution is said to be physically stable when there are no signs of conformation changes such as gel formation/aggregation, change in CD spectra showing alpha helix structure and/or the solution is clear in terms of particulate matter immediately after preparation and on storage at various storage conditions such as room temperature, 2-8^0 C for a period of about 6 months. It is important to note here again that any change in the conformation of hormone is unacceptable as it is found to provide less potent protein or provide less therapeutic effects, upon administration (See by Kathy L.De Jong, Bev Incledon, Chrostopher M.Yip, Michael R. Defellippis, *Biophys J Biofast*, June 9, 2006). The micellar solution of the present invention is suitable for parenteral administration. By the term 'suitable for parenteral administration' it is meant that the solution comprises the excipients in amounts that are safe and are approved by regulatory authority like USFDA, for parenteral use.

In one embodiment of the present invention, there is provided a micellar solution comprising glucagon in therapeutically effective amounts and distearoyl-phosphatidylethanolamine covalently bonded to PEG (PEG-DSPE) and, optionally, one or more stabilizers and pharmaceutically acceptable vehicle, wherein the pH of the solution is at about 5-7.5. The ready-to-use solution of the present invention comprises glucagon in the concentration ranging from about 0.5 mg/ml to about 5 mg/ml. Further, it comprises pegylated distearoyl-phosphotidylethanolamine in the concentration ranging from 4 mg/ml to 40 mg/ml. In one preferred embodiment, the molecular weight of pegylated distearoyl-phosphotidylethanolamine ranges from about 300 to 5000 Daltons, particularly, about 2000 & 5000 Daltons.

In one embodiment, ready-to-use glucagons solution of the present invention comprises pegylated distearoyl-phosphotidylethanolamine having molecular weight of about 300 to 5000. It is used in an amount of 1 mg/ml to 8 mg/ml. It was found that addition of a polar aprotic solvent such as dimethyl sulphoxide, allows decrease in the amount of pegylated distearoyl-phosphotidylethanolamine required to solubilize the glucagon. In some embodiment,polar aprotic solvent constitutes 5% to 15% v/v of the total volume.

In one preferred embodiment, the micellar solution further comprises a stabilizer which has a tendency to reside in the hydrophobic portions of the micelle. Preferably, the stabilizer is selected from the group consisting of fatty acid or a zwitterions choline derivative. The term fatty acids includes aliphatic (saturated or unsaturated) monocarboxylic acids derived from or contained in esterified form, in an animal or vegetable fat, oil or wax. Examples of fatty acids or its salts that may be used in the compositions of the present invention include but are not limited to fatty acids or its salts having 'n' number of carbon atoms wherein 'n' ranges from about 4 to about 28. The fatty acid may be a saturated fatty acid or an unsaturated fatty acid, and their salt and combinations thereof. The saturated fatty acid and its salts may be selected from butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, sodium caprylate, sodium laurate, sodium myristate, sodium palmitate and the like and/or mixtures thereof. The unsaturated fatty acid and its salts may be selected from myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, alpha linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, docosahexaenoic

acid, sodium oleate, sodium arachidonate and the like and/or mixtures thereof. In an embodiment in which the stabilizer is a zwitterion choline derivative selected from the group consisting of dimyristoyl phosphotidylcholine, egg phosphotidylcholine, dipalmityol phosphotidylcholine, distearoyl phosphotidylcholine, hydrogenated soya phosphotidylcholine, dilauryl phosphotidylcholine, dioleaoyl phosphotidylcholine and their-lyso products. In one embodiment, the solution of the present invention comprises dimyristoyl phosphotidylcholine in the concentration ranging from about 1 mg/ml to 2 mg/ml. In another embodiment, the micellar solution comprises caprylic acid as a stabilizer in the concentration ranging from about 0.1 mg/ml to 3 mg/ml, particularly, 1 mg/ml. Addition of these stabilizers improves the stability of glucagon in that the solution can be stored at room temperature for a period of about six months.

The ready-to-use solution of the present invention further comprises pharmaceutically acceptable vehicle which may comprise one or more acids, buffers, preservatives, tonicity adjusting agents and mixtures thereof. The buffers that may used in the pharmaceutically acceptable vehicle are selected from the group consisting of histidine, succinate, phosphate, citrate and the like and mixtures thereof. The tonicity imparting agents that may be added to the micellar solution may be selected from the group consisting of mono-saccharides like dextrose, sucrose, mannitol, lactose and glycerine and mixtures thereof.

The inventors of the present invention however found surprisingly and unexpectedly, that glucagon was solubilized and stabilized with the help of a pegylated distearoyl-phosphotidylethanolamine alone or in combination with a detergent like dimyristoyl phosphotidylcholine or a fatty acid such as caprylic acid, pH was adjusted to about 5.0 to 7.5, preferably, pH of about 6 to 7.

While the present invention is disclosed generally above, additional aspects are further discussed and illustrated with reference to the examples below. However, the examples are presented merely to illustrate the invention and should not be considered as limitations thereto.

EXAMPLE A

Effect of pH on the glucagon solution was studied. A bulk solution of glucagon (1mg/ml) was prepared using 8mg/ml MPEG-DSPE2K having molecular weight of 2000. This solution was divided in 3 portions & pH was adjusted to 3.0, 6.0, & 9.0. After storage at 40°C/75% RH for 3 days, these solutions were analyzed for assay of glucagon. Table 1 shows the data of the solutions indicating physical and chemical stability.

Table 1: Effect of pH on assay (HPLC) of aqueous glucagon solution after 3 days at 40°C

pH	3.0	6.0	9.0
Appearance	Turbid	Clear	Clear
Assay	73.39	107.30	76.58

From the data, it is evident that glucagon along with MPEG-DSPE is physically unstable at acidic pH (pH 3.0) & chemically unstable at basic pH (pH 9.0). At pH 6.0 (near to the isoelectric point 6.2 of glucagon), the aqueous solution of glucagon was found to be more stable.

Further, the effect of pH was studied in a narrow pH range. A bulk solution of glucagon (1mg/ml) was prepared using 8mg/ml MPEG-DSPE2K. pH was adjusted from 5.5 to 7.5 using citrate buffer & sodium hydroxide solution. After storage at 30°C/65% RH for 1 month, these solutions were analyzed for assay of glucagon & related substances.

Table 2: Effect of pH on physicochemical stability of aqueous glucagon solution

pH	Time	Physical appearance	HPLC Assay (%)	Related substance (%)				
				Glucagon sulphoxide	D-trp(25)-glucagon	D-phe(22)-glucagon	Single max.	Total
5.5	Initial	Clear	96.31	0.28	ND	0.22	0.68	2.42
	1M/30°C	Clear	93.77	0.59	0.02	0.04	0.98	5.16
6.0	Initial	Clear	95.86	0.26	ND	0.21	0.68	2.50
	1M/30°C	Clear	91.17	0.64	0.03	0.03	1.01	4.16
6.5	Initial	Clear	97.25	0.23	ND	0.20	0.64	2.32
	1M/30°C	Clear	94.61	0.83	ND	0.2	1.05	4.22
7.0	Initial	Clear	96.03	0.27	ND	0.21	0.67	2.46
	1M/30°C	Clear	92.62	0.91	ND	0.01	1.40	4.93
7.5	Initial	Clear	94.91	0.21	ND	0.21	0.67	2.32
	1M/30°C	Clear	90.35	0.73	ND	0.01	1.52	5.22

The results (Table 2) showed similar physicochemical stability of glucagon from pH 5.5 to pH 7.5 in solution form prepared using mPEG-DSPE2000. Thus it may be concluded from these preliminary findings, that when the glucagons solution is maintained at a pH range of 5.0 to about 7.5 and pegylated distearyl- phosphatidyl ethanolamine, a stable solution is obtained.

EXAMPLE 1-2

An aqueous solution of glucagon was prepared as follows:

Table 3: composition of the glucagons solution

Ingredients	EXAMPLE 1	EXAMPLE 2
	Quantity in milligrams	
Glucagon	1	1
mPEG-DSPE 2000	8	8
Sucrose	50	80
Histidine	-	1.5 to adjust to pH 6.0
Lactic acid solution (10%)	qs to adjust to pH 6.5	-
Sodium bicarbonate (8 mg/ml)		
Water for Injection	q.s to 1 ml	

mPEG-DSPE 2000 and sucrose were dissolved in purified water at room temperature. pH of this solution was adjusted to 6.5 using lactic acid and/or sodium bicarbonate solution (Example 1) or to a pH of 6.0 with histidine

Required amount of glucagon was dissolved in the above solution using magnetic stirrer at room temperature. This solution was filtered through 0.8/0.2μ PVDF filter in aseptic area and packed in 5ml USP-Type I glass vials. The final product was kept for stability study as per ICH guidelines at 2-8°C, 25°C/60%RH & 40°C/75%RH. Results of the stability are given below.

Table 4: Results of the stability testing of composition of example 1.

Stability Condition	Time	Description	Assay in %	Related substance (%)		Absorbance at 420 nm (AU)	% T at 650 nm
				Single	Total		
	Initial	Clear solution	101.19	0.53	1.40	0.011	99.812
2-8°C	1M	Clear solution	102.71	0.59	2.05	0.004	99.947
25°C/60%RH	1M	Clear solution	97.57	0.58	4.16	0.005	99.591
40°C/75%RH	1M	Clear solution	74.83	2.96	17.49	0.090	97.325
2-8°C	2M	Clear solution	94.66	0.52	3.0	0.006	99.927
25°C/60%RH	2M	Precipitation.	81.82	1.12	8.36	0.025	97.328
40°C/75%RH	2M	Precipitation	66.16	3.82	31.42	0.0570	29.506
2-8°C	3M	Clear solution	95.49	0.60	3.24	0.009	99.701
25°C/60%RH	3M	Precipitation	85.22	1.09	8.93	0.248	61.279
40°C/75%RH	3M	Precipitation	39.36	6.62	31.75	0.636	26.875
2-8°C	6M	Precipitation	97.01	0.72	3.21	0.076	92.430
2-8°C	12M	Precipitation	80.06	0.59	4.12	0.917	19.258

Composition of Example 2 was found to be not stable at room temperature as turbidity started appearing on 33 day when stored at room temperature. Therefore, it may be concluded that when glucagon was formulated into a solution by use of mPEG-DSPE wherein the pH is adjusted to a specific range of 5 to 7.5, the solution was found to be stable under 2-8°C for a period of three months only. However, the solution was not stable when stored at room temperature. The room temperature stability was further improvised by addition of a stabilizer such as fatty acid or dimyristoyl phosphotidylcholine.

EXAMPLE 3 -5

Table 5: composition of the present invention

Ingredients	EXAMPLE		
	3	4	5
Glucagon	1 mg	1 mg	1 mg
mPEG-DSPE 2000	8 mg	8 mg	-
mPEG-DSPE 5000	-	-	7 mg
Sucrose	50 mg	50 mg	50 mg
Dimyristoyl phoshotidylcholine	2 mg	-	-
Caprylic acid	-	1 mg	0.1 mg
Histidine	1 mg	1 mg	1 mg
Water for injection	q.s to 1 ml	q.s to 1 ml	q.s to 1 ml
Lactic acid solution (10%)	qs to adjust to pH 6.5	qs to adjust to pH 6.5	qs to adjust to pH 6.5
Sodium bicarbonate (8 mg/ml)	qs to adjust to pH 6.5	qs to adjust to pH 6.5	qs to adjust to pH 6.5

Example 3: Glucagon and Dimyristoyl phoshotidylcholine (DMPC) were mixed together in water for injection at room temperature, till the mixture becomes translucent. Then specified amount of mPEG-DSPE 2000 was added to this mixture and stirred until it becomes clear. Sucrose and histidine were added and dissolved in solution. pH was adjusted between 6-6.5 and volume was made up with water for injection. This solution was filtered through 0.2μ PVDF filter in aseptic area. The filtered bulk was filled in 1ml pre-filled syringes (with attached needle) & stoppered. The final solution was kept for stability study as per ICH guidelines at 2-8°C & 25°C/60%RH.

Example 4: mPEG-DSPE 2000 was dissolved in WFI. Glucagon was added to this solution & dissolved by continuous stirring at room temperature. Sucrose, histidine & caprylic acid were added one by one & dissolved. pH was adjusted between 6.0-6.5 & volume was made up with WFI. This solution was filtered through 0.2μ PVDF filter in aseptic area. The filtered bulk was filled in 1ml pre-filled syringes (with attached needle) & stoppered. The final solution was kept for stability study as per ICH guidelines at 2-8°C& 25°C/60%RH.

Example 5: mPEG-DSPE 5000 was dissolved in purified water at room temperature. Required amount of glucagon was dissolved in the above solution using magnetic stirrer at room temperature. Sucrose, histidine & caprylic acid were added & dissolved one by one in the above solution with continuous stirring. pH of this solution was adjusted to 6.5 using sodium bicarbonate solution and/or lactic acid solution if needed. A clear colourless solution was formed. This solution was filtered through 0.8/0.2μ PVDF filter in aseptic area and packed in 5ml USP-Type I glass vials.

Table 6: Stability results of solution of example 3 and example 4

Example No.	Time M (month) RT (Room temperature)	Physical appearance
Example 3	1M/RT	Clear solution
	5M/2-8°C	Clear solution
	5M/RT	Clear solution
	6M/RT	Clear solution
	10M/2-8°C	Clear solution
Example 4	RT/1M	Clear solution
	40°C/1M	Clear solution
	5M/2-8°C	Clear solution
	5M/RT	Clear solution
	6M/2-8°C	Clear solution
	6M/RT	Clear solution
	9M/2-8°C	Clear solution

Glucagon solution of Examples 3 & 4, were found to be stable. Without wishing to be bound by any theory, this effect said to be due to stabilization of micelles by fatty acid like caprylic acid or zwitterions choline derivative like DMPC in combination with mPEG-DSPE. Histidine is added in this solution to act as buffer. Thus, it may be concluded that mPEG-DSPE can improve physical & chemical stability of glucagon solution. But, precipitation starts appearing in glucagon solution containing mPEG-DSPE alone within 6 months, even when stored at 2-8°C. However, addition of a stabilizer like caprylic acid or DMPC can further improve physical & chemical stability of glucagon solution micellar solution.

Further, the solution similar to example 3 and example 4 were subjected to physical characterization (%Transmittance at 650nm & absorbance at 420nm), bioassay and chemical assay of glucagon as well as content of related substance at initial time point and at various storage conditions such as 2-8°C and 25°C/60%RH for three months. The physical stability of the hormone in the solution form in terms of conformation is confirmed by performing bioassay of the glucagon after the solution is stored at six months at room temperature. The procedure for the bioassay was as per USP monograph for glucagon injection. The results are tabulated in table 7 and table 8, respectively.

Table 7: Chemical and biological stability data for solution as per example 3

Stability Condition	Time	HPLC assay of Glucagon	Bioassay of Glucagon	Related substance					Absorbance at 420nm (AU)	%Transmittance at 650nm
				Glucagon sulphoxide	D-trp(25)-glucago	D-phe(22)-glucagon	Single max.	Total		
INITIAL		106.84	1.194	0.07	ND	0.03	0.86	1.96		
25°C/60% RH	1M	98.53	1.145	0.11	ND	ND	0.55	2.79	0.096	92.931
25°C/60% RH	2M	103.0	-	0.07	ND	0.02	0.86	5.55	0.246	91.780
2-8°C	3M	105.80	-	0.102	ND	1.132	0.239	1.965	0.045	98.180
25°C/60% RH	3M	91.78	-	0.074	ND	0.304	2.160	8.231	0.206	89.244
2-8°C	6M	104.63	-	0.112	ND	1.015	0.878	4.807	0.06	96.70

Table 8: Chemical and biological stability data for solution as per example 4

Stability Condition	Time	HPLC assay of Glucagon	Bioassay of Glucagon	Related substance					Absorbance at 420nm (AU)	%Transmittance at 650nm
				Glucagon sulphoxide	D-trp(25)-glucagon	D-phe(22)-glucagon	Single max.	Total		
INITIAL		106.70	1.09	0.17	ND	0.17	0.77	2.21		
25°C/60% RH	1M	101.05	0 .89-1.096	0.21	ND	ND	0.96	3.04	0.027	97.218
25°C/60% RH	2M	89.11	1.162	0.89	ND	1.21	1.21	7.02	-	-
2-8°C	3M	101.47	1.049-1.068	0.36	ND	ND	0.97	3.01	0.027	97.900
25°C/60%	3M	90.98	1.02	1.03	ND	0.33	1.67	7.84	0.025	98.221

				Related substance						
RH										
2-8°C	6M	102.24	1.298-1.409	0.490	ND	1.240	0.490	3.960	0.012	98.682
2-8°C	9M	102.66	0.983-1.013	0.75	0.01	1.19	1.28	5.823	0.02	97.852

EXAMPLE 6

Effect of aprotic solvents like dimethyl sulfoxide: mPEG-DSPE 3 mg/ml was dissolved in water for injection. Glucagon was added to dimethyl sulfoxide (equivalent to 10% of total volume) & dissolved by continuous stirring at room temperature. The solution of glucagon was slowly added to the solution of mPEG-DSPE with continuous stirring. pH was adjusted between 6.0-7.0 & volume was made up with WFI. This solution was filtered through 0.2μ PVDF filter in aseptic area.

Table 9: Stability results of solution of example 6

Time	Physical appearance	Total Related substances
3M/2-8°C	Clear solution	-
2M/RT	Clear solution	3.656%
3M/RT	Clear solution	4.551%

Micellar solution with less mPEG-DSPE with small amount of polar aprotic solvent was found to be chemically stable for three months at room temperature.

We claim:

1. A stable ready-to-use solution comprising therapeutically effective amounts of glucagon and pharmaceutically acceptable vehicle.

2. A stable, ready-to-use solution as claimed in claim 1 wherein the vehicle comprises pegylated distearoyl-phosphotidylethanolamine and the pH of the solution is about 5 to about 7.5.

3. A stable ready-to-use solution as claimed in claim 2 wherein glucagon concentration ranges from 0.5 mg/ml to 5 mg/ml.

4. A stable ready-to-use solution as claimed in claim 2 wherein the pegylated distearoyl-phosphotidylethanolamine has molecular weight of 300 to 5000 and is present in amount ranging from 1 mg/ml to 40 mg/ml.

5. A stable ready-to-use solution as claimed in claim 1 wherein the vehicle comprises at least one of a solvent or one or more stabilizers.

6. A stable ready-to-use solution as claimed in claim 5 wherein the solvent is selected from dimethyl sulfoxide, dimethylformamide, dioxane or hexamethylphosphorotriamide.

8. A stable ready-to-use solution as claimed in claim 5 wherein the stabilizer is a fatty acid or zwitterionic choline derivatives dimyristoyl phosphotidylcholine or mixtures thereof.

9. A stable ready-to-use solution as claimed in claim 5 wherein the solution is stable at room temperature for a period of about six months.

10. A stable ready-to-use solution as claimed in claim 1 wherein the solution is stable at 2-8 ^{0} C for a period of about three months.

INTERNATIONAL SEARCH REPORT

International application No.
PCT / IN 2011/000321

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 38/26*** (2006.01); ***C07K 14/605*** (2006.01); ***A61K 9/08*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
A61K, C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009114959 A1 (INSTITUTE OF PHARMACOLOGY AND TOXICOLOGY, CHENGDU YIPING PHARMACEUTICAL SCIENCE & DEVELOPMENT CO., LTD.) 24 September 2009 (24.09.2009) abstract (WPI; Acc.No.: 2009-P03130)	1-5, 8
X	US 6384016 B1 (KAARSHOLM, NIELS C.) 07 May 2002 (07.05.2002) claim 1	1,3,5
X	CA 2206736 C (ASTRA AKTIEBOLAG) 22 May 2007 (22.05.2007) claims 1,10,11	1,5,8
X	US2007087957A (KIDRON) 19 April 2007 (19.04.2007) claims 15,18	1,5,8

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2011 (30.09.2011)	10 October 2011 (10.10.2011)
Name and mailing address of the ISA/AT Austrian Patent Office Dresdner Straße 87, A-1200 Vienna Facsimile No. +43 1 534 24-535	Authorized officer KRENN M. Telephone No. +43 1 534 24-435

INTERNATIONAL SEARCH REPORT

International application No.
PCT / IN 2011/000321

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004038864 A1 (BALSCHMIDT, PER ET AL.) 26 February 2004 (26.02.2004) claims 1,12	1,5,6
A	US 2005112188 A1 (ELIAZ, ROM E, ET AL.) 26 May 2005 (26.05.2005) whole document	1-8

INTERNATIONAL SEARCH REPORT

International application No.
PCT / IN 2011/000321

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 9,10
because they relate to parts of the national application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

It is insufficient to restrict the technical disclosure of a claim to the assertion that a certain product is stable for a certain time without disclosing which feature(s) has caused said stability.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is only those claims for which fees were paid, specifically claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Box No. IV **Text of the abstract (Continuation of item 5 of the first sheet)**

The present invention provides a stable ready-to-use solution comprising therapeutically effective amounts of glucagon together with pegylated distearoyl-phosphatidylethanolamine.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT / IN 2011/000321

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