ABSTRACT

NOVEL FORMULATION FOR GONADOTROPINS

The present invention relates to a stable composition for gonadotropins. It provides a composition useful for stabilization of gonadotropins while preventing aggregation, dissociation, fragmentation and formation of oxidized species variants in solution for injection. Thus, it prevents instability of protein or polypeptide molecules caused due to aggregation or fragmentation or oxidation during or after formulation. Also, it provides a pharmaceutical composition of gonadotropins, which can be therapeutically used for the treatment of various indications either in single-dose form or in multi-dose form.

Field of the invention

The present invention relates to stable pharmaceutical compositions of gonadotropins. It provides a formulation composition useful for stabilization of gonadotropins by preventing aggregation, fragmentation and formation of oxidized species variants during and after formulation. It also provides a pharmaceutical composition of gonadotropins, which can be therapeutically used for the treatment of various indications either in single-dose form or in multi-dose form.

Background of the invention

Therapeutic proteins or polypeptides pose a number of challenges for pharmaceutical scientists regarding their formulation and delivery. Maintaining the physical and chemical stability of protein or polypeptide molecules in solution is important to retain the biologically active conformation of the molecule, which results in providing the desired level of potency and safety of the pharmaceutical preparation for injection comprising the protein or polypeptide molecules. Lack of physical and chemical stability may lead to significant degradation or irreversible modifications of protein or polypeptide molecules during processing, manufacturing, transportation and storage. Protein aggregation or fragmentation in pharmaceutical preparation is associated with loss of efficacy, altered pharmacokinetics, reduced stability, limited product shelf-life, and induction of unwanted immunogenicity. Aggregation or fragmentation or oxidation of protein or polypeptide molecules in pharmaceutical preparation severely affects the potency of the drug product. Pharmaceutical preparation comprising such functionally compromised molecule significantly alters the efficacy, bioavailability, tissue distribution pattern and pharmacokinetic profile of the drug product with higher risk of immunogenicity. In pharmaceutical preparation of protein drug product, a number of excipients have been used with varying success to reduce such protein degradation or modification. However, each excipient has its own limitations, and in some cases, the more effective ones are less amenable to inclusion in final formulation. Therefore, it is always challenging to establish stable formulation of sensitive protein or polypeptide molecules with a mixture of suitable inactive ingredients or excipients of interest, for pharmaceutical use.

Here, the present invention provides pharmaceutical composition of proteins or polypeptides, preferably gonadotropins, which provides stable formulation of the said molecules for therapeutic use either in single-dose or multi-dose form.

Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Human chorionic gonadotropin (hCG) etc. are glycoproteins in nature and composed of two subunits, alpha and beta, which remain held together by non-covalent forces in protein structure. Glycosylation occurs on both alpha and beta subunits at specific sites on the polypeptide backbone. The alpha subunit is identical among the specified gonadotropins, while beta subunit is different for each of these glycoproteins. The beta unit is responsible for the specificity of the biological activity. The subunit alone has no known biological activity. It is the formation of heterodimer that provides the biological activity of the protein molecules. The present invention aims to deliver novel composition for therapeutically effective amount of FSH or its variants, which provides stable formulation of FSH or its variants for pharmaceutical use either in single-dose or multi-dose form.

EP 1928413 application provides an aqueous formulation of a human follicle stimulating hormone (hFSH), comprising a therapeutically effective amount of hFSH, and glycine, methionine, a non-ionic surfactant and a phosphate buffer as stabilizers. Non-ionic surfactants are selected from poloxamer and polysorbates, preferably polysorbates.

WO2011/108010 provides a formulation comprising human gonadotropin or its variant with buffer system selected from the group consisting of acetate, lactate, carbonate and bicarbonate or their combination at a pH in the range of 6.5 to 9.0. Further, it includes ampholytes, sugars, polysorbates, antioxidants and preservatives.

US 5929028 discloses a liquid gonadotropin-containing formulation characterized in that the formulation comprises a gonadotropin and stabilizing amounts of a polycarboxylic acid or a salt, thereof, and of a thioether compound.

We, hereinafter, provide various formulations of the desired proteins or polypeptides, preferably gonadotropins, more preferably FSH or its variants, in which the said proteins or polypeptides remain adequately stable without undergoing further aggregation or fragmentation or oxidation or any other modifications during and after formulation. The formulations disclosed, hereinafter, can be stored for longer period of time, under suitable storage conditions and provide better stability.

Summary of the invention

The present invention provides a liquid stable formulation containing therapeutic amount of gonadotropins, preferably FSH or its variants for the purpose of single-use or multiple-use.

In one aspect, the present invention provides a liquid stable formulation containing a therapeutic amount of FSH or its variants and suitable excipients, optionally, selected from buffers, stabilizer(s),

surfactant(s) and suitable additives with other excipients, optionally, selected from antioxidants, amino acids, and preservatives.

In an embodiment, the present invention provides novel liquid stable formulation, which can optionally be lyophilized, comprising of suitable gonadotropins in suitable buffer(s), one or more suitable stabilizers, and other excipients, which are optionally selected from suitable surfactants, preservatives, and antioxidants.

In another aspect, the present invention provides a liquid stable formulation, which comprises of FSH or its variants with suitable buffer(s) and other excipients, optionally, selected from one or more stabilizers, surfactants and tonicity agents, antioxidants, amino acids, and preservatives.

In a further aspect, such formulations can also be, optionally, lyophilized comprising a therapeutic amount of FSH or its variants and other excipients, optionally, selected from one or more stabilizers, surfactants, tonicity agents, antioxidants, and amino acids. Lyophilization can be performed by a skilled person using the techniques available in the art, which includes various steps like freezing, annealing, primary drying and secondary drying.

In yet another aspect, the present invention provides a liquid stable formulation, which comprises of about 5 µg / mL to 200 µg / mL of FSH or its variants and suitable buffers at a concentration of about 5 mM to 100 mM, optionally suitable stabilizers with a concentration of about 0.005 % to 10%, optionally suitable surfactants at a concentration of about 0.001% to 5%, and optionally, preservatives at a concentration of about 0.01% to 1 %, for therapeutic use either in single-dose or multi-dose form.

In another aspect, the present invention provides a liquid stable formulation, which optionally can be in lyophilized form comprising of about 5 µg / mL to 200 µg / mL of FSH or its variants and suitable buffers at a concentration of about 5 mM to 100 mM, optionally suitable stabilizers with a concentration of about 0.005 % to 10 %, optionally suitable surfactants at a concentration of about 0.001 % to 5 %. The said lyophilized preparation is reconstituted in suitable diluent, preferably, in the presence of suitable preservatives at a concentration of about 0.01 % to 1 %, for therapeutic use either in single-dose or multi-dose form.

In one of the embodiments, the present invention provides a liquid formulation buffered between pH 5 to 9.

In another embodiment, the present invention provides a liquid formulation, which can be used for parenteral administration. Parenteral administration includes intravenous, subcutaneous, intra

peritoneal, intramuscular administration or any other route of delivery generally considered to be falling under the scope of parenteral administration and as is well known to a skilled person.

In another embodiment, the present invention provides a liquid formulation which stabilizes the protein or polypeptide molecule in solution by preventing any further degradation of the desired protein or polypeptide, during and after formulation. Generally, a stable formulation is the one which retains the physical stability, chemical stability and biological activity over a period of time, upon storage.

In a further embodiment, the present invention provides a process for the preparation of liquid stable formulation of FSH or its variants, which can be therapeutically used for the relevant indications.

Detailed description of the present invention

The present invention provides novel liquid stable formulation, which can optionally be lyophilized, comprising of suitable amount of therapeutic protein(s), preferably gonadotropins in suitable buffer(s), one or more suitable stabilizers, and other excipients, which are optionally selected from suitable surfactants, preservatives, and antioxidants. The present formulation stabilizes the gonadotropins during and after formulation and prevents any further degradation or modification of protein or polypeptide, while maintaining the active biological conformation of the protein or polypeptide during and after formulation. In such embodiment the protein is gonadotropin. In a preferred embodiment the gonadotropin is selected from FSH, LH, hCG or any suitable combinations thereof. In a more preferred embodiment, gonadotropin is FSH or its variants. The term FSH refers to Follicle stimulating hormone, LH refers to Luteinizing hormone & hCG refers to human chorionic gonadotropin hormone.

In some embodiments, the FSH or its variant is generally present in a therapeutic amount of up to 200 µg / mL. In a preferred embodiment the therapeutic amount is about 5 µg / mL to 100 µg / mL. In a more preferred embodiment the therapeutic amount is about 5 µg / mL to 50 µg / mL.

The liquid formulation comprises a suitable buffer along with other pharmaceutically acceptable excipients, which stabilizes the pharmaceutical preparation. Suitable buffers, which can be used, are selected from those which are known in the art and can be found in literature. In an embodiment, the suitable buffers comprise but are not limited to histidine, arginine, citrate, succinate, acetate, phosphate, tromethamine buffers and the like or their suitable mixtures.

In a preferred embodiment, the suitable buffer comprises of phosphate buffer or succinate buffer.

The buffers are generally used in concentrations of about 5 mM to 100 mM. In a preferred embodiment, the buffer concentration is about 10 mM to 50 mM.

In an embodiment, the liquid formulation maintains a pH value ranging from about pH 5 to about pH 9 depending on the FSH or its variant being used. In a preferred embodiment, the buffer used maintains the pH of the formulation in the range of about pH 6 to pH 8. In a more preferred embodiment, the pH is maintained to about pH 7.

The liquid formulation further comprises suitable surfactants, which are pharmaceutically acceptable excipients used to protect the protein formulations against various stress conditions, like agitation, shearing, exposure to high temperature etc., and reduce the surface interaction e.g., liquid-air or liquid-solid interfaces, during and after formulation.. The suitable surfactants include but are not limited to polyoxyethylensorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers (e.g. Brij), alkylphenylpolyoxyethylene ethers (e.g. Triton-X), polyoxyethylene-polyoxypropylene copolymer (e.g. Poloxamer, Pluronic), octanoic acid (caprylate), sodium dodecyl sulphate (SDS) and the like. In a preferred embodiment, the suitable surfactant is polyoxyethylenesorbitan-fatty acid esters (Tweens). In a preferred embodiment, the polyoxyethylenesorbitan-fatty acid esters are polysorbate 20, (sold under the trademark Tween 20™) and polysorbate 80 (sold under the trademark Tween 80™). In another preferred embodiment, the suitable surfactant is polyethylene-polypropylene copolymers, which are sold under the names Pluronic (R) F68 or Poloxamer 188™. In another preferred embodiment, the suitable surfactant is alkylphenolpolyoxyethylene esters, which are sold under the trade name Triton-X.

The surfactants are generally used in concentrations of about 0.001% to 5%. In a preferred embodiment, surfactant concentration is about 0.01% to 1%.

The liquid formulation further comprises one or more pharmaceutically acceptable or suitable stabilizer(s), which protect the active pharmaceutical ingredient from chemical and/or physical degradation during processing, manufacturing, transportation, storage and application. In an embodiment, the stabilizers include but are not limited to suitable sugars, amino acids, polyols, cyclodextrines and the like or suitable derivative or mixtures, thereof.

In one such embodiment, the sugar is a monosaccharide or an oligosaccharide. Monosaccharide sugars include but are not limited to glucose, fructose, galactose, mannose, sorbose, ribose, deoxyribose, dextran, dextrin and the like or amino sugars, like neuraminic acid or N-acetyl glucosamine and the like. An oligosaccharide includes but is not limited to sucrose, trehalose, lactose, maltose and raffinose and the like or suitable mixtures, thereof.

In another embodiment the polyols which can be used as stabilizers include but are not limited to mannitol, sorbitol, glycerol, arabitol, polyethylene glycol, propylene glycol and the like or suitable combinations thereof. In a preferred embodiment the suitable polyol is sorbitol or mannitol.

In another embodiment, polyethylenieimine can also be used as a stabilizer. In a preferred embodiment, the stabilizer is selected from suitable sugars, polyol and combination thereof. In an embodiment the stabilizer is present in amount about 0.005 % to about 10 %.

In a more preferred embodiment, the stabilizer is Polyethylene glycol (PEG) or Polyethyleneimine.

In another embodiment, cyclodextrines or derivatives thereof, which can be used as stabilizers, include but are not limited to α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, or their hydroxypropylated, hydroxyethylated, ethylated or methylated derivatives thereof or Sulfobutyl ether beta-cyclodextrin (SBE-beta-CD) or branched cyclodextrins or cyclodextrin polymers or suitable mixture thereof. In a preferred embodiment the suitable cyclodextrin variant is hydroxypropylated cyclo beta-dextrin (HP-β-CD).

In a preferred embodiment, the cyclodextrin or its derivative is present in amount of about 0.2 % to about 10 %.

In another such embodiment, the amino acids which can be used as stabilizers or antioxidants include but are not limited to arginine, glycine, lysine, histidine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophan, methionine, serine, proline, cysteine / cystine and the like or suitable combination of any of the above. In a preferred embodiment, the suitable amino acid is methionine or cysteine or glycine or tryptophan or suitable combination thereof.

In an embodiment, the amino acid is present in amount of about 0.01 % to 10 %.

Here, a skilled person can also use ascorbic acid or EDTA or combination, thereof, as an antioxidant(s) separately or in combination with other antioxidant(s) in the said formulation.

In one of the embodiments, the stable liquid formulation may further comprise of preservatives selected from hydroxybenzenes (phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol and the like), paraben(methyl, ethyl, propyl, butyl and the like), sodium benzoate, benzyl benzoate, benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. In a preferred embodiment, the preservative is selected from phenol, paraben, sodium benzoate, benzyl benzoate and mixture thereof.

In a preferred embodiment, the preservative is present in amount about 0.01% to 1%. In a more preferred embodiment, the preservative is present in amount of 0.01% to 0.5%.

In another embodiment, the liquid formulation optionally comprises tonicity agents such as sodium chloride or potassium chloride. In a preferred embodiment, the tonicity agent is sodium chloride, which is present in amount about10 mM to about 150 mM.

Phosphoric acid or sodium hydroxide can be used in a suitable amount to adjust the desired pH of the formulation.

The formulation may additionally further comprise one or suitable other excipients, which are well known to a person skilled in the art.

In some embodiments, the liquid formulation maintains the storage stability in terms of not allowing any further protein degradation or modifications as compared to the initial.

In some embodiments, the liquid formulation maintains the stability during the process of formulation.

In one of the embodiments, the stable liquid formulation with said excipients can be prepared for combination of FSH and LH or FSH and hCG or LH and hCG.

To estimate the level of aggregates and fragmented species variants of FSH or its variant initially and in the final formulation, analytical HP-size exclusion chromatography was performed. To analyze oxidized species variants or purity of desired protein a person skilled in the art can use reversed-phase HPLC. In-vivo or in-vitro biological assay can be performed to check the biological activity of the desired protein. A person skilled in the art can use other analytical tools/techniques known in the art to check the physico-chemical as well as biological properties of the desired protein.

The said analytical methods used in the present invention are well known to a skilled person.

The following non-limiting examples describe the different formulations, which can be prepared as per the present invention. It will be appreciated that other excipients may be added to these formulations and these are within the scope of a person skilled in the art.

Example 1

Active Ingredient	
FSH	44 µg / mL
Inactive Ingredients	
Sodium phosphate	10 mM

Sucrose	6 %
Polyethylene Glycol	0.1 %
EDTA	0.01 %
Phenol	0.3 %

Follitropin alfa was prepared as per the technique known in the art and formulation is performed in sodium phosphate buffer, further comprising sucrose, polyethylene glycol, EDTA and phenol at desired concentrations, as described above. The pH of the formulation medium was maintained at around pH 7.0. If required, pH of the formulation can be adjusted using O-phosphoric acid or sodium hydroxide. Excipients were added to the protein solution from respective stock solutions to adjust the final concentration and volume was made up to the desired level with sterile water or Water for Injection. The formulated bulk was distributed in suitable container-closure systems (like vials, cartridges, syringes etc.) for storage. Similarly, a person skilled in the art can also formulate the composition for Follitropin beta. Upon formulation, samples were analyzed for the presence of aggregates or fragmented species variants by HP-Size exclusion chromatography. A person skilled in the art can analyze said parameters at various temperature conditions like Real-Time storage condition (between +2 °C and +8 °C), Accelerated storage condition (about +25 °C) or stressed condition (higher temperature).

Example 2

Active Ingredient	
FSH	44 µg / mL
Inactive Ingredients	
Sodium phosphate	10 mM
Sucrose	6 %
Cysteine	0.01 %
Polyethylene Glycol	0.1 %
EDTA	0.01 %
Phenol	0.3 %

Follitropin alfa was prepared as per the technique known in the art and formulated in the presence of sodium phosphate buffer further comprising sucrose, Cysteine, polyethylene glycol, EDTA and Phenol at a desired concentration as described above in the formulation composition. pH of the formulation medium was maintained around pH 7.0. If required, pH of the formulation can be adjusted using O-phosphoric acid or sodium hydroxide. Excipients were added to the protein solution from respective stock solutions to adjust the final concentration and volume was made up to the desired level with sterile water or Water for Injection. The formulated bulk was distributed in suitable container-closure systems (like vials, syringes etc.) for storage. Upon formulation, samples were analyzed for the presence of aggregates or fragmented species variants by HP-Size exclusion chromatography. A person skilled in the art can analyze said parameters at various temperature conditions like Real-Time storage condition (between +2 °C and +8 °C), Accelerated storage condition (about +25 °C) or stressed condition (higher temperature).

Example 3

Active Ingredient	
FSH	44 µg / mL
Inactive Ingredients	
Sodium phosphate	10 mM
Sucrose	6 %
Methionine	0.01 %
Polyethylene Glycol	0.1 %
EDTA	0.01 %
Phenol	0.3 %

Follitropin alfa was prepared as per the technique known in the art and formulated in the presence of sodium phosphate buffer further comprising sucrose, methionine, polyethylene glycol, EDTA and Phenol at a desired concentration as described above in the formulation composition. pH of the formulation medium was maintained around pH 7.0. If required, pH of the formulation can be adjusted using O-phosphoric acid or sodium hydroxide. Excipients were added to the protein solution from respective stock solutions to adjust the final concentration and volume was made up to the desired level with sterile water or Water for Injection. The formulated bulk was distributed in suitable container-closure systems (like vials, syringes etc.) for storage. Upon formulation, samples were

analyzed for the presence of aggregates or fragmented species variants by HP-Size exclusion chromatography. A person skilled in the art can analyze said parameters at various temperature conditions like Real-Time storage condition (between +2 °C and +8 °C), Accelerated storage condition (about +25 °C) or stressed condition (higher temperature).

Various compositions can be prepared using similar process as mentioned in the preceding Examples. The compositions that can be prepared for the FSH or its variant protein as per the present invention are mentioned in the below Table 1.

Table 1

Example 1	**Example 2**	**Example 3**
5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol	5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Cysteine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol	5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol
Example 4	**Example 5**	**Example 6**
5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.01 – 5 %	5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA	5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol

Table 1		
Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol	0.1% - 0.5 % Phenol	0.01 – 5 % EDTA 0.1% - 0.5 % Phenol
Example 7 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 5 – 100 mM Methionine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol	**Example 8** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 5 – 100 mM Cysteine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol	**Example 9** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 5 – 100 mM Tryptophan 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol
Example 10 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.001 - 1% Polysorbate 20	**Example 11** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Tryptophan 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol	**Example 12** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.1 – 10 % Tryptophan 00.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Phenol

Table 1		
0.01 – 5 % EDTA 0.1% - 0.5 % Phenol		
Example 13 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 –10% Sucrose 0.1 – 10 % Methionine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben	**Example 14** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Cysteine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben	**Example 15** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben
Example 16 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA	**Example 17** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben	**Example 18** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben

Table 1		
0.1% - 0.5 % Paraben		
Example 19 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben	**Example 20** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Cysteine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben	**Example 21** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Tryptophan 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben
Example 22 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine	**Example 23** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Tryptophan 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA	**Example 24** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.1 – 10 % Tryptophan 00.001 - 1% Polysorbate 20

Table 1		
0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Paraben	0.1% - 0.5 % Paraben	0.01 – 5 % EDTA 0.1% - 0.5 % Paraben
Example 25 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate	**Example 26** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Cysteine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate	**Example 27** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate
Example 28 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.01 – 5 % Polyethylene Glycol	**Example 29** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium	**Example 30** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA

Table 1

0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate	Benzoate	0.1% - 0.5 % Sodium Benzoate
Example 31 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate	**Example 32** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Cysteine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate	**Example 33** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Tryptophan 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate
Example 34 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium	**Example 35** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Tryptophan 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate	**Example 36** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.1 – 10 % Tryptophan 00.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Sodium Benzoate

Table 1		
Benzoate		
Example 37 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate	**Example 38** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Cysteine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate	**Example 39** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate
Example 40 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl	**Example 41** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate	**Example 42** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.1 – 10 % Tryptophan 0.01 – 5 % Polyethylene Glycol 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate

Table 1		
Benzoate		
Example 43 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate	**Example 44** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Cysteine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate	**Example 45** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Tryptophan 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate
Example 46 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl	**Example 47** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Tryptophan 0.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate	**Example 48** 5 – 200 µg / mL FSH 5 – 100 mM Sodium phosphate buffer of pH 7.0 0.1 – 10 % Sucrose 0.1 – 10 % Methionine 0.1 – 10 % Cysteine 0.1 – 10 % Tryptophan 00.001 - 1% Polysorbate 20 0.01 – 5 % EDTA 0.1% - 0.5 % Benzyl Benzoate

Table 1		
Benzoate		

Similar formulations can be prepared using 5 –100 mM of acetate buffer (sodium acetate – acetic acid) or of succinate buffer or of citrate buffer (sodium citrate-citric acid) or of Phosphate buffered saline or of Arginine buffer or of citrate-phosphate buffer or of histidine buffer and the like with pH range of about pH 5.0 to pH 9.0.

Similar formulation can be prepared using 0.01 % to 10 % of raffinose or of trehalose or of sorbitol or of dextran or of cyclodextrin or of mannitol.

Similar formulation can be prepared using 0.001% to 5% of pluronics (poloxamers) alone or in combination with Polyethylene Glycol or polysorbates.

Similar formulation can be prepared using ascorbic acid in a suitable concentration.

Similar formulation can be prepared further using suitable tonicity agents in suitable concentrations.

Similar formulation can also be prepared for other gonadotropins, like LH or its variants and hCG or its variants or their suitable combinations thereof. A skilled person can prepare similar formulation for combination of gonadotropins selected from LH or its variant and FSH or its variant, hCG or its variant and FSH or its variant.

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