

LIQUID BUFFERED GDF-5 FORMULATIONSRELATED APPLICATIONS

This application is a non-provisional filing of a provisional application U.S. Pat. App. No. 61/044,518.

5 FIELD OF THE INVENTION

The invention relates to liquid formulations of bone morphogenetic proteins for improved stability, handling, and storage. More specifically, the invention relates to liquid formulations comprising GDF-5 in a biologically isotonic acidic solution having a pH of from about 4.0 to about 5.5, having improved protein
10 stability during handling and delivery at body temperatures.

BACKGROUND

GDF-5 is a member of the Bone Morphogenetic Proteins (BMP), which is a subclass of the TGF- β superfamily of proteins. GDF-5 includes several variants
15 and mutants, including mGDF-5 first isolated from the mouse by Lee (US Pat No 5,801,014). Other variants include MP52, which is the patented name (WO 95/04819) for the human form of GDF-5, which is also known as hGDF-5 and also as LAP-4 (Triantfilou, et al. *Nature Immunology* 2, 338-345 (2001)); also CDMP-1, an allelic protein variant of hGDF-5 (WO 96/14335); also rhGDF-5, the
20 recombinant human form manufactured in bacteria (EP 0955313); also rhGDF-5-Ala83, a monomeric variant of rhGDF-5; also BMP-14, a collective term for hGDF-5/CDMP-1 like proteins; also Radotermin, the international non-proprietary name designated by the World Health Organization; also HMW MP52's, high molecular weight protein variants of MP52; also C465A, a monomeric version wherein the
25 cysteine residue responsible for the intermolecular cross-link is substituted with alanine; also other active monomers and single amino acid substitution mutants including N445T, L441P, R438L, and R438K. For the purposes of this application

the term "GDF-5" is meant to include all variants and mutants of the GDF-5 protein, and rhGDF-5 is the exemplary member having 119 amino acids.

All members of the BMP family share common structural features including a carboxy terminal active domain and share a highly conserved pattern of cysteine
5 residues that create 3 intramolecular disulfide bonds and one intermolecular disulfide bond. The active form can be either a disulfide-bonded homodimer of a single family member or a heterodimer of two different members (see Massague, et al. *Annual Review of Cell Biology* 6:957 (1990); Sampath, et al. *Journal of Biological Chemistry* 265:13198 (1990); Celeste et al. *PNAS* 87:9843-47 (1990);
10 U.S. Pat. No. 5,011,691, and U.S. Pat. No. 5,266,683). The proper folding of the GDF-5 protein and formation of these disulfide bonds are essential to biological functioning, and misfolding leads to inactive aggregates and cleaved fragments.

The production of BMP's from genetically modified bacteria, and of GDF-5 in particular, utilizes plasmid vectors to transform *E. coli* to produce monomer
15 GDF-5 protein in high yield (see for example Hotten US 6,764,994 and Makishima US 7,235,527). The monomer is obtained from inclusion bodies, purified, and refolded into homodimers of GDF-5 protein to produce the biologically active dimer of the GDF-5 protein. The steps leading to this utilize various pharmaceutically unacceptable materials to modify the solubility in order to enable the separation
20 and purification of the GDF-5 protein.

The degradation of proteins in general has been well described in the literature, but the storage and solubility of bone morphogenetic proteins, particularly GDF-5 has not been well described. BMP-2 is readily soluble at concentrations greater than 1 mg/ml when the pH is below 6, and above pH 6 the
25 solubility can be increased by the addition of 1 M NaCl, 30% isopropanol, or 0.1 mM heparin (Ruppert, et al *Eur J Biochem* 237, 295-302 (1996). The solubility of GDF-5 is much more limited than that of BMP-2, and GDF-5 is nearly insoluble in physiological pH ranges and buffers. GDF-5 is only soluble in water at extreme pH (Honda, et al, *Journal of Bioscience and Bioengineering* 89(6), 582-589 (2000)).

GDF-5 is soluble at an alkaline pH of about 9.5 to 12.0, however proteins degrade quickly under these conditions and thus acidic conditions are used for preparation of GDF-5 protein.

The use of bone morphogenetic proteins has been well described in the case of BMP-2 and the growth of bone. GDF-5 has more activity in other areas of musculoskeletal development than BMP-2, and indeed in other areas of cellular biochemistry and regulation. The use of GDF-5 in these other areas presents fertile ground for potential treatments of various diseases and medical conditions. A major challenge for storage, handling, and delivery to target tissues is the stability of the GDF-5 protein molecule. Bulk GDF-5 protein is typically stored at sub-zero temperatures and lyophilized products are stored at 2-8C to protect the protein from degradation, but liquid formulations are required for many uses, including delivering liquid product in an implantable drug delivery pump.

In the past we have shown that a high amount of excipient, such as 60% trehalose, could be used to protect the GDF-5 protein at body temperature for extended periods of time. These formulas however were not isotonic and must be diluted before reaching the injection site. Biocompatible formulations of the GDF-5 protein present great challenges to obtain reasonable solubility and concurrent stability of the GDF-5 protein. Thus there is a need for improved formulations for the storage, handling, and delivery of GDF-5 protein solutions.

SUMMARY OF THE INVENTION

The present invention is directed to formulations of buffered isotonic GDF-5 protein solutions having improved storage and handling properties at body temperatures, providing for stability of the GDF-5 protein molecule. A preferred embodiment of the invention includes 0.1 mg/ml rhGDF-5 in a 5-10 mM acetate buffer at pH 4.5 -5 with 10% trehalose as an excipient, providing approximately 90% retention of the GDF-5 protein after 32 days storage at 37 degrees Celsius.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the stability of 0.1 mg/ml rhGDF-5 in 5 mM acetate buffer solution at pH 4.5 after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.

- 5 Figure 2 shows the stability of 0.1 mg/ml rhGDF-5 solution in 5 mM acetate buffer solution at pH 4.5 with 10% trehalose, as evidenced by HPLC.

Figure 3 shows the stability of 0.1 mg/ml rhGDF-5 in 5 mM acetate buffer solution at pH 4.5 with 10% trehalose and 0.1% HO-ectoine after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.

- 10 Figure 4 shows the stability of 0.1 mg/ml rhGDF-5 in 5 mM acetate buffer solution at pH 4.5 with 10% trehalose and 0.1% ectoine after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.

- Figure 5 shows the stability of 0.1 mg/ml rhGDF-5 in 5 mM acetate buffer solution at pH 5.0 with 10% trehalose after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.
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Figure 6 shows the stability of 0.1 mg/ml rhGDF-5 in 5 mM acetate buffer solution at pH 5.0 with 10% trehalose and 0.1% GABA after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.

- Figure 7 shows the stability of 0.1 mg/ml rhGDF-5 in 5 mM acetate buffer solution at pH 5.0 with 10% trehalose, 0.1% taurine and 0.01% TEA-HCl after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.
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Figure 8 shows the stability of 0.1 mg/ml rhGDF-5 in 5 mM acetate buffer solution at pH 5.0 with 10% trehalose, 0.1% betaine, and 0.1% ectoine after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.

- 25 Figure 9 shows the stability of 0.1 mg/ml rhGDF-5 in 10 mM acetate buffer solution at pH 4.5 with 10% trehalose after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.

Figure 10 shows the stability of 0.1 mg/ml rhGDF-5 in 10 mM acetate buffer solution at pH 4.5 with 0.01% TEA-HCl and 0.1% betaine after 32 days storage at 37 degrees Celsius, as evidenced by HPLC.

5 Figure 11 shows an HPLC chromatogram depicting the stability of 0.1 mg/ml rhGDF-5 in 10 mM acetate buffer solution at pH 5 with 10% trehalose 32 days storage at 37 degrees Celsius, as evidenced by 90% main peak retention.

10 Figure 12 shows an HPLC chromatogram depicting the degradation of 0.1 mg/ml rhGDF-5 in 5 mM maleate buffer solution at pH 4.5 with 10% trehalose after 32 days storage at 37 degrees Celsius, as evidenced by <70% main peak retention.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this application the definitions of the following terms will
15 be useful to clearly and concisely point out the subject matter of the claimed invention. The term "Growth and Differentiation Factor 5" (herein referred to as "GDF-5") as used herein is understood to include all synonyms, variants and mutations of the GDF-5 protein molecule, including, but not limited to GDF-5, mGDF-5, hGDF-5, MP-52, LAP-4, radotermis, CDMP-1, C465A, and rhGDF-5,
20 wherein rhGDF-5 is the exemplary member of the group. It is also understood to include monomeric GDF-5 proteins, which have also been shown to be biologically active.

 The term "room temperature", herein abbreviated as "RT" or "R.T.", is understood to mean the ambient temperature of an ordinary office or laboratory,
25 being from about 18 to about 25 degrees Celsius. The term "body temperature" as used herein is understood to mean the average body temperature of a mammal, being from about 34 to about 40 degrees Celsius, and generally accepted to be about 37 degrees Celsius for humans.

The term "bulk" as used herein when referring to "bulk protein" or "bulk solution" is understood to mean a purified solution of GDF-5 in from about 1 to about 10 mM HCl and stored at about -80 degrees Celsius, and is equivalent with the terms "stock", "stock protein", and "stock solution".

5 The term "buffer" is understood to have its common meanings in the literature, that as a noun being an acid and conjugate base pair capable of providing a liquid with the capacity to maintain a stable pH upon the addition of acid or base, and the term "buffer" as a verb is understood to describe the action of maintaining the pH of a solution by the addition of a buffer. Exemplary buffer
10 conjugate bases include, but are not limited to, acetate, lactate, tartarate, maleate, citrate, and combinations thereof, wherein acetate is the exemplary member of the group.

 The terms "isotonic", "isotonicity", and "biological isotonicity" as used herein have their common meanings in the literature and refer to a solution being or
15 having an osmolarity equivalent to that of human blood plasma, and is about 290 mosm/L with a range of about +/- 20 mosm/L.

 The term "excipient" as used herein is understood to mean any extra ingredient or additive other than a buffer that is added to a protein composition. Exemplary excipients include trehalose, sucrose, raffinose, glucose, mannitol,
20 TMAO, TEA-HCl, taurine, β -alanine, betaine, ectoine, HO-ectoine, GABA, and combinations thereof.

 The term "TMAO" as used herein is understood to mean trimethylamineoxide; the term "TEA-HCl" as used herein is understood to mean triethylaminehydrochloride; the term "GABA" as used herein is understood to
25 mean gamma-amino butyric acid.

 The term "tonicity adjuster" as used herein is understood to mean a solute that is added to a protein solution for the purpose of making the solution isotonic. The solute may also have other chemical or biological properties in addition to modifying the tonicity of the solution. Exemplary tonicity adjusters include salts,

such as NaCl, and certain carbohydrates, including but not limited to trehalose, sucrose, raffinose, glucose, and mannitol. Note that these tonicity adjusters may also be considered excipients, however not all excipients are useful as tonicity adjusters. The amount of some excipients that would need to be added in order to
5 affect tonicity would create other undesirable effects and thus negate their utility as a tonicity adjuster. However, in very small quantities these other excipient materials provide useful and desirable properties without substantially affecting the tonicity of the solution.

The term "HPLC" as used herein is understood to have its common
10 meaning of High Pressure Liquid Chromatography, also known as High Performance Liquid Chromatography, and also includes the terms "reversed phase HPLC" or "rp-HPLC".

Since its discovery and the subsequent development of recombinant human forms, GDF-5 has been stored in a 10 mM HCl solvent system at -80
15 degrees Celsius to preserve the GDF-5 protein structure. GDF-5 is less soluble than other BMP's, including BMP-2, for which the bulk of the scientific literature is directed to. There are few reports, if any, available on the solubility and stability of GDF-5. The preparation and isolation of GDF-5 monomer protein and the subsequent refolding into dimer presents a separate set of issues and problems
20 than that of the handling and storage of the bioactive dimer. Working with the mature dimer GDF-5 protein in biocompatible formulations presents a different set of problems, and the literature yields very little physicochemical information regarding the solubility and stability of the GDF-5 dimer protein.

The broad spectrum of activity of GDF-5 in musculoskeletal development
25 and also the expression of GDF-5 receptors in various tissues presents significant opportunities for therapies and treatment. One major drawback to using GDF-5 for treatment is the fragile nature of the protein. We have investigated the use of a number of different buffer systems and excipients in order to improve the stability

of GDF-5 protein solutions during storage, handling, and delivery, and herein describe useful formulations for working with this protein.

In the past we have shown that a high amount of excipient, such as 60% trehalose, could be used to protect the GDF-5 protein at body temperature for extended periods of time. These formulas however were not isotonic, and required dilution before use. Additionally, these solutions were quite viscous and were potentially problematic to handle.

We undertook several studies of various GDF-5 solutions to investigate buffer systems and excipients for the improved storage and handling of GDF-5 protein solutions having biological isotonicity and stability, particularly at body temperature. The stability of the GDF-5 protein was determined by reversed phase HPLC (herein referred to simply as "HPLC"), and the percent retention of the main peak was used as a measure of the stability or degradation of the GDF-5 protein. Our past results have shown that this is a reliable method of determining the stability of the GDF-5 protein molecule, and degradation of the GDF-5 protein is readily observed by the reduction of the main peak and the appearance of extraneous peaks due to oxidation, deamidation, aggregation, cleavage and fragmentation. These results were also confirmed in the present experiments.

For example, figure 11 shows an HPLC chromatogram of 0.1 mg/ml rhGDF-5 in 10 mM acetate buffer solution at pH 5.0 after 32 days storage at 37 degrees Celsius, showing 90% main peak retention and also the presence of minor additional peaks. In contrast, figure 12 shows the degradation of 0.1 mg/ml rhGDF-5 in 5 mM maleate buffer solution at pH 4.5 with 10% trehalose after 32 days storage at 37 degrees Celsius, showing only 66% main peak retention and the presence of additional peaks. We also evaluated the solutions based on the visual clarity of the solutions, with a hazy or cloudy solution being evidence of poor solubility and/or aggregation of the GDF-5 protein.

Numerous compounds have been shown to be useful in other protein formulations, including buffer systems of acetate, lactate, maleate, citrate,

succinate, phosphate, and tartrate, and other excipients such as trehalose, mannitol, sucrose, raffinose, trimethylamine oxide, trimethylamine hydrochloride, triethylamine hydrochloride, beta-alanine, alanine, glycine, taurine, betaine, ectoine, HO-ectoine, L-proline, potassium aspartate, sodium aspartate, heat shock
5 protein, dextran, cyclodextrins, glycine, arginine, PEGs, pluronics, lipids, phospholipids, diacylglycerols, vitamin E-acetate, and PEG-vitamin E succinate. However, there is no known evidence or literature of the use or benefit of these excipients with GDF-5.

Our experiments were designed to be isotonic solutions with the goal of still
10 maintaining the solubility and stability of the GDF-5 protein molecule. A GDF-5 protein concentration of 0.1 mg/ml was used for all experiments. The results showed that a 5 mM acetate buffer solution at pH 4.5 could be used to maintain a clear liquid that showed approximately 90% retention (stability) of the GDF-5 protein HPLC main peak after 32 days storage at 37 degrees Celsius (see figure
15 1).

The addition of other excipients did not enhance or detract from the GDF-5 protein stability under the experimental conditions studied, but these additional excipients may provide other benefits under other conditions. Thus, the addition of up to 0.2%, or possibly even up to about 0.5% of these other excipients may have
20 little affect on the stability of the protein under the present study conditions, but may enhance the solubility or stability of the protein under other conditions. Increasing the pH of the acetate buffer to 5.0 did not detract from the stability of the GDF-5 protein (see table 2). Thus, a formulation of 5 mM acetate buffer at a pH of from about 4.2 to about 5.3 with 10% trehalose added to adjust the tonicity
25 and one or more excipients at 0.1 wt. % of taurine, betaine, b-alanine, TMAO, GABA, ectoine, HO-ectoine, 0.01 wt. % of TMAO or TEA-HCl, and various combinations thereof provided for protection of the GDF-5 protein after 32 days storage at 37 degrees Celsius (See table 1&2, and also figures 1-8).

We then tested a similar series of formulations and excipients at pH 5.5, however all of these samples were hazy, indicating poor solubility and/or aggregation of the GDF-5 protein (see table 3). No HPLC scans were performed on these samples.

5 We also tested the stability of the 0.1 mg/ml GDF-5 protein solution in several 5 mM glycine-HCl buffers ranging from pH 3.9 to 5.0. The samples were tested with and without 10% trehalose to adjust the tonicity, and also with various combinations of the excipients taurine, betaine, b-alanine, and TEA-HCl. All of these samples yielded hazy solutions (see table 4), and were not further tested by
10 HPLC. Similarly, we also tested the stability of the 0.1 mg/ml GDF-5 protein solution in other buffer systems, including a 5 mM citrate buffer at pH 4.4 with 10 % trehalose, a 0.25 mM HCl solution at pH 3.5 with 10 % trehalose, and a 0.25 mM HCl solution at pH 3.5 without trehalose. All of these solutions were also hazy, indicating poor solubility and/or aggregation of the GDF-5 protein, and no HPLC
15 scans were performed on these samples (see table 4). Thus, it is surprising that an acetate buffer system at higher pH can provide superior solubility and protection of the GDF-5 protein molecule than other commonly known buffers used for biocompatible formulations at a lower pH, when lower pH is known to enhance the stability of GDF-5.

20 We then further tested the stability of the 0.1 mg/ml GDF-5 protein solution in a 10 mM acetate buffer system at pH 4.5 and also at pH 5.0 with selected combinations of excipients. All of these solutions were clear, and subsequent testing after storage at 37 degrees Celsius showed that these formulations afforded protection of the GDF-5 protein molecule, as evidenced by retention of
25 approximately 85% of the main peak after 32 days storage at 37 degrees Celsius (see tables 5 & 6). Further testing in 10 mM acetate buffer at pH 5.0 with 10% sucrose instead of trehalose added as a tonicity adjuster, and with various other excipients added also gave positive results, yielding nearly 90% retention of the main peak by HPLC (see table 7).

Although many of the excipients and combinations of excipients tested did not exhibit superior results under the study conditions of time, temperature, pH, and osmolality tested, they also did not exhibit detrimental effects upon the GDF-5 protein stability. Thus, they may show benefits in other systems, methods, or conditions, such as in a specific biological tissue, organ, or cell type, or over a longer time period or at other temperatures.

It is an object of the present invention to provide a formulation of GDF-5 protein in a biocompatible buffer system that provides for improved protein stability during handling and storage at body temperature, as evidenced by the retention of at least 80% of the HPLC main peak. It is another object of the present invention to provide an isotonic solution of GDF-5 protein that is stable during storage and handling at body temperature, as evidenced by the retention of at least 80% of the HPLC main peak. It is another object of the present invention to provide an isotonic solution of GDF-5 protein having an acetate buffer with a pH of from about 4.3 to about 5.2, and further having 10 weight percent of a tonicity adjuster selected from the group consisting of trehalose, sucrose, raffinose, glucose, mannitol, and combinations thereof, that is stable during storage and handling at body temperature, as evidenced by the retention of at least 80% of the HPLC main peak.

It is another object of the present invention to provide a method of preserving a solution of GDF-5 protein by providing an acetate buffered solvent system having biological isotonicity and a pH of from about 4.2 to about 5.3, wherein the GDF-5 protein is stabilized during handling and storage at body temperature up to and including 37 degrees Celsius for up to 32 days, as evidenced by the retention of at least 80% of the HPLC main peak.

The following examples are meant only to be illustrative in nature of the present invention, and not to be limiting in scope. One skilled in the art would easily conceive of other embodiments that would be considered within the scope of the present invention.

Example 1

Bulk frozen rhGDF-5 protein solution was thawed overnight. A portion of the stock solution was used in a 5 mM acetate buffer at pH 4.5 and tested with 10% trehalose to adjust the tonicity of the solution to be isotonic and various other excipients to evaluate the stability of the GDF-5 protein as evidenced by the clarity of the solution and by HPLC analysis of the main peak. Data were collected after 5, 11, 22, and 32 days storage at 37 degrees Celsius. Control samples having buffer only and buffer plus 10% trehalose were included in the experiment. The results are shown below in table 1. Surprisingly, the buffer alone provided nearly equivalent protection of the GDF-5 protein as compared with the other samples. None of the other excipients tested showed any noticeable improvement or reduction of the stability of the GDF-5 protein. Figure 1 shows a graph of the HPLC main peak % and the % recovery of concentration of the control sample with acetate buffer at pH 4.5 alone, showing approximately 90% retention (stability) of the GDF-5 protein after 5, 11, 22, and 32 days at 37 degrees Celsius. Figure 2 shows a similar graph of the HPLC main peak % and the % recovery of concentration of the sample with acetate buffer at pH 4.5 plus 10% trehalose, also showing approximately 90% retention of the GDF-5 protein after 32 days at 37 degrees Celsius. Figures 3 and 4 show similar graphs of the HPLC main peak % and the % recovery of concentration of the samples with 10% trehalose plus 0.1% HO-ectoine or 0.1% ectoine, respectively, also showing approximately 90% retention of the GDF-5 protein after 32 days at 37 degrees Celsius. See table 1 below for a complete listing of the various combinations of excipients tested and the results obtained. Note that all of the values are nearly equivalent, showing that the acetate buffer at pH 4.5 is able to maintain the stability of the GDF-5 protein at about 90%, with or without the addition of other excipients.

Table 1. Data showing the stability of 0.1 mg/mL rhGDF-5 in 5 mM acetate buffer at pH 4.5, also tested with 10% trehalose and other excipients.

Sample ID	Other Excipients	pH	Clarity	HPLC Analysis, % main peak				
				Time 0	Day 5	Day 11	Day 22	Day 32
RR303LN-73-56	0.01% TMAO	4.4	Clear	96.35	94.56	93.58	91.90	89.25
RR303LN-73-57	0.1% ectoine	4.3	Clear	95.83	94.68	93.41	91.73	88.67
RR303LN-73-58	0.01%TMAO/0.1% ectoine	4.4	Clear	95.92	95.88	93.49	91.87	88.98
RR303LN-73-59	0.1% HO-ectoine	4.3	Clear	96.27	94.42	93.42	91.54	89.35
RR303LN-73-60	0.01% TMAO/0.1% HO-ectoine	4.4	Clear	96.16	95.68	93.59	91.64	89.32
RR303LN-73-61	0.1% taurine/0.1% betaine	4.3	Clear	96.07	94.39	93.21	91.15	89.68
RR303LN-73-62	0.1%taurine / 0.1% ectoine	4.3	Clear	95.53	94.65	92.96	90.95	88.96
RR303LN-73-63	0.15 taurine / 0.1% HO-ectoine	4.3	Clear	95.82	94.73	93.33	91.25	89.00
RR303LN-73-64	0.1% taurine / 0.01% TEA-HCl	4.3	Clear	95.81	94.72	93.11	91.39	89.51
RR303LN-73-65	0.1% taurine / 0.1% b-alanine	4.6	Clear	96.36	95.00	93.54	91.36	89.30
RR303LN-73-66	0.01% TMAO / 0.1% taurine	4.4	Clear	95.21	94.42	93.15	91.56	89.09
RR303LN-73-67	0.01% TEA-HCl / 0.1% b-alanine	4.6	Clear	96.14	94.76	93.51	91.73	89.34
RR303LN-73-68	0.01% TEA-HCl / 0.1% betaine	4.3	Clear	95.23	93.70	93.30	91.84	89.50
RR303LN-73-69	0.1% b-alanine / 0.1% betaine	4.6	Clear	96.49	94.18	93.17	91.67	89.07
RR303LN-73-70	0.1% b-alanine / 0.1% ectoine	4.6	Clear	96.12	94.47	93.34	91.31	88.54
RR303LN-73-71	0.1% b-alanine / 0.1% HO-ectoine	4.6	Clear	95.63	95.05	93.59	91.55	89.23
RR303LN-73-72	0.1% betaine / 0.1% ectoine	4.3	Clear	95.72	94.01	93.18	91.08	88.55
RR303LN-73-73	10% trehalose only	4.3	Clear	96.09	94.93	93.57	91.89	89.26
RR303LN-73-74	No trehalose or other excipients	4.3	Clear	95.52	94.33	93.51	92.06	89.09

Example 2

- 5 In this experiment rhGDF-5 was also formulated at approximately 0.1 mg/mL in 5 mM acetate buffer, but at pH 5.0 instead of pH 4.5 as in example 1. 10% trehalose was added to adjust the tonicity, and various other excipients were added as indicated in table 2. These formulations were also stable at 37 degrees

Celsius for 32 days as shown in table 2. Figure 5 shows a graph of the HPLC main peak % and the % recovery of concentration of the sample having 5 mM acetate buffer at pH 5.0 and 10% trehalose, showing approximately 90% retention of the GDF-5 protein after 32 days storage at 37 degrees Celsius. Figure 6 shows a

5 graph of the HPLC main peak % and the % recovery of concentration of the sample having acetate buffer at pH 5.0 and 10% trehalose with 0.1% GABA, also showing approximately 90% retention of the GDF-5 protein after 32 days storage at 37 degrees Celsius. Figure 7 shows a graph of the HPLC main peak % and the % recovery of concentration of the sample having acetate buffer at pH 5.0 and

10 10% trehalose with 0.1% taurine and 0.01% TEA-HCl, also showing approximately 90% retention of the GDF-5 protein after 32 days storage at 37 degrees Celsius. Figure 8 shows a graph of the HPLC main peak % and the % recovery of concentration of the sample having acetate buffer at pH 5.0 with 10% trehalose, 0.1% betaine, and 0.1% ectoine, also showing approximately 90% retention of the

15 GDF-5 protein after 32 days storage at 37 degrees Celsius.

Table 2. Stability of 0.1 mg/mL rhGDF-5 in 5 mM Acetate buffer at pH 5.0, also tested with 10% trehalose and other excipients.

Sample ID	Other excipients	pH	Clarity	HPLC Analysis, % main peak				
				Time 0	Day 6	Day 14	Day 21	Day 34
RR303LN-76-75	0.01% TMAO	4.9	Clear	96.43	96.14	95.45	93.15	92.35
RR303LN-76-76	0.1% ectoine	4.8	Clear	96.42	95.50	94.59	93.13	90.15
RR303LN-76-77	0.01%TMAO / 0.1%ectoine	5.0	Clear	95.86	95.50	94.68	92.97	91.04
RR303LN-76-78	0.1% GABA	5.1	Clear	96.17	95.00	94.36	92.57	91.09
RR303LN-76-79	0.01% TMAO/0.1% GABA	5.2	Clear	95.41	95.84	94.30	93.09	91.21
RR303LN-76-80	0.1% taurine/0.1% betaine	4.8	Clear	95.63	95.95	94.16	92.50	90.28
RR303LN-76-81	0.1%taurine / 0.1%ectoine	4.9	Clear	95.94	95.93	94.15	92.34	90.22
RR303LN-76-82	0.15 taurine / 0.1% GABA	5.1	Clear	96.34	95.63	94.74	92.02	91.01
RR303LN-76-83	0.1% taurine / 0.01% TEA-HCl	4.8	Clear	95.59	95.22	93.81	91.56	90.15
RR303LN-	0.1% taurine / 0.1% b-	5.0	Clear	95.85	94.95	94.38	91.18	90.33

76-84	alanine							
RR303LN-76-85	0.01% TMAO / 0.1% taurine	4.9	Clear	95.81	94.14	93.96	91.84	91.09
RR303LN-76-86	0.01% TEA-HCl / 0.1% b-alanine	5.0	Clear	95.44	96.16	93.80	91.90	90.18
RR303LN-76-87	0.01% TEA-HCl / 0.1% betaine	4.8	Clear	95.99	95.82	93.54	91.88	90.47
RR303LN-76-88	0.1% b-alanine / 0.1% betaine	5.0	Clear	95.58	95.72	94.49	91.61	90.74
RR303LN-76-89	0.1% b-alanine / 0.1% ectoine	5.0	Clear	95.27	95.46	94.50	92.39	90.39
RR303LN-76-90	0.1% b-alanine / 0.1% GABA	5.2	Clear	95.48	95.88	93.89	91.51	91.24
RR303LN-76-91	0.1% betaine / 0.1% ectoine	4.8	Clear	95.93	95.16	94.27	92.98	90.44
RR303LN-76-92	10% trehalose only	4.8	Clear	95.90	96.33	93.60	91.61	90.74

Example 3

Similar to examples 1 and 2, rhGDF-5 was formulated at 0.1 mg/mL in 5 mM acetate buffer at pH 5.5 with 10% trehalose and other excipients, and all of these protein solutions all hazy (see table 3). Additionally, 5 mM citrate buffer at pH 4.5, glycine-HCl buffer at pH 4.5, and 0.25 mM HCl solvents were also evaluated. These formulations were also observed to be hazy, as shown in table 4.

- 10 Table 3. Stability of 0.1 mg/mL rhGDF-5 in 5 mM acetate buffer at pH 5.5, and also with 10% trehalose and other excipients.

Sample ID	Other Excipients	pH	Clarity	HPLC Analysis, % main peak			
				Time 0	Day 11	Day 20	Day 32
RR303LN-79-98	10% trehalose only	5.3	Hazy	NA	NA	NA	NA
RR303LN-79-99	0.1% taurine / 0.1% betaine	5.3	Hazy	NA	NA	NA	NA
RR303LN-79-100	0.1% taurine / 0.1% b-alanine	5.4	Hazy	NA	NA	NA	NA
RR303LN-79-101	0.01% TEA-HCl / 0.1% b-alanine	5.4	Hazy	NA	NA	NA	NA
RR303LN-79-102	0.01% TEA-HCl / 0.1% betaine	5.3	Hazy	NA	NA	NA	NA

Example 4

rhGDF-5 was formulated at 0.1 mg/mL in glycine and citrate buffers at various pH with 10% trehalose and other excipients, and all of these protein solutions were hazy (see table 4).

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Table 4. Stability of 0.1 mg/mL rhGDF-5 in various buffers with 10% trehalose and other excipients.

Sample ID	Other Excipients	pH	Buffer	Clarity	HPLC Analysis, % main peak			
					Time 0	Day 11	Day 20	Day 32
RR303LN-79-108	10% trehalose only	3.9	5 mM gly-HCl	Hazy	NA	NA	NA	NA
RR303LN-79-109	0.1% taurine / 0.1% betaine	5.0	5 mM gly-HCl	Hazy	NA	NA	NA	NA
RR303LN-79-110	0.1% taurine / 0.1% b-alanine	5.0	5 mM gly-HCl	Hazy	NA	NA	NA	NA
RR303LN-79-111	0.01% TEA-HCl / 0.1% b-alanine	4.9	5 mM gly-HCl	Hazy	NA	NA	NA	NA
RR303LN-79-112	0.01% TEA-HCl / 0.1% betaine	3.9	5 mM gly-HCl	Hazy	NA	NA	NA	NA
RR303LN-79-114	10% trehalose only	4.4	5 mM Citrate	Hazy	NA	NA	NA	NA
RR303LN-79-115	10% trehalose only	3.5	0.25 mM HCl	Hazy	NA	NA	NA	NA
RR303LN-79-116	No trehalose	3.5	0.25 mM HCl	Hazy	NA	NA	NA	NA
RR303LN-90-117	10% trehalose only	4.45	5 mM tartrate	hazy	NA	NA	NA	NA
RR303LN-90-119	10% trehalose only	4.32	5 mM lactate	clear	95.88	93.15	91.35	89.52
RR303LN-90-120	10% trehalose only	3.85	5 mM maleate	clear	95.62	72.07	66.33	66.35

Example 5

Similar to examples 1 and 2, rhGDF-5 was formulated at 0.1 mg/mL, but using 10 mM acetate buffer at pH 4.5 for example 5. The samples had 10% trehalose added to adjust the tonicity and were tested with various other excipients. These formulations were also stable at 37 degrees Celsius for 32 days as shown in table 5. Figure 9 shows a graph of the HPLC main peak % and the % recovery of concentration of the sample having 10 mM acetate buffer at pH 4.5

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and 10% trehalose, showing approximately 87% retention of the GDF-5 protein after 32 days storage at 37 degrees Celsius. Figure 10 shows a graph of the HPLC main peak % and the % recovery of concentration of the sample having 10 mM acetate buffer at pH 4.5 with 0.01% TEA-HCl and 0.1% betaine, also showing approximately 87% retention of the GDF-5 protein after 32 days storage at 37 degrees Celsius.

Table 5. Stability of 0.1 mg/mL rhGDF-5 in 10 mM Acetate buffer at pH 4.5 with 10% trehalose and other excipients.

Sample ID	Other Excipients	pH	Clarity	HPLC Analysis, % main peak			
				Time 0	Day 11	Day 20	Day 32
RR303LN-79-103	10% trehalose only	4.4	Clear	95.27	93.00	91.18	87.38
RR303LN-79-104	0.1% taurine / 0.1% betaine	4.4	Clear	95.34	92.92	90.96	86.27
RR303LN-79-105	0.1% taurine / 0.1% b-alanine	4.5	Clear	95.30	93.12	91.42	85.97
RR303LN-79-106	0.01% TEA-HCl / 0.1% b-alanine	4.5	Clear	94.97	94.32	91.19	86.41
RR303LN-79-107	0.01% TEA-HCl / 0.1% betaine	4.3	Clear	95.34	93.00	91.74	87.27

Example 6

Similar to example 5, rhGDF-5 was formulated at 0.1 mg/mL in 10 mM acetate buffer, but at pH 5.0 for example 6. The samples had 10% trehalose added to adjust the tonicity and were tested with various other excipients. These formulations were also stable at 37 degrees Celsius for 32 days as shown in table 6.

Table 6. Stability of 0.1 mg/mL rhGDF-5 in 10 mM Acetate buffer at pH 5.0, also tested with 10% trehalose and other excipients.

Sample ID	Other excipients	pH	Clarity	HPLC Analysis, % main peak			
				Time 0	Day 10	Day 20	Day 32

RR303LN-90-118	10% trehalose only	4.97	Clear	96.15	93.66	91.63	89.26
RR303LN-90-122	0.1% taurine/0.01%TEA-HCl	4.97	Clear	96.00	93.70	91.84	89.18
RR303LN-90-123	0.1%taurine / 0.1% b-alanine	5.04	Clear	95.64	93.31	91.30	88.40
RR303LN-90-124	0.15 taurine / 0.1% betaine	4.96	Clear	96.13	93.68	91.10	88.86
RR303LN-90-125	0.1% taurine / 0.1% ectoine	4.95	Clear	95.87	93.62	91.96	89.54
RR303LN-90-126	0.01% TEA-HCl / 0.1% b-alanine	5.01	Clear	96.02	93.51	91.65	88.79
RR303LN-90-127	0.01% TEA-HCl / 0.1% betaine	4.91	Clear	94.27	93.84	91.26	89.38
RR303LN-90-128	0.01% TEA-HCl / 0.1% ectoine	4.91	Clear	95.81	92.85	91.72	89.50
RR303LN-90-129	0.01% TEA-HCl / 0.1% betaine	4.98	Clear	95.64	93.54	91.50	89.14
RR303LN-90-130	0.1% b-alanine / 0.1% ectoine	4.98	Clear	95.37	93.52	91.49	88.96
RR303LN-90-131	0.1% ectoine	4.89	Clear	95.83	93.97	91.31	89.87
RR303LN-90-132	0.1% betaine	4.89	Clear	95.88	94.09	91.68	89.44
RR303LN-90-133	0.1% b-alanine	4.99	Clear	96.04	93.77	91.47	89.16

Example 7

Similar to example 6, rhGDF-5 was formulated at 0.1 mg/mL in 10 mM acetate buffer at pH 5.0. The samples in example 7 had 10% sucrose instead of trehalose added to adjust the tonicity. All of the samples yielded clear solutions and approximately 90% retention of the main peak by HPLC (see table 7).

Table 7. Stability of 0.1 mg/mL rhGDF-5 in 10 mM acetate buffer at pH 5.0, with 10% sucrose and other excipients.

Sample ID	Other excipients	pH	Clarity	HPLC Analysis, % main peak			
				Time 0	Day 10	Day 20	Day 32
RR303LN-90-121	10% sucrose only	4.98	Clear	95.87	93.47	91.83	88.74
RR303LN-90-134	0.1% taurine	4.95	Clear	95.73	94.59	91.69	89.12
RR303LN-90-135	0.1% ectoine	4.94	Clear	95.69	94.11	92.03	89.22
RR303LN-90-136	0.01% TEA-HCl/0.1% b-alanine	5.01	Clear	95.92	94.40	91.46	89.00

Example 8

Similar to Example 2, rhGDF-5 formulations were prepared at a higher concentration of 0.2 mg/mL rhGDF-5 in 5 mM or 10 mM sodium acetate buffer at pH 5 with 10% trehalose. The formulations were tested under different storage temperatures including 37, 40 and 5°C. The stability results are presented in Table 8.

Table 8. Stability of 0.2 mg/mL rhGDF-5 in 5 mM or 10 mM acetate buffer with 10% trehalose at various storage temperatures

Sample ID	Acetate buffer, pH 5	Storage temp	pH	Clarity	HPLC Analysis % main peak					SEC % aggregate	
					Time 0	Day 13	Day 20	Day 34	7 months	Day 34	7 months
RR303LN-108G	5 mM sodium acetate	37°C	5.00	Clear	95.01	93.40	92.02	89.48	-	2.33	-
RR303LN-108J	10 mM sodium acetate	37°C	4.98	Clear	95.46	93.01	91.56	89.09	-	2.07	-
RR303LN-108G	5 mM sodium acetate	40°C	5.03	Clear	95.01	92.89	89.89	86.43	-	2.79	-
RR303LN-108J	10 mM sodium acetate	40°C	4.93	Clear	95.46	92.18	89.77	85.47	-	2.33	-
RR303LN-108G	5 mM sodium acetate	5°C	4.99	Clear	95.01	-	-	95.51	92.45	0.49	0.61
RR303LN-108J	10 mM sodium acetate	5°C	4.96	Clear	95.46	-	-	95.66	92.53	0.36	0.49

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All of the samples yielded clear solutions. The samples stored at 37°C for 34 days still had approximately 90% retention of the rhGDF-5 protein as determined by HPLC. The rhGDF-5 protein recovery from the 40°C was slightly lower at approximately 85% recovery. No significant changes were observed with the 5°C samples for at least 7 months.

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The results indicate that rhGDF-5 formulated in acetate buffer with or without other excipients could be stored at 5°C for long term storage. The formulation could be delivered under the body temperature for over a month. The isotonic liquid rhGDF-5 formulation is more stable at high temperatures, 5 and 37°C than has been shown previously.

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CLAIMS

We claim:

1. A composition consisting essentially of a 0.1 to 0.2 mg/mL GDF-5 protein solution in a 5 to 10 mM acetate buffer having a pH of from about 4.2 to about 5.3.
2. The composition of claim 1 further comprising one or more excipients selected from the group consisting of trehalose, sucrose, raffinose, glucose, mannitol, and combinations thereof.
3. The composition of claim 2 wherein the solution is isotonic.
4. The composition of claim 2 wherein the excipient is trehalose.
5. The composition of claim 2 wherein the excipient is sucrose.
6. The composition of claim 2 further comprising an excipient selected from the group consisting of TMAO, TEA-HCl, taurine, β -alanine, betaine, ectoine, HO-ectoine, GABA, and combinations thereof.
7. The composition of claim 6 wherein the excipient is present in an amount of from about 0.01 to about 0.5 weight percent.
8. A method of stabilizing a solution of GDF-5 protein comprising the steps of:
 - a. providing a sample of a 0.1 to 0.2 mg/mL GDF-5 protein, and
 - b. adding a 5 to 10 mM acetate buffer having a pH of from about 4.2 to about 5.3, thereby providing for a stabilized solution of GDF-5.
9. The method of claim 8, further comprising the step of adding one or more excipients selected from the group consisting of trehalose, sucrose, raffinose, glucose, mannitol, and combinations thereof.
10. The method of claim 9, wherein the excipient is added in an amount sufficient to render the protein solution isotonic.
11. The method of claim 9, wherein the excipient is trehalose.
12. The method of claim 9, wherein the excipient is sucrose.

13. The method of claim 9, further comprising the step of adding one or more excipients selected from the group consisting of TMAO, TEA-HCl, taurine, β -alanine, betaine, ectoine, HO-ectoine, GABA, and combinations thereof.

14. The method of claim 13 wherein the excipient is present in an amount of from about 0.01 to about 0.5 weight percent.