Effects of Different Buffers and pH on the Stability of Recombinant Human Growth Hormone

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Recombinant Human Growth hormone (rHGH) is a pharmaceutical single-chain polypeptide that contains 191 amino acids, with two disulfide bonds. The objective of this study was to determine the effects of different buffers and pH on the stability of rHGH and select the best ones for increasing the stability of rHGH in liquid solution. rHGH were dissolved at 3.33 mg/ml in phosphate (5mM and 10 mM), L-histidin (10 mM), sodium citrate (10 mM) and ammonium hydrogen carbonate (10 mM) buffers with the pH of 6.0, 6.25, 6.5 and 7.0 containing 2.5 mg/ml phenol. Stability of rHGH in the presence of different buffers and pH was evaluated by a size-exclusion chromatography (SEC) to determine the changes of the rHGH monomer to dimer, oligomer, and high molecular mass (Dimer & HM) and RP-HPLC to determine chemical decomposition and deamidation of rHGH. The effect of different pH on aggregation and precipitation, due to the agitation of rHGH solution, was evaluated by UV spectroscopy and Light Scattering Particle size analysis methods. Analysis of stability studies at 4, 25 and 37°C revealed that pH variation had a significant effect on rHGH stability. As the pH shift from 6 to 7 changes of protein to Dimer and H.M. decreased at the contrary changes of rHGH to related protein is increased. Altogether the results indicates that histidine or citrate buffer at 10 mM concentration and pH 6.25 to 6.5 provides better stability both regards to its physical and chemical stability.

Key words: Recombinant Human Growth Hormone; rHGH; Stability; Somatropin; Buffers, pH.

Human Growth hormone (HGH) or Somatropin is a key hormone involved in the regulation of not only somatic growth, but also in the regulation of metabolism of proteins, carbohydrates and lipids. Growth hormone initiates its anabolic effect by binding to specific cell surface receptors[1].

The most prevalent form of pituitary HGH is a single-chain polypeptide containing 191 amino acids, internally cross-linked by two disulfide

bonds[2]. The disulphide bridge formed between Cys53 and Cys165 results in a major loop, while the disulphide bridge between Cys182 and Cys189 results in a minor loop[3-4]. Approximately 55% of the polypeptide backbone exists in a right-handed -helical conformation. The molecular mass is 22 kD, with pI near 5.3. [5].

Until the mid-1980s, the only source of HGH was from human cadaver tissue, and is called as pituitary HG, or pit-HGH. Pit-HGH was removed from the US market when its use was linked to deaths from a slow virus infection known as Creutzfeldt-Jakob syndrome[2].

These days Somatropin is produced by a method based on recombinant DNA (rDNA)

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technology. By convention, for the purpose of labeling somatropin preparations, 1 mg of anhydrous somatropin (C990H1528N262O300S7) is equivalent to 3.0 IU of biological activity[6]. When it is prepared as a lyophilized powder, it must be contains not less than 910 µg of somatropin per mg, calculated on the anhydrous basis[7].

rHGH is indicated in Growth Hormone deficiency in children[8], Prader-Willi syndrome, to treat growth failure in Turner's syndrome[9], chronic renal failure[10], and also for short children born small for gestational age[11].

It is known that the highly purified proteins are time-unstable. During process manufacturing, the peptide or protein is exposed to several types of stresses. Also, production of the pure protein itself prior to its formulation also exposes the protein to several stress situations. These stress situations can be loosely defined as pharmaceutical processing. These include the generation of extensive air–water interfaces because of the turbulence in mixing tanks, foaming, adsorption to filters or tubing, and other unique situations, such as exposure to light, organic solvents, or heavy metals[12].

A therapeutic product must have the correct chemical structure and be free of harmful contaminants to be both safe and effective. Structure in protein therapeutic products, however, implies not only the correct sequence of amino acids but also the proper folding of that amino acid chain in three-dimensional space[13].

Protein aggregation is therefore controlled by both conformational stability and colloidal stability, and, depending on the solution conditions, either could be rate limiting. To successfully stabilize protein against aggregation, solution conditions need to be chosen not only to stabilize the protein native conformation but also to stabilize protein against attractive intermolecular forces. During development of formulations for therapeutic proteins, the latter goal is often achieved empirically during preformulation studies, where ionic strength, pH, and buffer type are optimized to minimize precipitation and other adverse events (e.g. deamidation)[14].

In the recent liquid formulations of rHGH, buffers; such as, histidin, citrate, phosphate and sometimes ammonium are used. The objective of this study was to determine the effects of different buffers and pH on the stability of rHGH and select the best ones for increasing the stability of rHGH in liquid solution. For this purpose, first rHGH was characterized for its purity, assay, peptide mapping according to European Pharmacopoeia (EP). Then rHGH was formulated in different buffers and pH and stability studies were conducted. Changes of the rHGH protein to dimer, oligomer, and high molecular weight aggregates (Dimer & HM) were determined by a size-exclusion chromatography method. Chemical decomposition and deamidation of rHGH (related proteins) were characterized by RP-HPLC methodology.

MATERIALS AND METHODS

Materials

Recombinant Human Growth Hormone (from bacterial fermentation of *Escherichia coli* strain) was purchased from Hospira, Adelaide Pty Ltd., Australia. Somatropin reference standards (CRS) were purchased from Strasburg Cedex 1f-67029 France, EP Pharmacopeia. Disodium hydrogen phosphate, Sodium dihydrogen phosphate, L-Histidin, Sodium citrate and Ammonium hydrogen carbonate analytical grade purchased from Merck Co. Germany. Phenol was purchased from Sigma (St. Louis, MO).

All other chemicals were of reagent grade and were used as received. Finish product vials made from type I borosilicate glass were purchased from Nova Ompi Italy and colorobutyl rubbers, aluminum rings and Flip-off caps were purchased from Helvoet Pharma, Germany.

Methods

Preparation of rHGH formulations

Different formulations of rHGH were prepared in phosphate, L-histidin, sodium citrate and ammonium hydrogen carbonate buffer containing 2.5 mg/ml phenol according to Table 1. For this purpose phosphate (5mM and 10 mM), L-histidin (10 mM), sodium citrate (10 mM) and ammonium hydrogen carbonate (10 mM) buffers with the pH of 6.0, 6.25, 6.5 and 7.0 containing 2.5 mg/ml phenol were prepared. Then the lyophilized rHGH were dissolved at 3.33 mg/ml in different buffers with slow stirring at room temperature. The final protein solutions were filter sterilized using 0.22 µm Durapore Millipore sterile filtration model and filled into sterile 2 mL vials under sterile air

condition. Finally the vials were rubbered and capped under sterile air condition and stored at refrigerator.

Stability studies

For stability studies, the samples were placed in $37 \pm 0.1°C$ incubator, room temperature ($25 \pm 3°C$) and refrigerator ($4 \pm 2°C$). Then the samples were analyzed every two weeks by size-exclusion chromatography and reverse phase RP-HPLC.

Analysis by size-exclusion chromatography method

The amount of monomeric rHGH, dimer and related substances of higher molecular mass; such as, oligomer and polymer (Dimer & HM) were determined by size-exclusion chromatography (SEC) according to EP (6). A set of HEWLETT PACKARD HPLC (1100 SERIES) with UV detector and Alltech (Macrospher GPC 100 A, ID 7.5mm , 300×7 mm, Biosep- SEC-S 2000, USA) column was used for SEC. The flow rate was 0.6 ml/min and the mobile phase was 0.063 M phosphate buffer (pH 7.0) containing 3% 2-propanol. Injection volume was 20 µl. Assay of protein monomer, Dimer & HM was detected at 214 nm.

Analysis by RP-HPLC chromatography:

According to EP (6), chemical changes of protein to oxidized and deamidated forms (related proteins) in each formula during storage were assessed every two weeks by RP- HPLC. A set of HEWLETT PACKARD HPLC (1100 SERIES) with UV detector and ALTECH (Prospher 300A, 250 × 4.6 mm) C4 column was used. Mobile phase was 1-propanol (29%), 0.05 M tris-hydrochloride buffer solution pH 7.5 (71%) and the flow rate was 0.5 ml/min. The injection volume was 20 µl, and detection was conducted at 220 nm wave length.

Analysis by UV spectrophotometer method

Content of protein in solution was determined by UV spectrophotometer, according to Hospira assay formula: protein content = $(A276^{nm} - 2A333^{nm})*1.28$, in this formula "A" is absorbance and λ max of somatropin is 276 nm (13).

Analysis of particle formation by light scattering particle analysis method

Effect of different pH on the particles creation, due to the agitation of rHGH solution was evaluated by a light scattering particle analysis method. Particulate numbers indicate the degree of aggregation in the solutions. In this method, the samples were incubated at 50 °C and shaked at 150 RPM in an orbital shaker (GFL, Germany) for 35 hours. Then the entire vials content were transferred to Erlenmeyer and diluted 1 to 5 with particle free water and number of 1 to 25 µm particles was measured by light scattering analyzer (Klotze, Germany).

Statistical analysis

Statistical analysis of the results was carried out using unpaired Student t-test.

RESULTS

Changes of rHGH monomer to Dimer & HM

Analysis results in 4 °C: To evaluate the stability of rHGH regarding to its change to Dimer & HM; the native monomer and the aggregate forms of different formulations in pH 6.0, 6.25, 6.5 and 7.0 were determined by SEC-HPLC (Figure 8). Figure 1 shows the effect of different buffers in the stability of rHGH regarding its changes to Dimer & HM at 4°C. The amount of rHGH monomer of formulations in the pH 7.0 at Ammonium Hydrogen Carbonate, Citrate and Histidin buffers was around 98%; however, in the phosphate buffer pH 7.0 especially with 5 mM potency was near 90%. Among different formulations, the ones with pH 7.0 and 10 mM concentration (H7, C7 and A7 formulations) had the best effect. There were no statistically significant differences in the amount of rHGH monomer among Phosphate, Ammonium Hydrogen Carbonate, Citrate and Histidin buffers (10 mM) ($P > 0.05$); however, the amount of rHGH monomer in phosphate buffer 5 mM was decreased significantly compared to the other buffers ($p < 0.05$).

Analysis results in 25°C

Figure 2 shows the effect of different buffers in the stability of rHGH regarding its changes to Dimer & HM at 25°C. The amount of rHGH monomer of formulations in the pH 7.0 at Ammonium Hydrogen Carbonate, Citrate and Histidin buffers was around 95%; however, in the phosphate buffer pH 7.0 especially with 5 mM potency was near 86%. Among different formulations, the ones with pH 7.0 and 10 mM potency (H7, C7 and A7 formulations) had the best effect. There were no statistically significant differences among 10 mM buffers ($P > 0.05$), but in

evaluation of rHGH in Phosphate buffer 5 mM and the others with 10 mM concentration, there were a significant difference (P< 0.04). Therefore, rHGH is more stable at 10 mM concentration rather than 5mM.

Analysis results in 37 °C

Figure 3 shows the effect of different buffers in the stability of rHGH regarding its changes to Dimer & HM at 37°C. The amount of rHGH monomer of formulations in the pH 7.0 at Ammonium Hydrogen Carbonate, Citrate and Histidin buffers was around 92%; however, in the phosphate buffer pH 7.0 especially with 5 mM potency was around 81%. Among different formulations, the ones with pH 7.0 and 10 mM concentration (H7, C7 and A7 formulations) had the best effect. There were no statistically significant differences between different buffers at the same pH (P> 0.05), but in evaluation of rHGH in Phosphate buffer 5 mM and the others with 10 mM concentration, there were a significant difference (P< 0.01). So that rHGH is more stable at 10 mM rather than 5 mM.

Changes of rHGH to related proteins

To evaluate the stability of rHGH regarding to its change to oxidized and deamidated forms (related proteins), different formulations were analyzed by using RP-HPLC (Figure 9).

Figure 4 shows the effect of different buffers and pH on the stability of rHGH regarding its changes to related proteins at 4°C. The amount of related proteins of formulations in the pH 6.25 at Ammonium Hydrogen Carbonate 25%, Citrate and Histidin buffers was approximately 28%. Among different formulations, the Ammonium Hydrogen Carbonate with pH 6.25 and 10 mM concentration (A6.25 formulation) had better effect. There were no statistical significant differences between different buffers (P> 0.2). In all buffers; except Ammonium Hydrogen Carbonate; as the pH increased amount of Related Protein increased too. The amount of related proteins at pH 6 was minimum.

Figure 5 shows the effect of different buffers and pH on the stability of rHGH regarding its changes to related proteins at 25°C. The amount

Table 1. The composition of the liquid rHGH formulations in different buffers and PH.In the above formulations, P designated for Phosphate buffer formulations, H forHistidin buffer formulations, C for Citrate buffer formulations, A for Ammonium hydrogen carbonate buffer formulations. The numbers after Capital letters indicate the pH of solution. The number 5 before P indicate the molarities

PH	rHGH mg/ml	Phenol mg/ml	Formulation of Phosphate Buffer 5 mM	Formulation of Phosphate Buffer 10 mM	Formulation of L-histidin buffer 10 mM	Formulation of Sodium citrate buffer 10 mM	Formulation of Ammonium hydrogen carbonate buffer10 mM
6.0	3.33	2.5	5P6	P6	H6	C6	A6
6.25	3.33	2.5	5P6.25	P6.25	H6.25	C6.25	A6.25
6.5	3.33	2.5	5P6.5	P6.5	H6.5	C6.5	A6.5
7.0	3.33	2.5	5P7	P7	H7	C7	A7

Table 2. rHGH stability at different PH, in Histidin 10mM buffer solutions. The values are means ± standard deviations (n = 3).In the above Formulations H indicated as Histidin buffers

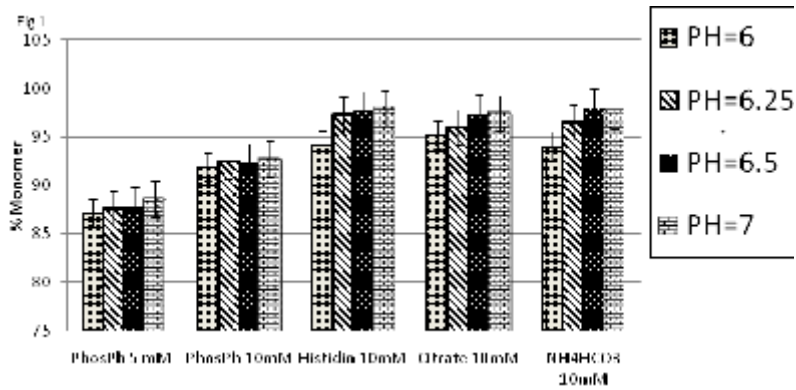| Formulation	PH	rHGH mg/ml (before incubation)	rHGH mg/ml (after incubation)	DecreasedrHGH mg/ml concentration
H6	6.0	3.271±0.005	1.074±0.062	2.197±0.061
H6.25	6.25	3.322±0.007	1.264±0.069	2.058±0.073
H6.5	6.5	3.338±0.006	1.341±0.047	1.998±0.047
H7	7.0	3.263±0.004	1.438±0.061	1.825±0.056

Fig. 1. Changes of rHGH monomer to Dimer and H.M. in different buffers and pH.
The samples stored for 4 weeks at 4°C. The figure represent the amount of native protein (monomer) remained in the protein solution. The values are means ± standard deviations (n = 3).
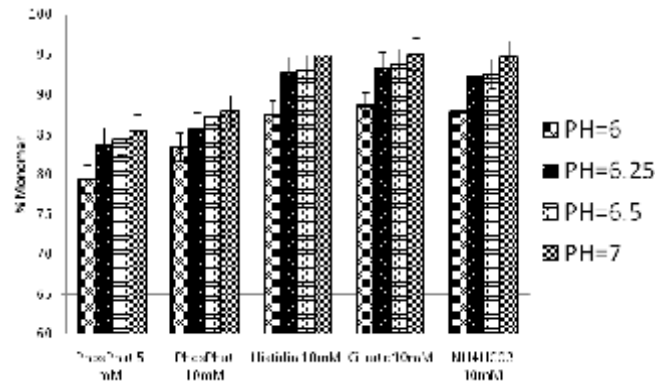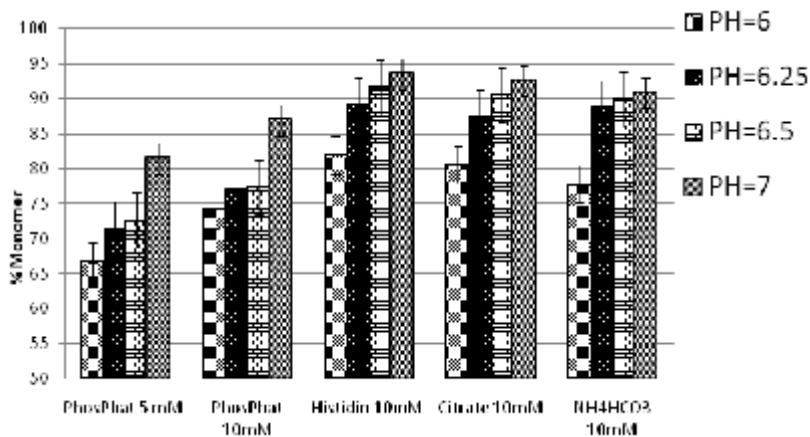

Fig. 2. Changes of rHGH monomer to Dimer and H.M. in different buffers and pH.
The samples stored for 4 weeks at 25 °C. The figure represent the amount of native protein (monomer) remained in the protein solution. The values are means ± standard deviations (n = 3)
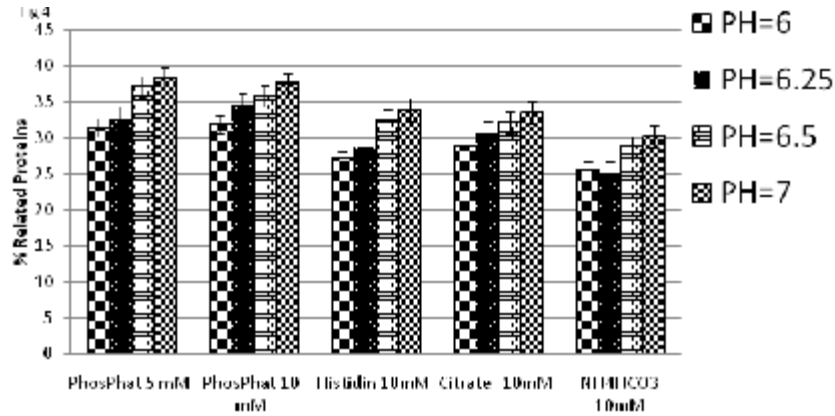

Fig. 3. changes of rHGH monomer to Dimer and H.M. in different buffers and pH. The samples stored for 4 weeks at 37 °C. The figure represent the amount of native protein (monomer) remained in the protein solution. The values are means ± standard deviations (n = 3)

Fig. 4. Changes of rHGH monomer to related proteins in different buffers and pH.
The samples stored for 4 weeks at 4 °C. The values are means ± standard deviations (n = 3)
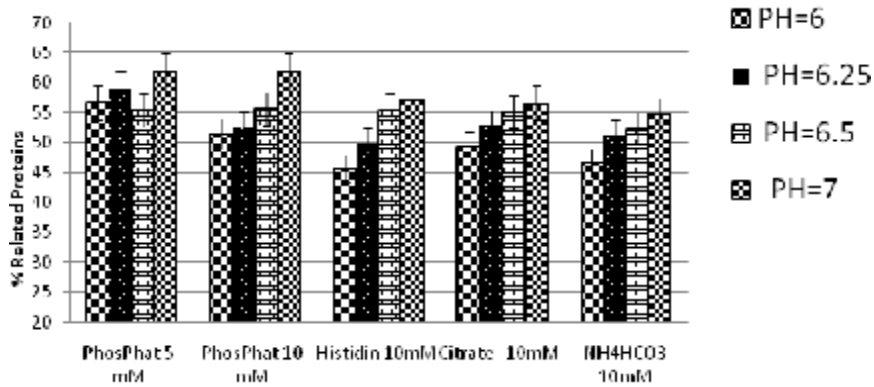

Fig. 5. Changes of rHGH monomer to related proteins in different buffers and pH. The
samples stored for 4 weeks at 25 °C. The values are means ± standard deviations (n = 3)
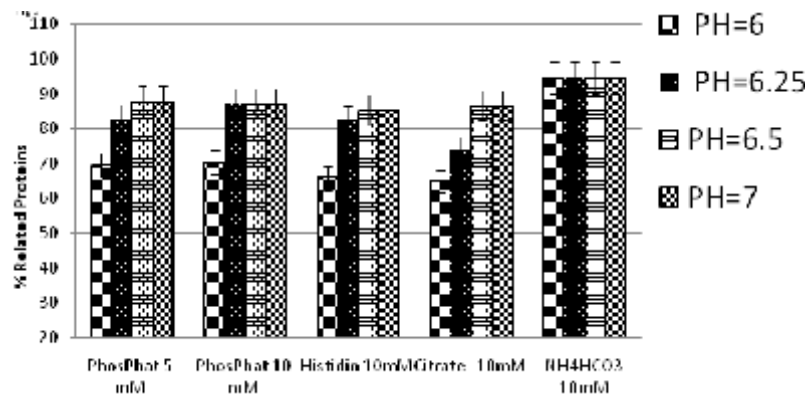

Fig. 6. Changes of rHGH monomer to related proteins in different buffers and pH.
The samples stored for 4 weeks at 37 °C. The values are means ± standard deviations (n = 3)
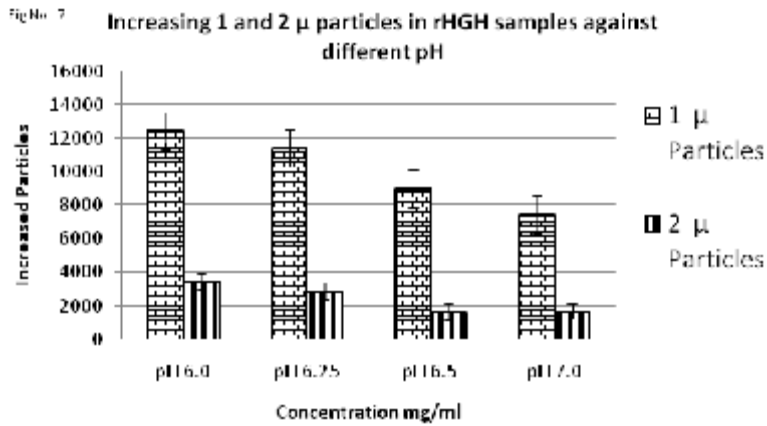
Fig. 7. The effect of different pH on the rHGH aggregation in Histidin 10 mM solutions. Samples were incubated at 50 °C and shaked at 150 RPM for 35 hours. The values are means ± standard deviations (n = 3)
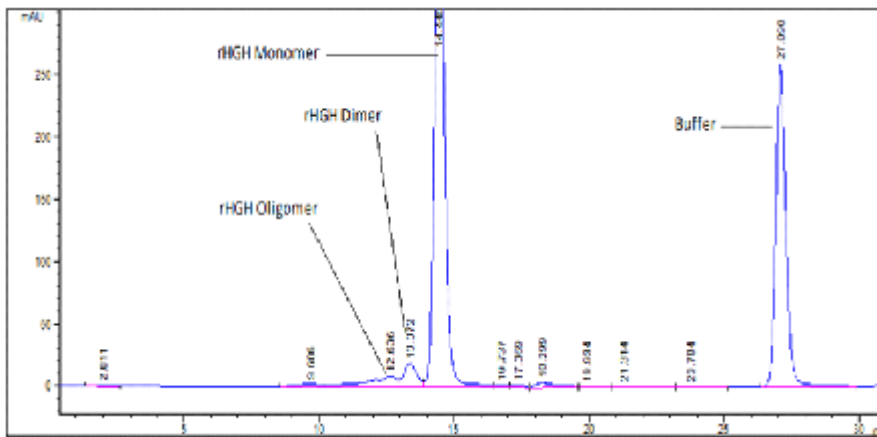

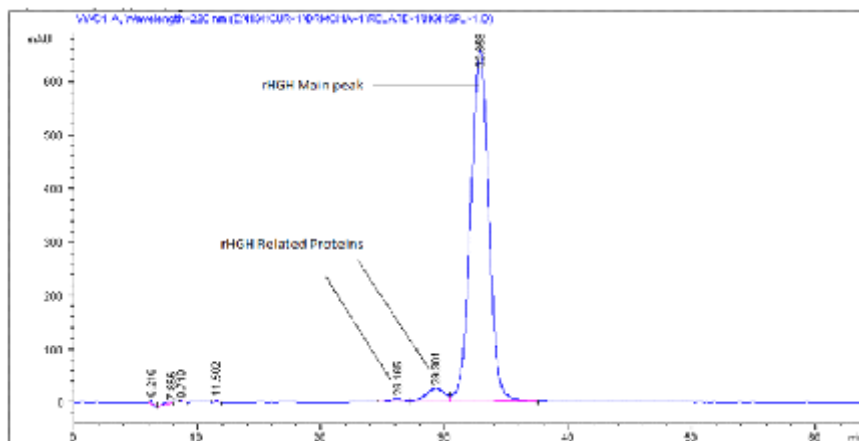**Fig. 8.** Sample chromatogram of rHGH by size exculsion chromatography (SEC-HPLC)

Fig. 9. Sample chromatogram of rHGH by RP-HPLC

of related proteins of formulations in the pH 6.0 at Histidin buffer was approximately 46%. Among different formulations, the ones with pH 6.0 and 10 mM concentration (H6, C6 and A6 formulations) had better effect (p<0.04). There were no statistical significant differences between different buffers at the same pH (P> 0.05), and also in evaluation of rHGH in Phosphate buffer 5 mM and the others with 10 mM concentration, there were no significant effects (P>0.52).

Figure 6 shows the effect of different buffers and pH in the stability of rHGH regarding its changes to related proteins at 37°C. Approximately in most samples; pH 7 and 6.5; all the rHGH changed to related proteins. Interestingly 100% of rHGH in all Ammonium Hydrogen Carbonate samples (N6, N6.25, N6.5 and N7) changed to related proteins. Significantly better effects (p<0.05) was seen with Phosphate, Histidin and citrate buffers at pH 6.0.

Analysis by UV spectrophotometery

For further rHGH stability studies only Histidin 10 mM buffer and phenol 2.5 mg/ml was used (Formulations H6, H6.25, H6.5 and H7)**.** Concentration of rHGH in all sample determined by UV absorbance at 276 and 333 nm at time zero, according to Hospira protocol as explained in materials and methods([1]). The samples were incubated at 40 °C and agitated at 150 RPM in an orbital shaker (GFL Germany) contemporary for 30 hours then the entire vials content were transferred to polypropylene Eppendorf tubes and centrifuged for 20 min at $5000 \times g$. Then the amount of decreased protein concentration was determined at the supernatant of samples.

Figure 7 shows the effect of different pH on creation of 1 and 2 μ particles in rHGH solutions. The number of 1 and 2 μ particles (aggregated proteins) in the protein solution increased significantly. The number of 1 μ particles increased more than 2 μ particles. The number of particles increased when the pH of solution increased from 6.0 to 7.0. The pH 7.0 had the most stabilizing effects.

DISCUSSION

Regard to rHGH, like most large proteins the term "stability" refers to the physical and chemical stability and also maintenance of biological potency[1].Physical instability refers to a change in the secondary, tertiary, or quaternary structure of a protein and includes denaturation, aggregation, precipitation, or adsorption to surfaces. Chemical instability involves covalent modification of the protein via bond formation or cleavage. Chemical instability is an outcome of reactions such as hydrolysis, deamidation, oxidation, disulfide exchange, β-elimination, and racemization[12].

In this study physical or conformational stability of rHGH in aqueous solution due to the changes of native protein to Dimer and higher molecular mass and changes of rHGH to related proteins investigated, along this, ionic strength, pH, and buffer was considered.

Protein aggregation is arguably the most common and troubling manifestation of protein instability, encountered in almost all stages of protein drug development. Protein aggregation, along with other physical and/or chemical instabilities of proteins, remains to be one of the major road barriers hindering rapid commercialization of potential protein drug candidates. Although a variety of methods have been used/designed to prevent/inhibit protein aggregation, the end results are often unsatisfactory for many proteins. The limited success is partly due to our lack of a clear understanding of the protein aggregation process ([2]).

Protein molecules can often undergo self-association by physical or chemical forces to form dimers, trimers, tetramers, or higher oligomers. This self-association or aggregation is a common problem during formulation development and pharmaceutical processing. Generally, aggregation is a two-step process. The first step involves unfolding of the protein molecule, thereby exposing the buried, hydrophobic amino acid residues to the aqueous solvent. In the second step, the hydrophobic residues of the unfolded protein molecules undergo association, leading to protein aggregation. Such association takes place in order to minimize the unfavorable exposure of hydrophobic residues in the unfolded protein to water. In accordance with this mechanism, aggregation is a polymolecular, concentration-dependent process that obeys higher-order kinetics ([11])

Normally rHGH has a concentration of about 1.34 mg/ml in lyophilized dosage forms, but in liquid dosage forms higher concentration is used[3-5]. Higher concentration of rHGH (commonly 3.33 mg/ml) in the treatment of GH deficiency make it more acceptable for patients, because injection volume decreases[6]. Thus, In this study rHGH with the concentration of 3.33 mg/ml in each formulation was used. One of the most common antimicrobial preservatives in peptide and protein products is phenol[7], so as preservative, Phenol 2.5 mg/ml was used.

The pH of the product is one of important parameter that influence the rHGH stability. In formulation of protein pharmaceutical drugs patient compliance due to pH of the product is also important. The pH of the protein formulation can be critical to its stability and bioactivity. The net charge on a protein is zero at its isoelectric point PI, positive at pH below PI, and negative at pH above PI. The solubility is the lowest at PI; thus, buffering at pH very close to PI is not desirable[10]. In some therapeutic proteins pH has a strong effect on aggregation of proteins. Some Protein solutions are physically stable at narrow pH ranges only, like as low molecular weight urokinase[8], rhGCSF[9] and insulin[10]. Commercial rHGH products in the market have a pH range about 6 to 7, well above the PI of rHGH (5.3); therefore, in this study pH of 6, 6.25, 6.5, and 7.0 was selected. The effects of above pH on the stability of rHGH regarding to changes of native protein to Dimer & H.M. and related proteins was evaluated.

Changes of rHGH to Dimer & H.M. at different pH

Stability of rHGH in aqueous solutions with different buffers and pH is time and temperature dependent (Fig 1, 2 and 3). So that, changes of rHGH to Dimer and Higher molecular mass, significantly increased at high temperature especially at acidic pH. Results at 4 °C show that changes of native protein to Dimer, H.M and aggregated forms are very limited. There were no statistical significant difference between buffers and different pH. Meanwhile the result confirmed that the reduction of rHGH monomer in Phosphate 5mM is significantly more than the other buffers with 10 mM strength. It seems 5 mM concentration does not provide enough ionic strength to properly dissolve the rHGH protein. There were statistically significant differences

between different pH of formulations at 25 and 37 °C (P< 0.05). Almost in all formulations when the pH shifted from 6 to 7, changes of protein to Dimer and H.M. increased and the stability of rHGH was higher at pH 7.0. In another word rHGH was more unstable in acidic pH of 6.0, The reason could be that this pH is near to isoelectric pH of the protein (PI 5.3).

Changes of rHGH to Related proteins at different pH

Deamidation by direct hydrolysis or via a cyclic succinimide intermediate, oxidation of the methionine residues and cleavage of peptide bonds are the main degradation reactions of rHGH (26). Deamidation especially takes place at the Asn and rHGH solutions are easily oxidized ([11]).The chemical degradation of rHGH was measured by RP-HPLC as rHGH related proteins according to EU Pharmacopoeia.

The stability of the proteins regards to deamidation is dictated by pH. Also, the best choice of pH to avoid deamidation may be dictated by the mechanism of deamidation. If the mechanism is general acid/base catalyzed, then a pH of 6.0 will minimize deamidation. Deamidation that proceeds through cyclic imide intermediate is base catalyzed, and acidic pH would thus be desirable in this case[10].

There were no significant effect between different buffers at the same pH (P>0.08) but between the different pH there were a significant effect so that changes of rHGH to related proteins in pH 6 is less than the others (p<0.04).

Stability study at 4 °C confirmed that there were no significant effect between different pH (p>0.2) and also between the different buffers but at accelerate conditions; 25 °C a significant effect was observed (P<0.05) Fig 5.

Result (figure 5) indicate that as the pH shift from 6 to 7, changes of protein to related proteins increases and the stability of rHGH decreased. Therefore, acidic pH (pH 6) is better to avoid deamidation of rHGH. This finding is in agreement with the results of Jacob S and et al, that indicate deamidation of asparginine residues to aspartate or isoaspartate via succinimide intermediates (positive to negative charge), occurs in many proteins and peptides in neutral to basic pH, and is a major cause of spontaneous degradation and loss of amino acid sequence homogeneity[12].

Changes of rHGH to Particulate Matter at different pH

rHGH denatures at the air-liquid interface, especially under high shear. This is due to the fact that rHGH is prone to be adsorbed to the air-liquid interface[28]. To further investigate, the effect of different pH on the aggregation and particle creation of rHGH solution due to the agitation and shaking, the stability studies was conducted and the number of produced particles was evaluated by a light scattering particle analysis method. The result showed that in accelerated conditions (the high temperature of 50 °C with shaking) at all pH, creation of 1 and 2 µ particles increased sharply. As the pH shift from 7 to 6 significantly the number of produced particles increased (p< 0.03). The amount of larger particles like as 5, 10 and 25 µ, before and after incubation, was not change significantly (Figure 7). Light scattering particle analysis and UV spectrophotometer analysis (table 2) confirmed our previous results of SEC (Figure 1, 2 and 3).

CONCLUSION

The results of this study employed to use an specific buffer and pH in order to minimize the low stability of rHGH in aqueous formulations. Stability of rHGH in aqueous solutions with different buffers and pH is time and temperature dependent. So that changes of rHGH to dimer and higher molecular weights, significantly increased at high temperature especially at acidic pH. As pH shift from 6 to 7, changes of rHGH to dimer, higher molecular weight and aggregated forms reduced and protein physical stability increased. At the contrary when pH shift from 6 to 7, changes of rHGH to related proteins increased and protein chemical stability decreased. Buffers at 10 mM concentration had a better stability effects compared to 5 mM. Changes of rHGH in different buffers at the same condition (with the same pH, temperature, and molarities) were not significant. Therefore, aggregation of rHGH was not found to be dependent on the nature of the buffer but was related to the pH and ionic strength of buffer. Stability of rHGH in liquid formulation is pH dependent; therefore in formulation of rHGH we must select the best pH so that maximum physical (with minimum Dimer and H.M.) and chemical

stability (with minimum creation of related proteins) earned. Altogether the results indicates that histidine or citrate buffer at 10 mM concentration and pH 6.25 to 6.5 provides better stability both regards to its physical and chemical stability.

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