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RESEARCH PAPER

Degradation Kinetics of Somatostatin in Aqueous Solution

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ABSTRACT

The degradation kinetics of somatostatin (somatotropin release inhibiting factor), a cyclic tetradecapeptide, was investigated as a function of temperature, pH, ionic strength, buffer type, and buffer concentration. In addition, the effect of different container materials in which the solutions were stored and the presence of an antimicrobial agent for in vitro use was examined. The degradation of somatostatin followed first-order kinetics under all investigated conditions. The pH-stability profile showed a well-defined stability optimum around pH 3.7. The degradation was accelerated at higher buffer concentrations, phosphate buffer being significantly more detrimental than acetate buffer. The ionic strength and the drug concentration had virtually no effect on the degradation rate. When general purpose glass vials were used as storage containers, degradation was faster due to release of alkali from the container material. The solution properties, i.e., pH, buffer type, buffer capacity, and the experimental setup such as container material and sterile conditions need to be carefully selected or maintained, in order to avoid accelerated degradation.

Key Words: Chemical stability; Peptides; Somatostatin.

INTRODUCTION

Somatostatin, or SRIF (somatotropin release inhibiting factor), is a cyclic tetradecapeptide inhibiting the secretion of several endogenous hormones, including growth hormone, glucagon, and insulin.[1] Like most other peptide drugs, somatostatin has a very short in vivo half-life [1.1–4.9 min[2]], limiting the suitability of this substance for long-term clinical use. The search for long-acting analogs has resulted in the synthesis of various molecules, octreotide being the substance with the largest therapeutic success so far. However, due to its effects on carbohydrate metabolism, somatostatin alone or in combination

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with other hormones may present interesting opportunities in the treatment of diabetes, but the optimum mode of administration has yet to be defined.

Despite the large number of reports dealing with the stability of peptide drugs such as ACTH,[3] calcitonin,[4] gonadorelin,[5,6] insulin,[7] (LH-RH),[8] nafarelin,[9] and secretin[10] to name just a few, very little is known about the stability of somatostatin. Analogs of this drug have been investigated before,[11–13] but, to our knowledge, no stability studies have been performed with the parent molecule somatostatin, except for one publication reporting the loss of peptide in solutions for total parenteral nutrition.[14] Systematic investigations are, therefore, needed in order to gain more information on factors influencing the stability of this particular drug and of peptides in general. These data are essential for rational dosage form development, the design of stability studies, and shelf-life predictions, and they should result in a facilitated and accelerated development of suitable formulations, a prerequisite to obtain the desired pharmacological effect.

In this study, the chemical stability of somatostatin in aqueous solution as a function of the most important storage parameters (temperature, pH, ionic strength, and buffering conditions) was investigated. In addition, we attempted to assess the effect of different container materials in which the solutions were stored and the addition of an antimicrobial agent for in vitro use. Quantitative information on these factors is relevant for the development of conventional and long-acting dosage forms for somatostatin and other peptide drugs.

EXPERIMENTAL

Materials

The following materials were used as received: Somatostatin acetate as a freeze-dried solid in sterile ampoules (Dr. Willmar Schwabe Pharmaceuticals, Karlsruhe, Germany), high-performance liquid chromatography (HPLC)-grade acetonitrile and phosphoric acid (EM Science, Gibbstown, NJ), analytical grade buffer salts (Fisher Scientific, Fair Lawn, NJ), and sodium azide.

Methods

The drug concentration was determined with a stability indicating HPLC procedure: LC-600-HPLC-pump, SIL-9A autoinjector, SPD-6A UV-detector, CR-601 integrator (Shimadzu, Kyoto, Japan); ET 250/8/4, Nucleosil 300-5 C18 column (Macherey and Nagel, Düren, Germany), equipped with a Vydac I-218TP guard column (Vydac, Hesperia, CA). Solvent A: 94.5% water, 5% acetonitrile, 0.5% phosphoric acid; solvent B: 49.5% water, 50% acetonitrile, 0.5% phosphoric acid; gradient: from 38% B to 70% B within 30 min; flow rate 0.9 mL/min; ultraviolet-detection at 210 nm. The retention time of somatostatin under these conditions was approximately 16 min. Peptide solutions of known concentrations (0.01–0.20 mg/mL) were used to generate calibration curves. The HPLC method was checked with respect to linearity ($r^2 > 0.99$) sensitivity (5×10^{-3} mg/mL), precision [$\pm 3\%$ relative standard diviations (RSD)] and accuracy (approx. $\pm 10\%$ RSD).

As the initial standard storage condition, somatostatin acetate was dissolved in 10 mmolar phosphate buffer, pH 7.4, concentration = 0.20 mg/L, made isotonic with NaCl and stored in a dark constant temperature oven at $60 \pm 0.5^{\circ}$C. Depending on the factor to be investigated, the temperature, pH, drug concentration, ionic strength, buffer species, and buffer concentration were varied. The buffer solutions used in this study and their respective pH values are summarized in Table 1; the applied levels of the investigated factors are listed in Table 2. The pH of the buffer solutions was measured at the appropriate temperature, and, for the pivotal experiments, the pH also was measured after degradation. The drug solutions were stored as individual samples ($V = 0.5$ mL) in tightly closed polypropylene containers. Alternatively, when the effect of the container material was investigated, 3.0 mL of the peptide solution was placed in freeze-drying vials (Type I glass, (USP) XXII) closed with rubber stoppers or in standard glass vials of comparable size (Type NP, general purpose glass, USP XXII) sealed with PTFE-lined screw caps. Sodium azide

Table 1. Investigated buffer systems (buffer composition from[15] buffer concentrations are given in the text).

Buffer system	pH	Component A	Component B
Citrate/HCl	1.2	HCl	Sodium citrate
Citric acid/ phosphate	2.2–8.0	Citric acid	Na_2HPO_4
Acetate	6.0	CH_3COOH	$NaCH_3COO$
Phosphate	6.0, 7.4	KH_2PO_4	Na_2HPO_4
Borate/HCl	9.0	HCl	H_3BO_3/NaOH

Table 2. Summary of the degradation rate constants and degradation half-lives of somatostatin under various conditions (from Figs. 5–10).

Investigated parameter		k_{obs} (10^{-2} d^{-1})	t_{50} (d)
Temperature (°C)	23	0.4	157.5
(phosphate buffer	37	2.5	27.4
0.01 M, pH 7.4)	50	11.7	5.9
	60	27.5	2.5
	65	37.5	1.9
	70	76.2	0.9
	80	268.6	0.3
pH	1.2	29.9	2.3
($T = 60°C$)	2.2	4.9	14.1
	3.0	1.4	49.2
	4.0	0.7	106.6
	5.0	1.8	38.5
	6.0	6.4	10.8
	7.0	27.2	2.6
	8.0	97.6	0.7
	9.0	367.3	0.2
Acetate buffer	0.01	4.7	14.8
(pH 6.0, $T = 60°C$)	0.10	5.6	12.3
(M)	0.20	6.5	10.6
	0.30	6.5	10.6
Citrate/phosphate	0.01	4.4	15.7
buffer	0.10	6.2	11.2
(pH 6.0, $T = 60°C$)	0.20	7.2	9.6
(M)	0.30	7.7	9.0
Phosphate buffer	0.01	4.9	14.3
(pH 6.0, $T = 60°C$)	0.10	7.2	9.6
(M)	0.20	7.4	9.4
	0.30	8.2	8.4

(0.05% w/v) was added as an antimicrobial agent in order to prevent the growth of microorganisms. This common preservative for in vitro use had no direct effect on the chemical stability of the peptide. The whole container or appropriate sample volumes were removed from the ovens at suitable time intervals, stored in a freezer at −20°C and analyzed together in one HPLC run. A fresh standard solution was prepared for every set of samples.

Kinetic Methods

The initial peptide concentration (standard condition: 0.2 mg/mL) was taken as the 100% value, and the degradation was monitored for at least two half-lives, except for a few experiments (e.g., at pH 3.0 and pH 4.0, 60°C), where the peptide was exceptionally stable. The apparent first-order reaction constants (k_{obs}) were obtained from semilogarithmic plots by linear regression and used to calculate the degradation half-lives. The correlation was good (usually $r^2 \geq 0.99$) in all experiments.

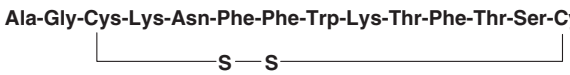

Figure 1. Amino acid sequence of somatostatin.

RESULTS AND DISCUSSION

The chemical stability of a peptide mainly depends on the chemistry of the side chains and on the structure of the backbone. Therefore, as the first step of the stability assessment, the primary sequence (Fig. 1) and the physicochemical properties of the particular substance should be closely examined. Somatostatin is a 14 amino acid peptide with a molecular weight of 1638 D (free base) containing two lysine residues, giving the peptide a positive charge at all pH values investigated in this study (pI ≈ 9.5). The two cysteine residues form a disulfide bridge and the asparagine presents a possible deamidation site. The peptide is easily soluble in aqueous buffers (solubility > 70 mg/mL) and to a much lesser extent in methanol and ethanol (>15 and ≈ 6 mg/mL, respectively[16]).

As a standard storage condition, a temperature of 60°C and 10 mmolar isotonic phosphate buffer pH 7.4 as the solvent was chosen. The results obtained in this experiment and preruns under various conditions gave an indication how to select the time intervals for sample collection. The somatostatin concentration decreased according to pseudo first-order kinetics at all investigated temperatures, pH values, and buffer concentrations.

The semilogarithmic first order plot for the degradation of the drug stored in different containers at 60°C is shown in Fig. 2. The reaction rate was slightly higher in standard glass vials (solvent: 10 mmolar phosphate buffer pH 7.4). The pH of these solutions at the end of the storage period was raised by approximately 0.1 pH units, indicating the release of alkali from the container material and an insufficient buffer capacity. Increasing pH values accelerate the degradation of somatostatin (shown below, Fig. 8). The pH of the drug solutions stored in polypropylene vials and high-quality glass vials remained unchanged and the degradation was, therefore, slightly slower. Similar results were found at a

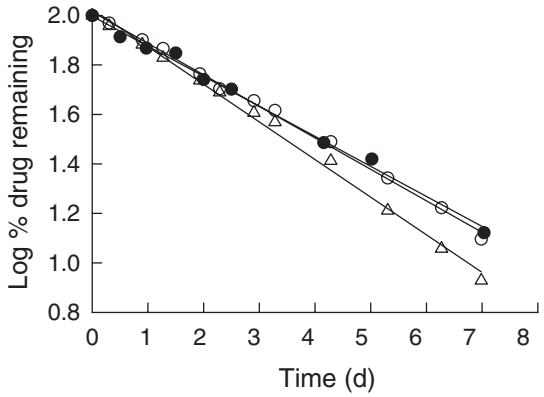

Figure 2. Effect of the type of container on the degradation of somatostatin in isotonic phosphate buffer 0.01 M, pH 7.4 at 60°C. (●) Type I glass vials (high hydrolytic resistance), (○) polypropylene tubes, (△) general purpose glass vials.

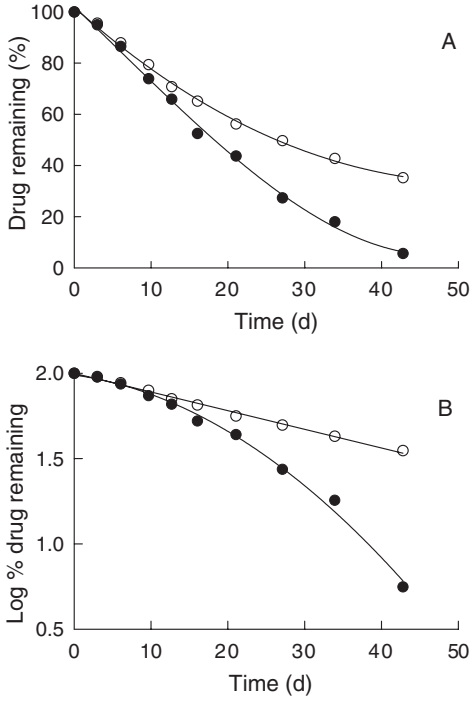

Figure 3. Effect of the presence of 0.05% g/V sodium azide as an antimicrobial agent on the degradation of somatostatin in isotonic phosphate buffer 0.01 M, pH 7.4 at 37°C. A: Linear plot. B: Semilogarithmic plot. (○) With sodium azide, (●) without sodium azide.

storage temperature of 37°C (data not shown). Replacing the air in the general purpose glass containers with nitrogen to prevent a possible oxidative degradation had no effect on the drug stability (data not shown).

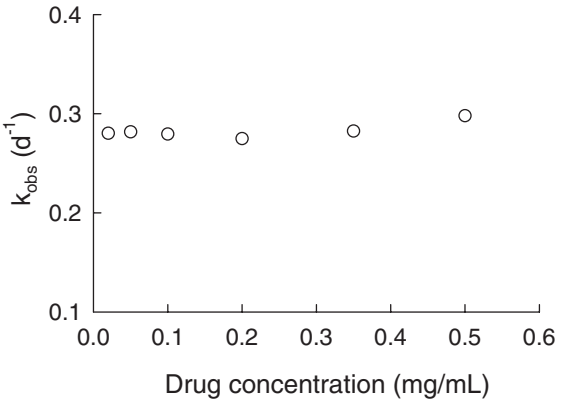

Figure 4. Effect of the somatostatin concentration on the observed pseudo first-order degradation rate constant in isotonic phosphate buffer 0.01 M, pH 7.4 at 60°C.

The degradation kinetics of somatostatin in the standard solvent (10 mmolar isotonic phosphate buffer pH 7.4) at 37°C with and without 0.05% w/V sodium azide is shown in Fig. 3. This substance is a widely used antimicrobial agent for in vitro use, preventing the growth of microorganisms. In the absence of sodium azide, the kinetics deviated significantly from first-order kinetics with increasing time and the degradation was accelerated, probably because of the susceptibility of the peptide against microbial degradation. The pH of the samples containing sodium azide was unchanged, whereas, the pH of the samples lacking the preservative and stored for more than 40 days was 0.14–0.24 units lower than the initial pH. This should have resulted in a slower degradation because somatostatin is more stable at lower pH values. Sodium azide had no effect on the degradation of the peptide at 60°C in the same solvent (data not shown). These results show that long-term stability studies at temperatures allowing the growth of microorganisms should be performed under aseptic conditions or a chemically inert preservative should be added. This is especially important when the in vitro release profiles of depot forms for peptides or proteins (e.g., microspheres) are investigated.

From Fig. 4, it can be concluded that the peptide concentration had no effect on the observed pseudo first-order reaction rate in the investigated range. A small increase in the reaction rate could be supposed from the k_{obs} at 0.5 mg/mL. Higher concentrations were not investigated, due to the limited amount of material that was available for the study.

Figure 5 and Fig. 6 (Arrhenius plot) illustrate the effect of the storage temperature on the degradation

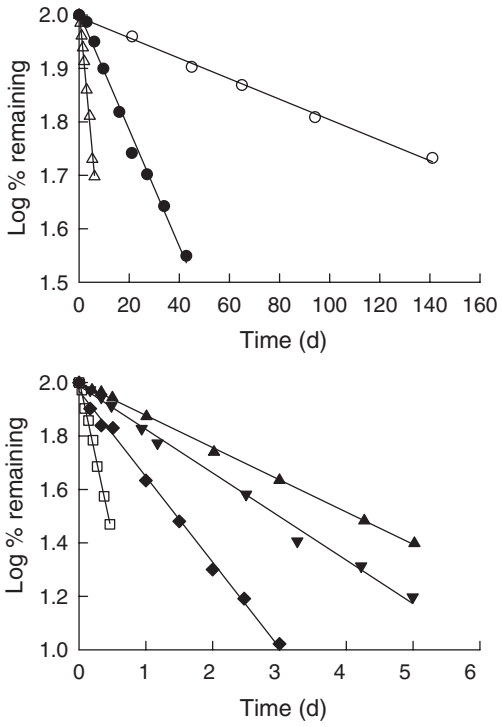

Figure 5. Effect of the temperature on the degradation of somatostatin in isotonic phosphate buffer 0.01 M, pH 7.4. (○) 23°C, (●) 37°C, (△) 50°C, (▲) 60°C, (▼) 65°C, (♦) 70°C, (□) 80°C.

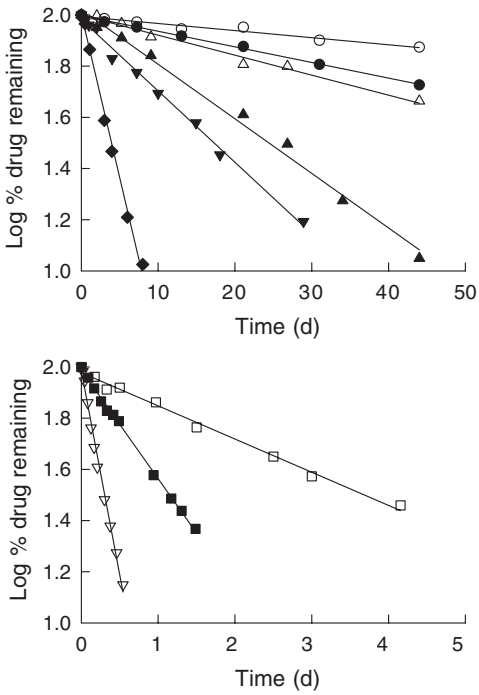

Figure 7. Effect of the pH on the degradation of somatostatin at 60°C (ionic strength = 0.5 adjusted with NaCl; pH 1.2 citrate/HCl buffer; pH 2.2–8.0 citric acid/phosphate buffer; pH 9.0 boric acid buffer). (○) pH 4.0, (●) pH 3.0, (△) pH 5.0, (▲) pH 2.2, (▼) pH 6.0, (♦) pH 7.0, (□) pH 1.2, (■) pH 8.0, (▽) pH 9.0.

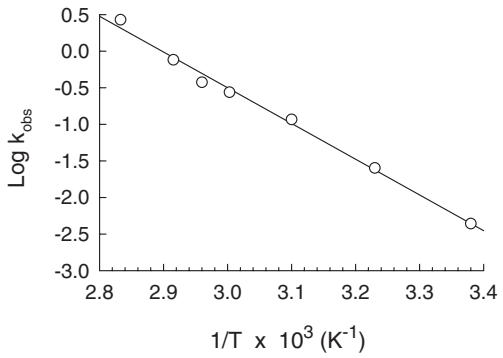

Figure 6. Arrhenius plot for the degradation of somatostatin in isotonic phosphate buffer 0.01 M, pH 7.4.

of somatostatin at pH 7.4. The respective rate constants and degradation half-lives are summarized in Table 2. From the slope of the linear Arrhenius plot ($r^2 = 0.994$, temperature range 23–80°C), an activation energy of 93.5 kJ/mol (22.4 kcal/mol) could be calculated. This value is comparable with activation energies found for other peptide drugs (at a different pH).[5,6] Extrapolation of the Arrhenius plot to a temperature of 4°C gives a k_{obs} of $3.3 \times 10^{-4}\,d^{-1}$ and a t_{90} of approximately 320 days at pH 7.4. At room temperature, the t_{90} will be around 26 days under these solution conditions.

The effect of the pH on the degradation kinetics of somatostatin at 60°C is shown in Figs. 7 and 8. The degradation followed pseudo first-order kinetics at all investigated pH values. The data obtained at pH 3.0–5.0 are somewhat less reliable because the degradation was recorded for only one half-life or less due to the good stability of the peptide. Both branches of the pH-rate profile are linear (basic part, $r^2 = 0.998$; acidic part, $r^2 = 0.998$), although three different buffer systems (effect of buffer species, Fig. 9) had to be used to cover the desired pH range. The slope of both branches is different from unity (pH 1.2–3.0, slope = −0.74; pH 4.0–9.0, slope = 0.56), indicating general acid and base catalysis (also confirmed by the accelerating effect of the buffer concentration, as shown in Fig. 9), the pH stability optimum was found around pH 3.7. The optimum is remarkably well

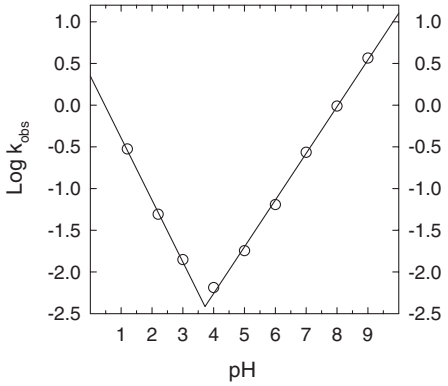

Figure 8. pH/rate profile of somatostatin at 60°C.

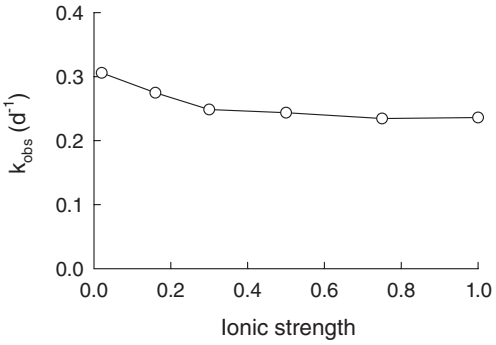

Figure 9. Effect of the ionic strength on the degradation rate constant of somatostatin in phosphate buffer 0.01 M, pH 7.4 at 60°C (ionic strength adjusted with NaCl).

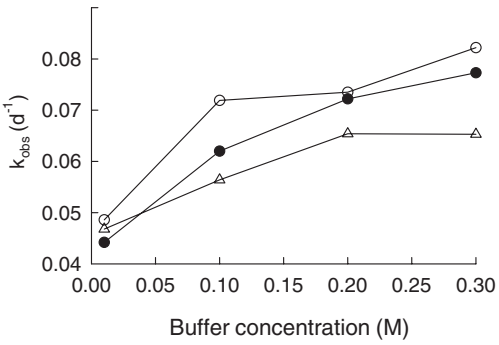

Figure 10. Effect of the buffer type and buffer concentration on the degradation rate constant of somatostatin at pH 6.0 and 60°C. (○) Phosphate buffer, (●) citrate/phosphate buffer, (△) acetate buffer.

defined, solvent catalysis seems, therefore, not very pronounced. This pH-stability optimum is similar to values reported in the literature for other peptides, which usually are found between pH 3–5 (salmon calcitonin, pH 3.3[4]; triptorelin, gonadorelin,[5] LH-RH,[8] nafarelin,[9] and octastatin[13] pH 4–5). Depending on the buffer type and the peptide drug, the profiles differ in shape significantly[10,13] and are often less pronounced.

Figure 9 shows the effect of the ionic strength on the degradation rate constants in 0.01 M phosphate buffer at 60°C and pH 7.4. The overall influence is small the degradation is slightly faster at a lower ionic strength.

No significant effect of the buffer type was found at a buffer concentration of 0.01 M at pH 6.0 (k_{obs} listed in Table 2). Increasing buffer concentrations accelerated the degradation, acetate buffer being less detrimental than citric acid/phosphate and phosphate buffers. A nonlinear relationship between the buffer concentration and k_{obs} was observed, which is probably due to the fact that the buffer concentrations used in our study are higher compared with other reports.[5,6,10,17]

No attempts were made to identify the structure of the degradation products, however, oxidation of the disulfide bridge could be ruled out as a major degradation pathway, since incubating the peptide with dithiothreitol (60°C, 15 min, pH 7.4, somatostatin/DTT ratio 1/5) resulted in a shift of the single peak of nondegraded somatostatin from a retention time of 15.6 min to single peak at 18.3 min. This peak was never seen in any of the degradation experiments. The main decomposition product of partially degraded somatostatin samples eluted at a retention time of 17.2 min. This product is probably chemically not very much different from the original molecule. This peak was by far the largest degradation peak in all experiments where the pH was above the stability optimum. Below the pH 4, the degradation pattern was completely different, showing several small peaks, most of them having a shorter retention time than somatostatin. This could either be caused by a different degradation mechanism or by further decomposition of the main degradation product by hydrolysis. Deamidation is probably the most common mechanism in Asn- and Gln-containing peptides. It can follow pseudo first-order kinetics and is favored by high temperatures, high pH, and phosphate buffers.[17] These effects also have been found in this study.

In conclusion, this study shows that the degradation of somatostatin follows first-order kinetics with a pH-stability optimum around pH 3.7, The ionic strength and the drug concentration have virtually no effect on the degradation rate. As reported for

other peptide drugs, phosphate buffers and a pH above 5 are especially detrimental and should be avoided. Acetate buffer with a low buffer concentration is a favorable solvent. Solution properties and the experimental setup such as container material and sterile conditions need to be selected or maintained carefully in order to avoid accelerated degradation.

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REFERENCES

1. Reichlin, S.N. Eng. J. Med. **1983**, *309* (24), 1495–1501, 1556–1563.
2. Kompella, U.B; Lee, V.H.L. In *Peptide and Protein Drug Delivery*; Lee, V.H.L., Ed.; Marcel Dekker, Inc.: New York, 1991; 391–484.
3. Bhatt, N.P.; Patel, K.; Borchardt, R.T. Chemical pathways of peptide degradation. I. Deamidation of adrenocorticotropic hormone. Pharm. Res. **1990**, *7* (6), 593–599.
4. Lee, K.C.; Lee, Y.J.; Song, H.M.; Chun, C.J.; DeLuca, P.P. Degradation of synthetic salmon calcitonin in aqueous solution. Pharm. Res. **1992**, *9* (11), 1521–1523.
5. Helm, V.J.; Müller, B.W. Stability of gonadorelin and triptorelin in aqueous solution. Pharm. Res. **1990**, *7* (12), 1253–1256.
6. Hoitink, M.A.; Beijnen, J.H.; Bult, A.; Houwen van der, O.A.G.J.; Nijholt, J.; Underberg, W.J.M. Degradation kinetics of gonadorelin in aqueous solution. J. Pharm. Sci. **1996**, *85* (10), 1053–1059.
7. Brange, J.; Langkjær, L.; Havelund, S.; Vølund, A. Chemical stability of insulin. 1. Hydrolytic degradation during storage of pharmaceutical preparations. Pharm. Res. **1992**, *9* (6), 715–726.
8. Powell, M.F.; Sanders, L.M.; Rogerson, A.; Si, V. Parenteral peptide formulations: chemical and physical properties of native luteinizing hormone-releasing hormone (LHRH) and hydrophobic analogues in aqueous solution.
9. Johnson, D.M; Pritchard, R.A.; Taylor, W.F.; Conley, D.; Zuniga, G.; McGreevy, K.G. Degradation of the LH-RH analog nafarelin acetate in aqueous solution. Int. J. Pharm. **1986**, *31*, 125–129.
10. Tsuda, T.; Uchiyama, M.; Sato, T.; Yoshino, H.; Tsuchiya, Y.; Ishikawa, S.; Ohmae, M.; Watanabe, S.; Miyake Y. Mechanism and kinetics of secretin degradation in aqueous solution. J. Pharm. Sci. **1990**, *79* (3), 223–227.
11. Krishnamoorthy, R.; Mitra, A.K. Kinetics and mechanism of degradation of a cyclic hexapeptide (somatostatin analogue) in aqueous solution. Pharm. Res. **1992**, *9* (10), 1314–1320.
12. Pourrat, H.; Barthomeuf, C.; Pourrat, A.; Cottier, P.-E.; Ibrahim, H. Stabilization of octastatin a somatostatin analogue. Preparation of freeze-dried products for parenteral injection. Biol. Pharm. Bull. **1995**, *18* (5), 766–771.
13. Jang, S.W.; Woo, B.H.; Lee, J.T.; Moon, S.C.; Lee K.C.; DeLuca, P.P. Stability of octastatin, a somatostatin analog cyclic octapeptide, in aqueous solution. Pharmaceutical Development and Technology **1997**, *2* (4), 409–414.
14. Montoro, J.B.; Galard, R.; Catalan, R.; Martinez, J.; Salvador P.; Sabin, P. Stability of somatostatin in total parenteral nutrition. Pharm. Weekbl. Sci. **1990**, *12* (6), 240–242.
15. Merck, A.G. *Tables for the Chemical Laboratory*; Darmstadt, Germany.
16. Herrmann, J.; Bodmeier, R. Somatostatin containing biodegradable microspheres prepared by a modified solvent evaporation method based on W/O/W-multiple emulsions. Int. J. Pharm. **1995**, *126*, 129–138.
17. Patel, K.; Borchardt, R.T. Chemical pathways of peptide degradation. II Deamidation of an asparaginyl residue in a model hexapeptide. Pharm. Res. **1990**, *7* (6), 593–599.