Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 251 (2003) 99–106
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

A new stressed test to predict the foreign matter formation of minodronic acid in solution

Katsutoshi Nakamura [a,*], Shigeharu Yokohama [a], Masataka Katsuma [a], Toyohiro Sawada [a], Takashi Sonobe [b]

[a] *Pharmaceutical Technology Laboratories and Novel Pharmaceutical Laboratories, Institute for Drug Development and Research, Yamanouchi Pharmaceutical Co., Ltd. 180 Ozumi Yaizu-shi, Shizuoka-ken 425-0072, Japan*
[b] *School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan*

Received 5 July 2002; received in revised form 11 October 2002; accepted 19 October 2002

Abstract

A formulation containing 0.5 mg/ml minodronic acid, 40 mM citrate, pH 4.5, and sodium chloride, stored in regular flint glass ampoules, was stable without particulate increase under high temperature conditions, such as 40 °C for 6 months, or 50 or 60 °C for 3 months. However, when stored at 25 °C, there was an increase in ≥ 2 μm particles at the 5-month timepoint. This demonstrated that long-term stability cannot simply be predicted by the evaluation of samples just stored at higher temperatures. Therefore, a new stressed test was designed which is useful in the rapid selection of formulations that are stable and without particulate increase. Since the particulate matter is apparently a complex of minodronic acid and aluminum ions leaching from ampoules, samples were placed at 80 °C for up to 4 weeks to accelerate aluminum leaching. Although no particulate increase was observed directly after storage at 80 °C, 4 freeze–thaw cycles following the storage caused a drastic particulate increase. The evaluation of samples subjected to the freeze–thaw cycles indicated that the following formulation modifications have inhibitory effects on particulate generation: (1) addition of meglumine, diethanolamine, mannitol, or glycerol to the formulation; (2) increase of citric acid concentration; (3) decrease of minodronic acid concentration. These modifications also worked well for samples stored at 25 °C for 6 months, and particulate increase did not occur. This method is a powerful tool for predicting the stability of minodronic acid in solution.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Minodronic acid; Parenteral formulation; New stressed test; Particle; Complex; Aluminum ion

1. Introduction

To increase the efficiency of formulation studies ensuring the sufficient stability of finished products, pharmaceutical scientists frequently conduct 'accelerated testing' or 'stressed testing', placing a candidate formulation under exagger-

* Corresponding author. Tel.: +81-54-627-5110; fax: +81-54-629-7615
E-mail address: nakamurk@yamanouchi.co.jp (K. Nakamura).

0378-5173/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0378-5173(02)00594-X

ated storage conditions such as high temperatures. This testing is designed to increase the rate of chemical or physical degradation of drug products and quickly obtain information on stable formulations. Without the accelerated testing, the time-consuming, long-term testing of each formulation, stored under actual storage conditions, would be necessary, limiting the number of formulations screened.

Minodronic acid hydrate, [1-hydroxy-2-(imidazo[1,2-*a*]pyridin-3-yl)ethylidene] bisphosphonic acid monohydrate, is a new bisphosphonate that is expected to be clinically useful in the treatment of osteoporosis and hypercalcemia. In animal studies, this compound inhibits bone resorption with 100-fold greater potency than pamidronate (Kudo et al., 1992), and has 10-times greater efficacy than incadronate disodium, disodium cycloheptylaminomethylenediphosphonate monohydrate (Kudo et al., 1990, 1992).

In the process of formulation development, 'accelerated testing' was performed in order to gather the basic information on the stability of minodronic acid in solution. Preliminary studies indicated that 10 mM or higher citrate buffers, with pHs ranging from 3 to 5, optimized the stability of minodronic acid (Nakamura et al., 2001). Based on those results, a citrate buffer (40 mM, pH 4.5) formulation containing 0.5 mg/ml minodronic acid and sodium chloride to adjust the osmolarity was chosen and further evaluated under high temperature conditions, such as 40 °C for 6 months, or 50 or 60 °C for 3 months (Nakamura et al., 2002). These tests also demonstrated a good stability with no drug content loss and no increase in the amount of insoluble particles. It was thought that this formulation would also be stable at lower temperatures such as 25 °C, a standard storage condition for drug products during their distribution. Contrary to this expectation, the number of particles in the formulation increased at the 5-month timepoint when it was stored at 25 °C (Nakamura et al., 2002). The particulate matter was thought to be a complex between minodronic acid and aluminum ions apparently leaching from the surface of the glass ampoules, as other bisphosphonates form complexes with metal ions (Claessens and van der Linden, 1984; Lamson et al., 1984). It was suggested that the complex formation was exothermic and general 'accelerated testing' could not predict stability in terms of particulate generation.

Although there is some controversy about the clinical significance of particulate matter (Borchert et al., 1986), in general, a lower number of particles represents higher product quality (Gallelli and Groves, 1993). Needless to add, if the particles in the formulation further multiply and grow to a point of visual haze or precipitate, it is not acceptable at all.

The major purpose of the present study was to design a new stressed test so that stable formulations with no particulate increase, when they are stored at 25 °C, can be selected in a precise way. Secondly, strategies to reduce or inhibit particulate increase were investigated using the new stressed test.

2. Materials and methods

2.1. Materials

Minodronic acid hydrate was synthesized by Yamanouchi Pharmaceutical Co., Ltd. All other ingredients and reagents were of analytical grade.

Regular flint glass ampoules were obtained from Japan Glass Industry Co., Ltd (Tokyo, Japan). Daikyo Resin CZ vials and Formulation No. 777 rubber closures, which are made of plastic containing an extremely small amount of metal ions, were from Daikyo Seiko, Ltd (Tokyo, Japan).

2.2. Preparation of the minodronic acid solution

The necessary amount of each ingredient was dissolved in water for injection at approximately 80% of the final volume. The pH value of the formulation was adjusted to 4.5 with a 1 N sodium hydroxide solution. The minodronic acid solution was brought to the final volume with water and filter-sterilized through a 0.2 μm filter. The formulation was filled into clean containers (an

ampoule or a vial). The ampoules were hermetically sealed and the vials were stoppered with rubber closures and sealed with aluminum seals. Then, the samples were sterilized in an autoclave at 115 °C for 30 min.

2.3. Sample storage conditions and treatment to measure generated particulate

As a storage condition adopted regularly, samples were stored in a temperature-controlled incubator or in a room set at a designated temperature. At appropriate time intervals, the samples were withdrawn and, if necessary, allowed to stand until they reached room temperature before the analysis of the amount of particles in the samples.

To attain the rapid evaluation of formulation stability, some treatments after storage to detect generated particulate were examined. Sample shaking was performed with a mini SS-80S EYELA shaker (Tokyo Rikakikai Co. Ltd; Tokyo, Japan) placed in a room with a temperature maintained at around 5 °C. A freeze–thaw cycle of samples was conducted as follows: samples were frozen completely at −30 °C and then thawed at ambient temperature. This cycle was repeated if necessary. The samples were allowed to stand until they reached room temperature before the amount of particles in the samples was analyzed.

2.4. Particle counting

The number of particles in the minodronic acid samples was determined with a HIAC/ROYCO Model 4100 particle counter, a Model 3000 syringe controller, an HRLD-150 sensor (Pacific Scientific, Menlo Park, CA) and a DPU-411 Type II thermal printer (Seiko Instruments Inc., Chiba, Japan). A sample collection probe was inserted directly into each open container, and the solution within was drawn into the HIAC system and analyzed.

3. Results and discussion

3.1. Development of a new stressed test to choose stable formulations of minodronic acid in solution

As a major purpose of this study, a convenient new stressed test was designed to efficiently and reliably predict the tendency on particulate increase during 25 °C storage. Because a formulation containing 0.5 mg/ml minodronic acid, 40 mM citrate, pH 4.5, and sodium chloride, stored in regular flint glass ampoules, was stable without particulate increase under high temperature conditions. However, when stored at 25 °C, particles increased at the 5-month timepoint (Nakamura et al., 2001). In the process of formulation screening, unstable formulations should be accurately ruled out. However, 'accelerated testing' generally conducted was useless in these formulation studies.

Initially, the design was to use the unstable formulation mentioned above (the control sample) and explore the cause of the increase in particles. The particulate matter was apparently a complex of minodronic acid and aluminum ions, the latter thought to leach from glass ampoules (Hoiberg, 1989; Pavanetto et al., 1989; Nakamura et al., 2001). Since aluminum ions are one of the contributing factors in the generation of this complex, it was thought that enhancement of aluminum ion leaching would hasten the appearance of the complex. However, just storing the formulation at 80 °C for up to 4 weeks did not cause an increase in particulate matter (Table 1). Subsequent to the storage at 80 °C for 3 weeks, the samples were allowed to stand at 5 °C with or without shaking in hope of particulate increase. However, particles did not increase during a 14-day observation (Table 1).

However, interestingly enough, freeze–thaw cycles after 80 °C storage of samples worked well and induced a drastic particulate increase (Table 1). Five to six cycles of freeze–thaw were necessary to detect particulate increase in samples stored at 80 °C for 3 weeks. Regarding samples stored at 80 °C for 4 weeks, three to four cycles were enough to cause particulate increase.

In contrast, an increase in particulate matter was not observed in samples stored at 5 °C for 4

Table 1
The effect of sample handling after storage on the amount of ≥ 2 μm particles in minodronic acid solution stored in glass ampoules

Storage conditions	Sample handling after storage	Amount of particles
Initial	No treatment	−
5 °C, 4 weeks	4 F–T[a] cycles	−
25 °C, 6 months	No treatment	+
80 °C, 3 weeks	No treatment	−
	5 °C, 14 days	−
	5 °C, 14 days shaking	−
	3 F–T cycles	+
	5 F–T cycles	+++
	6 F–T cycles	+++
80 °C, 4 weeks	No treatment	−
	2 F–T cycles	++
	3 F–T cycles	+++
	4 F–T cycles	+++

The symbols indicate particle counts per milliliter as follows: −, 0–99; +, 100–999; ++, 1000–9999; +++, ≥10 000.
[a] F–T, freeze–thaw.

weeks, even though four cycles of freeze–thaw were conducted (Table 1). In addition, no particulate increase was detected in samples in plastic containers having an extremely low amount of metal, even though they were placed at 80 °C for 4 weeks and subsequently frozen and thawed four times (Table 2). These results demonstrated that the particulate formation in this system requires not only a sufficient amount of aluminum ions in solution but also the freeze–thaw cycles. The complex forcibly created by this stressed test might not be exactly the same with the complex appeared during the 25 °C storage. However, the both complexes have similar chemical compositions containing aluminum ions that were leached from glass containers and, naturally, it is temperature dependent. It was suggested that the release of aluminum ions from glass ampoules is the key factor in forming the particulate matter both at 25 °C and elevated stressed condition.

Table 2
The amount of ≥2 μm particles in minodronic acid solution stored in plastic containers

Storage conditions	Sample handling after storage	Amount of particles
Initial	No treatment	−
25 °C, 6 months	No treatment	−
80 °C, 4 weeks	4 F–T[a] cycles	−

The symbols indicate particle counts per milliliter as follows: −, 0–99; +, 100–999; ++, 1000–9999; +++, ≥10 000.
[a] F–T, freeze–thaw.

One remaining question is why the complex could appear as detectable particles when the freeze–thaw cycles were performed. One speculation is that the reaction causing complex formation was enhanced at low temperatures because at high temperatures aluminum ions were released from glass containers but the complex was least formed due to its exothermal nature (Nakamura et al., 2002). Therefore, −30 °C might be low enough to allow the reaction among minodronic acid and aluminum ions that were leached at the higher temperature. Additionally, the repetition of the freeze–thaw cycle might stimulate complex formation through the phase change between solid and liquid.

Taking the results into account, it was decided that foreign matter stability of formulations should be evaluated by the above new stressed test: after four cycles of freeze–thaw following the storage at 80 °C for 4 weeks. After the four cycles, the amount of generated particulate reached almost constant. Then, stability formulation screening was conducted at the four cycles condition.

The amount of particles in the control sample increased markedly after subjecting it to the new stressed test. The amount of particles detected by this method was much larger than that detected in samples stored at 25 °C for 6 months (Table 1), probably because at higher temperature more aluminum ions were leached from the ampoules. This implies that the way the sample is handled following the new stressed test is more stressful to formulations than sample storage at 25 °C. Therefore, there is a probability that formulations ruled out as unstable by the new stressed test might be stable if stored at 25 °C. However, it is thought that the formulations that can withstand this stressful test are more robust than those that cannot withstand it.

3.2. Inhibitory effect on particulate generation in minodronic acid solutions

As the second purpose of this study, stable formulations were investigated through the new stressed test. The stability of formulations to be examined was compared to the unstable formulation in flint ampoules containing 0.5 mg/ml minodronic acid, 40 mM citrate, pH 4.5, and sodium chloride to adjust the osmolarity (the control sample).

3.2.1. Addition of various ingredients to the minodronic acid solutions

For the initial test, the sodium chloride used to adjust the osmolarity was not added to the formulation (the citrate formulation), and the amount of particles was counted after the new stressed test. The particulate matter in this solution increased in the same manner (Table 3). The results show that the particulate increase occurs irrespective of the existence of sodium chloride in solutions.

Next, separate formulations were made by adding the following ingredients to the citrate formulation: arginine HCl, sodium gluconate, sucrose, meglumine, diethanolamine, mannitol, and glycerol (Table 3). In solutions containing ariginine HCl, the amount of particles increased drastically after the new stressed test. The formulations containing sodium gluconate or sucrose became discolored after storage at 80 °C for 4 weeks, without being subjected to any freeze–thaw cycles. Therefore, these two formulations were not evaluated for particulate matter. In contrast, there was little, if any, increase in the amount of

Table 3
Preventive effect of various ingredients on generation of ≥2 μm particles in minodronic acid solutions

Component	Sample handling	Amount of particles
Citrate+NaCl 0.7% (w/v) (control sample)	Initial	−
	80 °C, 4 weeks+4 F–T[a] cycles	+++
	25 °C, 6 months	+
Citrate	Initial	−
	80 °C, 4 weeks+4 F–T cycles	+++
Citrate+arginine HCl 40 mM	Initial	−
	80 °C, 4 weeks+4 F–T cycles	+++
Citrate+sodium gluconate 40 mM	Initial	−
	80 °C, 4 weeks	(discolored)
Citrate+sucrose 6.8% (w/v)	Initial	−
	80 °C, 4 weeks	(discolored)
Citrate+meglumine 40 mM	Initial	−
	80 °C, 4 weeks+4 F–T cycles	−
	25 °C, 6 months	−
Citrate+diethanolamine 40 mM	Initial	−
	80 °C, 4 weeks+4 F–T cycles	−
	25 °C, 6 months	−
Citrate+mannitol 3.7% (w/v)	Initial	−
	80 °C, 4 weeks+4 F–T cycles	+
	25 °C, 6 months	−
Citrate+glycerol 1.9% (w/v)	Initial	−
	80 °C, 4 weeks+4 F–T cycles	−
	25 °C, 6 months	−

The symbols indicate particle counts per milliliter as follows: −, 0–99; +, 100–999; ++, 1000–9999; +++, ≥10 000.
[a] F–T, freeze–thaw.

particles in solutions containing meglumine, diethanolamine, mannitol, or glycerol (Table 3). It is assumed that the inhibitory effects exerted by meglumine and diethanolamine might be derived from the speculation that the positively charged amines of these two ingredients and the negatively charged phosphate groups of minodronic acid electrostatically interact and block the access of aluminum ions to minodronic acid. Regarding the inhibitory effect of mannitol and glycerol on particulate generation, it is known that mannitol forms complexes with Fe, Al and Cu and that sorbitol, an isomer of mannitol, also forms chelates with many di- and trivalent metal ions (Kibbe, 2000). Therefore, these compounds or polyols might form complexes with aluminum ions and sequester aluminum ions from minodronic acid.

The formulations containing meglumine, diethanolamine, mannitol, or glycerol were allowed to stand at 25 °C and the amounts of particles were examined (Table 3). No particulate increase was observed after storage at 25 °C for 6 months. In terms of particulate increase, the results obtained from the new stressed test correlate well with those obtained from samples stored at 25 °C. Consequently, it is concluded that this new stressed test accurately predict promising formulations in a shorter period of time than currently used accelerated testing.

Table 4
Preventive effect of citrate concentrations on generation of ≥2 μm particles in minodronic acid solutions

Component	Sample handling	Amount of particles
Citrate 40 mM + NaCl 0.7% (w/v) (control sample)	Initial	−
	80 °C, 4 weeks + 4 F–T[a] cycles	+++
	25 °C, 6 months	+
Citrate 100 mM + NaCl 0.2% (w/v)	Initial	−
	80 °C, 4 weeks + 4 F–T cycles	−
	25 °C, 6 months	−

The symbols indicate particle counts per milliliter as follows: −, 0–99; +, 100–999; ++, 1000–9999; +++, ≥10 000.
[a] F–T, freeze–thaw.

3.2.2. *Increase of citrate concentration and decrease of minodronic acid concentration in the formulation*

The citrate concentration of the formulation was increased to 100 mM and the effect on particulate generation was evaluated. In this case, the concentration of sodium chloride was also adjusted so that the osmolarity of the

Table 5
Effect of minodronic acid concentrations on generation of ≥2 μm particles in minodronic acid solutions containing 40 mM citrate and 0.7% sodium chloride (pH 4.5)

Minodronic acid concentration	Sample handling	Amount of particles
0.20 mg/ml	Initial	−
	80 °C, 4 weeks + 4 F–T[a] cycles	−
	25 °C, 6 months	−
0.30 mg/ml	Initial	−
	80 °C, 4 weeks + 4 F–T cycles	−
	25 °C, 6 months	−
0.35 mg/ml	Initial	−
	80 °C, 4 weeks + 4 F–T cycles	−
	25 °C, 6 months	−
0.40 mg/ml	Initial	−
	80 °C, 4 weeks + 4 F–T cycles	+
	25 °C, 6 months	−
0.45 mg/ml	Initial	−
	80 °C, 4 weeks + 4 F–T cycles	++
	25 °C, 6 months	−
0.50 mg/ml (control sample)	Initial	−
	80 °C, 4 weeks + 4 F–T cycles	+++
	25 °C, 6 months	+
1 mg/ml	Initial	−
	80 °C, 4 weeks + 4 F–T cycles	+++
	25 °C, 6 months	++

The symbols indicate particle counts per milliliter as follows: −, 0–99; +, 100–999; ++, 1000–9999; +++, ≥10 000.
[a] F–T, freeze–thaw.

formulation was similar to human blood. It was found by the new stressed test that particulate generation was suppressed by increasing the citrate concentration (Table 4). The increased citrate concentration also worked for samples placed at 25 °C for 6 months and no particulate increase was detected. It was speculated that citrate exerts a chelating effect (Hasegawa et al., 1982a,b,c,d,e) and stabilizes minodronic acid by scavenging aluminum ions from the solution.

Regarding the quantitative components used for the control sample, the concentrations of minodronic acid were gradually changed and evaluated for the tendency to generate particles (Table 5). Based on the results of the new stressed test, the particulate generation was suppressed by decreasing the concentrations of minodronic acid. Similarly, this was confirmed in samples stored at 25 °C for 6 months. It was found that decreasing the concentration of minodronic acid in the solutions generated less particulate matter.

4. Conclusions

A particulate increase was observed in the formulation containing 0.5 mg/ml minodronic acid, 40 mM citrate, pH 4.5, and sodium chloride after storage in regular flint glass ampoules for 5 months at 25 °C, even though it was stable without particulate increase under higher temperature conditions of up to 60 °C. A new stressed test was designed that is useful for rapid and accurate selection of stable formulations. No particulate increase was observed just after storage at a much higher temperature of 80 °C. Next, following storage at 80 °C, samples were allowed to stand at 5 °C with or without shaking. However, no particulate increase was detected. Further investigation revealed that freeze–thaw cycles following the storage at 80 °C caused a drastic particulate increase. Therefore, formulations were evaluated after four freeze–thaw cycles following the storage of samples at 80 °C for 4 weeks. As a result, it was demonstrated that the following formulation modifications have preventive effects on particulate generation: (1) addition of meglumine, diethanolamine, mannitol, or glycerol to the formulation; (2) increase of citric acid concentration; (3) decrease of minodronic acid concentration. These modifications worked well and no particulate increase was recognized even after 6 months at 25 °C. This prediction method is useful for choosing promising formulations efficiently.

References

Borchert, S.J., Abe, A., Aldrich, D.S., Fox, L.E., Freeman, J.E., White, R.D., 1986. Particulate matter in parenteral products: a review. J. Parenter. Sci. Technol. 40, 212–241.

Claessens, R.A.M.J., van der Linden, J.G.M., 1984. Stability constants of tin(II) and calcium diphosphonate complexes. J. Inorg. Biochem. 21, 73–82.

Gallelli, J.F., Groves, M.J., 1993. USP perspective on particle contamination of injectable products. J. Parenter. Sci. Technol. 47, 289–292.

Hasegawa, K., Hashi, K., Okada, R., 1982a. Physicochemical stability of pharmaceutical phosphate buffer solutions. I. Complexation behavior of Ca(II) with additives in phosphate buffer solutions. J. Parenter. Sci. Technol. 36, 128–133.

Hasegawa, K., Hashi, K., Okada, R., 1982b. Physicochemical stability of pharmaceutical phosphate buffer solutions II. Complexation behavior of Al(III) with additives in phosphate buffer solutions. J. Parenter. Sci. Technol. 36, 168–173.

Hasegawa, K., Hashi, K., Okada, R., 1982c. Physicochemical stability of pharmaceutical phosphate buffer solutions III. Gel filtration chromatography of Al(III) complex formed in phosphate buffer solutions. J. Parenter. Sci. Technol. 36, 174–178.

Hasegawa, K., Hashi, K., Okada, R., 1982d. Physicochemical stability of pharmaceutical phosphate buffer solutions IV. Prevention of precipitation in parenteral phosphate solutions. J. Parenter. Sci. Technol. 36, 210–215.

Hasegawa, K., Hashi, K., Okada, R., 1982e. Physicochemical stability of pharmaceutical phosphate buffer solutions V. Precipitation behavior in phosphate buffer solutions. J. Parenter. Sci. Technol. 37, 38–45.

Hoiberg, C.P., 1989. Aluminum in parenteral products: overview of chemistry concerns and regulatory actions. J. Parenter. Sci. Technol. 43, 127–131.

Kibbe, A.H., 2000. Handbook of Pharmaceutical Excipients, third ed.. American Pharmaceutical Association; Pharmaceutical Press, Washington, DC; London, UK, p. 327, 517.

Kudo, M., Abe, T., Motoie, H., Nagao, Y., Kawamuki, K., Ouchi, N., Kawashima, H., 1992. Pharmacological profile of new bisphosphonate, 1-hydroxy-2-(imidazo[1,2-*a*]pyridin-3-yl)ethane-1,1-bis(phosphonic acid). Bone Miner. 17, 170.

Kudo, M., Abe, T., Kawamuki, K., Yamaoka, E., Isomura, Y., Takeuchi, M., Kawashima, H., 1990. Effect of YM175 on experimental hypercalcemia and tumor-induced osteolysis in rats. J. Bone Miner. Res. 5, S166.

Lamson, M.L., Fox, J.L., Higuchi, W.I., 1984. Calcium and 1-hydroxyethylidene-1,1-bisphosphonic acid: polynuclear complex formation in the physiological range of pH. Int. J. Pharm. 21, 143–154.

Nakamura, K., Tanaka, T., Saito, K., Yokohama, S., Sonobe, T., 2001. Stabilization of minodronic acid in aqueous solution for parenteral formulation. Int. J. Pharm. 222, 91–99.

Nakamura, K., Yokohama, S., Sonobe, T., 2002. Failure of stability prediction for minodronic acid injectable by accelerated stability testing. Int. J. Pharm. 241 , 65–71.

Pavanetto, F., Genta, I., Conti, B., Modena, T., Montanari, L., 1989. Aluminum, cadmium and lead in large volume parenterals: contamination levels and sources. Int. J. Pharm. 54, 143–148.